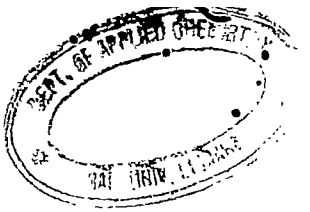
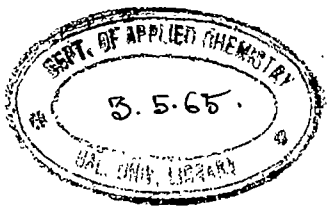
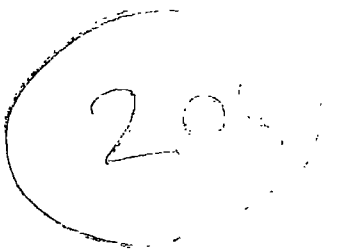
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NATURE

A WEEKLY JOURNAL OF SCIENCE







Volume 204

OCTOBER 3, 1964, to DECEMBER 26, 1964

LONDON

MACMILLAN (JOURNALS), LTD.

NEW YORK: ST. MARTIN'S PRESS, INC.



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Printed in Great Britain by FISHER KNIGHT & CO. LTD., The Gainsborough Press, St. Albans,
and published by MACMILLAN (JOURNALS), LTD., at St. Martin's Street, London, W.C.2,
and ST. MARTIN'S PRESS, INC., 175 Fifth Avenue, New York 10010, N.Y., U.S.A.
Saturday, March 20, 1965

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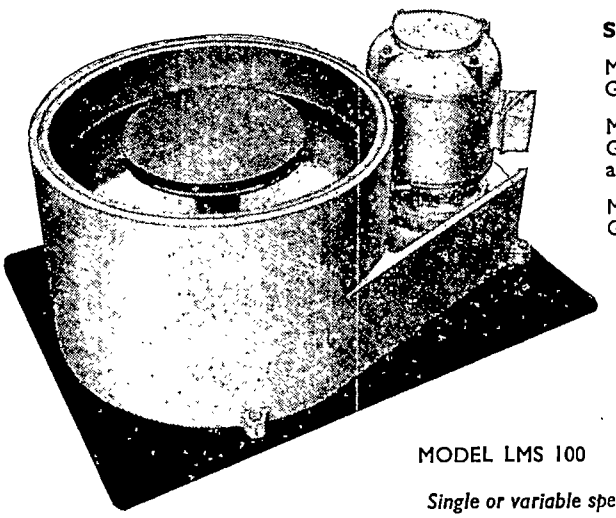
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A NEW IMAGE FOR LIBRARY SERVICES

THE first research report to be published by the Research Institute for Consumer Affairs is not, as its title might suggest, about the London Library but about the use which is made by the Londoner of seven borough libraries—those of Chelsea, Finsbury, Hampstead, Lewisham, Stepney, Stoke Newington and Wandsworth—chosen to represent the metropolis as a whole*. It is thus essentially a complement to the Roberts Report on the Public Library Service, and to the subsequent report of the Working Party on Standards of Public Library Service, dealing with the subject from the point of view of the user (*Nature*, 200, 615; 1963). Its ultimate recommendations are based on the assumption that an efficient library service should concern itself with the kinds of people whose needs are to be met, the numbers who are being and who should be served, and the way in which the service is working in practice. Librarians are likely to reach more people more effectively if they are guided by knowledge about the people they are serving or failing to serve.

In a statement sent to the Ministry of Education, the Association of Special Libraries and Information Bureaux noted that the Roberts Committee and the Working Party all assumed in their reports that the purpose of the whole library system is to satisfy readers, and observed that little attempt seems to have been made to find out how far readers are, in fact, satisfied. Mr. B. Groombridge, in his report, bases his findings on the facts elicited from a sample of more than 1,300 adult Londoners, and the core of the report deals with a representative 500 of these. It recognizes that over the past forty years substantial progress has been made in the extent and the standards of public library service, and that even to raise standards throughout Britain to a level comparable with that achieved by the seven boroughs surveyed would, in many areas, represent substantial progress.

Nevertheless, while the popular prestige of public libraries is immense, the present demands made on them are relatively light, and the regard in which they are held should be taken as an asset, as goodwill with which to make more progress, rather than as a sign that enough has been achieved. Apart from the fact that illiteracy prevents many people from using what libraries have to offer, nearly half the adult population have never been enrolled as members of a public library and actual users probably number no more than one in three. Moreover, the libraries' potential public is limited by the stimulus or the damage that it has received from other sectors of social life, and it should also be remembered that to go regularly to a public library even to borrow light fiction is to exercise more initiative and to show more cultural resourcefulness than most people do.

Mr. Groombridge accordingly maintains that, while possibly making public libraries more efficient, too much concern with standards and too little with the people for whom the service is intended is not likely to make them more effective. Concern for the extent and nature of holdings, for example, must be balanced by some regard to issues per head because that is a criterion of the service relation between library and user. A public library in its truest sense should reflect the heterogeneity of the public to be served, and not only supply the needs of a few special sections of that public. It may well be expected to have regard to the needs of the student population, for example, but it must be able and ready to respond to the increasing demands which the decrease of illiteracy and the expansion of further education are certain to bring.

From the point of view of public awareness, Mr. Groombridge puts publicity first. A high standard of public service may be the best form of advertisement for any library, but the fact remains that many potential readers have no idea what the public library has to offer them, or even of its existence. A continuing and many-sided publicity campaign is plainly needed. Librarians cannot afford to neglect any device aimed at making choice more efficient, or at making their professional knowledge fully and easily available to the people who read. The, he thinks, may be the biggest single transformation needed to increase the effective contact of libraries with the public.

With this more creative and less bureaucratic relation between the library staff and the public, added to the existing goodwill, the question of stock is likely to be approached in the right way. No worse criticism could be made of a public library than that the books a reader wants are not available from it, but Mr. Groombridge found that nearly a quarter of those who had ceased to use a public library had done so for this reason, and a third of those using the borough libraries made this criticism to a lesser or greater degree. This, moreover, is the essential test of efficiency that should be applied to any branch library, but making allowances for the obvious limitations of such a unit. Again, on the question of reference, it is not simply a matter of holdings but of the attitude and availability of the library staff, and the establishment of the more creative relation already suggested could be a decisive factor.

While finance is important, it is not the sole or, possibly, even the most decisive factor, and Mr. Groombridge makes other suggestions which could help to bring libraries more effectively before the public. The conception that libraries are for readers is fundamental, but libraries should be wary of the assumption that readers must of necessity go to the library itself. Already some services are rendered to local voluntary groups, or to dependent groups such as the elderly and the housebound, but a more imaginative look at such extra-mural services, particularly in the context of expanding further education, is plainly desirable. Libraries cannot, indeed, meet these wider needs without more money for staff as well as for books, and on a scale which will enable them to attract and retain professional staff of the calibre needed. Whether, in the end, public libraries will be able to nourish adequately the literacy of a society in which standards of education are steadily rising will ultimately depend not on the imagination and ability of the professional librarian but on the standards and resources which the public are willing to provide.

It should not be overlooked that the survey on which Mr. Groombridge's report is based was supported by a

* *The Londoner and His Library.* By Brian Groombridge. Pp. viii + 124. (London: The Research Institute for Consumer Affairs, 1964.) 15*s*.

grant from the Library Association. The discussion at the annual conference of the Library Association at Birmingham on July 6 on "Library Services for Students" is closely related to the general theme of the report. This is especially true of the papers dealing with students' needs by Mr. A. Rhys Hughes, and with public libraries by Mr. E. A. Clough. It is also true to some extent of the other two papers—Mr. J. A. Dearden considered "Further Education College Libraries", and Mr. D. T. Ricknell discussed the memorandum of evidence submitted by the Library Association in February to the Committee on Libraries set up by the University Grants Committee and the Minister of Education.

Mr. Rhys Hughes, as president of the National Union of Students, was concerned with presenting the student's point of view. While challenging the view that all students could spend £30 each year on books, he suggested that some students might welcome more tutorial advice on buying books; he also stressed the importance of residential provision in relation to the use of libraries. His comments on guidance and on library hours were constructive and should be noted by the Committee on Libraries. He did not regard it as sound that the local public library should, during university vacation, be the student's main source of books. This is something that might well be examined as a particular aspect of library co-operation, both between university libraries and between these and the public libraries.

Mr. Clough was concerned with the magnitude of the potential demand on public libraries, not simply that coming from university or college students, but from the part-time students and private-study students whose numbers may well approach a million within the next twenty years. There is no doubt that both in London and the provinces, public library authorities must increase their services to students, particularly their reference services, quite apart from a possible doubling of the library services in the universities and colleges, and this expansion should be started now. Public library services are an important part of the educational fabric of Britain and this should be recognized at the national as well as at the local level. Moreover, as Mr. Clough observed, what is needed is an immediate increase, of the order of £1·5 million a year, for the provision of reference library services and other services to students.

Mr. Dearden was content to indicate how the libraries of the colleges of further education, including those of the teacher training colleges, fit into the general pattern, and how they are affected by the standards prevailing elsewhere, both in provision of books and services and in respect of salaries of staff. The problem has to be seen as a whole and the contribution which any particular section makes should not be considered in isolation. It is perhaps from this point of view that the memorandum submitted by the Library Association and discussed by Mr. Ricknell is of prime importance.

Much of this memorandum is concerned with the points made by Mr. Hughes, Mr. Clough and Mr. Groombridge, and it demonstrates convincingly the way in which the university libraries and the service they render are linked with the library services in Britain. It is recognized that the prime function of the library in an institution of higher education is to support the teaching and research of the institution itself and that the service customarily given to others should not be allowed to override this prime purpose. Nevertheless, the value of the technical advisory services offered to advanced research workers by industrial libraries and others is noted in the Library Association's memorandum. The memorandum suggests that, although a considerable specialist staff would be needed, such technical information services should receive very high priority in the colleges of advanced technology.

The memorandum consists of replies to some twenty-four questions put by the Committee on Libraries, six of which are specifically concerned with the provision of books for undergraduate students. Here, Mr. Ricknell points out that the old conviction that students should buy their own text-books and that the university or college library should not multiply copies of standard works is no longer tenable and flies in the face of common sense. The difficulty is that inadequate book funds may compel libraries to curtail the range of their research material if a large part of this fund has to be used for duplicate copies of standard material. The Library Association believes that students should be encouraged to buy a reasonable number of essential books for themselves, including the prescribed texts. Moreover, it directs attention to the value of improved reading lists provided by the teaching staff and of an officially recognized bookshop on the campus. The Association also suggests that the whole system of students' grants for books should be reinvestigated, but, apart from a reservation on the introduction of earmarked book tokens, Mr. Ricknell adds nothing beyond the suggestion of a controlled experiment in the use of a subscription library of text-books in at least one university.

The Library Association insists that undergraduates should be trained to use the main collection in the university library, whatever special provision is made for their needs. It puts all its experience and authority behind the centralization of book selection and acquisition within a university in the university library, and questions the relative value of departmental libraries and of those at the halls of residence. Not surprisingly the memorandum comments trenchantly on the provision of seating accommodation in university libraries and on hours of opening. It suggests that there should be accommodation for not less than 25 per cent of student places, and attributes the overcrowding of public libraries by students to shortcomings in the accommodation and accessibility of the university and college libraries.

There is also a reference to the vacation problem, on which the Committee on Libraries put a leading question, though here, again, there is still the problem of adequate reading space. Public libraries recognize that they have a part to play in meeting students' needs for both reference and lending, especially during vacation, but universities should also do all they can in this respect, if necessary by extending the opening hours of their reading rooms during vacation. Mr. Hughes suggested that reciprocal arrangements for vacation reading might be made between universities and other institutions of higher learning, and between the university attended by a student and that of his or her home town or area.

The extension of opening hours is linked with the provision of adequate staff, and Mr. Ricknell also points out that it is also linked with the provision of canteen facilities. Once again we are back to adequate finance and, in general, the memorandum from the Association fully supports the argument of the memorandum from the librarians of the new universities as to the need for much more generous provision for both books and periodicals. Even for a university of 3,000 undergraduates, 700 post-graduates and 350 academic staff it visualizes a basic library stock of at least 25,000 volumes, involving a capital

expenditure of some £500,000, and annual expenditure on acquisitions of at least £37,500.

Coming so soon after the memorandum from the librarians of the new universities, this independent, but authoritative, endorsement of the bulk of the argument of that memorandum is impressive. It demonstrates convincingly that what we should be looking for is not economy but an assurance that the provision being made for the academic libraries, whether in the new universities, the established institutions, or in the colleges of technology, is sufficiently generous, alike in respect of books, of periodicals, of accommodation and of staff and their terms of service. The range and scope of the questions put to the Library Association by the Committee on Libraries at least suggest that that Committee is now aware of the magnitude of the issues. The advice it has already received should go far to ensure that an imaginative report is forthcoming, with recommendations meeting the situation, provided that the Committee is sufficiently resolute to grasp the opportunities confronting it.

MAN'S DEVELOPING DILEMMA

Automation and the Future of Man

By S. Demczynski. Pp. 238. (London: George Allen and Unwin, Ltd., 1964.) 32*s*.

THE author of this book is obviously a well-read and highly intelligent man. His experience as a prisoner in a forced labour camp behind the Iron Curtain and his present occupation with automatic machinery and data processing equipment have caused him to ponder deeply on the problem of the moral nature of man. For, if it were true that future developments in cybernetics would reduce almost to nothing the distinction between the functioning of man and of machines and if, as seems likely by the end of the century, life were to be produced in a test-tube, does this not spell the beginning of the end of man's distinctive position in the world? Moreover, will not the increasing tendency, especially in the social sciences, to interpret human behaviour in deterministic terms destroy the basis for any moral code?

It is a pity that the author should have chosen such a cliché for a title and that he should have tried to cover so vast a field. The result is inevitably patchy. By far the best chapters are in the second part in which he considers the problems of free-will and determinism and of the relationship between mind and brain in the light of modern scientific knowledge.

Mr. Demczynski's solution to the dilemma posed by our increasing knowledge of the physiological processes of thought is the concept of increasing complexity of the system. In a similar way as matter is now known to be transmutable into energy, so matter when connected intimately with the mind tends towards the formation of extremely complex systems and so changes its own nature. Such complex systems exhibit freedom of choice and therefore make value discussions meaningful. If this concept is to be accepted it has to overcome two obstacles which the author faces, but, not surprisingly, does not resolve. The first is that it is in contradiction to the second law of thermodynamics by which entropy increases. His only answer to this is to suggest that there exists an opposing tendency in Nature corresponding to the Freudian concept of the will to live in contrast to the will to destroy and die. But this is a metaphysical concept which still leaves us seeking for a first cause for which the non-religous philosophers of the world have been searching through the centuries. The second obstacle is the problem posed by the increasing complexity of machines themselves. Here his solution is itself contradictory, for while he admits that machines may have the potential possibilities of men he believes that in some way this admission will enable the capacities of men to be clearly seen as superior. The comparison of man as he is with the best machines now available can only raise the status of the former.

These ideas are still a long way from the possibility of scientific investigation and they must remain a matter of belief and related as such to personal values. The author is a convinced humanist. To defy rationality, he writes, is to condemn in the end the whole effort of human civilization. Attracted to existentialism because of its support for the individual's freedom of will, he nevertheless realizes that a socially useful ethic cannot be built on the basis of complete subjectivity.

In the end his logical thinking joins his generous emotions in producing a dislike of a society the only motive of which is economic gain. This leads him to socialism and the need for planning.

Unfortunately, just when his industrial experience and analytical mind could have made a real contribution to the practical problems of society in an age of rapid technological change, the argument becomes discursive and leads to some rather unthought-out conclusions: for example, that government should be in the hands of an educated and socially conscious oligarchy—meaning the professional middle class.

Although he appears to believe that there is nothing to be hoped for between total disarmament and total destruction he dismisses the "few hundred thousand deaths due to increased leukæmia" caused by fall-out from nuclear tests, with very little argument, as necessary for the preservation of national independence and the Western way of living. All this is much beneath the level of the rest of this interesting attempt to face some of the philosophical problems raised by man's excessive ingenuity.

AUSTEN ALBU

SOCIAL CHANGE

On the Theory of Social Change: How Economic Growth Begins

By Prof. Everett E. Hagen. (Study from the Center for International Studies, Massachusetts Institute of Technology.) Pp. xvii+557. (London: Tavistock Publications (1959), Ltd., 1964.) 63*s*.

THE sub-title of this book is: "How Economic Growth Begins", and the author is concerned with a hypothesis about the changes that take place as a 'traditional' society moves towards modern entrepreneurial methods. What factors promote and what factors impede such a process?

So far as impediments are concerned, Prof. Hagen dismisses the familiar economic arguments—inability to save, lack of capital, lack of 'know-how', lack of markets and inadequate transportation. His arguments are compelling and he adduces evidence to show that economic growth has in fact taken place in the absence of these advantages. Of course the presence of adequate markets, adequate transport systems, capital and the rest of it are a help, but if they are provided from outside sources they will be useless unless the inhabitants for whose use they are intended have the right attitude of mind. His argument is similar to that of McClelland, who has put forward the view that material aid to underdeveloped countries is far less important than the inculcation of the 'achievement motive'.

Prof. Hagen's thesis involves a more complicated analysis. Underdeveloped countries have a 'traditional' culture, which is passed on from generation to generation by their child-rearing practices. This is treated in some detail; the upshot is that children are brought up in an 'authoritarian' atmosphere, in which they learn the rules

of their society from dominating adults, whose ways seem to them to be unpredictable. Indeed, the adults themselves feel surrounded by mysterious forces over which they have no control. All this makes the traditional way of life important as a safeguard against ever-present threats. In such a world, how can one dare to innovate?

The innovator, on the other hand, must be brought up by nurturing parents, so that he will have a sense of security and will find the world a predictable and orderly affair which he can manipulate with impunity—he has, after all, found in his own experience as a child that he can bring about minor changes in a predictable and desired direction by means of his own efforts.

It will be seen that Prof. Hagen draws heavily on the work of those social anthropologists who relate culture patterns to child-rearing methods, and this is a central part of his theory.

Now the traditional type of culture is stable; it has lasted so long in its various forms because it caters for the psychological needs of those who 'carry' it. How then can change come about? Here we come to the second part of Prof. Hagen's hypothesis. In a traditional society everyone has his place and a status, however lowly it may be, which is respected. Change comes when some section of the society has the respect withdrawn to which it feels entitled. The reaction to this withdrawal of respect is varied, but a broad pattern emerges. The members of the affronted group—the men, that is to say—feel angry, dejected and apathetic. The old culture has let them down, so they 'retreat'. The only way they can retain some status is by behaving more dictatorially in their homes, but this is a hollow gesture. Their children and their wives see through it. After a generation or so the women seem to play a part. Their nurturance becomes more important than the inculcation of obedience to a failure. They tend their children with care and affection, partly because they are disenchanted with their fathers and husbands. Thus the stage of childhood security is set and creativity becomes possible.

Such is the theory, and now several case studies are examined in the light of it. The course of English history is outlined and attention is directed to the fact that in the fifteenth century the upper classes started to withdraw respect from the common people, and evidence is produced to show that many of the early innovators sprang from just that stock. Next Japan, where the Tokugawa Shogunate demoted several sections of Japanese society, and there is some evidence that it was from those circles that entrepreneurs derived. In Colombia it was the inhabitants of an unpromising area that have made the greatest strides, and they were despised, because they worked with their hands, by people who lived in other and more fertile districts. Clifford Geertz compares two Indonesian towns, and produces supporting evidence.

In the case of colonies the withdrawal of status respect may be said to be universal throughout the whole of the conquered community. The blow was shattering and the ensuing apathy traumatic. In some cases some of the indigenous population came to 'identify themselves with the aggressor' and ape his Western ways, but this was all on the surface, and they did not acquire—and did not want to acquire—the driving force which is needed for administrative competence. Burma is taken as a case in point, and the case of Burma is followed by an analysis of the Sioux on two reservations in America.

How far has the case been made out? The second part of the hypothesis is not rejected. It seems plausible and well worth pursuing. But this is only part, and in a sense a minor part, of the theory. To detect the social origins of the innovators is essential, of course, but according to the main theory evidence is required for a change in child-rearing practices, and this, alas, is not forthcoming. The only cases of which we have some information—Japan and Bali, where one of the Indonesian towns is situated—present difficulties. The Japanese boy, as described by Ruth Benedict, has a permissive childhood, but when he goes to school he finds that he has to preserve the 'face' of his family in a highly structured society; and the insecurity of the Balinese child, as described by Mead and Bateson, seems scarcely conducive to creativity, if Prof. Hagen's hypothesis is correct. Perhaps, of course, there have been changes since these investigations were made, but it is surprising, since he lays such store by child-rearing practices, that Prof. Hagen has not dealt with them in some detail.

This does not, of course, mean that he is wrong, and he confesses that we do not know enough about these matters. However, if a change in child-rearing practices is so important, and if such changes are, as they must be, quite apparent, it ought not to be difficult to get evidence from the underdeveloped countries that are now achieving independence. Prof. Hagen believes that several—as many as four—generations may have to pass before such changes bear fruit, but clearly the next step is to make careful investigations into changing methods of dealing with children, and the new nations, after all, provide laboratory conditions.

W. J. H. Sprott

AN ANALYSIS OF EMPATHY

On the Problem of Empathy

By Edith Stein. Translated by Waltraut Stein. Pp. xxi+113. (The Hague: Martinus Nijhoff, 1964.) 14.50 guilders.

IT is wholly impossible to give an adequate account of this book without some preliminary reference, of a biographical nature, to the remarkable woman who wrote it.

Edith Stein was a Jewess, born in Breslau in 1891. She became assistant to Edmund Husserl in 1916 when he was at Freiburg-im-Breisgau. The present work is a translation of her doctoral thesis, with the omission of the historical introduction. In 1922 she embraced the Roman Catholic faith, and ten years later became a Carmelite nun. She took refuge at Echt (Holland) during the Nazi persecutions, and perished at Auschwitz in 1942. Such in brief was the dramatic life of devotion of the authoress. Without some knowledge of this background a reading of these pages, difficult as they are, would be almost meaningless.

One might think that a definition or at least a description of empathy would occur early in the discussion, but this is not the case: probably in the academic tradition of the period it was needless for the scholars of phenomenology with whom Edith Stein was so closely associated. Philologically, empathy is an attempt to translate the German *einfühlung*, or 'feeling-in', roughly paralleled by sympathy or 'feeling-with', and it is just at this point that a basic divergence from normal psychological methodology occurs. We are asked to consider a fundamental distinction between the living body (*Leib*) and the material body (*Körper*). The former takes its place, so to say, as the origin of co-ordinates of the spiritual world, whereas the latter is apprehended as a physical entity.

It is manifest that deep thinking along these lines will throw up innumerable problems in natural philosophy as well as in the cultural sciences (*Geisteswissenschaften*), more particularly aesthetics. In fact, an earlier investigator, Theodor Lipps (1851–1914), had noted a tendency for a human subject to 'copy' the outstretched arms of a statue in an attempt to emphathize *in* (not *with*) the essence of the object revered. Further, the connexion with axiology is close, since value-judgments may enter at once when the empathic bond is firmly established.

In the light of these general considerations, the pattern of the dissertation may become a shade clearer. First

appears the essence of acts of empathy, then the constitution of the psycho-physical individual, and lastly, empathy as the comprehension of mental persons. To students of Husserl it will seem remarkable how well Edith Stein has mirrored the master's aversion from psychologism, namely the insidious intrusion of purely subjective tenets into domains essentially logical. This volume is not very likely to appeal to experimental scientists, but it has significance for them if they explore the field of 'personal knowledge' as Polanyi has conceived it.

On the level of human experience, there can be little doubt that the attainment of a high degree of empathy is something which can be recognized as akin to a species of genius. An obvious example is the fusion of the whole personality of a great actor with that of his hero, or even with situations encountered by him. Less potent, but equally intriguing, are the cases in which master and pupil have shared a common intellectual quest, reaching perhaps a common goal. In these instances agreement is not necessary at all stages of the journey; what is important is that the thread of 'in-feeling' should never be strained beyond the elastic limit. It appears that empathic potential can build up substantially, and maybe lead to a break-through in discovery, or to the birth of new ideas.

Even in the workaday world, history produces vestiges of empathic reactions when, for example, an artist has exceptional respect for and understanding of his materials and so is likely to bring about a result of greater sensibility than one lacking, or largely lacking, in this quality. The phrase 'mastery over materials' is often heard and is misleading if it connotes coercion into inappropriate uses. Indeed, one of the outstanding features of a great work of art is the perfect fitness of the substances involved in reaching the final cause (in the Aristotelian sense) which the artist had in mind.

In brief, the background of this investigation is strictly phenomenological, which, however, does not destroy—in fact it enhances—its value to the philosophical psychologist. It is impossible to waive (in the manner of Husserl's 'reduction') sensations. They are just as surely given as judging, willing and perceiving.

A word of appreciation is due to the translator, who is a grand-neice of Edith Stein. She has reproduced the spirit of the thesis well, although there are one or two lapses from idiomatic English. An excellent point is the marginal pagination of the original text. Edith Stein served with the Red Cross in World War I and experienced empathy in the field; she was to live to write her great *Kreuzeswissenschaft* later on, and to seal it with her death.

F. I. G. Rawlins

NUTRITIONAL VALUE OF PROTEINS

Valeur Nutritionnelle des Protéines

Symposium organisé le 15 Novembre 1962 sous l'Egide de la Délégation Générale à la Recherche Scientifique et Technique. (*Annales de Biologie Animale, Biochimie, Biophysique*, Vol. 3, Numéro Hors-série 1.) Pp. 133. (Paris: Institut National de la Recherche Agronomique, 1963.) 11.50 francs.

THIS small volume contains the papers read at a symposium held under the auspices of the Délégation Générale à la Recherche Scientifique et Technique in Paris during November 1962. It contains some twenty papers organized in seven sections, and reflects work carried out on nutritional aspects of proteins in a number of research laboratories throughout France during 1961 and 1962.

In the first section there is a report of work carried out at the Metabolism Laboratory of the Institut National de la Recherche Agronomique on the amino-acid composition of proteins in animal and human foods, using the chromatographic technique of Moore and Stein.

Some new techniques are reported in the section on the digestion of proteins and the intestinal absorption of amino-acids. Because of the importance of the correct balance of amino-acids arriving simultaneously at the point of protein synthesis, the rate of digestion of protein was investigated, the distribution in the alimentary tract of the pig being determined at intervals; followed by an interesting account of work on the changes in amino-acid concentration in the portal vein during digestion. The use of different methods of estimation of protein quality was considered. For example, the microbiological method of Ford, using *Streptococcus zymogenes*, was found to give satisfactory correlation only for animal foods, not for soya oil-cake.

The effect of heat-processing of herring meal used for animal feeding was next considered at the symposium. The fish-meal heated at 60°, 90° and 130° C was examined in different laboratories by various methods: there was not, however, agreement on the degree of protein damage at different temperatures. One laboratory reported on the remarkable ability of a small supplement of fish protein in quadrupling the rate of growth of rats being fed with gluten as the source of protein in the feed.

The significance of the ratio of calories to protein was emphasized in the next section, in which the work of various laboratories led to the view that the biological value of the proteins is an important factor, affecting the spontaneous caloric intake of both rats and chicks in growth experiments. The protein value of various African foods was considered, in relation to the fight against kwashiorkor in West Africa. These foods included three types of African beans, and the traditional methods of preserving fish were also reviewed.

This useful symposium concluded with three papers concerned with biochemical aspects of proteins: the relationship between the significance of gliadin in cœliac disease; the isolation of α-lactalbumin from cows' milk; and investigations of glyco-proteins in human and cows' milk.

The organizers are to be congratulated on this valuable contribution to our understanding of the subject—the book will be of great interest to all workers in the field.

M. Spencer

NEUTRON DIFFRACTION

The International Encyclopedia of Physical Chemistry and Chemical Physics

Topic 11: The Ideal Crystalline State, Vol. 1: Applications of Neutron Diffraction in Chemistry. Edited by M. Blackman. Pp. xi + 141. (Oxford, London, New York and Paris: Pergamon Press, 1963.) 42*s*.

THE de Broglie wave-length of a thermal neutron, that is, a neutron the kinetic energy of which corresponds to room temperature, is of the order of a few Ångströms, which is about the same as the distance between atoms in a solid or liquid. Thus neutrons may be used, just like X-rays, to provide information about the structure of solids and liquids. As they have no charge neutrons cannot be accelerated or focused; hence it is necessary to start with a powerful source for diffraction experiments. Such sources have become available in the past two decades with the advent of the nuclear reactor.

Unlike X-rays, which are scattered by the electrons in the atom and which are, therefore, only scattered weakly by atoms of small atomic number, neutrons are scattered mainly by the nucleus itself. The scattering from different nuclei varies in an irregular manner throughout the Periodic Table. It is this difference which makes neutrons such a useful complement to X-rays. In particular, the

scattering of neutrons by hydrogen is quite appreciable; so neutrons may be used to locate the hydrogen atoms in substances where these are present alongside heavy atoms. A second application of neutron diffraction is the investigation of chemical ordering in compounds or alloys which contain elements that are near neighbours in the Periodic Table. Such elements have similar scattering properties for X-rays but are often quite different for neutrons.

Applications of Neutron Diffraction in Chemistry by Prof. G. E. Bacon is devoted in the main to a description of these two classes of experiments.

The first chapter gives the principles and general experimental methods of neutron diffraction. Then follow chapters on the investigation of hydrogen in various inorganic and organic compounds, and also of carbon, nitrogen and oxygen in certain heavy element compounds. Experiments on alloys of transition metals and on spinel structures, which have been extensively studied by neutron methods, are further described.

The neutron has a magnetic moment and so, in addition to being scattered by the nucleus, it is scattered by the electrons in those atoms that have a resultant magnetic moment. This property means that neutrons can be used to study the ordering of the spins that occurs in ferro-, antiferro- and ferri-magnetic materials. Although not primarily of chemical interest, a chapter on such investigations is included. This particular field has proved a triumph for neutron methods. We now know that, in addition to the relatively simple magnetic structures mentioned here, other more complicated structures, such as spiral structures ($Mn\ Au_2$) and what might be called an alternating umbrella structure (Cr Se), occur in Nature.

The book is written by one who has done much work in the field. The text is straightforward, the diagrams are clear and helpful and there are copious references to the original work. The book is well produced.

G. L. Squires

GENETICS OF BACTERIA AND BACTERIOPHAGE

The Genetics of Bacteria and their Viruses

Studies in Basic Genetics and Molecular Biology. By Dr. William Hayes. Pp. xii+740+25 plates. (Oxford: Blackwell Scientific Publications, 1964.) 84*s*. net.

ONLY some twenty years have passed since the discoveries which ushered in the era of intensive investigation of the genetics of micro-organisms—the demonstration by Avery and his colleagues that the active principle in the type-transformation of the *Pneumococcus* was deoxyribonucleic acid; Luria and Delbrück's fluctuation test, and, a little later, Tatum and Lederberg's discovery of sexuality in bacteria.

Yet bacteria and their viruses are now perhaps the most fruitful experimental material for fundamental investigation in genetics, and Dr. Hayes has needed more than 600 well-filled pages to provide what he rightly describes as a moderately advanced text-book of the genetics of bacteria and bacteriophage. In his preface, Dr. Hayes states that in teaching microbial genetics to graduates in a variety of subjects, including the physical sciences, he found the main barrier to their perception was their ignorance of classical genetics and of genetical terminology. For this reason, among others, he provides in the first section of *The Genetics of Bacteria and their Viruses* a brief account of the observations and ideas of classical genetics, including Mendel's laws, and of the theory of genetical analysis.

He next proceeds to the examination of recombination and mapping, especially as applied to bacteria and phages; and to a short account of the specialized genetical systems of fungi. Part 3 deals with biochemical genetics, with some examples drawn from higher organisms illustrating the generality of the phenomena under consideration. Part 4 is devoted to the analysis of genetical fine structure, considered theoretically and taking as examples the transductional analysis of the loci concerned in synthesis of tryptophan and of histidine in *Salmonella* and Benzer's analysis of the r_{II} locus of phage *T*4. Part 5 concerns bacterial mutation and the expression of mutations. Part 6, of some 140 pages, is devoted to the physico-chemical mechanisms of heredity, including the structure and function of DNA and RNA, and the evidence of how they function to embody, and express, genetic information—in fact the genetic aspects of 'molecular biology'.

In his preface, the author says that his limited knowledge and experience of the methods of chemistry and physics have necessitated an approach which may appear naïve to the expert; be this as it may, I, as a non-expert, found the account here, as elsewhere, admirably clear and comprehensible. The second half of the book deals systematically with various genetic systems; bacteriophage, including physiology and the phenomenon of lysogeny; the structure of the 'chromosome', in phage and bacteria; recombination systems (transformation, transduction, conjugation) occurring in bacteria; genetic aspects of control mechanisms; and sex factors and other episomes.

The style throughout is pleasant and easy to follow; in those sections in which I felt competent to judge, the material was well chosen, all the more important facts, including those only very recently published, being included, so far as space permitted. The book is illustrated with numerous text figures, in many of which the use of red ink clarifies the representation of, for example, crossing-over; and also by 25 well-chosen and well-reproduced photographs. In the bibliography, of some 900 items, the citations include the titles of the papers and the text page numbers where they are cited; features which should greatly enhance the value of the book.

Dr. Hayes has performed a most valuable service in selecting and collating the essentials from the vast number of publications on this subject; and his book can be cordially recommended.

B. A. D. Stocker

ANIMAL NOISES IN THE SEA

Marine Bio-acoustics

Proceedings of a Symposium held at the Lerner Marine Laboratory, Bimini, Bahamas, April 11–13, 1963. Edited by William N. Tavolga. Pp. xii+413. (London and New York: Pergamon Press, 1964.) 100*s*.

IN spite of the rapid development of air transport, the Atlantic Community still depends very largely on sea transport to carry its food, oil and raw materials. This fact is not lost on our potential enemies, who have built large submarine fleets. This in turn has stimulated the Western countries to put a great deal of effort into developing anti-submarine systems. Unfortunately the sea is a very difficult medium to work in, particularly with regard to the detection of underwater objects at long range. Electromagnetic waves (including light) are rapidly attenuated, and the only wave motion which travels reasonably well is sound, and then only at comparatively low frequencies. The problem is further complicated by the presence of reflecting boundaries at the surface and sea-bed, and by refraction of the sound by gradients of temperature and salinity, mostly in the vertical plane.

Marine animals are important to this problem in two ways. First, they make sounds which interfere with our systems. Secondly, they encounter similar problems themselves, and many of the more advanced vertebrates have evolved highly sophisticated detection systems

which may contain clues which would help us. After all, as one of the contributors to *Marine Bio-acoustics* points out, man's ear is probably atrophied compared with that of animals, but even we can estimate by ear the size of a room in which we are speaking, and we are remarkably good at picking out the sounds we wish to hear from a noisy background.

The subject is also, of course, extremely interesting from an academic point of view, and has important civilian applications. For example, some recent South African work on the hearing of sharks is part of a long-term programme to develop shark-repellent systems. However, most of the money is undoubtedly made available, particularly in the United States, because of the potential military importance.

The subject is at present in an exciting state. The considerable amount of work which has been done so far has raised a number of interesting questions, without, in many cases, providing more than a hint at the answers. For example, some of the sounds picked up are almost certainly used for echo-location purposes, but are of a complex form the purpose of which we do not fully understand.

A symposium on "Marine Bio-Acoustics" was held at the Lerner Marine Laboratory, Bimini, Bahamas, during April 11–13, 1963. *Marine Bio-acoustics* incorporates 23 of the papers presented at the symposium, together with the discussion. The papers are collected into five categories. The first deals with a large underwater acoustic-video system installed at the Lerner Marine Laboratory, the second category deals with physical and acoustic aspects, the third with biological sound producers in the sea, the fourth with marine fishes as acoustic detectors, and the fifth with the special acoustic problems of cetacean research. The book forms a very useful survey of the present state of this subject.

All the contributors to this symposium were from American organizations. Although the United States has almost a monopoly in this field, some useful work is being done in other countries. M. J. TUCKER

JORDAN'S FISH GENERA

The Genera of Fishes and a Classification of Fishes

By David Starr Jordan. Pp. xvi+800. (Stanford, California: Stanford University Press; London: Oxford University Press, 1963.) 140*s*. net.

D. S. JORDAN'S four classic papers entitled "The Genera of Fishes" and their taxonomic index "A Classification of Fishes" have been out of print and difficult to obtain for more than thirty years. Their reappearance as a single, moderately priced volume will receive a wide welcome.

Jordan practised as a taxonomist during a period when there was no international agreement on nomenclatural procedure. His monumental work on the fishes of north and middle America brought him face to face with innumerable problems of nomenclature and it seems likely that this experience more than any other was responsible for the "Genera". In effect, these papers were an attempt to bring some stability into zoological nomenclature. Further, as Prof. Myers emphasizes in his admirable introduction to the reprint, the "Genera" had the immediate purpose of bringing the acceptance and application of fish generic names into accordance with the Paris 'Rules' (1905) by selecting a nomenclatural type species for every genus. In this respect the "Genera" was perhaps a little premature. It was published before methods of applying the 'Rules' were widely understood and before zoologists had benefited from the International Commissions' *Opinions* published in 1920. Thus, the terms Jordan used to indicate the different methods by which a type species name was designated or recognized were not so exact or mutually exclusive as the future workers required. Consequently, these categories have never been adopted and have gradually fallen into disuse.

Be that as it may, Jordan's "Genera" is still an essential first reference to be consulted in any nomenclatural tangle; present-day fish taxonomists should gratefully recognize its importance in the history of their subject. Few works in zoology can have contributed so much to the stability of nomenclature or done so much of the essential spadework for succeeding workers.

Each of the four parts of the "Genera" deals chronologically and by alphabetically arranged authorship with the generic names proposed during the period it covers. For each genus a full bibliographical reference is given, and a type species selected according to the scheme defined in the second part. The total period covered is from 1758 to 1920. In the first part Jordan was assisted by his colleague, E. B. Evermann, who, it is thought, was largely responsible for the format and layout; later parts were the sole responsibility of Jordan. Inevitably in a work of this scope there are mistakes and omissions, but one cannot help but be impressed by their infrequency.

"A Classification of Fishes" was first published three years after the fourth part of the "Genera" (which covered the period 1881–1920). It had a dual purpose: to list in their families all generic names of fishes, living and fossil, published since the inception of binominal nomenclature and, secondly, to produce a new arrangement of fishes in their natural groups.

As a classification it was not entirely satisfactory, particularly since it contained few definitions of the groups involved and little discussion of their interrelationships. It was, in fact, largely a re-interpretation of Regan's classification. Unfortunately, Jordan did not justify the emendments he made and the modern student must remain ignorant of, and intrigued by, Jordan's reasons for disagreeing with Regan in those places where the two systems diverge. As a taxonomic bibliography the "Classification" remains unique and, despite the limitations imposed on it by the growth of ichthyological literature, an invaluable reference work. It is particularly valuable for the newcomer wishing to 'place' some genus he has encountered for the first time. The principal limitation to its use in this way was the lack of a single alphabetical index covering both the "Genera" and the "Classification". The four separate parts of the "Genera" each had an index, as did the "Classification". In the reprint edition, however, the several indexes have been omitted, to be replaced by a single comprehensive index based on a manuscript compiled by L. P. Schultz and the late H. M. Smith. The new index, now available for the first time, is a great help, for it serves also to correct misspellings in the text, to place genera omitted by Jordan from the "Classification" although included in the "Genera", and, finally, to put in their correct families some genera misplaced in the original publications.

Prof. Myers has greatly enhanced the value of the reprint by his informative introduction which not only reviews the historical importance of Jordan's five great works but also sets them in their present perspective and thus instructs in their correct use. Prof. Myers regrets that it has not been possible to revise, correct and bring up to date the work so as to cover the forty years of research since its publication. His remarks on this subject illustrate both the vigorous growth of ichthyology and particularly the awful stature of David Starr Jordan: "However, no one has been found with both the ability and the time to carry through such an extensive revision". It is with humility that modern zoologists should remember that the "Genera" and the "Classification" were but five works from a bibliography of more than 1,900 titles of which 645 were papers devoted to fishes; the others covered such diverse subjects as education, international affairs, philosophy, poetry and even children's books.

P. H. GREENWOOD

Analytical Chemistry

Edited by Carl E. Crouthamel. Vol. 3, Part 4: Pp. 95–162. 20*s*. net. Vol. 3, Parts 5–7: Pp. vii + 163–265. 30*s*. net. (Progress in Nuclear Energy, Series 9.) (London and New York: Pergamon Press, 1963.)

PART 4 of the third volume of *Analytical Chemistry* has the title "Critical Review of the Analytical Methods of the Titanium, Vanadium and Chromium Transition Element Groups" and is by R. Z. Bachman and C. V. Banks of the Institute for Atomic Research and Iowa State University. The authors point out that their contribution is limited to a consideration of "the so-called 'wet' methods of chemical analysis . . . reported (from) 1950 into 1961. Spectrochemical, X-ray fluorescence and radiochemical methods are deleted intentionally . . .". This severely restricts the utility of the article and perhaps denies its claim in the title to be a critical review of the analytical methods. In fact, while it is very useful to have a compilation of methods for separation and determination of these elements and a list of 574 references, the review is not really critical even in the aspects it covers, in that it lists the articles but does not, in general, guide the user.

Part 5, "Chemical Monitoring of Heavy Water Moderated Research Reactors", is by L. E. Smythe; while Part 6, "Recent Advances in the Analytical Chemistry of Beryllium", is by L. E. Smythe and T. M. Florence. The authors, from the Lucas Heights establishment of the Australian Atomic Energy Commission, write from several years' experience of both problems, and the result is comprehensive and authoritative. The beryllium article is much more critical than that on titanium, etc., which forms Part 4, and is consequently of more value to the reader, and, even though no claim is made for complete coverage, articles published in 1963 are mentioned, thus giving a much more up-to-date picture than that of Part 4.

Part 7, "A Large-Scale Facility for Chemical Research with Intensely Radioactive Materials", is by D. C. Stewart of the Chemistry Division of the Argonne National Laboratory. This is a useful description of that Division's new "hot laboratories" for chemical research or analytical service in direct support of research programmes. Not many readers will be called on to design such laboratories, costing more than four million dollars, but all who have to handle sizable amounts of radioactivity will find much of interest and value in this article. Perhaps we can look forward to another paper in a few years' time reviewing the operation of these facilities, with any second thoughts?

A. A. Smales

L'Électronique dans les Appareils de Contrôle Nucléaire

Tome 1. Par Georges Nicolo. Pp. 521. (Paris: Éditions Eyrolles, 1963.) 110 F.

THIS work is devoted to a discussion of electronic circuits used in nuclear reactor control installations. The author includes in this first volume extensive chapters on nuclear particle detectors (64 pp.); general circuit theory (113 pp.); compensation of valve heater voltage variations (38 pp.); stabilized voltage and current circuits (64 pp.); linear d.c. amplifiers (84 pp.); and logarithmic d.c. amplifiers (64 pp.); with, in addition, brief chapters on the elements of nuclear physics, the effects of radiation on materials, and applications of transistors. There is little indication of what is to be included in the second volume.

This is an elementary and uninspired book, with little of interest to the specialist. As an introduction to the subject, there is much to disagree with in the balance of the contents. Ionization chambers and counters, which are highly important in the context of reactor control, are given only a passing glance, whereas the relatively unimportant scintillation counter is treated at length. A fuller discussion of modern semi-conductor circuit techniques could have been hoped for, at the expense of some cuts in the space allotted to obsolescent electronic valve circuits and to the interminable, dull chapters on balanced heaters and stabilized supplies.

The most vehement protest must be made, however, at the quite deplorable number of mistakes both in the text and in the diagrams, mismatching of text and diagrams, misprints in the formulæ, sudden unexplained changes in symbolism, errors in simple algebra, and obscure algebraic derivations which seem almost calculated to confuse and infuriate the reader.

J. F. Hogg

Mushrooms and Other Common Fungi of the San Francisco Bay Region

By Robert T. and Dorothy B. Orr. (California Natural History Guides: 8.) Pp. 71 + 8 plates. (Berkeley and Los Angeles: University of California Press; London: Cambridge University Press, 1962.) 12*s*.; 1.50 dollars.

THIS small booklet, intended for the amateur, consists of a general introduction to the study of the larger, fleshy fungi, with brief descriptions of certain genera and notes on a few of the more common representative species occurring in the area. There is also a very much simplified key to a limited number of agaric genera.

The work is illustrated with line drawings which are remarkably effective and with very poor, and in some instances decidedly misleading, coloured photographs of fungi in their natural habitats. It is a pity that all authors and publishers have not as yet realized that when coloured photographs are used to illustrate works concerned with the identification of fungi they must not only be of a very high quality but must also be selected with a view to showing the diagnostic characters of the species. One cannot help feeling that many of the subjects depicted would have been better portrayed by a good black-and-white photograph. Particularly misleading are the illustrations of *Cantharellus cibarius* and *Helvella crispa*. In fact it seems very doubtful if the fungus shown under the latter name is correctly identified since it should be creamy-white, not brown! Similarly the illustrations of *Hygrophorus psittacinus* and *Lactarius deliciosus* would scarcely pass for the European species. The photograph of *Armillaria mellea* is so poor that it is unlikely that an amateur would be able to recognize it, and likewise that of *Tricholoma personatum*, where the characteristic lilac colour of the stem is completely lacking. Other photographs are so badly reproduced that the diagnostic features are completely obscured, as, for example, the gills of the *Russula* and the teeth of *Hericium coralloides*.

While this booklet contains much useful information and may stimulate its readers to go out into the field and look at mushrooms, they will, I am afraid, be liable to become thoroughly frustrated when they try to name their collections, on account of the relatively few species covered and the poor quality of the coloured plates.

D. A. Reid

Antibiotic-producing Microscopic Fungi

By V. I. Bilai. Pp. vii + 215. (Amsterdam, London and New York: Elsevier Publishing Company, 1963.) 60*s*.

THIS book is an English translation of МИКРОСКОПИЧЕСКИЕ ГРИБЫ-ПРОАУЦЕНТЫ АНТИБИОТИКОВ by V. I. Bilai, published at Kiev in 1961, unchanged apart from a modernization of the bacterial nomenclature in Table 17. After a brief account of the occurrence of microscopic fungi in Nature, and an assessment of the biological significance of the antibiotics they produce, the text deals successively with genera of fungi involved and the chemistry of their products. The longest chapter is, naturally, devoted to *Penicillium*, with taxonomy and illustrations based on those in the well-known monograph of Raper and Thom. There follow shorter chapters on *Aspergillus*, *Cephalosporium*, *Trichoderma*, *Trichothecium*, *Alternaria*, *Fusarium* and a few miscellaneous fungi. A brief final chapter discusses antibiotic properties of the toxins of fungi which cause mycotoxicoses in man and his animals.

R. W. G. Dennis

THE PERSONALITY OF BOTANY: THE RECIPROCAL RESPONSIBILITY OF BOTANY AND THE COMMUNITY*

By Prof. H. GODWIN, F.R.S.
University of Cambridge

My own acquaintance with the International Botanical Congresses began with the Cambridge Congress of 1930 at which Prof. Sir Albert Seward was president, and we had the support of the great Cambridge botanists, Sir Arthur Tansley, Dr. F. F. Blackman and Prof. F. T. Brooks, then general secretary of the Congress. It heightens my sense of pride and of obligation to be succeeding such distinguished teachers, and I am sorry that they, like many other friends of past congresses, Prof. Skottsberg, president of the Stockholm Congress, among them, are now lost to us. It is, however, of the essence of science that their contributions, made with a lifetime of exacting observation and thought, constitute a permanent part of the edifice of knowledge and have placed the world lastingly in debt to them. The thought of these losses reinforces the pleasure with which we welcome at this Congress such internationally honoured botanists as Prof. Bohumil Němec of the Charles University of Prague, now past his ninetieth year but still enviably active.

The growing complexity and extent of botanical science and the vast increase in attendance at congresses have forced some changes on our organization. The Montreal Congress recommended a reduction in the number of constituent sections, and this suggestion accorded with a widely expressed belief that scientific congresses in future should not attempt to cover at one time the whole field of knowledge that they represent, but should rather concentrate on those areas of greatest activity and importance and discuss where notable advances are being made. This policy, warmly endorsed by the International Union of Biological Sciences and followed with great success by the International Congresses of Genetics and Biochemistry, is that which we have adopted.

We have concentrated, therefore, on organizing symposia on all the most active and rapidly developing fields of botanical science. If the old structure of sections has been submerged, this implies no intention to suppress or play down any section of the botanical field of interest, but rather a determination to link all the fields together to their common advantage. The organization in symposia has involved the need to ensure for each meeting a nucleus of opening speakers, but a large and substantial amount of time should always be left for general discussion from the floor: if this is not done the major aims of the Congress are frustrated. The symposial organization has necessarily involved some restriction on the number of persons able to read formal papers at the Congress: to meet such cases there has been a generous provision for annotated exhibits with preprinted synopses: over 3,000 ft. of bench space has been allocated for this purpose. At every section also there has been provided facility for the meeting and discussion together of interested groups of specialists. We cannot afford a form of Congress organization which ignores the present pace of scientific advance.

Botanists were among the first scientists to organize international congresses, and it is now exactly one hundred years since the meeting in Brussels of the first International Horticultural and Botanical Congress under the direction of Eduoard Morren, rector of the Botanical Institute of the University of Liège: the overwhelming support for the tenth International Congress of Botany indicates the maintained vitality and continued growth of the subject through the century.

This large attendance, from sixty-nine countries, testifies not only to the vigour of botanical science, but also to appreciation of the many and evident advantages of the historic city of Edinburgh. Scotland and its fair capital have, moreover, both played a very important part in the development of botanical science. Already in 1670 Dr. Andrew Balfour and Dr. Robert Sibbald had rented a portion of the Royal Gardens at Holyrood House for the making of a Physic Garden, and two other such gardens were shortly established in the City, although by 1700 they all were under the direction of James Sutherland, the professor of botany. The enfranchisement of the subject from its dependence on medicine was indicated by the creation in 1768 of a chair of botany separated from that of materia medica, and the first holder of that post, John Hope, was a gifted botanical teacher, foreshadowing, in his classes of dissection and other practical work, the great biological advances to be made by Thomas Henry Huxley and his London colleagues a hundred years later.

During the long period in which directorship of the Royal Botanic Garden and the chair of botany were vested in one post under the Crown it is interesting to note that, just as the two Hookers, father and son, directed the Royal Botanic Gardens at Kew for altogether fifty-four years, likewise in Edinburgh, between 1848 and 1921, the two Balfours, John Hutton Balfour and his son Isaac Bayley Balfour, controlled the fortunes of the Edinburgh chair for no less than sixty-eight years. Charles Darwin was himself initially an Edinburgh student and the elder Balfour's reign covered the period of publication and discussion of the Darwinian theory. Correspondingly, it was the role of the younger Balfour to bring to Edinburgh and establish there the modern schools of botanical thought fostered in the great German university departments in the latter part of the nineteenth century. Under this leadership and that of their successor, Sir William Wright-Smith, the Edinburgh Department and Garden built up its present outstanding reputation, notably associated with the collections of new species of flowering plants brought back from China and other parts of the Far East by such great botanical collectors as Forrest, Farrer, Wilson and Kingdon-Ward.

In thus recalling the greatness of Edinburgh botanists of the past it would be unseemly if I were not to refer to one of the greatest of Scottish botanists, Prof. F. O. Bower, for many years holder of the Glasgow chair, a great and inspiring teacher whose own research on the evolutionary relations of the non-flowering vascular plants brought him world-wide acclaim. Scarcely less important was the way in which he assembled the powerful Scottish team of palæontologists, Lang, Gwynn-Vaughan and Kidston among them, who paralleled in northern Britain the work of Scott, Oliver, Williamson and Seward in the south. Scottish botanists and Scottish gardeners played no small part in exploring the far parts of the world for plants of economic importance, scientific interest and horticultural value; many, fostered by the traditional Scottish love of education, have pursued distinguished careers overseas helping to guide and develop less fortunate countries—England, I should add, among them.

* Substance of the Presidential Address to the tenth International Congress of Botany held in Edinburgh during August.

We meet, then, in one of the great traditional centres of Scottish botany, and the Congress derives great pleasure from this: it is likewise deeply grateful to the two scientists who are heirs to this great tradition, Dr. Harold Fletcher, Regius keeper of the Royal Botanic Garden and our general secretary, and Prof. Robert Brown, the distinguished plant physiologist who is head of the University Department of Botany.

I chose to make the main theme of my address "Botany and the Community" because there is far too little realization by people in everyday life, by Governments, by the main mass of scientists, and even by botanists themselves, of how extremely important to the life of mankind the study of botany is. It is not understood in general what is meant by botany, nor how botanical science shares, and in some directions leads, advances in scientific thought, nor that it now is the basis of plant technologies of the greatest economic importance to the world.

People accept, as they must, the truth that virtually all the food supply of the world originates in the photosynthetic processes of green plants, and that the fossil fuels such as coal, petroleum and natural gas release in their combustion energy derived from the same process operating in past ages. This truth has been dulled by familiarity, but it remains one of the inescapable realities binding botanical science to the community of men. It has been this primary food relationship that has bound man to the plant kingdom from Palæolithic and Mesolithic times when the human being was a hunter, fisher and collector, dependent for survival on his capacity to comprehend his environment and successfully to recognize and use its resources. It has not been sufficiently noticed how much more close became the ties between man and the plant kingdom with the onset of what Gordon Childe called the "Neolithic Revolution".

With the cultivation of crops, their storage and regular yield, came the possibilities of fixed human settlements, the development of village, town and city life and all the attributes of civilization that arise therefrom. It is one of the most exciting outcomes of recent co-operation between botanists, archæologists and geophysicists that we can now point with certainty to the origins of agriculture in the Middle East in the seventh and eighth millenia before Christ, and can recognize the affinity between the grains the Neolithic farmers then grew and the wild progenitors of these crops. It has been recognized from Darwin's time onward that human selection, intentional and unconscious, must have continually modified the original stock of wild plants to confer on them the qualities which farmers require, that is to say ready germination, simultaneous ripening, easy harvesting, high yield and similar properties. It was not long before it was also understood that the associated weed plants must have suffered a similar selection and that many species, in the course of time, passed in one direction or the other, over the boundary between the useful crop plant and the undesired weed. What had escaped our notice, until it was recently pointed out by Prof. C. D. Darlington, is that the process of natural selection was not confined to the crops and weeds, but that from the onset of Neolithic agriculture the units responding to selection pressure were the new symbiotic systems themselves, the ecosystems constituted in various environments by man plus his crops and domestic animals. Just as crop plants were selected because they suited man, so conversely an efficiently adapted husbandry favoured given races of men. This was without doubt selection for botanical ability. It is not too much to say that built into the genetic make-up of all the races of men derived from this ancient agricultural background is a sympathy for and an understanding of plant life. One might speculate that many traits, recognizable still even in our present urban existence, stem from the same antique sources, among them the love of gardens and affection for the countryside.

Even possibly love of such poetry as I quote from Chaucer:

> "of alle the floures in the mede,
> Than love I most these floures whyte and rede,
> Swiche as men callen daysies in our toun.
> To hem have I so greet affecciouu,
> As I seyde erst, whan comen is the May,
> That in my bed ther daweth me no day
> That I nam up, and walking in the mede
> To seen this flour ageln the sonne sprede,
> Whan hit upryseth erly by the morwe;
> That blisful sighte softneth al my sorwe."

With these deep instinctual roots and with the basic nutritional dependence of human beings on plant growth, it is all the more to be regretted that there is so indistinct and erroneous a public idea of what the botanist of to-day is like and what his work amounts to. Let me briefly consider what has happened.

As the late Dr. Charles Raven has so convincingly shown, the change from the old world of superstition, alchemy and magic to that of the natural sciences must be sought in the seventeenth century, when the 'new philosophers' arose in Europe, and when the Royal Society of London and the great German Society 'Leopoldina' were founded. Between 1650 and 1705 flowered the genius of John Ray, prince of British botanists, whose works over the whole biological field provided the basis, and much more, for the nomenclatural and organizational leadership given to the world by the great Swede, Linnæus.

At the early meetings of the Royal Society and kindred bodies there were not lacking pioneer essays in the field of experimental botany, as witness the publications by Malpighi, Stephen Hales, Grew and others, including the much-neglected holder of my own chair in Cambridge, Richard Bradley; but, as we now see, the physical sciences had not then achieved the level of development that could make them effective tools in biological research. Meanwhile, on the foundations of the learning of materia medica there blossomed the great age of enquiry into the riches of the plant and animal life of the world. It was facilitated by expanding transport and increased exploration, by the general spread of education and by the introduction of such biological research tools as the microscope. By the discovery, description and publication of the floras of vast new areas of the Earth, and by the recognition and examination of classes of organisms hitherto not known, methodical and exact observation was developed and an intensive concern with plants was fostered. The mainspring of this activity remained (as it began) in man's natural instinct of curiosity and his conviction, to use John Ray's title, of "The Wisdom of God manifested in the Works of the Creation". The plant collectors of the nineteenth century, from the greatest of international explorers to the humblest working-men naturalists, jointly gave rise to the popular picture of 'the gentle botanist' searching the forest and shore with his black vasculum and hand-lens: such a man could well subscribe to the view of John Gerard, the herbalist, who wrote, with strange appropriateness to to-day: "Who would dangerously look up at the planets, that might safely looke downe at plants?".

It was this period and this tradition, fortunately by no means abandoned to-day, that witnessed the great triumphs of inductive science. From the detailed particular observation of a multiplicity of organisms and structures in relation to their environment arose the revolutionary hypothesis of evolution by natural selection, and such lesser but still great triumphs as the demonstration by Hofmeister of the underlying equivalence of the life-cycles throughout archegoniate and higher plants.

It was altogether natural that the testing and elaboration of the principles of evolution should now pre-occupy the minds of biologists: the systematic comparison of

organisms throughout the plant and animal kingdoms formed the basis of biological training, and palæontology expanded vigorously in the expectation of discovering 'missing links' in the evolutionary chains. Although our university and school text-books still bear the strong impress of this phase of biological development, it has now come to occupy a much smaller part of our active interest than formerly it did. It is not so much that the problems have been solved, or interest in them diminished, but rather that the problems have proved too difficult of solution by methods now available, and enquiry has turned to more immediately rewarding topics.

It was the new problems and the new approaches to botanical science originating at the close of the nineteenth century and gathering impetus and importance throughout the twentieth that have transformed the aspect of botany, and given the botanist of the 1960's his present-day scientific personality. Put briefly, this modern development of botany is a constant progression towards the experimental and quantitative investigation of plant life, towards the investigation of all botanical processes in terms of causation and mechanism, an approach which, I need scarcely add, involves the application to biological problems of all the resources available from the physical sciences as well as those of related biological disciplines.

Although in the second half of the nineteenth century some advance had been made in this direction, especially by the German plant physiologists, the opinion persisted that 'vital' phenomena obeyed laws other than those of the physical sciences. The contrary view, urged with particular skill by Dr. F. F. Blackman in the first decade of this century, is now completely taken for granted, and it has been triumphantly vindicated by such discoveries as those of Crick, Kendrew, Watson and Watkins, of the molecular constitution of the chromosomes and the manner of their operation in the living cell. At last the cycle of development has now brought the physical sciences to a condition in which they can be effectively applied to the intensely complex phenomena of the living cell, as we can well see in the rapid development of the fields of plant physiology, plant biophysics and biochemistry which bring into their service all the complex techniques and elaborate apparatus of modern biology.

Not only to plant physiology are these exacting standards and techniques of the physical sciences applied: plant geneticists use radioactive elements both to induce mutations and to trace the processes of inheritance, and even so unlikely a subject as biogeography—called by Darwin "that grand subject, that almost keystone to the laws of creation", is using the techniques of radiocarbon measurement and of mass-spectrometric oxygen isotope assay to obtain absolute dates and palæotemperatures for the floras of past times, while its own discoveries are linked with the progress of palæomagnetic research into the fascinating history of the movements of continents about the Earth's surface. As botanists increasingly accept the dictum long since propounded by Lord Bacon that "Many parts of nature can neither be invented with sufficient subtilty, nor demonstrated with sufficient perspicuity, nor accommodated into use with sufficient dexterity, without the aid and intervening of mathematics", so we now find that problems of the natural ranges of plant species are successfully attacked by the use of electronic computers; we have had an *Atlas of the British Flora* produced by the application of mechanical data processing, sorting and printing, and in universities and research institutes highly exacting standards of statistical procedure are commonly used in analyses of plant growth and crop yield.

Plant taxonomy, the discipline which provides the essential nomenclatural basis for botanical science, has profited from improved microscopic techniques, from the findings of genetics and cytology, and from biochemical investigations based on the powerful use of chromatography, so that the phenols, tannins, alkaloids, to mention but a few, can be identified in their varied occurrence throughout families, genera and species of the higher plants.

Herbaria of dried plants came to be the standard equipment of plant taxonomists because the vast arrays of facts concerned in taxonomy could only be co-ordinated and correlated when convenient re-examination of the plant material could be arranged for. There never was any doubt that living specimens of plants provided yet more features of importance for taxonomic investigation, but although type collections of living micro-organisms have come into being, it has never been possible to maintain more than small specialist collections of living plants for taxonomic examination over long periods of time. Now, however, the recording and analysis of many features of living plants can be facilitated by mechanical and electronic means, which place at the disposal of the taxonomist vastly more facts than he has hitherto been able to encompass, and which create an effective agency both of storage and retrieval of taxonomic data.

How far, we may ask, can the same devices be used for assessing the information, how far can they work out correlations between the attributes that have been coded, and how helpful can they be in indicating classifications that arise from such correlations? These are questions that arouse passions in the bosoms of the most 'gentil' of botanists, nor can we expect any early agreement on such issues. Meanwhile, what I take to be an altogether outstanding service has been done, both to botanical taxonomy and that of all other organisms, by a group of biologists examining afresh the philosophical basis on which both classification and taxonomy rest. It is their contention, that I think is scarcely to be denied, that many natural classifications can usefully and properly be made of any group of organisms, and that not only has a phyletic classification no claim to be called the only natural classification, but also that phyletic classification is so difficult to determine objectively that it ought no longer to dominate systems that are primarily needed simply for purposes of nomenclature. Each natural system of classification according to them has its own validity, and it is correct and useful just so far as it discharges the purpose for which it has been set up. Prof. H. Bondi has written:

> "Now what is clear from all that has been said about the nature of science is that scientists must be able to *communicate* with each other. One cannot stress too strongly that the mystic who sees things clearly in his own mind, but cannot transmit this information to others, is not a scientist. The making of discoveries, the original act of thinking up a new theory, is in no sense a more valuable contribution than its transmission to others. It is only by making known what one thinks that it can be checked and cross-checked by others, and it is in this process of communicating for the purposes of checking that the essence of science lies."

It is the purpose of international congresses to foster this communication and clarification.

We now turn from consideration of the nature and present pre-occupations of the botanist of to-day to those relationships with the everyday life of the world which are implied in the title of my address. Any survey of the world's present food position must commence from the generally accepted opinion that the world population will have doubled by the end of this century, and that by A.D. 2000 food production must correspondingly double if it is to do no more than maintain the world's present inadequate standards of nutrition. All biologists will agree that the final solution to this problem of expanding populations must be limitation on the rate of production: failing this the population pressures must ultimately result in famine, revolution and war. Meanwhile, however, we can, by the extension and improvement of the techniques of food production, do an immense amount to

overtake the world's needs and contribute to the relief of hunger and starvation.

It is not generally understood how large a part the botanist has already played in the improvement of the practices of agriculture, horticulture, fishing and forestry, nor how vital is the maintenance and development of the great fields of plant technology that are associated with these industries. The nature and scope of these plant-technological activities are admirably outlined in the pamphlet written by Prof. A. J. Riker on behalf of the U.S. National Academy of Sciences and distributed to all members of this Congress. Although written in respect of botanical activity in the United States *, this pamphlet, *Some Opportunities and Challenges in the Plant Sciences*, has relevance throughout the botanical world. It is especially appropriate that attention should be paid to such issues during this Congress in view of the organization under the auspices of Unesco of the International Biological Project within which biologists in all countries will concentrate on increasing the organic productivity of the world and conserving its natural resources.

We now take almost for granted the services of plant physiology to the investigation of crop growth, of mycologists and plant pathologists to the constant protection against fungi and bacteria of food growing in the field or kept in storage; we have accepted and almost forgotten that the origin of the vast new industry of weed control by selective herbicide was in the botanical investigation of the natural growth substances of plants. In our present plight the potentialities of the breeders of crop plants can scarcely be over-estimated. The botanists of the new world, by the introduction of hybrid corn, at one step immensely increased the production of maize, and a recent symposium on wheat genetics revealed the astounding virtuosity of the plant breeders, who, basing their work on the fundamental cytogenetic investigations of the past, locate and transfer from species to species, even from genus to genus, the genes controlling yield, adaptability and disease resistance. As the organic chemist can now claim to tailor the molecule, so the wheat geneticists tailor the chromosomes of that cereal on which so much of mankind depends for food. It is a far cry from the selection of wild grasses by the Neolithic agriculturists of the 'Fertile Crescent' eight thousand years ago to the wheat genetic conference in Lund; but the mutual dependence of man on the cereal crop has become not looser but more compelling. The challenges grow greater, not less, and great fields of enquiry lie open before us. To mention two only, the productivity of the oceans on one side, and that of the arid deserts on the other, have barely yet been affected by scientific research. The Governments of the world carry a great responsibility to maintain and extend botanical research both pure and applied: they will neglect it at their peril.

It is evident that biological science and botanical science are indispensable to our complicated material civilization and that technical advances depend on maintaining them, but this is not the only reason why the world requires them. Sir Arthur Tansley, writing of the whole discipline of science, said that its practice involved "intellectual, ethical, æsthetic and ultimately spiritual values of a high order". He sought for scientific teaching to take a permanent and unquestioned place in the education of peoples throughout the world, not merely because it lies at the root of successful technology and material prosperity, but also because it has cultural values of its own, values not conferred by the older traditional systems that we still largely follow. He wrote, and all would, I believe, endorse his view: ". . . There can be no doubt that the scientific habit of mind, with its insistence on preliminary careful and accurate survey of facts and its constant testing of theory by fresh observations and experiments, is the master-key to the devising of successful techniques, not only in the material but in the social and political spheres", and he continues: "Equally with literature and art, science has its own contribution to make to the development of the mind, its own distinctive quality engendered by the habit of dispassionate objective investigation and of respect for objective truth".

The need constantly to distinguish ruthlessly between what is and what is not known conditions an ability in all educated scientists to recognize the limits of established knowledge and to admit 'we do not know' in the all-too-frequent situations where this is called for. The exercise of such standards in pursuit of the biological sciences is still more exacting than in the physical sciences; but the aptitude, so far as it can be acquired, has then even more relevance in its applications to everyday life, since we are ourselves not the least complex of living organisms. For this reason I suggest that there has to be a substantial biological content in the education of any and every cultured man or woman, and for such purposes I rate the botanical content equally valuable with that of other biological disciplines.

I have attempted to express something of the responsibility which botanists must accept towards the pressing needs of the world: they will be better able to accept and discharge these responsibilities by meeting together as in the tenth International Congress of Botany. Likewise, this Congress permits us to point to the reciprocal responsibility of peoples and Governments to sustain and encourage botany both at the level of research and as part of a balanced general education.

Finally, allow me to recall that Sir J. J. Thomson, one of the most eminent of physicists, towards the end of his life remarked that if he were to start again he would study botany, for he considered that subject to be at a stage of development comparable with that of physics in the days of his youth The recent progress not only of botany, but also of biology, confirms that great physicist's insight: the field of botanical research is open to the ablest of scientific minds and will provide problems for them not only of the greatest intrinsic interest, but problems which in practical application are vital to the prosperity and even to the future existence of mankind.

* Reprint from *AIBS Bull.*, 12, No. 2 (April 1962).

THE FUTURE OF BOTANY

IT is something of a paradox that, at a time when the learned journals dealing with the plant sciences are struggling to cope with the demand for the publication of an increasing flow of high-class contributions, when botanists are bombarded by a growing complex of review articles and "Recent Advances" and when thousands of botanists can gather at a Congress devoted to innumerable discussions of developments in their subject which are often spectacular, there is still felt in many quarters a profound disquiet concerning both the support at present being given to botany and the prospects for the future.

In a memorandum prepared for the U.S. National Academy of Sciences and collated by Prof. A. J. Riker of the Department of Plant Pathology, University of Wisconsin[1], the case is made that the examination of plants has conferred on mankind so many benefits now considered indispensable, and that the future of civilization will be so conditioned by the rate at which plant science develops, that it is inconceivable that the fullest support

should not be given to botany and to botanists here and now. In point of fact, however, in most parts of the world at least, and the United Kingdom is one of them, botany is invariably, of the scientific disciplines, the one to receive the least support either by way of research facilities or of new buildings and—much the more important—to be the least well provided with students of the requisite quality in the requisite numbers.

At the tenth International Botanical Congress held recently in Edinburgh occasion was made to look into the extent of this public neglect of botany and into the possible reasons for it, with particular concern for the responsibility which botanists themselves must accept for the evils of the present situation, at a general meeting, under the title: "Botany; the Present Crisis and Future Prospects".

At the meeting concerned, four main speakers, Prof. W. O. James (Great Britain), Prof. K. V. Thimann (United States), Prof. A. J. Riker (United States) and Prof. T. S. Sadasivan (India), dealt in turn with various aspects of the problems and afterwards there was a lively discussion among the large number of botanists attending. As a result of this meeting it is abundantly clear that there is among botanists world-wide disquiet about the situation of botany. The reasons for this disquiet are, as might have been anticipated, not completely identical in the 'developed' countries and in the 'underdeveloped'. By and large, the underdeveloped countries look to the developed countries for help in establishing the modern expensive lines of investigation, and their needs will not be discussed further here, for representations have already been made elsewhere in another context. If, however, the developed countries are to accept the challenge of the underdeveloped, as they must, this can only be done if their own botanical institutions are flourishing abundantly. This is all the more reason why the developed countries should look to their own households.

Perhaps the major point which was made during the discussion, and this came out again and again from both the developed and the underdeveloped countries, was the faulty image of botany prevailing in the schools, among non-botanists even in universities, and presented to the public. This is the long-standing concept of an amiable individual, vasculum on shoulder and lens in hand, pottering ineffectively among the flowers of the field. It is to a large extent the modesty of botanists which has allowed this image to persist so long, and this was pointed out most forcefully, particularly by Prof. Thimann. In this, as in many other matters, the meeting warmly supported views already so admirably expressed by Prof. Godwin in his address (p. 10 of this issue).

It is self-evident that living plants have all the complexity found in the inanimate world together with many special tricks of their own. It therefore follows that in addition to the well-established, and quite indispensable, 'classical' approaches to botany, the whole range of techniques and lines of thought characteristic of modern physics and chemistry are vital also in plant science. These are indeed assimilated into botany as rapidly as they become available wherever and whenever finances permit, as eloquently testified in the general lectures at the Congress and in very many papers read in the scientific sections. Far from being a 'soft option' for students, botany is the most demanding of the sciences and therefore the most rewarding, with requirements ranging from meticulously careful observation, collection and classification of field results to the most exacting physical and chemical determinations in the laboratory and interpretation reaching as far as established physical principles will allow. Several speakers made the point that botany therefore comprises a range of interests which will meet the intellectual needs of a wide range of student interests. The trouble seems to be that the interest of really good students is not caught at a sufficiently early age, if it is caught at all, so that, with comparatively rare exceptions, they pass to other disciplines unaware of this rapidly developing subject.

Part of the neglect of botany in this and in other ways clearly stems also from a common ignorance of the debt owed by the community to applied botany, and this again may be in part a consequence of the undue modesty of botanists. To quote examples given by Prof. Thimann, among the many which could have been documented and some of which were documented by Prof. Godwin in his address (p. 12), how many now recall that the whole rubber industry dated from plants of *Hevea brasiliensis* grown by Sir Joseph Hooker in the greenhouses at Kew Gardens, of which he was director, and sent by him to Ceylon, where they thrived; or that the distribution of seeds from this and other plants in South East Asia was made possible only by the investigations of the botanist Ridley? How many recall, or even know, that streptomycin was brought to light by the botanist Waksman, chloramphenicol by the botanist Burkholder, and the tetracyclines by Duggar? Very many such modern drugs are, in any event, not synthetic but 'botanical', and this makes it difficult to express any sympathy for the present-day neglect of botany by organized medicine with its consequential encouragement to schools to minimize the need for extensive botanical teaching.

There is no doubt that the next major breakthrough in science will be in biology, and in the view of Prof. Riker, and many of us, botany will at least play its fair part. If this breakthrough is to come quickly and effectively, all the developed countries will need the right people in the right numbers to meet the challenge both in their own communities and in those less well favoured. Only a far-sighted national policy providing university entrants of high quality in greater numbers than now, and giving botany departments ample facilities both for teaching and research at the exacting standard demanded, can achieve these ends; and it is not obvious that Britain has any policy at all. This was a point instanced by Prof. James and many other speakers.

The view was expressed, moreover, over and over again that a national policy should be based not only on the practical value of botany, which is undoubted, or on the intellectual challenge it gives to its exponents; note should be taken of the value of botany in bringing man close to his natural surroundings and in exercising his mental capacities to any limit they can reach; it is a discipline to which all young people should be exposed as part of their necessary education. Perhaps the best way of inducing intelligent young people to expose themselves to the challenge of botany is for professional botanists themselves to present to them the excitement of working in this expanding field, and opportunities should be made, and taken, to this end.

Finally, concern was expressed by several speakers, including Prof. Sadasivan, that botanists are not at present being adequately employed either in industry or in research associations. It is quite obvious that physicists and chemists are employed in considerable numbers in duties which properly call for botanists. Some of them are indeed coming into botany as professional scientists working on fundamental problems in botany, and these are to be welcomed and encouraged by every possible means. But they bring dangers; dangers arising, for example, from an unrecognized complexity in their experimental material and from unawareness of the wide variations of which the immediate material may be only one. There is no avoiding the need for botanists trained in the standard methods of botanical investigation and experienced in the application to plants of modern physical and chemical, and even mathematical, methods. Industry should, moreover, become aware of the versatility of well-trained botanists and their consequent value in research laboratories as well as in the field. R. D. PRESTON

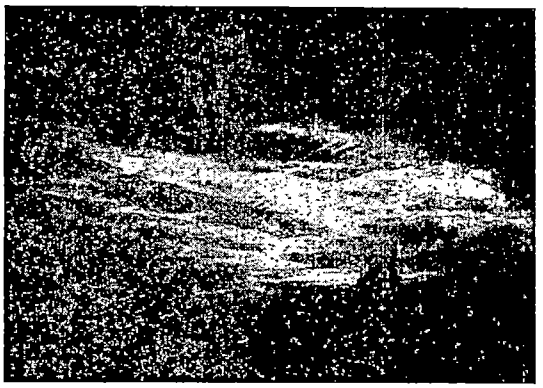
[1] *Some Opportunities and Challenges in the Plant Sciences, AIBS Bull.*, **12**, No. 2 (April 1962).

NOCTILUCENT CLOUDS IN THE SOUTHERN HEMISPHERE

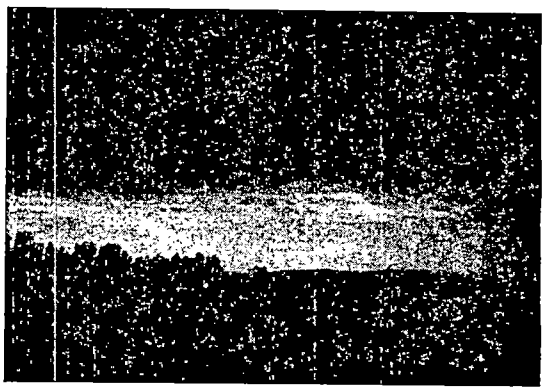
By BENSON FOGLE
Geophysical Institute, University of Alaska, College, Alaska

NOCTILUCENT clouds are occasionally seen in high latitudes during summer months. They are a beautiful sight to behold, as those who have observed them will readily affirm. Several photographs of the clouds appear in Fig. 1.

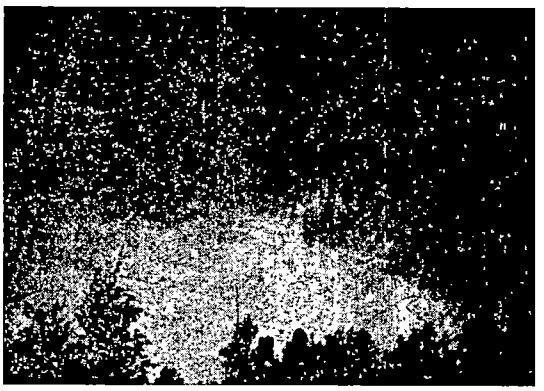
The study of noctilucent clouds was begun in the year 1885 by Jesse in Germany and Tseraskii in Russia. Since then hundreds of observations have been made from Alaska, Canada, Europe, and Russia. During this same period, however, only three displays have been reported



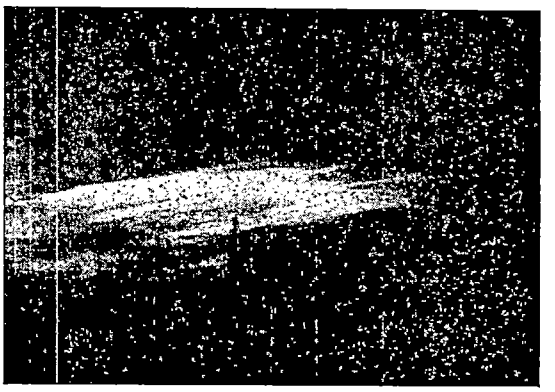
July 27/28, 1957. Anchorage
(by J. Zoller)



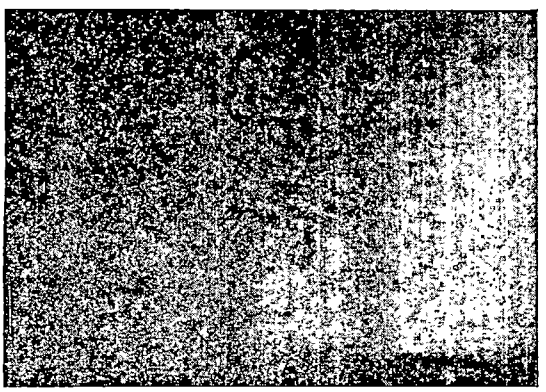
August 13/14, 1962. College
(by Y. Gotaas)



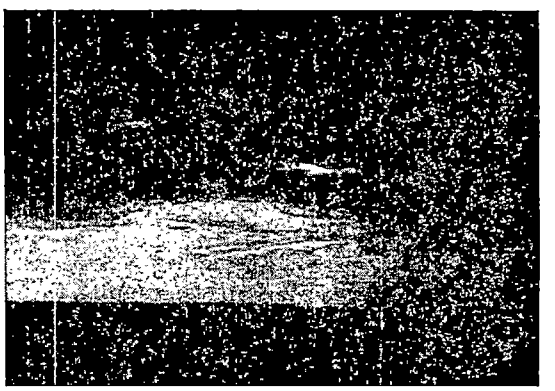
August 6/7, 1963. College
(by C. Wilson)



August 11/12, 1963. College
(by B. Fogle)



August 11/12, 1963. College
(by B. Fogle)



August 12/13, 1963. College
(by C. Deehr)

Fig. 1. Photographs of noctilucent clouds observed over Alaska

from the southern hemisphere, and this scarcity of reports, along with the uncertainties associated with them, cast considerable doubt on the occurrence of noctilucent clouds in southerly latitudes.

Characteristics of Noctilucent Clouds

Among the characteristics established during the past 80 years are the height, cloud particle size, the number of particles/c.c., time of year and time of day when they can be observed in the northern hemisphere, and the temperature of the mesopause when the clouds are present. The cloud height is between 79 and 87 km, the average being 82 km[1]. The temperature at this altitude, when noctilucent clouds are present, is about 130° K (N.A.S.A. News Release No. 62–248).

Statistics on the occurrence of noctilucent clouds over the northern hemisphere show that they occur most often during July and August, although they have been observed as early as March and as late as October. Most of the observations have been made after the summer solstice. Naturally occurring noctilucent clouds have been observed only in the latitude range of 45°–77°, the optimum region being 50°–65°. They can be observed visually only during the twilight periods of the day when the Sun is between 6° and 16° below the horizon. The particle density in noctilucent clouds has been estimated to be between 1 and 10^{-2} particles/cm^3 (ref. 1). The average radius of these particles has been determined to be about $1{\cdot}4 \times 10^{-5}$ cm (ref. 2). The results of rocket-sampling experiments conducted in Sweden during 1962 showed that: (*a*) the particles in noctilucent clouds ranged in diameter from 5×10^{-6} cm to 5×10^{-5} cm; (*b*) the larger particles were coated with a volatile material believed to be ice; (*c*) the dust nuclei contained traces of nickel and iron, indicating that they are of extra-terrestrial origin; (*d*) the particle density at the mesopause was found to be greater by a factor of 100–1,000 when noctilucent clouds were present than when they were absent[3].

Southern Hemisphere Observations

Since 1885 there have been only three reports of noctilucent clouds from the southern hemisphere. The first report was made by a ship's captain about the year 1886[4]. His ship was located in the Beagle Channel (54·5° S., 68° W.) off Punta Arenas, Chile, at the time the observation was made. The exact date, time, direction, and characteristics of this sighting were not given in the report. The second sighting was reported by Herr Stubenrauch[4] in December 1888, from Punta Arenas, Chile (53·5° S, 71° W.). Again, the exact date, time, direction, and characteristics of this sighting were not given. The third sighting was reported by Mr. W. Holman on the night of December 4–5, 1962 (private communication). His observation was made from the U.S.N.S. *Eltanin*, which, at the time of the observation, was located south-east of Tierra del Fuego at 55° S., 58·8° W. Mr. Holman attempted to photograph the clouds but was unsuccessful. Since none of these observations was well documented, there is some doubt as to their reliability. Thus the question of whether noctilucent clouds do occur in the southern hemisphere is still an open one. If they do occur, there are the supplementary questions of their frequency of occurrence, height and latitudinal distribution.

Based on the observational data from the northern hemisphere, which gives a maximum of noctilucent cloud activity in the summer months of June, July, and August, one would expect that, if they occur in the southern hemisphere, they would most likely be seen in the months

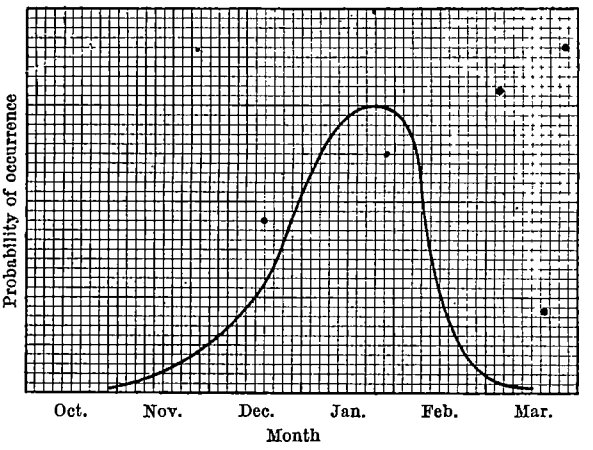


Fig. 2. Expected probability of occurrence of noctilucent clouds in the southern hemisphere

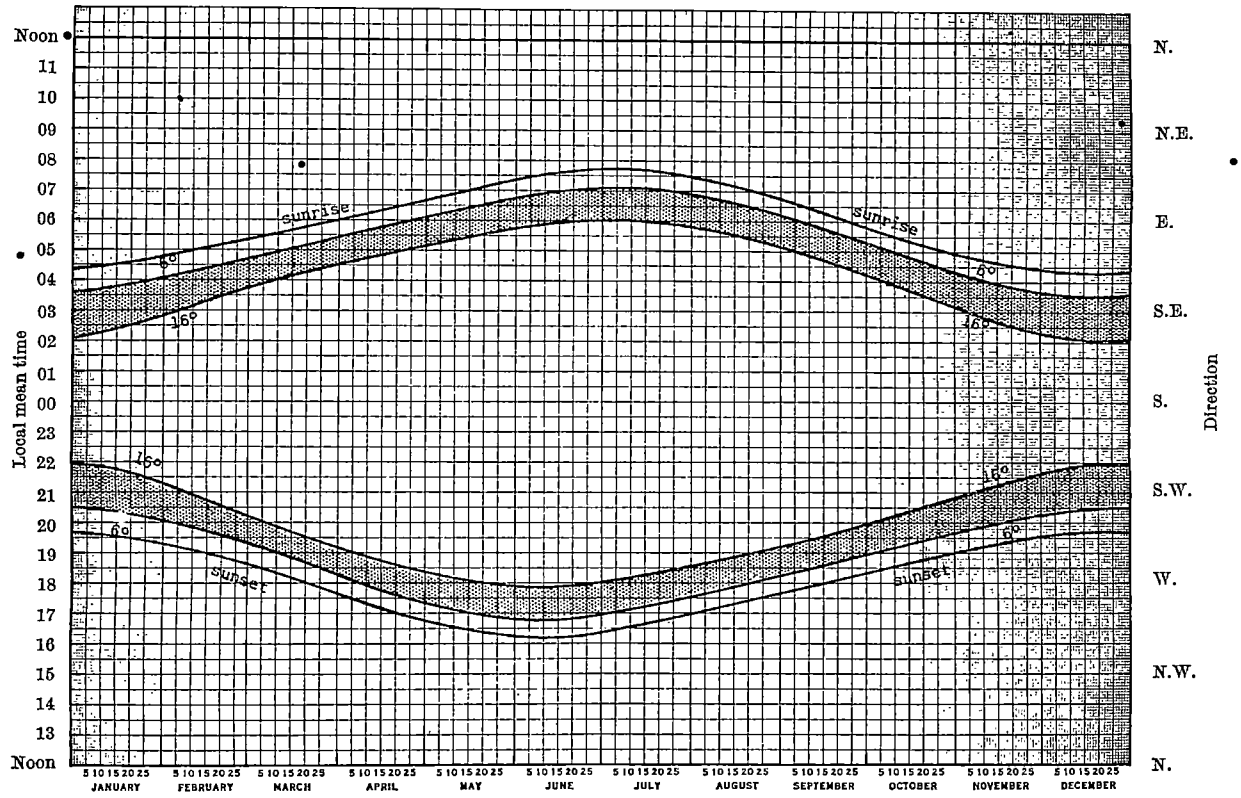
of December, January, and February (Fig. 2). Observers in the latitudes 45°–70° S. should stand the best chance of seeing these clouds. The times for observing noctilucent clouds from these latitudes are shown in Fig. 3.

In contrast to the northern hemisphere, there is very little inhabitable land and a corresponding scarcity of people in the region 45°–70° S. There are, however, some locations in these latitudes that are well situated for observing noctilucent clouds. These locations are tabulated in Table 1.

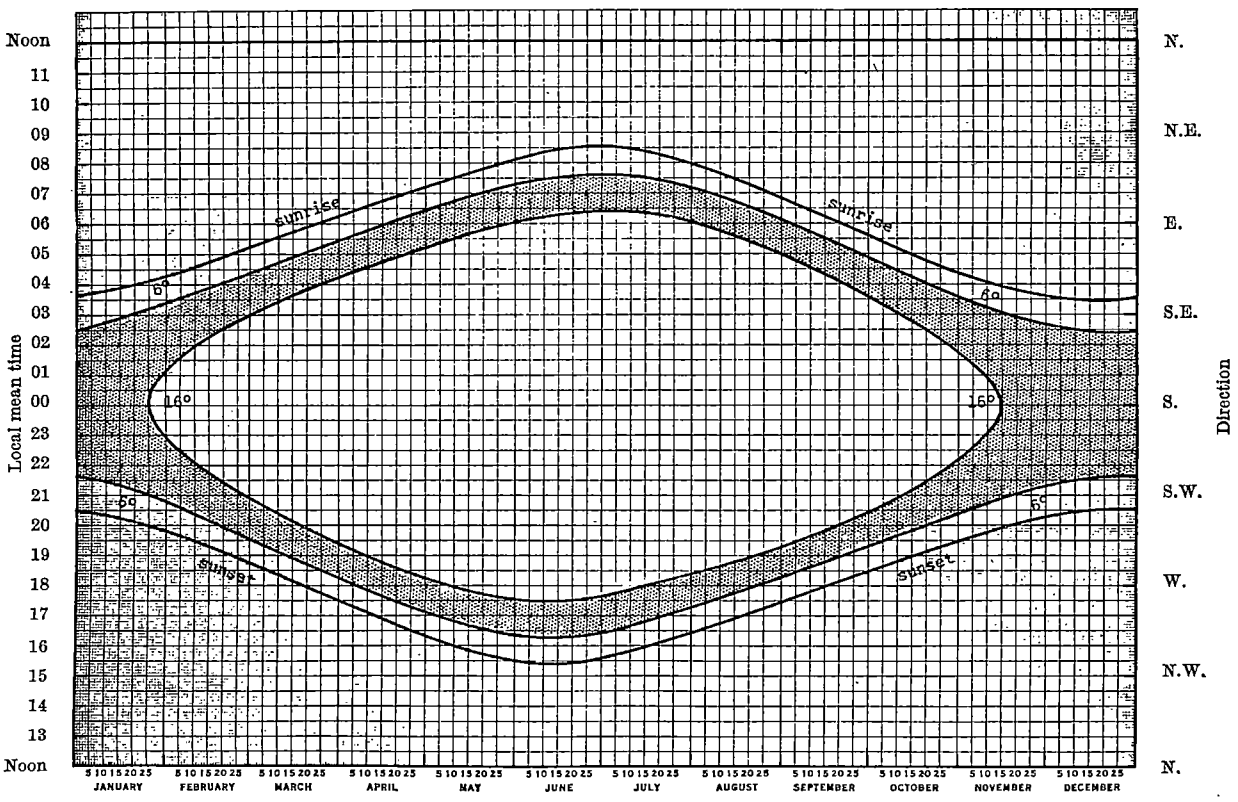
Table 1. Southern Hemisphere Stations that are well situated for Observing Noctilucent Clouds

Station	Sponsor country	Geographic co-ordinates Latitude	Longitude
1. Lauder	New Zealand	45·0 S	169·7 E
2. Invercargill	New Zealand	46·4	168·3 E
3. I. Crozet	France	46·5	52·0 E
4. Bluff Hill	New Zealand	46·6	168·4 E
5. Marion Is.	South Africa	46·8	37·9 E
6. Port aux Francais	France	49·0	70·0 E
7. Auckland Is.	Australia	51·0	166·0 E
8. Port Stanley	United Kingdom	51·7	57·9 W
9. Campbell Is.	New Zealand	52·5	169·1 E
10. Heard Is.	Australia	53·0	74·0 E
11. Punta Arenas	Chile	53·5	71·0 W
12. McDonald Is.	Australia	54·0	74·0 E
13. Grytviken	United Kingdom	54·3	36·5 W
14. Macquarie Is.	Australia	54·5	158·9 E
15. Ushuaia	Argentina	54·8	68·3 W
16. South Georgia	United Kingdom	55·0	37·0 W
17. Orcadas del Sur	Argentina	60·8	44·7 W
18. Deception	Argentina	63·0	60·7 W
19. Esperanza	Argentina	63·4	57·0 W
20. Teniente Matienzo	Argentina	65·0	60·0 W
21. Argentine Is.	United Kingdom	65·3	64·3 W
22. Wilkes	Australia	66·3	110·5 E
23. Dumont d'Urville	France	66·7	140·0 E
24. Mawson	Australia	67·6	62·9 E
25. Davis	Australia	68·6	78·0 E
26. Sanae	South Africa	70·3	02·4 W

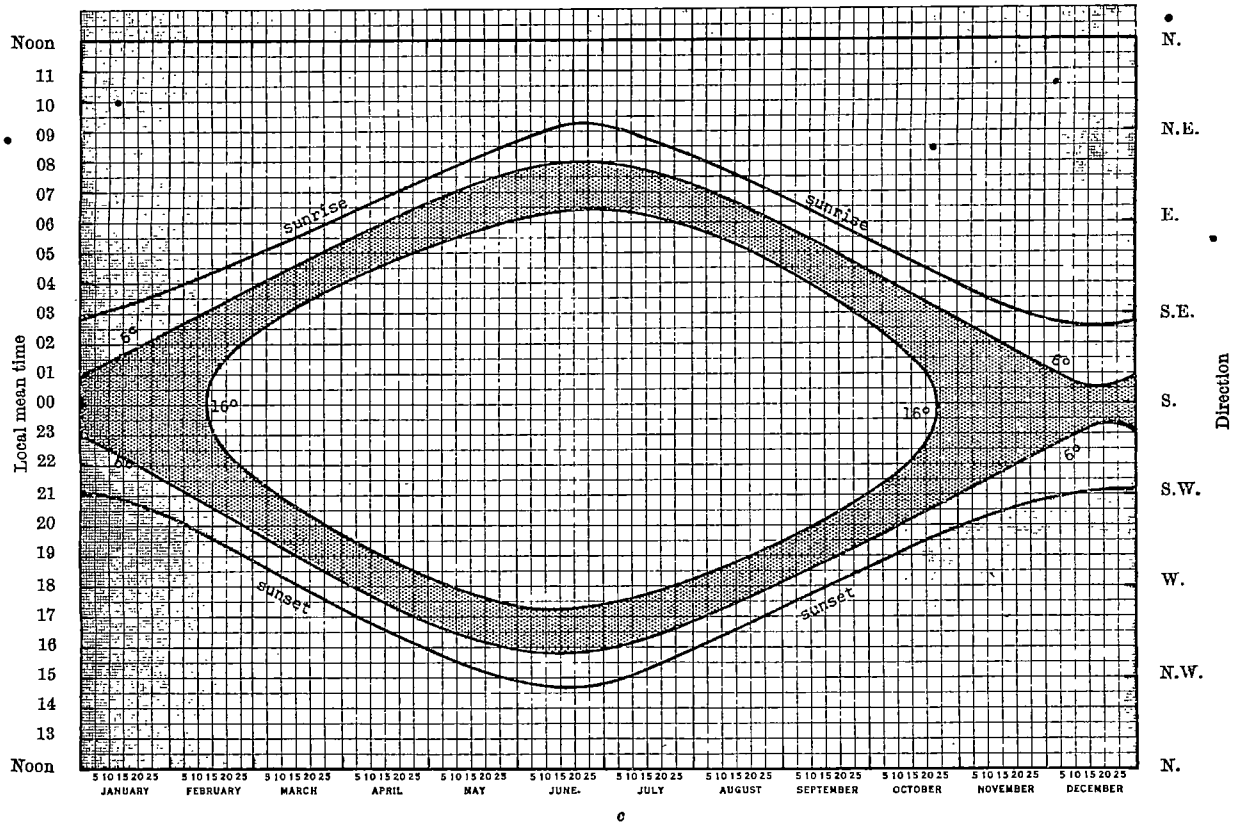
To answer the question about noctilucent clouds in the southern hemisphere, an effort is being made to establish a network of observers in those regions to keep a watch for these clouds. The weather observers at the U.S. Antarctic bases are already participating in this programme, but they are unfortunately outside the optimum latitude range for seeing the clouds frequently. A noctilucent cloud observation manual has been prepared[5] to familiarize these observers with the phenomenon, and is available on request to me.


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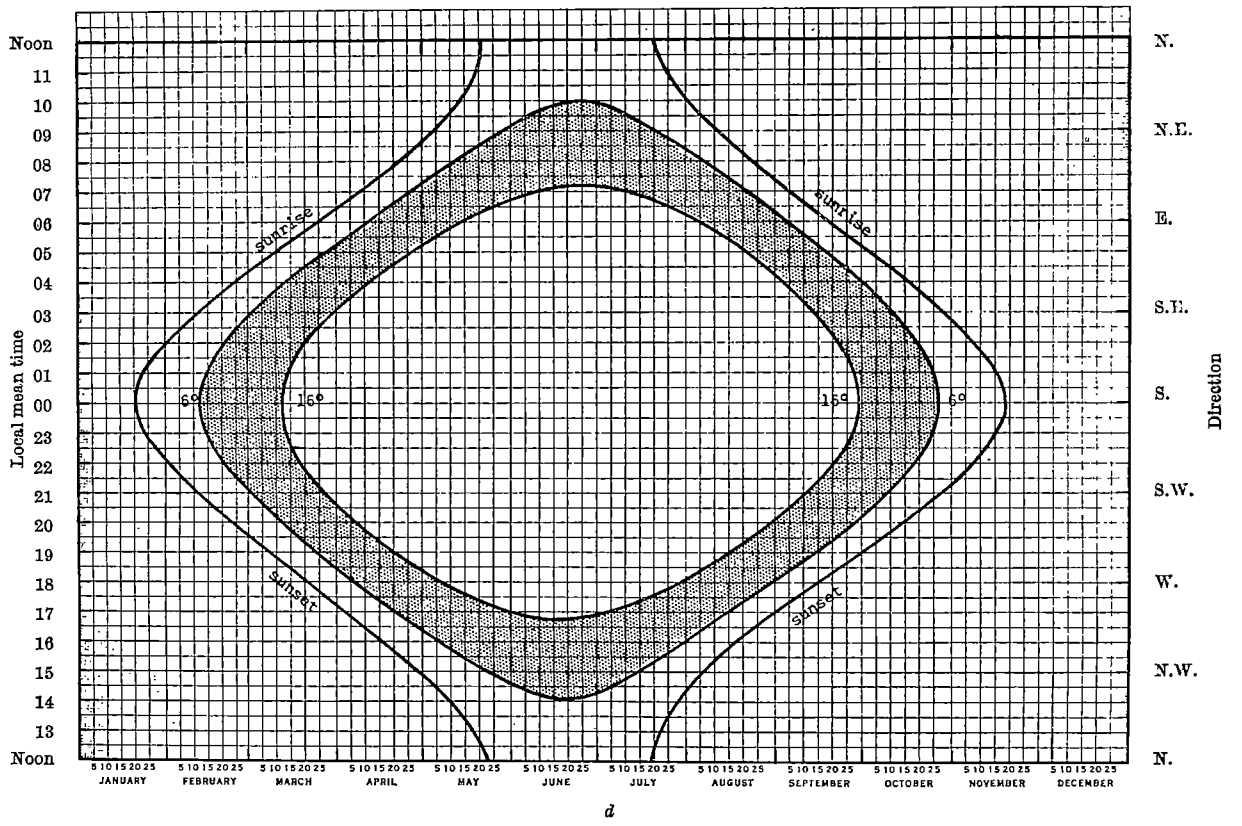
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JUNE
JULY
AUGUST
SEPTEMBER
OCTOBER
NOVEMBER
DECEMBER

b



c



d

Fig. 3. *a*, Noctilucent cloud observation periods (shaded area on curves) for 45° S. *b*, Noctilucent cloud observations (shaded area on curves) for 55° S. *c*, Noctilucent cloud observations (shaded area on curves) for 60° S. *d*, Noctilucent cloud observations (shaded area on curves) for 70° S

The purpose of this article is primarily to direct attention to the need for more observations. This work is being supported by the National Science Foundation grant GP–1759.

[1] Ludlam, F. H., *Tellus*, 9, 341 (1957).
[2] Witt, G., *J. Geophys. Res.*, 65, 925 (1960).
[3] Soberman, R., *Sci. Amer.*, 208, 51 (1963).
[4] Jesse, O., *Astro. Nach.*, 121, 111 (1889).
[5] Fogle, B., *Cloud Report* No. 1*a*, Geophys. Inst., Univ. Alaska (1964).

A NEW JOINT AMERICAN–AUSTRALIAN ASTRONOMY CENTER

By Prof. T. GOLD* and Prof. H. MESSEL†
Cornell–Sydney University Astronomy Center

Introduction

A NUMBER of important research programmes in astronomy would be considerably more effective if they were carried out in both the northern and southern hemispheres by one and the same group of scientists freely using a variety of integrated major astronomical instruments. It is likely that future developments in astronomy will increase the need for such observations—and, in fact, one may expect that some of the more significant work in this field of research will result from such large-scale programmes. These programmes will require the efforts of a very large group of scientists with experience in a number of astronomical and related fields.

Astronomical research in universities has suffered in the past from the fact that astronomers have usually worked in groups of 'sub-critical' size. This has sometimes led to difficulties in regard to staff recruitment, attraction of young postgraduate workers to the field, to the difficulty of raising adequate funds for the construction of a major facility and also of making full use of the facility after completion. Considering these difficulties in conjunction with the rapidly increasing capital costs and size of astronomical installations, plus their running and maintenance at widely separated geographical positions, it is evident that no present single university could effectively undertake a programme of the type envisaged here. This applies to efforts within any one single country and even more so if one considers operating in both hemispheres.

The problems for universities, arising from running a large basic research facility at a great distance from a university centre, are well known. Especially important is the danger of a resulting intellectually isolated research facility which then loses touch with university teaching—and finally fails to provide for the adequate training of postgraduate students.

However, it is now generally agreed that research and postgraduate education of young scientists are intimately related—and hence the function of the universities, whether it be in the costly field of radio astronomy or some other. This fact is vital and has not always been taken sufficiently into consideration when deciding on the administrative abode of important astronomical installations. The resultant world-wide shortage of young astronomers, and especially radio astronomers, is well known and requires urgent efforts to rectify it.

As a helpful step in this direction it is proposed that scientists working in the astronomical and related fields at Cornell University and the University of Sydney, Australia, pool their already individually considerable resources in regard to manpower and equipment in a joint university astronomy programme spanning both hemispheres.

The only major initial costs associated with the scheme are those concerned with travel and living expenses. These, however, will be small in relation to the capital invested in the installations themselves and their running costs, which amount to approximately two million dollars annually at present.

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† H. Messel, School of Physics, University of Sydney, Australia.

The Joint Programme

At present both universities have major new astronomical installations either completed or nearing completion and a number in an advanced planning stage.

The University of Sydney stellar intensity interferometer under Prof. R. Hanbury Brown, at Narrabri, New South Wales (Fig. 1), has commenced operations, and first results on the angular size of Vega have been published. The University's large Mills Cross under Dr. B. Y. Mills, at the Molonglo Observatory at Hoskinstown, New South Wales, is nearing completion and observations will be under way by the end of 1964. The capital cost of these two Sydney School of Physics facilities alone will be more than two million dollars. Major financial support has been or is being provided for these projects by the U.S. Air Force Office of Scientific Research, the U.S. National Science Foundation, the Department of Scientific and Industrial Research of Great Britain, the Nuclear Research Foundation within the University of Sydney and Mr. S. E. Chatterton of Sydney, Australia.

In addition the University of Sydney's Electrical Engineering School, with Prof. W. N. Christiansen as its head, has a field station at Fleurs, about thirty miles west of Sydney. This field station—which was given by the Australian Commonwealth Scientific and Industrial Research Organization—contains the 1,420-Mc/s grating cross with sixty-four 19-ft. paraboloids, commonly known as the Criss Cross, the original 1,500-ft. × 1,500-ft. Mills Cross operating on 85 Mc/s, and the Shain Cross operating on 19 Mc/s. Additions to the Criss Cross are being built and the Shain Cross is at present undergoing modifications.

The giant 1,000-ft. radio and radar instrument in Arecibo, Puerto Rico (Fig. 2), has just been completed at a cost of nine million dollars as part of the Advanced Research Projects Agency 'Project Defender'. Known as the Arecibo Ionospheric Observatory, it has been constructed and is being operated by Cornell University under contract with the U.S. Air Force's Cambridge Research Laboratories and Office of Scientific Research. Although designed primarily for the investigation of the ionosphere, it may have even greater basic scientific interest in radar and radio astronomy, owing to the great power and gain required for the ionospheric programme. Observations with it are under way, under the direction of Prof. W. E. Gordon, and already many important new results have emerged.

In addition to the astronomical instruments, both universities have closely related cosmic astronomy installations which would be included under the proposed arrangement. The installations, which are supported largely by the U.S. Air Force Office of Scientific Research and the Nuclear Research Foundation (Sydney), are under the direction of Prof. K. Greisen of Cornell University and Prof. C. B. A. McCusker of the University of Sydney. Further, both the university groups have direct control of, or call on, a very wide range of necessary supporting staff and facilities. For example, the University of Sydney School of Physics recently installed a new high-speed computer in order to be able to cope with the large amount of data which will be forthcoming from its new astronomical instruments.

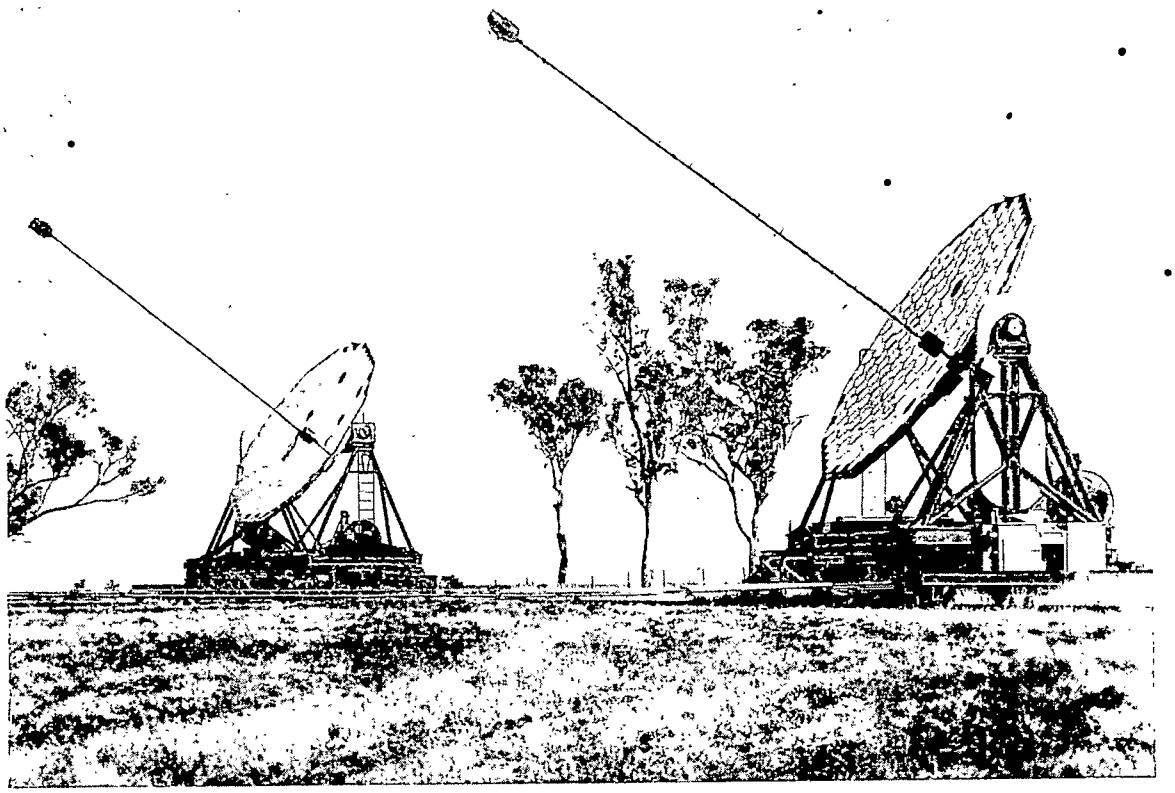


Fig. 1. The two mirror units of the stellar intensity interferometer at the Narrabri Observatory some 350 miles from Sydney

Members of the Centre agree to pool for the use of the Centre all the astronomical facilities—and facilities closely associated with these—under their direction or control. At Cornell the scientists concerned are the members of the Cornell Center for Radiophysics and Space Research under the direction of Prof. T. Gold; at the University of Sydney those concerned are the members of the Chatterton Astronomy and Falkiner Nuclear Departments in the School of Physics under the direction of Prof. H. Messel and the Sydney University Electrical Engineering School under the direction of Prof. W. N. Christiansen. The joint operation is proposed under the direction of Prof. Messel and Prof. Gold, who will be the Centre's joint directors.

Senior members in Sydney include R. Hanbury Brown, W. N. Christiansen, C. B. A. McCusker, B. Y. Mills, A. E. Aitchison, I. S. Docherty, E. A. S. Finlay, A. G. Little, M. I. Large, C. T. Murray, H. D. Rathgeber, M. M. Winn, L. R. Allen, J. Davis, K. S. Imrie, W. B. McAdam, R. F. Mullaly, H. S. Murdoch, L. S. Peak, A. J. Turtle and R. Wielebinski.

At Cornell there are E. E. Salpeter, P. Morrison, K. I. Groisen, G. Pettengill, C. Hazard, F. D. Drake, M. Cohen, W. L'Axford, M. R. Kundu, M. O. Harwit, J. P. Delvaille and B. Hapke.

All research carried out by the members of the Centre under this operation or work which benefited from it will appear under the title "Cornell–Sydney University Astronomy Center".

Both the facilities and scientists of the two universities will, as at present, remain administratively under their respective control; however, the facilities will be pooled for use by the joint group. New installations in the future could be placed under the administrative control of one or other of the universities or, if dictated by geographical position, under their joint control. The mode of operation will not interfere with any research priority system which may be in effect at a facility, but it will merely increase the pool of staff available who may carry out such research.

It is envisaged under this plan that staff within the Centre would be completely interchangeable and that scientists from Cornell would spend considerable time in Sydney (Australia) and at the facilities of the Sydney group, and Sydney scientists would equally well spend considerable time in Ithaca (New York) and Arecibo (Puerto Rico). A knowledge of the different modes of operation and of various technical skills could thus be disseminated to the group as a whole. Technical and instrumental problems of any one facility would come to the attention of the entire group, and the knowledge, ingenuity and experience of all members would be available for their solution. Undergraduate and postgraduate lecture courses and seminars given by scientists of the sister institution would greatly expand the range of interests and knowledge both of faculty and students.

Graduate students would also be interchanged between the two institutions whenever appropriate for the research work on which they are engaged. They would, perhaps, be the most important beneficiaries of this scheme. In the first place, good prospective graduate students in both countries would more readily enter radio astronomy and associated fields if they saw the possibility of joining a group of such a wide range of knowledge and skills. They may develop a particular interest in the approach available in the other institution. As an example, at the present time insufficient training is available in the United States in such fields as phased arrays and interferometers and instruments of the Mills Cross type, and this is felt as a serious handicap in the development of new instruments in the United States. The Australian scientists do not have any planetary radar facility at their disposal. There are several other examples of this nature.

The scientific programme which would initially receive most attention under the joint operation would be the survey of discrete radio sources. This is the investigation of the statistical distribution of sources with the view of deriving information concerning the distribution in depth.

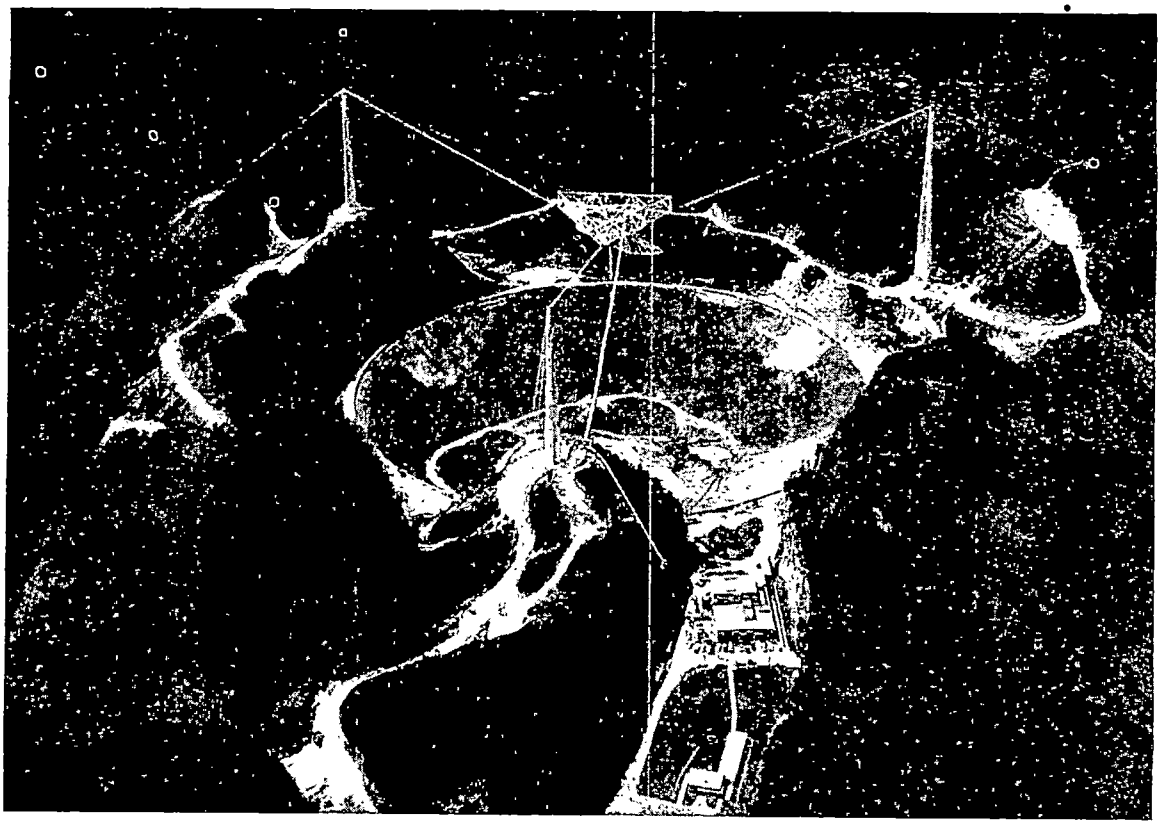


Fig. 2. Aerial picture of the giant radar radio telescope at Arecibo in Puerto Rico. The spherical dish antenna is 1,000 ft. in diameter and the movable feed arm is suspended on cables some 500 ft. above it from three concrete towers, two of which are 265 ft. in height and the third 365 ft.

It has been shown that such investigations have the possibility of giving information concerning the structure of the universe on the largest scale and, therefore, about cosmology.

Investigations of this kind have been made several times in the past, and have been severely disturbed by a variety of errors. It is now considered that such catalogues of sources ought to be published, so far as possible, only after each source has been investigated separately by two different instruments which are unlikely to suffer from similar deficiencies and which work on approximately the same frequency. A large area-filled instrument like a dish and an open area instrument like a Mills Cross make an ideal pair in this respect. It is proposed, therefore, to prepare a detailed catalogue of sources in the area of sky in which the Sydney Mills Cross and the Arecibo instrument overlap. All discrepancies between the results of the two instruments will be investigated in detail, and the final catalogue should be of a level of precision far exceeding that of any previous work.

Other investigations where a joint operation is clearly advantageous are those of extended sources and background structure where more detail can be described by using two such instruments than by either singly.

These examples of joint observation are those which come to mind in the present state of the instrumentation. It is expected, however, that many other types of observations will equally benefit as the subject advances and as the instrumental facilities available increase. Furthermore, one may look forward to new research projects coming forward as the full effect of the joint team effort comes to fruition.

The two universities have agreed to recognize research supervision extended by the sister institute and with the approval of the home research supervisor, and the time spent by a student on thesis work in the sister institute will be accepted toward the residence requirement.

The scientists concerned are agreed that such a joint operation would be most beneficial for their work and for the subject, for the reasons mentioned. An operation of this kind, where the advantages to research are conspicuous, may pave the way towards other combined university operations which tend to break down barriers imposed by geography and thus broaden the educational programme.

OBITUARIES

Dr. R. S. Creed

His many pupils will have read with regret of the death on July 7 at his home near Oxford of Dr. R. S. Creed, emeritus Fellow of New College, Oxford, and formerly demonstrator and lecturer in the Department of Physiology, University of Oxford. He was in his sixty-fifth year and is survived by his wife, herself a distinguished physiologist, a son who was elected to a Grocer's Company research fellowship in zoology at New College this year, and three daughters.

Dr. Creed went up to Trinity College, Oxford, in 1916 and, after service with the Royal Field Artillery in France and Belgium during the next two years, returned to take a first-class degree in the Honours School of Natural Science (Physiology) in 1921, winning a senior demyship at Magdalen and the Gotch Memorial Prize. He took his clinical training at St. Thomas's Hospital and gained the B.M., B.Ch. and Conjoint qualifications in 1923. He was elected to a fellowship at New College at the age of 27, and served the College for many years as steward, pre-

centor, editor of the college record, sub-warden, and as a Fellow of Winchester College. He took the D.M. in 1930 and was awarded the Radcliffe Prize in 1933. Between the two World Wars Dr. Creed was an active Territorial officer; he was called up for service with the Royal Army Medical Corps in 1939 and served overseas in the Middle East and Italy. He returned to Oxford in 1945 and from 1947 until 1960 was chairman of the management committee of the Warneford Hospital. In 1949 he joined the editorial board of the *Journal of Physiology*, at a time of rapid post-war expansion, and was chairman from 1951 until 1956, a position of particular responsibility in maintaining that *Journal's* high standards. He was president of the physiology section of the meeting of the British Association for the Advancement of Science in 1955.

Dr. Creed was a co-author of the *Reflex Activity of the Spinal Cord*, a physiological classic published in 1932 with Denny-Brown, Eccles, Liddell and Sherrington. He made a number of contributions to the analysis of inhibitory reflexes, and also to investigations of the latency of after-images and of the electro-retinogram with Ragnar Granit. He was particularly interested in the mechanism of hearing and the function of the vestibular apparatus. However, above all, he was a College tutor in the classic tradition who would listen to an essay without interruption, and then, by a few deft and disconcerting questions, demonstrate the value of succinct expression and critical evaluation. Many generations of New College medical undergraduates have owed him a lasting debt for his perceptive and friendly guidance. G. S. DAWES

Mr. Lauge Koch

THE Danish geologist Lauge Koch, who died on June 7 aged seventy-two, was a legendary figure among Arctic geologists (themselves noted for strong characters). His first geological expedition was in 1913; then he was back in Greenland in 1916 with the veteran explorer Knud Rasmussen with the Thule Expedition which was marooned in north-west Greenland until after the First World War ended. Despite the hardships of this expedition (on one journey they ate their starving dogs and a member died) he returned to the same district to lead his own expedition. I met him a good many years later when I joined his party in Scoresby Sound in 1926–27. He had undertaken all the work of planning, stocking the party for the year and choosing the men—the Danish geologist Rosenkrantz, two Eskimo hunters and myself as a specialist on fossil plants. It was Koch who made the long sledge journey in late winter; he left to us shorter journeys and detailed work.

Koch was a big strong fellow, happier on a dog sledge than anywhere else. His character had much strength and half-a-dozen bad sides of which he was cheerfully aware and which all could see; but for me it was saved by his robust and often generous sense of humour. Very likely, too, the partial language barrier made relations easier. I am sure men will long brighten the Greenland night by anecdotes of Lauge Koch, stories that may or may not be true, just as Koch's own stories were. So he would have wished it to be.

Koch continued to lead expeditions for many years. As he grew old he managed to avoid the most exacting physical exertions, but he remained very much the leader. He wanted to complete the picture of the geology of Greenland, and, while he tolerated detail by other hands, his part was to be painted with bold strokes. I doubt if he ever spent months working on one rock formation to perfect knowledge that was already fairly good.

It was natural that he should undertake the work of writing a comprehensive account of the geology of Greenland. Nevertheless, he was the wrong man to do this. Though he had done more than anyone else, his boldness with theory bordered on rashness; it is said he had not the patience to confirm facts, and, worse, that he failed to attribute to others what was due to them. I do not know the truth, but he had already antagonized many (a thing that troubled him not at all) and there was a published counter-blast, a libel action and a hideous row which split Danish geology for years.

No more pioneering work like Koch's remains to be done, and now an aeroplane can do in an hour what a dog sledge took a month to do. But when the next comprehensive work on the geology of Greenland is written I am sure that by far the longest list of entries will be under Koch's name. TOM M. HARRIS

NEWS and VIEWS

Research and Development, U.S. Coast and Geodetic Survey : Dr. John S. Rinehart

DR. JOHN S. RINEHART, former director of the Mining Research Laboratory, Colorado School of Mines, has been appointed to direct the Office of Research and Development of the U.S. Department of Commerce Coast and Geodetic Survey. He succeeds Dr. Christopher E. Barthel, jun., who left the Survey to become executive director of the Kansas Research Foundation at Topeka. As assistant director for research and development, Dr. Rinehart will be concerned with the expansion of the Survey's scientific responsibilities, especially in satellite technology, mineralogy, instrument development, metallurgy, astronomy, ballistics, and physics. Prior to the Second World War, he taught physics at Fort Hayes, Kansas State College, and at Wayne University, and during the War he was executive secretary of Division 4 of the National Defense Research Committee, which directed development of radio and photoelectric proximity fuses. Dr. Rinehart also directed a programme of ordnance research and development at the New Mexico School of Mines. Later, at the U.S. Naval Ordnance Test Station, China Lake, California, he carried out research on problems associated with fracture and deformation of materials subjected to high-speed impact and explosive forces. During 1954–55, Dr. Rinehart carried out research in the University of Cambridge. In July 1955 he joined the Smithsonian Astrophysical Observatory as its assistant director, where he participated in the satellite tracking programme; he was also a research associate in astronomy at Harvard University, where he was concerned with meteorite research. Dr. Rinehart established the Mining Research Laboratory, Colorado School of Mines, in 1958. Born in Kirksville, Missouri, Dr. Rinehart went to the Northeast Missouri State Teachers College, where he gained a B.S. degree in education and an A.B. in physics. He obtained his M.S. degree in physics at California Institute of Technology, and his Ph.D. in physics at the State University of Iowa.

Botany at Queen Elizabeth College: Prof. J. Edelman

DR. J. EDELMAN, who has been appointed to the chair of botany in the Department of Biology at Queen Elizabeth College in the University of London, graduated in botany at the Imperial College of Science and Technology in 1948. Already interested in plant biochemistry, he spent two years in Prof. (now Sir) Hans Krebs's Depart-

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ment at Sheffield, obtained his Ph.D. there in 1950, and then returned to the Imperial College to join Prof. F. G. Gregory's team in the Research Institute of Plant Physiology. During 1953–54 he held a Rockefeller Foundation research fellowship at Berkeley, where he was closely associated with Prof. W. Z. Hassid. In 1956 he was appointed to the academic staff of Imperial College as reader in plant physiology and, later, as reader in enzymology. Dr. Edelman's research has been concerned mainly with the enzymatic synthesis of polysaccharides, particularly of fructosans, and he has published many papers on various aspects of this problem. Other publications have dealt with some transformations of sucrose, and with dark-fixation of carbon dioxide in *Kalanchoë*. More recently, he has initiated research into the formation of 'induced' enzymes in mature plant tissues, and has shown that plant hormones (auxins) affect the induction. All plant biochemists will be interested in the future development of these investigations.

The Rock Carling Fellowship: Prof. W. V. Mayneord

Prof. W. V. Mayneord, professor emeritus of physics as applied to medicine, University of London, has been elected the third (1964) Rock Carling Fellow by the Nuffield Provincial Hospitals Trust. The fellowship was founded in 1961 as a memorial to the late Sir Ernest Rock Carling, for many years a Governing Trustee and chairman of the Medical Advisory Panel of the Nuffield Provincial Hospitals Trust. Each holder of the fellowship will seek to review in a monograph the state of knowledge and activity in one of the fields in which Sir Ernest had been particularly interested and which is within the purposes of the Trust. The arrangements provide that the monograph will be introduced by a public lecture given at a medical teaching centre in the United Kingdom. The subject of Prof. Mayneord's review is "Radiation and Health", and this will be introduced at a public lecture at the Medical School, University of Birmingham, on November 19. Admission will be by ticket only, for which applications should be made to the Secretary, Nuffield Provincial Hospitals Trust, 3 Prince Albert Road, London, N.W.1.

Botany in the University of Delhi: Prof. P. Maheshwari

A special volume of the *Journal of the Indian Botanical Society* has been issued in honour of Prof. P. Maheshwari on entering his sixtieth year (42A.. Edited by T. S. Sadasivan. Pp. xxxiv + 330. Madras 5: Indian Botanical Society, University Botany Laboratory, 1963. Rs. 32; 50*s*.; 7 dollars). Prof. Maheshwari has been professor and head of the department of botany in the University of Delhi since 1949. He is well known for his work on the morphology and embryology of vascular plants and the *in vitro* culture of reproductive organs. In addition to a complete bibliography of his published works the volume contains 37 invited contributions on mycology and plant pathology, algae, bryophytes, pteridophytes, angiosperm anatomy, morphology and taxonomy, palynology, embryology, cytology, genetics, plant breeding, palæobotany and *in vitro* culture.

India at Mid-passage

The Overseas Development Institute has collected four short papers discussing aspects of India's third Five Year Plan (*India at Midpassage*. London: Overseas Development Institute, Ltd., 1964. 6*s*.). In the first, Mr. W. Clark discusses the progress of the Plan (1961–66), and suggests that it has not received sufficient publicity, nor have the central planners adequately appreciated the immense difficulty of passing on new agricultural techniques to the mass of the populace. Mr. Clark believes that nothing could be more useful than continued private investment in India, but it is essential that the Aid India Consortium should accept responsibility for playing a definite part in the planned attack on poverty or in the plan for economic growth. The success or failure of India's planned effort to end its poverty hangs in the balance, and although the outcome rests in Indian hands the key factor of external assistance can be applied effectively or ineffectively. There is at present great danger that too little aid will be given just too ineffectively. It is therefore essential that the donors should accept in principle the aims and methods of the Indian Plan and judge the amount that is to be made available in terms of what is needed to achieve its objectives. In the second paper, Mr. R. Neild examines population policy in the context of long-term economic planning in India. He points out that any population policy is unlikely to do more than moderate the growth of the population over the next 10 or 20 years, and stresses the urgent need to expand programmes of family planning as fast as possible and to survey the results obtained so far. There is also the broader question of the priority and attention which should be given to population policy and economic planning. He concludes firmly that if the objective of Indian planning is to raise the living standards, far too little is now spent on population policy. E. F. Schumacher examines the problem of rural industries, including the need for a suitable technology and also for a suitable form of organization. He suggests that detailed design investigations should be undertaken on all industries envisaged for establishment in rural areas. K. B. Lall discusses Indo–British trade prospects.

Expansion of Higher Education in Britain

In a brief debate in the House of Commons on July 29 on scientific and technological education, opened by Dr. J. Bray, Sir Edward Boyle, the Minister of State for Education and Science, reaffirmed the Government's endorsement of the recommendations of the Robbins Report regarding the building-up of technological universities and the development of management studies. He thought that the Robbins figure of 45,000 full-time advanced students in technical schools other than the Colleges of Advanced Technology was an underestimate of what would be achieved, and insisted that the Government would plan firmly on the basis of 219,000 students in institutions of university status by 1973–74, and 390,000 full-time places in higher education in the full ten-year plan. Output of qualified scientists and technologists this year was estimated as 19,500, and in the present year 1,073 awards of Dip.Tech. had been made. Between 1953 and 1968 capital work to the value of £18·5 million would have been done at the Imperial College of Science and Technology and its numbers would have increased from 1,700 to 3,700. Between 1955 and 1967 the capital expenditure of the Manchester College of Science and Technology would exceed £6·5 million with an increase in numbers from 800 to 2,700, while the University of Strathclyde over the same twelve years would have had a capital outlay of £5·25 million. Between 1955 and 1966, capital allocations to the University of Durham would total about £5·75 million, of which a little less than £1 million would be for applied science; while in the same period capital allocations to the University of Newcastle upon Tyne would total £9 million, of which £3·7 million would be for engineering and applied science. Sir Edward Boyle also paid tribute to the contribution of the Sunderland and Rutherford Technical Colleges and of the Constantine College at Middlesbrough to the provision of higher technical education in the North-East.

Financing Education in Britain

A pamphlet recently issued by the National Union of Teachers looks at the system of educational finance in Britain (*The Financing of Education: A Discussion Document*. Pp. i + 38. London: National Union of Teachers, 1964. 2*s*.). It examines suggestions that have

been put forward for changes and modifications in the present system, or for its retention in its existing form in order to see whether it can be made to work or whether some new and better system can be found. The ways in which different sectors of the system are financed are reviewed together with the controls which have been used to deal with increasing expenditure on education (estimated in 1961–62 at £898 million for present expenditure and £163 million for capital expenditure, or 4·4 per cent of the gross national product). Some important points are suggested for consideration by the local and county associations of the National Union of Teachers. Emphasis is placed on the belief in the unity of education which runs through all the thinking of the Union, and on the recognition that in some fields it is the duty and right of individual teachers or groups of teachers to determine what is best in any particular circumstances. It is regarded as the right and duty of the Union to defend this freedom of professional judgment. Here the pamphlet suggests that, if the present grant system continues, there will, in effect, be a breakdown in local Government finance, at least as applied to education. Possible alternatives to the present grant system are: the devising of new techniques of rate assessment and levying that would mitigate the impact and social injustices of the present system; alternative or additional methods of raising local revenues; the reorganization of local education authorities into regional or larger or *ad hoc* authorities. The associations should also consider whether the Union should support a return to a system of specific grants; the transfer of payment of teachers' salaries to the Central Exchequer; the financing of different parts of the education system by different methods; a nationally financed system of education. Appended to the pamphlet are summaries of the main points made by Mr. J. R. Sampson, Mr. N. Morris, Mr. John Vaizey, Dr. Horace King, M.P., and Sir William Alexander before the Union's panel on educational finance.

Sociology in Professional Training

THE question of what should be included in a sociology syllabus is frequently raised and many who are responsible for introducing the subject have indicated that they would welcome advice on this matter. The appropriate syllabuses could, with obvious advantage, be drawn up as a result of consultations between members of the various professions and sociologists who specialize in particular branches of the discipline. For example, discussions could be held between training college principals and educational sociologists, town planning officers and urban sociologists, those concerned with management studies and industrial sociologists. With the view of improving sociology syllabuses in general the British Sociological Association has established a panel of members from various fields in sociology to discuss the needs of professional organizations and to give advice in formulating sociology syllabuses. Further information can be obtained from the Honorary General Secretary, British Sociological Association, Skepper House, 13 Endsleigh Street, London, W.C.1.

Family Planning Association

THE 33rd annual report of the Family Planning Association describes the success of the campaign to secure a large sum of money to extend the Association's activities overseas. Over the past four years the number of Family Planning Association clinics in Britain has increased from 300 to 409, oral contraceptives have been introduced as a normal method of birth control, training for lay workers has developed, training for doctors and nurses has been improved, advisory visits to clinics have been initiated and the procedure for the inspection of training clinics overhauled. A limited cancer smear service is now available, clergy courses are regularly arranged and relations with a number of universities are much closer. There were 409 clinics in 1963, compared with 392 a year earlier. During the year covered by the report more than 335,000 individual patients were seen, 13,670 of whom used oral contraceptives; 138 of the Association's clinics undertake cervical smear examination, an important contribution towards the early detection of cancer of the cervix.

Abnormalities of the Sex-Chromosome Complement in Man

KNOWLEDGE of human chromosome anomalies as causes of abnormal somatic and sexual development is relatively new, and is increasing rapidly on account of the technical advances that have been made. However, techniques are still time-consuming, and laboratories that specialize in this work can cope with only relatively small numbers of patients while, in general, fairly large numbers are required to obtain statistically sound information on a number of points concerning the types, origins and outcomes of human chromosome anomalies. Therefore, the publication of detailed reports by those laboratories which are able to make an efficient and accurate examination of persons with potential chromosomal anomalies is particularly welcome. In *Abnormalities of the Sex-Chromosome Complement in Man*, Dr. W. M. Court Brown and his colleagues have given as much valuable information about each case as they have been able to assemble during their painstaking work. (Privy Council—Medical Research Council Special Report Series, No. 305. Pp. viii+239+3 plates. London: H.M.S.O., 1964. 27*s*. 6*d*.)

The data have been collected in connexion with the Registry of Abnormal Human Karyotypes set up in 1959 in the Medical Research Council Effects of Radiation Research Unit in Edinburgh. The primary object of the Registry is to enable an investigation to be made of the long-term effects of the chromosomal abnormalities that have been detected in man. This report deals with 266 subjects with anomalies and discrepancies of the sex chromosomes—the total number of cases with sex-chromosome defects which have been registered from 1959 to the end of 1962. There are two introductory parts: in the first are described the techniques and methods of examination of human sex-chromosome abnormalities by means of sex-chromatin and metaphase-chromosome investigation, and a section is devoted to the recognition of sex-chromosome mosaicism; in the second part, the literature concerning chromosomal findings in the persons described in the main body of the report, which follows, is briefly and critically reviewed. The third and main part deals with the persons reported to the Registry; reasons for referral, history and general clinical findings are given and reference is made to publications in which the patient has previously been reported. The persons examined are grouped into three main sub-sections according to phenotype; 134 have a male phenotype, 128 are phenotypically female and 5 are true hermaphrodites. 99 of the 134 phenotypic males have an XXY sex-chromosome complement and 24 have sex-chromosome mosaicism. Of the 128 phenotypic females, 38 are XO, 22 are sex-chromosome mosaics, 33 are XXX and 26 (not 20 as stated on p. 129) have an XY complement, and of these, 20 are examples of testicular feminization. The report contains a great deal of factual information on practically all types of sex-chromosome anomaly discovered in man, and it is to be hoped that new material will be published in similar form as it becomes available from time to time.

Total Beta Activity in Global Fall-out in Australia during 1963

CONTINUOUS sampling of fall-out for the measurement of total β-activity was maintained through 1963 by the

Atomic Weapons Tests Safety Committee in an Australia-wide network of sampling stations. Daily sampling was carried out at 24 stations for the first five months of the year, after which the number of stations was reduced to 11. Monthly sampling, using two methods, was conducted at eight stations throughout the year. A report by R. J. I. Alsop, W. J. Gibbs, J. R. Moroney, D. J. Stevens and Prof. E. W. Titterton presents results for total β-activity measurement on all these samples (*Aust. J. Science*, 26; May 1964). The measurements were intended to provide information on tropospheric and early stratospheric fall-out over Australia from high-yield nuclear weapon tests in the atmosphere, carried out by the U.S.S.R. and the United States during 1961 and 1962. The most recent release of fission products into the atmosphere in sufficient quantity to be of significance in considerations of global fall-out occurred in late December 1962. Results reported complement data obtained by the Committee in its extensive and continuing survey of strontium-90 in the Australian environment. All stations exposed 6 in. × 12 in. gummed films continuously for the purpose of providing daily samples for the measurement of total activity. The gummed films were changed every 24 h; the radioactivity measurements were made at a central laboratory. Up to May 31, daily gummed film sampling was carried out at 24 stations. Only 22 of the 3,624 daily samples taken during this period showed activities above the minimum detectable level; even the higher activities were just above the minimum detectable level. The survey showed that levels of total β-activity in fall-out over Australia throughout 1963, as monitored by the daily and monthly sampling methods, were notably lower than in 1962. Towards the end of 1963 the total activity-levels were approaching those recorded before the resumption of nuclear weapon testing in 1961. However, nuclear weapon testing during 1961 and 1962 resulted in the injection of substantial quantities of strontium-90 and cæsium-137 into the stratosphere which will contribute to the low levels of these long-lived fission products expected in fall-out over Australia. There were indications during 1962 that some strontium-90 in fall-out over Australia at that time was arising from recent nuclear weapon testing. Efforts in the fall-out monitoring programme will now be devoted to the continuing assessment of the levels of these long-lived fission products in the Australian environment.

British Lichen Distribution Maps

The British Lichen Society now has a Distribution Maps Scheme to record the distribution of lichens within the British Isles. The Scheme is based on the 10-km square system of the National Grid. A record card listing 154 easily identified lichens, with one side blank for additions, is available for use in the field. Eleven members are mapping individual genera or species for early publication. A large number of species are confined to the Highland Zone of Britain, and many lichens have restricted distributions due to such factors as climate, soil conditions, forest clearance, survival of open communities, atmospheric pollution, etc. Maps showing the precise British distribution of lichens will therefore be of considerable interest, and will help to disentangle the various factors which affect the distribution of particular species. Full details of the Scheme can be obtained from the Society's mapping recorder, Mr. M. R. D. Seaward, Lynwode, Vicarage Avenue, Wrawby, Brigg, Lincolnshire. Details of the British Lichen Society itself can be obtained from the secretary, Mr. J. R. Laundon, Department of Botany, British Museum (Natural History), Cromwell Road, London, S.W.7.

Textile Research in Poland

The Politechnika Łódźka has published a further issue of its Scientific Bulletins, details of which were given earlier (*Nature*, 201, 1171; 1964). Issue No. 11 (1964) of *Włókiennictwo* contains contributions from the Department of Mathematics of the Chemical Faculty on statistical analysis, and from the Department of Textile Raw Materials and Metrology on the effects of changes in structure, caused by heat-setting, on the mechanical properties of polyethylene terephthalate fibres during stretching. There are also articles on the causes of irregularity in carded flax sliver, and on the analysis of cotton combing in relation to fibre length distribution.

Wellcome Trust Grant for Neurophysiological Research in Canada

A grant of £10,000 is to be made by the Wellcome Trust in London to Dr. V. C. Abrahams and Dr. E. P. Langworth, Queen's University, Kingston, Ontario. This will enable them to establish the necessary computer and tape system for their research work on the neurophysiology of the brain stem. Dr. Abrahams and Dr. Langworth, both of whom formerly worked at the National Institute of Medical Research, Mill Hill, London, went to Queen's University, in 1963, to establish a Neurophysiological Research Unit. Together they have drawn up plans for joint research with other university departments in a programme of research on communication theory, particularly on organization in the brain stem.

Carlsberg–Wellcome Travelling Research Fellowships

The Carlsberg Foundation of Copenhagen and the Wellcome Trust of London have announced that the Carlsberg–Wellcome travelling research fellowships for the year 1964–65 have been awarded to Mr. Jens Hyldgaard-Jensen, of the University of Copenhagen, and Mr. G. C. Coles, of the University of Cambridge. Mr. Hyldgaard-Jensen, recently a research scholar at the Royal Veterinary and Agricultural College, Copenhagen, will work at the Rowett Research Institute, Aberdeen, under the guidance of Dr. R. N. B. Kay, on problems of the physiology of feeding in cattle. Mr. G. C. Coles, a post-graduate student in the Department of Zoology at the University of Cambridge, will carry out research under Prof. T. Weis-Fogh at the Zoophysiological Laboratory at the University of Copenhagen.

Announcements

Prof. F. C. Steward, professor of botany in Cornell University, Ithaca, has been awarded the Stephen Hales Prize of the American Society of Plant Physiologists, for his distinguished work on the salt uptake of plants, the nitrogenous constituents of plant tissues, and plant growth regulators.

A joint meeting of the Challenger Society and representatives from the Marine Laboratories (Development Commissioners' Scheme) will be held at the Freshwater Fisheries Laboratory, Pitlochry, during October 14–15. Further information can be obtained from the Secretary, Challenger Society, National Institute of Oceanography, Wormley, Godalming, Surrey.

A symposium on "Concrete Quality", organized by the Cement and Concrete Association, will be held in London during November 10–12. Further information can be obtained from Dr. R. P. Andrew, Cement and Concrete Association, Wexham Springs, Slough, Bucks.

An international conference on "General Relativity and Gravitation" will be held in London at the beginning of July 1965. The main meetings will be held during July 1–10, but arrangements will be made so that those participants who wish may arrive from June 28 onwards in order to take part in discussions and informal seminars. Further information can be obtained from the chairman of the local organizing committee, Prof. H. Bondi, Department of Mathematics, King's College, Strand, London, W.C.2.

THE GLASGOW UNIVERSITY EXPLORATION SOCIETY EXPEDITION TO MINGULAY

By J. F. ROBERTSON, J. B. RANKIN and DR. D. R. BOWES
University of Glasgow

AND

A. M. HOPGOOD
University of St. Andrews

A GLASGOW University Exploration Society expedition to the uninhabited Island of Mingulay (Nat. Grid NL 056783), some twelve miles south of Barra in the Outer Hebrides, was made during June 21–July 14, under the leadership of Mr. J. F. Robertson. The Island is essentially made up of rocks of the Lewisian complex[1] and the geological investigations, undertaken by six of the expedition members under the direction of Dr. D. R. Bowes and Mr. A. M. Hopgood, are a continuation of Lewisian investigations being carried out in the Departments of Geology of the University of Glasgow and the University of St. Andrews. Topographically the dominant features of the island are the sheer cliffs, some more than 700 ft. high, on the western seaboard, and a large, sheltered eastward-facing glacially-carved amphitheatre which rises from a sandy bay on the eastern seaboard. A crofting settlement, almost completely abandoned in 1909, is situated in the lower parts of this amphitheatre largely on superficial deposits of boulder clay and sand. This 'fossilized' settlement, which has remained well preserved by virtue of its isolation, was investigated under the direction of Mr. J. F. Robertson and Mr. J. B. Rankin as part of the crofting investigations being carried out by the Departments of Geography and Archæology of the University of Glasgow.

The Lewisian rocks are dominantly banded gneisses of intermediate to acid composition. They show evidence of metamorphic reconstitution at hornblende granulite facies, and subsequent retrogressive effects, and are injected by many structurally controlled pegmatite veins. The effects of at least five periods of folding and associated deformation are displayed. In addition there is evidence of two periods of pseudotachylite development, one within the Lewisian sequence and the other associated with the Hebridean Thrust. In summary the geological sequence of events is:

(1) The formation of the prominent banding, associated with tight isoclinal folding of lithological layers, tectonic disruption and metamorphic reconstitution at hornblende granulite facies. Strong linear and planar elements developed with mineral growth axial plane to the fold structures. The first prominent elements of rock microfabric were formed at this stage.

(2) The development of asymmetrical folds which fold the banding. The fold axes plunge gently north while the axial planes trend approximately north-west–south-east and dip in a north-easterly direction at a moderately steep angle. The dip of the north-eastern limb is consistently much less than that of the south-western limb which is commonly near vertical or overturned. A secondary foliation is developed parallel to the axial plane. The largest folds observed are of this type and they control the gross attitude of the banding. Quartzofelspathic pegmatitic material developed and its uprise was controlled by the axial planes of these folds.

(3) Pseudotachylite was formed and injects gneisses and pegmatites. Bands of pseudotachylite breccia trend east-north-east–west-south-west and dip toward the south-south-east at approximately 30°.

(4) Open folding with much small-scale crenulation and the development of axial plane cleavage. Fold axes plunge gently towards the south-east and axial planes dip at a moderately steep angle to the south-west.

(5) Folding of variable intensity on approximately east–west trending axes which plunge gently eastwards. Interference structures resulting from the effects of these folds on the asymmetrical folds are common and much of the variation in attitude of the lithological layering is the result of this interference. The most common and the largest pegmatitic veins trend east–west and are emplaced parallel to the axial planes of these folds.

(6) Variable, very open folding with near horizontal axes generally trending between north-north-east and east-north-east.

(7) Thrusting, dislocation and pseudotachylite formation associated with the development of the Hebridean Thrust.

(8) Emplacement of dolerite dykes (of presumed Tertiary age).

Events 1–6 are considered to represent the Lewisian history of the rocks and they correspond, both individually and in sequence, with Lewisian events shown by the rocks of Barra investigated by one of us (A. M. H.). There is also considerable similarity to the Lewisian rocks of the mainland, particularly in the vicinity of Gairloch, investigated by one of us (D. R. B.), but correlation at this stage, in the absence of absolute age dates, is considered premature.

The main settlement, which was surveyed by plane table at a scale of 1 : 500, consisted of approximately thirty crofts. It was located on the seaward side of the main track joining the school and St. Columba's Chapel, both of which were set apart from the rest of the village. The quality of the dry stone masonry of the croft houses was generally good, entire walls being intact in some cases, some with stones up to three-quarters of a ton. All the croft houses were roofless and most were approximately 30 ft. long, 15 ft. wide and 5 ft. high (internal measurements) with doorways 4 ft. 6 in. high by 2 ft. 6 in. wide. Both doorways and windows were topped by stone lintels. The walls, which consisted of inner and outer stone shells with rubble between, were approximately 4 ft. thick and generally rounded at the corners. Most of the croft houses had a fireplace and chimney in the end wall, and, with the exception of two that were gabled, the houses were hip-ended. The chief area of crofting land, surveyed at a scale of 1 : 2,500, was located on the landward side of the main track on the southern flanks of the amphitheatre. Elongate rectangular croft strips extended down the well-drained slopes from the Lewisian outcrops to one of the small streams cut in the boulder clay. Drystone dykes, some in very good condition, bounded the strips of land—good quality pasture broken by rock outcrops towards the head-dyke. The remains of the smaller crofting settlement at Skipsdale, in the south-eastern part of the island, were also investigated.

The expedition could not have been arranged without the generous financial assistance of the University Court of the University of Glasgow, the Carnegie Trust for the Universities of Scotland and the Royal Scottish Geographical Society. Grateful acknowledgment of assistance is also made to Kodak, Ltd., Hanimex (U.K.), Ltd., the Northern Lighthouse Board, the keepers of Barra Head Lighthouse, the Inverness Education Committee, our many kind friends in Castlebay, Barra, and Prof. Ronald Miller, Dr. J. B. Caird and Mr. G. Petrie of the Department of Geography, and Dr. H. Fairhurst of the Department of Archæology, University of Glasgow.

[1] Jehu, T. J., and Craig, R. M., *Trans. Roy. Soc. Edin.*, 53, 419 (1923).

DEVELOPMENT OF NORTHERN AUSTRALIA

In his presidential address to the Section of Geography and Oceanography at the thirty-seventh annual congress of the Australian and New Zealand Association for the Advancement of Science, Prof. R. H. Greenwood (University of Queensland) analysed the conditions for accelerated development in Northern Australia (*Australian Journal of Science*, 26; April 1964).

The first point which he emphasized was the necessity for an enlarged market for Australian goods. Since the largest domestic market is in the south-east, and, since Australian transport costs are high, there is no purpose in seeking to replace existing production in the north. Additional markets may be created in four sectors —two domestic and two foreign. The domestic market is being constantly enlarged to keep in line with the increasing Australian population; however, it could—with benefit to the economy—be increased still more by replacing imports by home-produced goods. The latter offers the more appealing prospects, but in the main it depends on the ability to pioneer new techniques for the production of tropical crops that are normally labour-intensive (rubber, coffee, tea, copra, etc.) and to intensify the search for minerals and oil (which constitutes a particularly large item on the import bill). Overseas markets can be expanded either by seeking new buyers for the conventional products of the north (mainly beef, sugar and minerals), or by innovations such as exploiting newly discovered minerals, or by the large-scale production of tropical grains and other food crops and raw materials for Asian markets. In theory, there is a substantial potential in each of these market sectors. The major problem is high costs, and this is of such significance to the basic proposition that the second condition for accelerated development is the ability to reduce overall production costs, or (and this is not by any means the same thing) to reduce the price charged for goods produced in the north.

There are some ways in which small savings can be made. It would be an advantage to increase productivity in those parts of the Australian Commonwealth that are nearest to her customers. It is unsatisfactory that Australia should, in a geographical sense, face away from her customers. It is a significantly shorter haul from Darwin to Hong Kong, Madras or London than it is from Melbourne, and the shorter time of the sea voyage may be important for technical reasons as well as cost (for example, in the ability to deliver chilled beef to the United Kingdom). Land would be comparatively cheap and every advantage of large-scale commercial production could be reaped in agriculture. The largest element in the higher cost margin, however, relates to the establishment of essential services—water, power, transport and building —in areas remote from the main centres of production. The capital costs involved in setting the productive machine in motion are high and the benefits derived would be long-period, rather than short-period, benefits of a kind which reduce the attractiveness of the proposition to private enterprise. Moreover, public funds available for such capital developments are not unlimited.

This leads to the third condition for development. The benefits of capital investment in the north must be maximized. It would be essential to extract the utmost value from each project. In an area as big as tropical Australia, there is likely to be a measure of regional dispersal of public expenditure, each item adding different amenities in widely separated districts. The main item of public investment in the north should, however, be carefully planned to provide as many of the prerequisites for diversified development as possible. An ideal model would be the provision of a water storage that could be used both for irrigation, town supply and power generation to a nearby area with suitable conditions for the cultivation of an industrial crop which could be processed—at least in part locally—with road access to a not-too-distant port. The anticipated benefits would include the capacity of the original investment to bring about growth of ancillary and dependent activities in an exponential manner and, in particular, to stimulate the growth of small urban communities, the increasing needs of which would create opportunities for additional use of local land, water, power and other services. Such a deceptively simple model is not by any means easily created. For example, there is the temptation to start with the selection of a suitable dam-site and see what can be done with its surroundings. Governments in many parts of the world have been prone to build dams first and think of ways of using the water they retain afterwards.

The starting-point should be the selection of a crop with carefully evaluated market prospects. The next stage would be the location of one region in which market surveys indicate that the production of the crop is justified on a large scale. This should be a region with maximum nodality, with country capable of complementary rural production in different districts, with maximum access to a port, roads, railways and other services or with optimum means of creating such access. The logistics of geographical location are of immense importance, and the districts around Rockhampton and Darwin have great possibilities as regards the polarization of new developments. They are natural nodes to which easy lines of communication converge and around which varied resource patterns are disposed. They are of particular significance, as notably Australia lacks such naturally endowed nodal districts. Dam sites should only be looked into after the purpose has been satisfactorily defined of the new developmental scheme and the optimum location for its introduction agreed.

A GREAT PATHOLOGIST AND NATURALIST

Adolph Lutz, one of the world's greatest medical zoologists, was born in Rio de Janeiro in 1855*. His parents were Swiss, and when they returned to Berne in 1865, Adolph was educated in Switzerland. When his parents later returned to Brazil, Adolph was left behind to go to school in Basle. He had by then already decided to spend his life studying natural history, and in 1874 he began to study medicine at the University of Berne, taking courses also at Leipzig, Strasbourg and Prague, and studying biology as well. In 1879 he received his licence to practise medicine. Already he had published papers on the Cladocera of the neighbourhood of Berne and Leipzig. He went to Vienna and Paris for post-graduate studies, and then returned to Brazil, where he practised medicine from 1882 until 1892 in the province

* *Adolpho Lutz* (1855–1955): *Vida e Obra do Grande Cientista Brasileiro*. Pp. 55. (Rio de Janeiro: "Comissao do Centenario de Adolpho Lutz", Conselho Nacional de Pesquisas.)

of São Paulo and elsewhere. In 1885 and 1886 he went to Hamburg to study dermatology. In 1887 he moved to the city of São Paulo until the pandemic of yellow fever in Campinas in 1889, and he was later summoned to Hawaii to investigate leprosy. Although he was busy as a country doctor, Lutz found time to publish papers on helminthology and medicine, among which was a monograph on *Ankylostoma duodenale* and ankylostomiasis, and others on ascariasis and the mode of infection with the tapeworm *Taenia*. On the latter subject he experimented on himself. In 1886 he published a paper on the structure of the organism that causes leprosy, proposing to include it in a new genus called *Coccothrix*, including the causal organism of tuberculosis in the same family, but the name *Coccothrix* was not adopted, although the relationship of the two organisms is nowadays recognized. In 1885 he discovered a new disease of children, now known as pink disease, and he published further papers on leprosy, based on his observations made in Brazil. Working in Honolulu he published papers on dermatology, and in 1891 a paper in which he separated amoebic from bacillary dysentery.

In Honolulu he met and married an Englishwoman, Amy Fowler, who had gone there as a volunteer nurse. After a spell in San Francisco, he and his wife returned to Brazil in 1893, where two children were born to them. In the same year Lutz was appointed vice-director of the new Bacteriological Institute of the State of São Paulo, founded by Felix Le Dantec, and later he became its director. Here he did much work on yellow fever, malaria and typhoid fever, making possible the accurate diagnosis of these endemic diseases, and directing campaigns against them. Hearing that the transmission of yellow fever by mosquitoes had been proved, Lutz, together with his colleague Ribas, director of the State sanitary service, instituted, in spite of much opposition, measures against the mosquitoes. He also worked at this time (1899–1906) on cerebrospinal meningitis, plague and myiasis, and published papers on them. In 1897–98 he discovered the sylvan form of yellow fever in the area between São Paulo and Santos, and rightly ascribed the transmission of this to the forest mosquitoes rather than to *Aëdes aegypti* (*Stegomyia fasciata*).

In 1907–08 he discovered and described two new diseases caused by fungi, namely, a sporotrichosis of man and rats, and the disease later known as the mycosis of Lutz. From 1903 onwards Lutz began to work on medical entomology, publishing papers on the technique of the study of mosquitoes, on bloodsucking Diptera and on new species of Culicidae and Tabanidae.

He also did pioneer work on veterinary medicine in Brazil, investigating among other diseases equine trypanosomiasis. Later he was asked to join the staff of the Instituto Oswaldo Cruz, becoming chief of the Division of Medical Zoology. This post gave him time for work on medical entomology and helminthology, and for work on snakes and amphibia. He also wrote papers on Tabanidae and a monograph on the Simuliidae, parasitic Hippoboscidae and the forest flies and Ceratopogonidae, contributing chapters on insects of medical importance to the *Handbuch der pathogenen Organismen*.

From 1916 onwards he returned to work on helminthology, doing pioneer work on schistosomiasis in Brazil, studying the biology and ecology of the intermediate hosts involved. Later papers were on trematodes and scorpions in connexion with his lectures on poisonous animals. His earlier interest in leprosy was retained to the end of his life, and increasingly he regarded it as a disease transmitted by mosquitoes. Lutz travelled widely in Brazil in the course of his studies, and in 1928 he warned the country of the danger of outbreaks of malaria when *Anopheles gambiae* was carried from Africa to South America. It later caused, in north-eastern Brazil, one of the most disastrous outbreaks in the history of the disease.

In the course of his life Lutz named many insects, worms, molluscs and frogs, and many species were named in his honour. He was an honorary and corresponding member of many learned societies, and received many honours. On the first centenary of his birth the Brazilian Government issued a stamp with his portrait on it, and several towns in the State of São Paulo, where he did much beneficent work, named streets after him. His research extended over 61 years, and a complete edition of his publications, of which there are more than two hundred, is being planned.

G. LAPAGE

ROTHAMSTED EXPERIMENTS

IN 1919, Sir Alfred Hall wrote that the purpose of the Rothamsted Experiments was "to ascertain the conditions under which the plant grows and the soil supplies it with nutrient". Since then agricultural research has developed in many spheres, and it is clear from the annual report of the Rothamsted Experimental Station for 1963 * that at present the classical investigations, initiated by Lawes and Gilbert over one hundred and twenty years ago, form only a part of the manifold activities of the Station.

The report follows the same pattern as in previous years, consisting of reports from twelve research departments, the farms at Rothamsted and Woburn, Broom's Barn and Woburn Experimental Stations, and a summary of the work of the Soil Survey for England and Wales. In an admirable general report, the director, Mr. F. C. Bawden, has commented on a few of the subjects of research carried out, but emphasizes that his introduction is merely an indication of the range of work of the Station, and is in no way a summary. Similarly, in a short review it is possible to mention only a few of the items contained in the report, and I can only hope that the interested reader will explore the report further for himself.

Some of the most interesting and important scientific developments have come from the research of the Departments of Physics and Botany into the phenomena involved in crop physiology. Detailed investigations into the physical conditions within crops and the growth of species under different sets of conditions have been carried out to obtain information about the factors which limit the productivity of healthy crops receiving adequate water and nutrients. As the director pointed out, until such knowledge is available it will be impossible to say with certainty whether a variety is producing its maximum yield, or what conditions increase yielding capacity. The Physics Department has been concerned with the energy involved in photosynthesis, and carbon dioxide flux measurements. Already several important trends have emerged, and some earlier ideas have had to be modified. For example, the soil's contribution to carbon dioxide uptake is much less than had been thought. Evapo-transpiration studies have been continued, in particular the estimation of insolation from cloudiness records, and the calculation of net radiation from measured solar radiation, analysis of evaporation and records of heat balance. The value of these evapotranspiration measurements as a basis for assessing irrigation needs should be fully realized when the new irrigation scheme is installed at Rothamsted. In the Botany Department, controlled environment rooms have been used to examine the effects of varying temperature and humidity on plant growth. Apart from the controlled

*Lawes Agricultural Trust. Report of the Rothamsted Experimental Station for 1963. (Harpenden: Rothamsted Experimental Station, 1964.) 15s.

environment studies, other aspects of crop physiology, such as photosynthesis of ears of cereals, and dependence of the grain : leaf ratio on radiation, have been studied. These physiology studies, in conjunction with the work of the Physics Department, should yield fundamental information of far-reaching practical consequence.

In a year in which much has been written and spoken about the harmful effects of pesticides on Nature, it is gratifying to record that several departments have devoted considerable time and effort to pesticide evaluation. Although the investigations are of widely varying character, they have all been carried out with a common purpose—"to increase the efficacy of pesticides while decreasing the risk of unwanted side-effects". The Insecticides and Fungicides Department has been concerned with the search for effective new substitutes for the more persistent organic chlorine compounds and has experimented with organo-phosphorus compounds. Initial experiments have shown that yields of wheat in a wireworm-infested field were considerably increased by applying some of these latter compounds, but further work is required to determine whether their application is a commercial proposition. In the Entomology Department, the experiment on the long-term effect of DDT and aldrin has been continued. The extent to which insecticides such as aldrin, dieldrin and DDT accumulate in worms and other soil invertebrates is being studied. Results so far indicate that the concentration of dieldrin in earthworms is several times greater than in the soil itself. Another important aspect of these investigations is the re-colonization of sterilized soil by soil invertebrates which help to maintain soil fertility.

In agricultural entomology, although much time and effort has been spent in studying pests themselves, comparatively little work has been done on their relations with the growing plant, especially on the way in which pest populations of different sizes affect the growth and yield of plants at different stages in their development. Such an investigation has been commenced at Rothamsted on both root and seed crops.

The Biochemistry Department is pursuing its interest in the extraction of leaf protein as a contribution to the improvement of protein supplies in the underdeveloped countries. Three protein-extraction units have been set up in Lincolnshire, Lucknow and Jamaica, and a pulping machine has been sent to Makerere for an investigation into the prospects of using leaf protein in Uganda. The factors which control the suitability of a crop for leaf-protein extraction are the amount of protein which accumulates in the leaf and the readiness with which it can be extracted. Maize has been found to be particularly useful, as good extraction rates are obtained over a substantial part of the growing period, thus allowing continuity of production throughout the summer and autumn.

In the Chemistry Department, work on organic manures, soil structure and nutrient uptake has been continued. Attempts have been made to relate laboratory measurements of easily soluble nutrients to nutrient uptake in the field. Variations in such correlations in the past have been caused by variation in the structure of the same soil in different years. Ways of altering the soil structure have been tried in order to distinguish between the nutrient and physical effects of organic manures. Experiments carried out to investigate the way in which root growth and crop nutrition are influenced by soil structure have confirmed the theory that it is the rigidity of pore spaces in the soil rather than their size or continuity which affects root growth. Fertilizer placement experiments and work on nitrogen fertilizers, particularly ways of lessening losses of nitrate by leaching and denitrification, should prove to be of considerable practical significance.

The lay-out of the report, whereby the work of each department is recorded separately, often makes it difficult to deduce how much collaboration there has been between departments. The Plant Pathology report, however, includes an outstanding example of such collaboration, in the work on the cause of farmer's lung disease, which involved the Departments of Soil Microbiology, Plant Pathology and Biochemistry, and the Institute of Diseases of the Chest. Many of the problems in agriculture can only be tackled by interdepartmental co-operation, and it is hoped that the extent of such co-operation might be more clearly indicated in future.

The report contains two special reviews, on mobilization phenomena in soils and on the classic Park Grass Experiment. The character of these two reviews reflects the satisfactory blending of applied and pure scientific research conducted at Rothamsted. In his excellent review of mobilization phenomena, C. J. Bloomfield combines a summary of his own well-known work on the movement of iron in soils with a critical survey of the contributions of other workers in this field. Among the many important points he raises are the need to record the presence or absence of sulphate in investigations of the chemistry of waterlogged soils, and the difficulties involved in ferrous iron determinations in soils containing organic matter.

The report ends with a series of abstracts of papers published by the staff over the year. In all, two hundred and forty papers have been written, which is in itself a fair indication of the substantial contribution made by Rothamsted to present-day agricultural research.

D. A. MacLeod

THE ELECTRICAL RESEARCH ASSOCIATION

THE activities of the Electrical Research Association throughout the year 1963 are recorded in the forty-third report presented to the annual general meeting held on April 29*.

Perhaps the most notable development is the building up of a substantial research programme in the electronics field. This work, which is concentrated on thin films, is directed towards helping the manufacturer of thin-film devices first by accumulating information about the production of films, the effects of impurities and the factors determining the suitability of materials for particular applications. By providing information on manufacturing techniques such as methods of deposition and on the effect of different vacuum conditions on the performance of products, and by assistance in the analysis of faults occurring after manufacture, the Association should be able to provide much of the technical background required by industry.

Silicon monoxide, which is widely used for making insulating layers in cryatrons and microcircuits, is being investigated as its dielectric properties are known to be variable and dependent on uncontrolled deposition parameters. The evaporation of films and the investigation of their electrical properties are being carried out using an ultra-high-vacuum system in which the residual gas pressure can be reduced to 10^{-9} torr.

In relation to the deposition of thin metal films, an investigation is proceeding on the production, collimation and control of a beam of metal ions which can be directed at a suitable receiving surface. An arc discharge *in vacuo* between copper electrodes is being used to produce a high density of copper ions. It is thought that this technique will be of importance in the fabrication of thin-film

* The Electrical Research Association, 43rd Annual Report for the year ended 31st December, 1963. Pp. 92. (Leatherhead: The Electrical Research Association, 1964.)

components in which high standards of delineation and rate of deposition are necessary. Investigation of the conductivity of thin metallic films resolves naturally into that of three conduction mechanisms representing three stages in the formation of the film, namely: (1) conduction between discrete aggregates; (2) conduction between aggregates linked by metallic bridges; (3) conduction along a thin homogeneous plane parallel film. Work is in progress on all three conduction mechanisms, the last-named having already been investigated extensively.

A significant part of the Association's work on insulating materials is directed towards establishing or improving British Standards. For example, a proposed specification for capacitor tissue is nearly complete and will shortly be issued to members. A preliminary investigation is in progress on the use of non-polar plastics at a stress of about 500 kV/cm. A knowledge of the long-term effect of these high stresses and of the mechanism of failure in the absence of discharges is essential to the design of high-voltage plastic insulated cables. Theoretical work has continued on fundamental phenomena in dielectrics in the stable state and, in relation to the fundamental phenomena of instability, a paper on the theory of the stability of bubbles in insulating liquids in an electric field has been accepted for publication by the Royal Society.

Research on high-temperature plasmas has comprised further work on the magnetically constricted low-pressure d.c. arc. Probe and microwave techniques together with spectroscopic investigations have confirmed the part played by the cathode fall of potential in the production of the apparent high ion temperatures in excess of 2×10^{6}° K which are observed in the positive column of this low-power arc. This work has prompted a re-examination of existing theories of electrode mechanisms. The effect of electrode boundary layers and the mechanism by which current can be extracted from a flowing ionized gas constitutes one of the least understood problems of magneto-hydrodynamic power generation. A laboratory model of a novel form of plasma furnace has been built for the purpose of spheroidizing highly refractory particles.

Circuit breakers and the phenomena associated with current interruption continue to occupy an important place in the research programme. Particular attention is being directed to the further development of the 'synthetic' testing of circuit breakers. The demand for circuit breakers with still higher rupturing capacities implies increase in the size of the alternators and associated equipment of short-circuit testing stations on a scale which could be exceedingly costly. Synthetic testing which, in effect, supplies current and voltage for the test from two independent relatively low power sources, offers a possible alternative solution to the problem involving a much lower order of expenditure. While there is considerable evidence that synthetic methods can reproduce the severity of conventional testing it has still to be shown that synthetic circuits can stimulate adequately the many factors involved in the proving of circuit breakers. Among the items of research equipment developed for circuit breaker testing is a compact design of restriking voltage indicator.

The important fundamental investigation of possible binary and ternary alloys other than those of the silicon-iron binary series, which might offer improved characteristics in soft magnetic core materials, continues. This work is being carried on mainly at the British Iron and Steel Research Association Laboratories at Sheffield. In relation to transformer noise, tests are being made to ascertain what correlation exists between magnetostriction and core vibration. These are being made on transformer cores having similar magnetic properties but widely different values of magnetostriction.

An extensive investigation has been made of the over-voltages which occur in high-voltage transmission systems as a result of switching operations. A critical résumé has been prepared of the amplitudes, wave shapes and frequency of occurrence of switching over-voltages in extra-high-voltage transmission systems. An automatic surge oscillograph has been installed on one of the 275-kV transmission lines of the British Grid which is equipped with automatically reclosing circuit breakers in order to obtain information about the over-voltages produced by high-speed reclosure. The Association's facilities for power system analysis, particularly complex transient problems, have been extended by additions to the network analyser or, as it is now termed, the 'Integrated Computer'.

Transient problems have been dealt with relating to 50-c/s a.c. railway electrification, switching surges on high-voltage d.c. submarine cables, rate of rise of restriking voltage on large-scale systems and the fault contribution from large induction motors. The analyser is no longer used for load flow and stability investigations, as computer programmes are now available for the solution of those problems on digital machines.

The rating of cables constitutes an important part of the work of the division dealing with lines and cables for power supply. A field investigation on the thermal behaviour of buried cables is being made in co-operation with various area electricity boards, the Central Electricity Generating Board and the Cable Makers Association. This investigation is concerned with the influence of soil moisture migration on the power-rating of cables buried directly in the soil and with the exploration of methods of mitigating thermal run-away due to drying out in the summer. Experiments have been made with 'backfill' materials with good thermal properties and promising results have been obtained with impermeable cover and weak mortar bedding.

One section of the programme of work on intrinsic safety has been completed by the investigation of a further nine inflammable gases, and all inflammable gases and vapours of industrial importance are now included in a British Standard Specification. Work on the relationship between circuit current and the probability of ignition has shown that the safety factor at present used ensures that the probability of ignition is as low as $1 : 10^{8}$. Investigations concerned with rotating machines comprise an investigation of unbalanced magnetic pull and the continuation of work on tooth ripple phenomena in alternators.

Among the non-industrial applications, electrical heating takes quite an important place. Various aspects of off-peak floor heating are now being investigated. Field trials of floor heating in multi-story flats have reached a stage which enables definite recommendations to be made regarding floor structure, floor and building insulation and cable spacing. A related problem, that of the static electrification of carpets, has been investigated during the year and a report issued. There is increased general interest in problems of static electrification resulting from warmer and drier atmospheric conditions in buildings, the development of certain new industrial processes and the widespread use of very readily electrified plastic materials. Means of heat storage other than floor heating are being investigated, in particular the use of the lime-water reaction.

The Association's work related to agriculture and horticulture continues to be directed towards crop conservation, equipment in livestock husbandry and controlled environment for livestock together with instrumentation and control in horticulture and the horticultural applications of heat and light. Very encouraging results have been obtained with the dairy heat pump.

Quite outside the normal range of the Association's activities, although deriving directly from earlier work on arc physics, and on lightning discharge, are the contributions to astrophysics made by Dr. C. E. R. Bruce. It has been demonstrated that the types of absorption spectrum lines which have since 1877 been regarded as evidence of rapid stellar rotation often derive from discharge-generated vortices akin to ball-lightning. A theory of ball-lightning

has been developed which appears to be in accord with all known observations.

A significant trend in the financial support given to the Association is evidenced by the large increase, relative to the general subscription income, in the specific contributions by industrial groups to specially sponsored programmes. This, to a great extent, reflects the wish of members, especially the larger ones, to exercise a greater measure of control over the application of the funds which they provide than is possible if they simply contribute to general funds through membership subscriptions.

J. Greig

DISTRIBUTION AND MOTIONS OF OH NEAR THE GALACTIC CENTRE

By J. G. BOLTON, F. F. GARDNER, R. X. McGEE and B. J. ROBINSON

C.S.I.R.O. Division of Radiophysics, University Grounds, Sydney, Australia

MEASUREMENTS with the Australian 210-ft. telescope have shown[1] a wide and deep absorption by OH in the radio spectrum of Sagittarius *A*. The absorption occurred over a region the angular extent of which was much less than that of the whole Sagittarius *A* source, and it appeared likely that only the 'core' component[2,3] of the source was being absorbed. However, the original observation did not reveal the overall angular extent of the absorbing gas.

We have since observed OH absorption in other parts of the Sagittarius *A* complex at 1,667 Mc/s, 1,665 Mc/s and at 1,612 Mc/s, the frequency of one of the recently detected[4] satellite lines of OH. Absorption has been detected at all longitudes between $l^{II} = 358°\ 30'$ and $l^{II} = 2°\ 30'$, and for latitudes between $b^{II} = +10'$ and $b^{II} = -30'$. As was found[1,4] for the central source of Sagittarius *A*, only some of the components of the continuum radiation were absorbed, and the ratios of the intensities of the lines were smaller than the theoretical values. The OH is distributed in apparently discrete clouds with characteristic velocities. An unexpected result was that the velocities of the various clouds are constant over a range of galactic longitude, in contrast with the velocity distribution deduced from 21-cm measurements of neutral hydrogen.

Observations at intervals of 10′ arc were made at 1,665 and 1,667 Mc/s using a modified receiver with 48 channels[5] of band-width 37 kc/s. Absorption was found at radial velocities from +160 to −200 km/s. Partially resolved minima in the profiles at a number of discrete velocities can be traced continuously over one to two degrees in longitude. An example is given in Fig. 1; contours of the 'apparent opacity', at 1,667 Mc/s, of the gas with radial velocity near −135 km/s are superimposed on a map of the continuum at 1,670 Mc/s. (The continuum measurements were made during the present survey with a wide-band section of the receiver). The apparent opacity is defined as the amplitude of the observed absorption divided by the continuum temperature in the same direction; as in general only part of the continuum radiation is absorbed, this ratio will be a lower limit to the true opacity. The gas responsible for absorption near −135 km/s extends south of the galactic plane in a band 20′ arc wide, the opacity being appreciable between $l^{II} \doteqdot 359°\ 10'$ and $l^{II} \doteqdot 0°\ 30'$. No contours are shown for the weaker absorption outside this longitude range. The apparent opacity reaches peak values of about 0·4, corresponding to an optical depth of at least 0·5.

Clouds of similar extent are observed at a number of other velocities. At negative velocities the gas is found mainly south of the plane in the longitude range from 358° 30′ to 1°. For positive velocities the clouds are observed to lie close to the plane, in the longitude range $l^{II} = 359°\ 50'$ to $l^{II} = 2°\ 30'$. These clouds have dimensions of approximately 200 parsecs at the distance of the galactic centre.

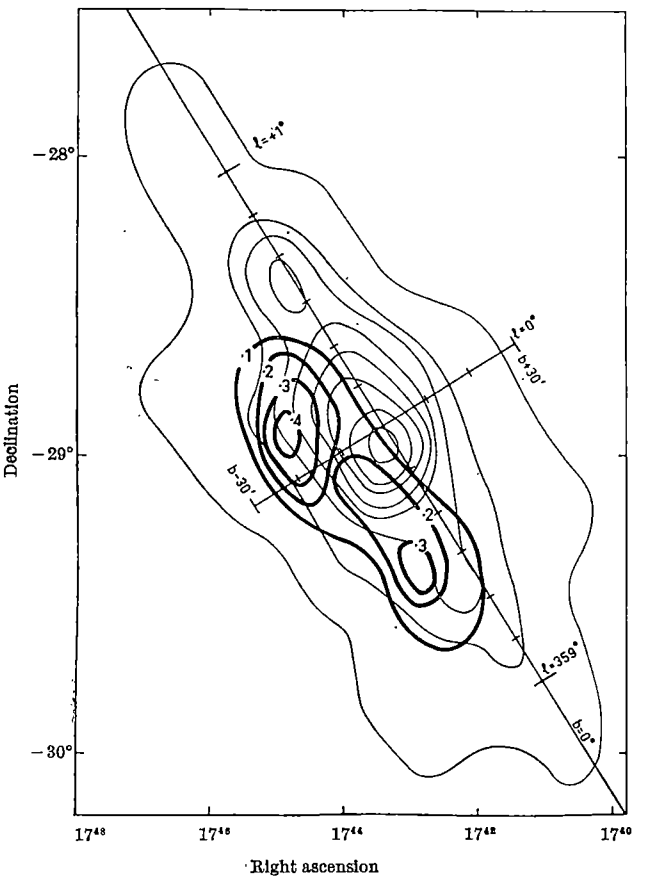


Fig. 1. Contours of the 'apparent opacity' at 1,667 Mc/s of the gas with a radial velocity near −135 km/s (1964·5 co-ordinates)

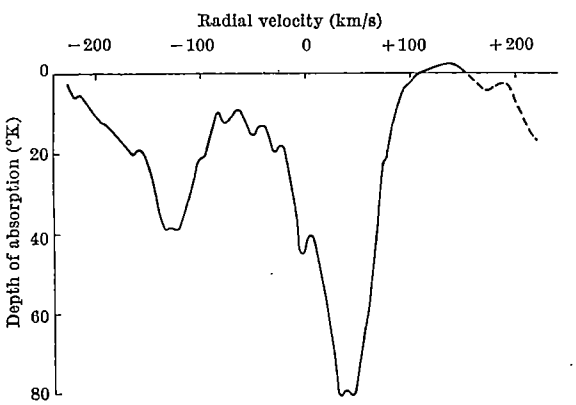


Fig. 2. Absorption profile of Sagittarius *A* at 1,667 Mc/s, measured with a band-width of 37 kc/s. For velocities greater than +150 km/s the profile overlaps that of the 1,665 Mc/s line

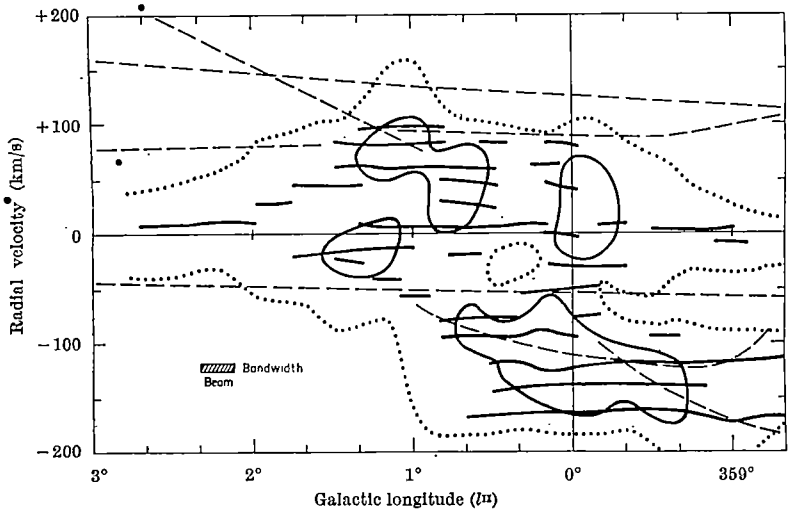


Fig. 3. Galactic longitude *v.* velocity diagram, showing for $b^{II} = -0° 10'$ the areas of strong 1,667-Mc/s absorption (enclosed by contours) and the boundaries of the observed absorption (dotted lines). The thick lines join discrete minima on the profiles, while the dashed lines represent the velocity variation of HI observed[7] at 1,420 Mc/s

Goldstein *et al.*[6], observing the central source of Sagittarius *A* with a 54′ arc beam, found an absorption at −120 km/s of intensity comparable to the deep minimum at +40 km/s. The complete profile for this source, observed with the 12′·5 arc beam at 1,667 Mc/s, is shown in Fig. 2. With the greater resolution the localized[1] absorption near +40 km/s is enhanced relative to that at high negative velocities. Both the strong absorption features are seen to be blends of several components not revealed in the earlier investigations[1,6].

An impression of the motions of the gas may be gained from the radial velocity *v.* galactic longitude plot at $b^{II} = -0° 10'$ given in Fig. 3. The diagrams for other latitudes are similar. The extent of the absorption is indicated by the four closed contours, delineating areas where the absorption is greater than 7° K, and by the dotted lines showing where absorption can no longer be detected. The thick lines running across the diagram join the radial velocities of discrete minima on the profiles; for clarity no attempt has been made to indicate the intensity at the minima. The resolutions in angle and radial velocity are shown on Fig. 3. For comparison, the dashed lines indicate the behaviour of the main emission features of neutral hydrogen, for $|V| > 50$ km/s, as deduced by Rougoor[7] from 21-cm observations with a beam-width of 34′ arc.

A striking characteristic of the diagram is the constancy of the velocity of the absorption features. A close correspondence between the OH and HI velocities is seen between +90 and 100 km/s, and near −50 km/s. For velocities greater than +100 km/s no marked OH absorption is seen; the hydrogen at these velocities also shows no appreciable absorption[8], and is believed to lie beyond the centre and to be moving away from it. However, the OH observed between 0 and +100 km/s must lie in front of the centre and be moving inwards.

At higher negative values, up to −170 km/s, the constancy of velocity of the several partially resolved minima of the OH profiles (Fig. 2) contrasts with the 21-cm observations. The latter show steep gradients of velocity with longitude, interpreted[7] as a "rapidly rotating disk" and a "contracting or expanding arm at −110 km/s". The presence of apparently discrete velocities conflicts with the model proposed by Rougoor and Oort[8] for the motions of the gas in the central region. It is clear that radial motion is as marked as any component of rotation about the centre. The 21-cm measurements have possibly suffered from confusion of a mixture of emission and absorption, and inadequate resolution. In this survey no OH emission greater than 2° K was found, so that the absorption stands out clearly.

[1] Robinson, B. J., Gardner, F. F., van Damme, K. J., and Bolton, J. G., *Nature*, **202**, 989 (1964).
[2] Biraud, F., Lequeux, J., and LeRoux, E., *Observatory*, **80**, 116 (1960).
[3] Moffet, A. T., *Astrophys. J. Supp.*, **7**, 93 (1962).
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[5] McGee, R. X., and Murray, J. D., *Proc. Inst. Rad. Eng. (Austral.)*, **24**, 191 (1963).
[6] Goldstein, S. J., Gundermann, E. J., Penzias, A. A., and Lilley, A. E., *Nature*, **203**, 65 (1964).
[7] Rougoor, G. W., *B.A.N.* (in the press).
[8] Rougoor, G. W., and Oort, J. H., *Proc. U.S. Nat. Acad. Sci.*, **46**, 1 (1960).

INTERACTION BETWEEN LOWER AND UPPER TROPICAL TROPOSPHERES

By C. R. V. RAMAN and Y. RAMANATHAN

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ATTENTION has been directed by a number of workers to the concentration of easterly winds in the upper troposphere (150–100 mb levels) over the tropics into maxima, exhibiting jet stream characteristics. Such maxima in easterlies generally occur and are most prominent in the northern summer (July and August) along the southern periphery of the immense anticyclones over south Asia and north Africa. Occurrence of easterly wind maxima has also been noted in the Caribbean. These are sporadic and have been described as the return current of west wind maxima around the periphery of the sub-tropical anticyclone over the North Atlantic (Bermuda high).

Upper divergence in the favourable regions of the east-wind maxima over the tropics has been utilized in explaining low-level weather sequences on a cause-and-effect basis. In a case study, Alaka[1] cites examples of vertical motion associated with an easterly jet being directly favourable for organized convection over the Caribbean and western part of the Gulf of Mexico. In explaining conditions leading to the onset of the summer monsoon over India, monsoon rains as well as intensification of monsoon depressions, Koteswaram[2] visualizes the pre-existence of favourable regions of vertical motions in the upper troposphere brought about by east-wind maxima, perturbations in the upper easterlies and their movements. Summarizing the applications of similar mechanisms, Reiter[3] states that "the Indian monsoonal rains and the rainy season in equatorial Africa are dependent on the 'Tropical Easterly Jet' and its position and not so much on the zenithal march of the Sun". These are instances of extension to the tropics and near

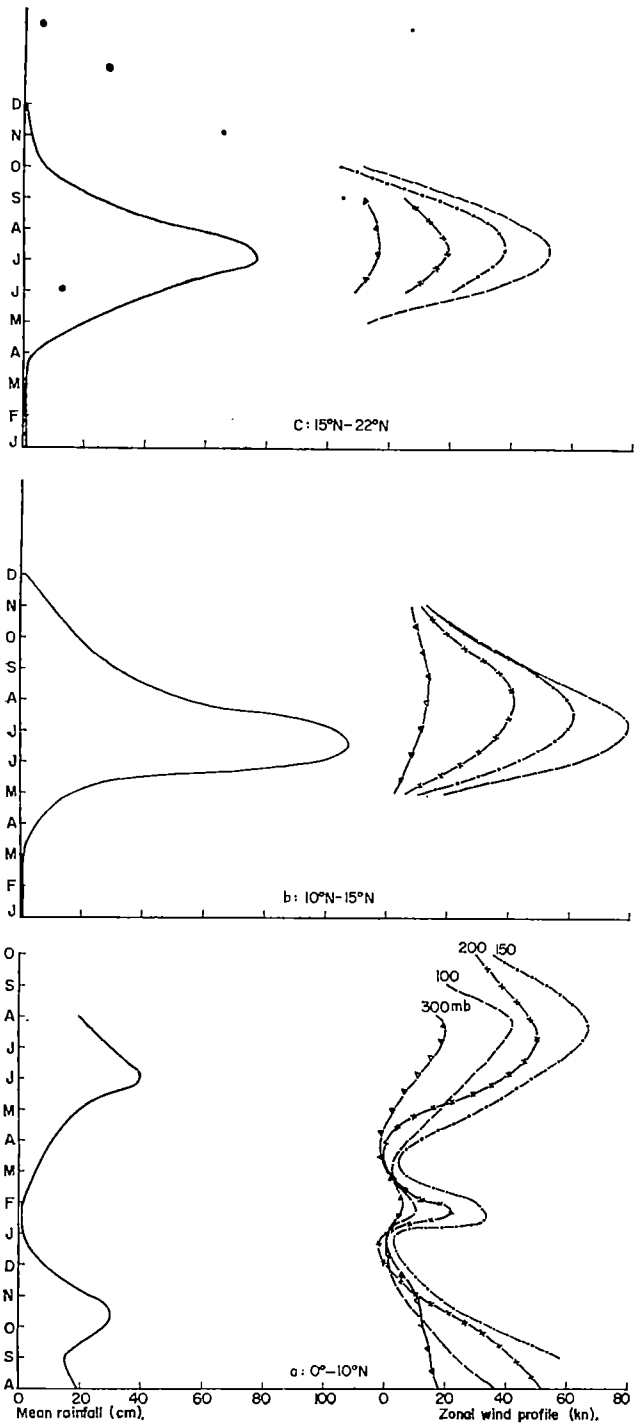


Fig. 1. Mean monthly rainfall distribution and profiles of upper tropospheric mean zonal components (east) along 77° E. (west coast of India)

equatorial regions of middle latitude concepts involving influence of changes in upper air on lower levels.

In an investigation of the upper easterlies during July 1957, Raman[4] directed attention to the absence of marked wave-type perturbations in the easterly current unlike the middle latitude westerlies. He also found that during the summer monsoon period, the upper tropospheric east wind maxima underwent a phase of generation and degeneration in an interval of five to nine days. Parthasarathy[5] pointed to the weakening of high-level easterlies over peninsular India during 'breaks' in the south-west monsoon when precipitation over the southern slopes of the Himalayas and sub-montane regions is copious but is practically absent over the plains and west coast of India. Again, reasons for the lateral displacements of east-wind maxima over south Asia during summer, occurrence of easterlies of jet magnitude over the equator, far removed from the sub-tropical anticyclones have remained obscure. Explanation is also necessary for the occurrence of east wind maxima of jet magnitude over tropical Australia and Africa, equatorward of the sub-tropical anticyclones in the southern hemisphere.

Towards a better understanding of some of these aspects, an examination was made of the behaviour of the upper tropospheric easterlies from south-west Pacific to equatorial West Africa during the IGY and IGC period (1957–59). It was found that the time sequence of the upper event to the lower effect was not always realized; for example, monsoon rains along the west coast of India did not appear always to result from changes in the upper troposphere. Instances were not uncommon when strengthening of the upper easterlies was noticed some time after occurrence of heavy rain along the west coast of India. Experience with daily analysis of extended weather charts (20° E. to 155° E. and 50° S. to 45° N.) at the International Meteorological Centre (IMC) during 1963 lent support to such a possibility, and suggested a re-thinking on the possible interaction between the lower and upper tropical tropospheres. In tropical areas, where the land–sea contrast is marked and convective processes are common, it became probable that weather sequences in the lower troposphere may precede and even cause significant changes in upper tropospheric circulations.

Climatological Aspects

As a first step towards investigation of such a possibility, the mean monthly rainfall at stations along the west coasts of Ceylon and India in the latitudinal belts 0–10° N., 10–15° N. and 15–22° N. was compared with the upper tropospheric winds along 77° E. (Fig. 1). The rainfall curve was obtained by averaging the mean monthly rainfall amounts of all available stations in each belt. In the near equatorial region (0–10° N.) (Fig. 1*a*), this curve exhibits a double peak, one during May–June and another during October–November. An explanation for this lies in the double traversal of the Sun in the near equatorial regions during its annual march and the activation of the near equatorial trough. The zonal components of available winds (east) in the layer 300 mb to 100 mb for Gan (00° 41′ S.), Colombo (06° 54′ N.) and Trivandrum (08° 29′ N.) also display a double peak, one during July–August and another during January–February. However, in the latitudinal belts (10–15° N. (Fig. 1*b*) and 15–22° N. (Fig. 1*c*), the monthly march of mean rainfall along the coast as well as the zonal wind in the upper troposphere display only single maxima. It is interesting to note that along 77° E. (typifying the Indian monsoon area) climatologically, the upper tropospheric wind is strongest in the latitudinal belt where the rainfall is also heaviest along the coast. In all the three zones, from the climatological point of view, the summer rainfall peak occurs first and the maximum east wind in the upper troposphere shortly thereafter. This fits with the observation of Raman and Dixit[6], who noticed a lag between low-level troughs and stabilization of upper anticyclones in the Indian monsoon area. However, it is felt that in the equatorial belt, the time gap between the rainfall peak in the transition months October–November and the wind maximum during January–February would get reduced when long period mean wind data for Colombo and Gan become available. Similar characteristics are observed along 100° E. (along the coasts of Arakan and Tenasserim) (Fig. 2); also along 30° E. and 10° E. (tropical Africa). In this context, it is interesting to note that Davies and Sansom[7] observed easterly wind maxima over Nairobi in two distinct epochs, July–September and December–March. Hay[8] found maxima in the upper tropospheric

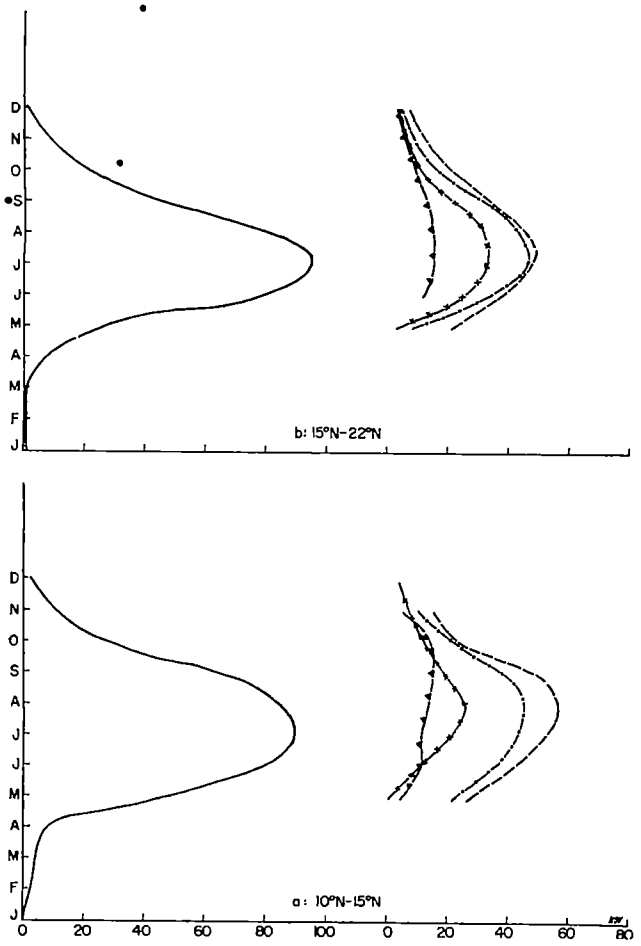


Fig. 2. Mean monthly rainfall distribution and profiles of upper tropospheric mean zonal components (east) along 100° E. (Arakan and Tenasserim coast)

easterlies over Singapore both during the months of south-west monsoon, June–August, and of the north-east monsoon, December–February. He, however, found that over Hong Kong (22° 18′ N.) east-wind maximum occurred only once in the year, and that in the summer months. A preliminary examination of upper wind data of Gan for three years reveals that strong upper tropospheric easterlies occur on individual days both during the northern and southern summer months.

Synoptic Examination and Quantitative Evidence

The climatological indication thus obtained was then put to a synoptic test. For this purpose, it was sought to examine the relationship, if any, between the precipitation and upper tropospheric zonal winds on a day-to-day basis, in the latitudinal belt 1° S.–22° N. during the mid-monsoon months, July–August of 1961, 1962 and 1963. To ensure homogeneity, rainfall data only of coastal observatory stations (numbering 22) along the west coast of India were utilized. Rainfall amounts for the 24-h period ending 0300 G.M.T. of each day were plotted against latitude of the recording stations and from the resulting curve the latitudes of maxima of rainfall along the coast were located. The latitude of east-wind maximum was arrived at from daily wind profiles of 200 mb, 150 mb, 100 mb and 70 mb along the mean meridian 77° E. Where data of 00 G.M.T. were not available, those of 12 G.M.T. were used in drawing the profiles. In order to assess quantitatively the interrelationship, if any, between the precipitation and easterly wind maxima, a set of correlation coefficients, as here listed, was computed.

The latitude of easterly wind maximum of each day was correlated with the latitude of highest rainfall along the west coast of India, recorded over a 24 h period ending 0300 G.M.T. of (i) previous day, (ii) next day, (iii) same day. Out of a total of 186 days in July–August 1961–63, wind data were available for comparative examination on about 100 occasions.

Table 1. Correlation between latitude of upper tropospheric east-wind maximum on a day with the latitude of maximum rainfall recorded at 0300 G.M.T. of (i) previous day, (ii) next day and (iii) same day along the west coast of India

Month/year	East-wind maximum of date with* (a) Previous date's precipitation maximum	(b) Next date's precipitation maximum	(c) Same date's precipitation maximum
July 1963	0·76	0·21	0·64
August 1963	0·90	0·23	0·54
July 1962	0·86	−0·11	0·21
August 1962	0·81	0·55	−0·06
July 1961	0·87	0·29	−0·27
August 1961	0·56	0·23	0·04

* (i) Total number of cases for which complete wind data were available for comparative study: 100.

The results are presented in Table 1.

It will be seen from the highly significant correlation coefficients in column (*a*) of Table 1 that precipitation maximum on a day seems to be associated with the concentration of upper tropospheric winds into a maximum probably on the next day only. The contribution of the day's maximum wind to the next day's precipitation maximum does not appear to be as much. Results for 100° E. for July–August 1963, given in Table 2, point to the same inference.

The general weakening of the east-wind maximum during the northern summer months over Saudi Arabia and Ethiopia, where cloudiness and rainfall are much less than along the west coast of India, the disappearance of the 'easterly jet' over the Sahara immediately to the west of Khartoum and its reappearance over Nigeria, which lies in the monsoon régime, then seems to follow a logical pattern.

The daily graphs of precipitation and the east-wind maxima along 77° E. and 100° E. bring out the following features. These are illustrated in Fig. 3 with the help of typical examples chosen out of a large sample. (i) Peakiness of 24-h rainfall along the coast on a day is reflected in the upper troposphere as a concentration of east wind into a maximum the next day. The latitude of east-wind maximum lies about 1–3 deg. south of the latitude of the recorded maximum of previous day's rainfall (Fig. 3*a*). This fits with Koteswaram's observation of the occurrence during 'breaks' of the axis of the maximum east wind at a more northerly location than usual, that is, closer and to the south of the rain excess region in the Himalayan slopes. (ii) The pattern of rainfall distribution along the coast with more than one maxima, as often occurs during the south-west monsoon, is largely reflected in the next day's wind profile as well (Fig. 3*b*). IMC charts also exhibited similar characteristics on a number of occasions, with well-defined maxima in easterlies near the equator, in the latitudinal belt 5–15° N. and farther north. Such occurrences were not confined to the monsoon months alone but were observed in other months as well. East-wind maxima were observed over Kenya, Malaysia, Indonesia and north Australia. Reconnaissance upper wind observations by the Indian Air Force during July–August 1959 also pointed to the occurrence of distinct east-wind maxima along different latitudes over south peninsular India. (iii) When rainfall along the coast is small and negligible, concentration of the easterlies on the next day is significantly absent on a number of occasions (Fig. 3*c*).

Table 2. Correlation between latitude of upper tropospheric east-wind maximum on a day with latitude of maximum rainfall recorded at 0003 G.M.T. of (i) previous day, (ii) next day and (iii) same day, along Arakan, Tenasserim and Malaysia coasts

Month/year	East-wind maximum of date with* (a)	(b)	(c)
July and August 1963	0·68	0·43	0·47
July 1963 alone	0·86	—	—

* Total No. of cases for which complete wind data available for comparative study: 46.

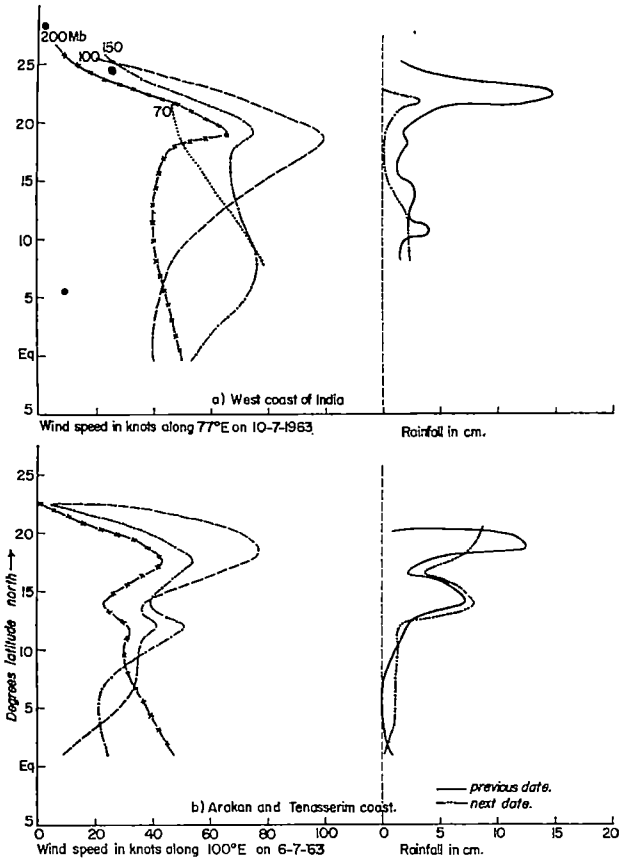


Fig. 3. Relation between summer monsoon rainfall along coast and upper tropospheric easterlies

An attempt was then made to synthesize the daily data into an average picture. With reference to the latitude of a day's east-wind maximum, discrete precipitation amounts from available data were extracted at one-degree intervals over a five-degree belt north and south, for the previous day and the next day. Mean monthly profiles for the period of study are presented in

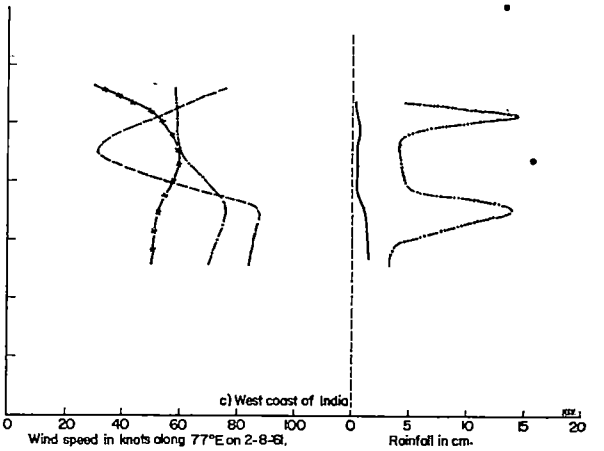


Fig. 4. It is clear that the axis of east-wind maximum lies on the average to the south of the previous day's recorded rainfall maximum (Fig. 4*a*). The average curves for the next day's rainfall do not exhibit any systematic or consistent relationship with the axis of maximum east wind (Fig. 4*b*). Herein it is pertinent to note Bond's[9] reference to the prior occurrence of strong surface pressure gradients, strong low-level westerlies and existence of mid-tropospheric vortices in describing easterly jet over Darwin. It seems possible now to offer a physical explanation to the observational evidence provided by Bond. Ramsey[10] has linked the position of the 'Intertropical front' with easterly jet over the far east and Indonesia.

While it is not possible to rule out the influence of the upper troposphere on conditions nearer ground, this investigation shows that there is a significant contribution to the upper level patterns by precipitation. This contribution probably results from the release of large amounts of latent heat from excessive cloudiness and copious precipitation. Such latent heat release may be expected to cause heating of the upper troposphere, advective transport of heated tropospheric air and consequent changes in upper tropospheric circulations[11]. This concept is being carefully considered in a few investigations in IMC. Further, quantitative assessments of the contribution of latent heat for production of kinetic energy are being made and the results will be published elsewhere.

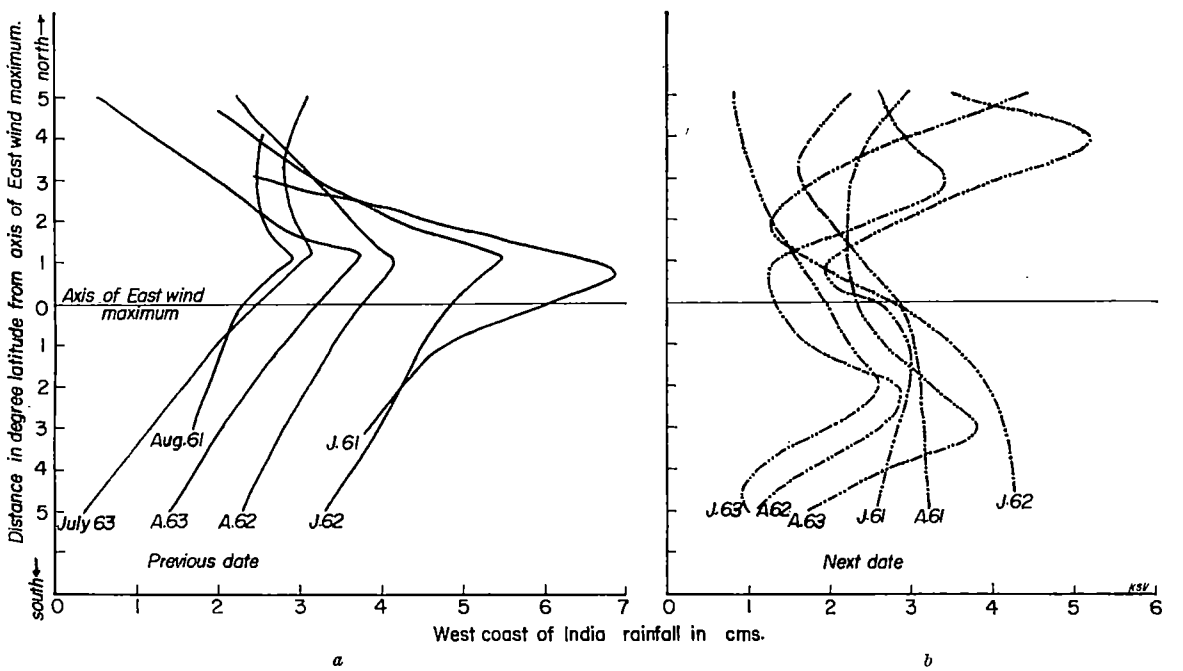


Fig. 4. Mean distribution of rainfall along west coast of India about the axis of strongest upper tropospheric easterlies in summer monsoon

The foregoing preliminary investigation provides a new point of view in the examination of low-latitude disturbances and strongly suggests that weather sequences in the lower troposphere may frequently cause changes in the upper tropospheric circulations.

We thank Dr. C. S. Ramage and Dr. P. R. Pisharoty for their advice.

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ELECTRICAL CONDUCTION IN THIN AGGREGATED METAL FILMS

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An electrical field effect has been observed between discontinuous aggregated thin evaporated metal films of gold, and the substrate on which they were deposited. The effect is similar to that observed by Cox[1] and Bonfiglioli and Malvano[2] for continuous films, but the magnitude of the change in resistance involved is much larger. It suggests that conduction in the aggregated films occurs by electron tunnelling through the substrate and not directly between the metal aggregates as suggested by Neugebauer and Webb[3] and Hartmann[4].

The gold films were prepared in a vacuum of about 10^{-6} torr; soda and borosilicate glasses were used as the substrates. The substrates were cleaned with detergent solutions and baked in vacuum at a temperature of 320° C for a period of 4 h; they were then cooled to 300° C and the films deposited. The electrical resistance of the specimens was monitored during deposition, and evaporation was stopped when the resistance fell to about 5×10^3 ohms. Measurements of the films were then carried out over a lower temperature range (20°–250° C) so that no further structural reorganization took place. The metal films were removed from the vacuum after the measurements were completed and were examined by electron microscopy. They were completely aggregated with an average particle size of 260 Å, and an average spacing of 52 Å.

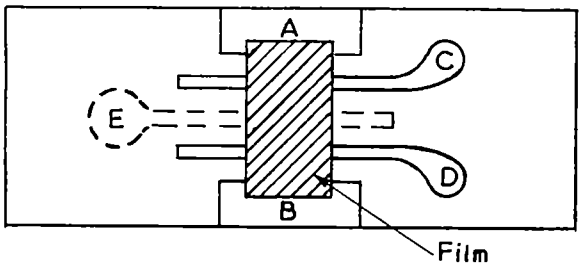


Fig. 1. Diagram of the evaporated film and the electrodes

The substrates carried fired-on electrodes, as shown in Fig. 1. *A* and *B* are current input and output electrodes, *C* and *D* are for voltage sensing. Electrode *E* was an extra electrode fired on to the back of the substrate. It was found that the resistance of the film between *C* and *D* could be varied, in a completely reproducible manner, by impressing a d.c. voltage on the bias electrode *E*. To remove uncertainties from leakage currents an a.c. signal at 50 c/s was used to measure the film resistance, and the driving potential was obtained from the ends of a centre-tapped transformer, the centre tap of which was earthed. The d.c. bias was supplied from a battery, one terminal of which was also earthed.

The variation of resistance as a function of bias voltage for the two glasses, and for a range of temperatures, is shown in Fig. 2. The changes in resistance of gold films on soda glass were small, but larger changes were observed using good-quality borosilicate glass (Corning No. 7740).

We have attempted to explain the results by applying the theory of amorphous dielectrics[5–7] to the system. If the bulk of the glass is assumed to contain a large number of trapping sites between the quasi-conduction and valence bands, then it can be deduced that the most likely distribution of traps is of a double exponential form, that is, many traps near the band edges and a few in the middle of the band gap. Thus the energy required to activate an electron from one trap to the trap of the next highest

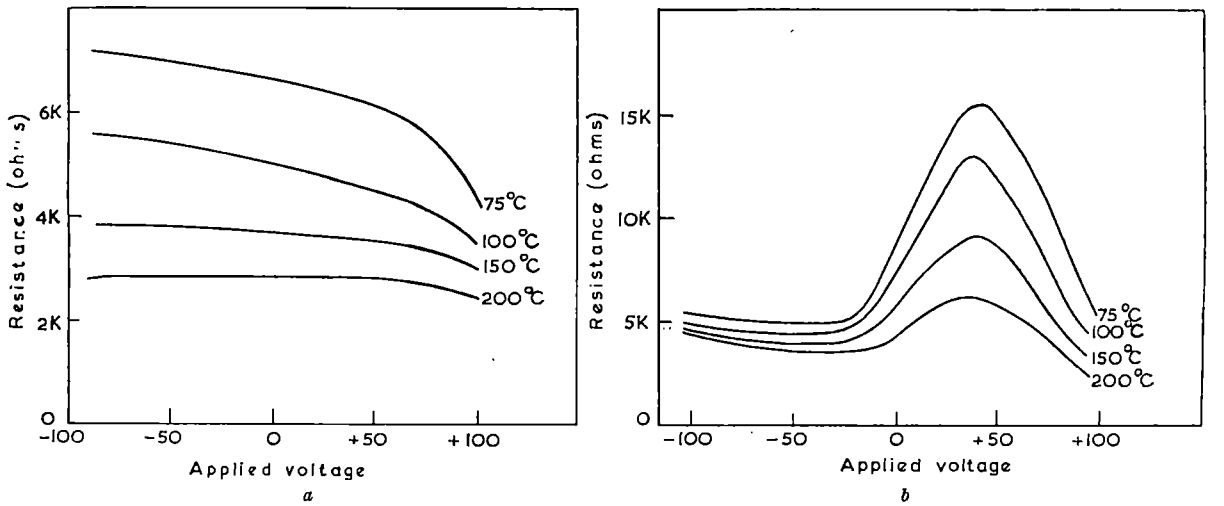


Fig. 2. The variation in resistance of gold films as a function of applied bias voltage and temperature. *a*, Soda glass 0·050 in. thick; *b*, borosilicate glass 0·020 in. thick

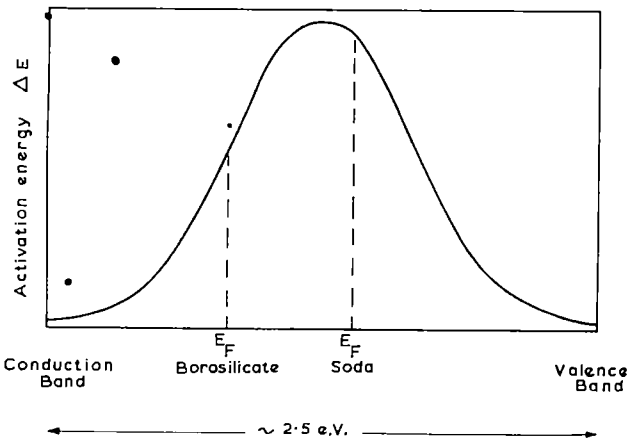


Fig. 3. The activation energy required for the transfer of an electron from trap to trap as a function of position in the normally forbidden energy gap

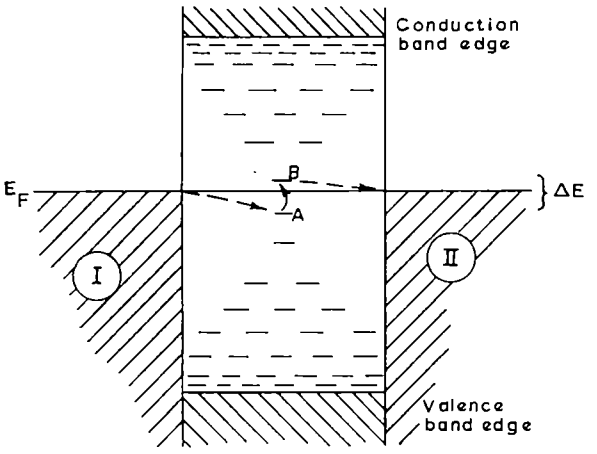


Fig. 4. Schematic energy diagram for the transfer of an electron from aggregate I to aggregate II by means of a trap jump A–B

energy, ΔE, will depend on the position of the trap in the band gap (Fig. 3). The energy diagram for the system is shown in Fig. 4, where I and II represent two adjacent metal particles separated by the glass. The Fermi-levels in the metal particles are shown at E_f, and the Fermi-level in the glass becomes aligned with these by charge transport between the metal and the bulk of the glass. As we are only considering the region just below the metal–glass interface we can neglect band bending effects. The applied potential between the aggregates is small (about 2×10^{-5} V) and can also be neglected. If a trap site were exactly located at the Fermi-level direct tunnelling could occur from the free thermal electrons above the Fermi-level in I, via the trap, with a much smaller effective impedance than for simple tunnelling between aggregates[8]. The process would be non-thermally activated. If, however, there is no trap in this position electron tunnelling into trap A can occur, as shown in Fig. 4, the small energy difference being lost to the glass lattice structure. Tunnelling from A into particle II cannot take place readily because there is no available energy-level within II at the energy value of A. Before this can happen the electron has to be activated into trap B, the nearest available trap of energy higher than E_f. The process then becomes thermally activated and a negative temperature coefficient of resistance would be expected, and is experimentally obtained for aggregated films.

The positions of the Fermi-levels within the glasses can be obtained from the d.c. activation energies (Fig. 3). On applying a positive bias to E the Fermi-level in the glass is lowered towards the quasi-valence band and the activation energy for conduction follows the curve shown in Fig. 3. As the Fermi-levels for the two glasses we are using lie on opposite flanks of the curve, we obtain agreement with the form of the experimental measurements shown in Fig. 2.

The resistance of the films was found to obey the usual relationship:

$$R = R_0 \exp (\Delta E/kT)$$

so that at any one temperature a change in the activation energy ΔE would give an exponential change in resistance. The activation energy was measured for various bias conditions and the calculated resistance values gave good agreement with the experimental results.

At present we are not able to calculate the number of trapping sites that are involved in the process, but it is possible to calculate the ratio of the number of active traps for the two glasses. At zero bias conditions the ratio of traps for the borosilicate and soda glasses are 5·0/12·5, whereas the sodium concentration in the borosilicate glass is 5 per cent and in the soda glass is in the range 10–15 per cent.

It appears that conduction in aggregated films occurs through the substrate, and is aided by the presence of trapping centres in the glass. Bonfiglioli and Malvano[2] found some evidence of interaction with surface traps on mica using evaporated films of a few ohms resistance, that is, a continuous structure. By using the discontinuous structure and a non-crystalline substrate we have enhanced the effect by some three orders of magnitude. We intend to use ultra-high-vacuum techniques to remove any uncertainties arising from surface layers and a wider range of glasses of known composition in order to test the experimental model we are proposing.

I thank Mr. G. Siddall and Mr. A. M. Cassie, of this laboratory, for their advice, and Mr. J. S. Cleaver for assistance in the electron microscopy investigation.

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DEOXYRIBONUCLEIC ACID IN PLANT NUCLEOLI

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THE view that nucleoli contain ribonucleic acid (RNA) but no deoxyribonucleic acid (DNA) is based mainly on staining properties and the results of autoradiography. In general, nucleoli are Feulgen-negative although Feulgen-positive inclusions have occasionally been found in both plant[1] and animal[2–4] cells. Furthermore, nucleoli exhibit little, if any, residual basophilia after extraction of the RNA by ribonuclease (RNase)[5]. It is also known that under conditions in which radioactive precursors of DNA, such as tritiated thymidine, are readily incorporated into the chromosomes, no comparable incorporation into the nucleoli takes place.

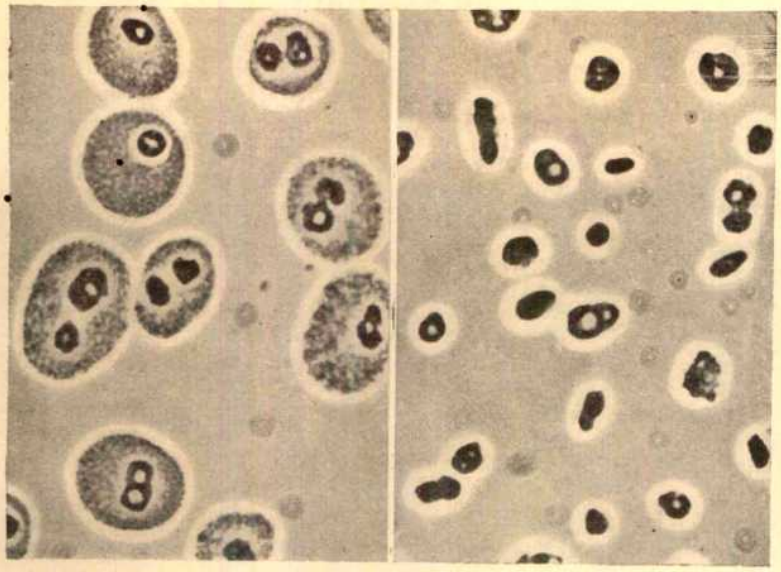


Fig. 1. Nuclei (left) and nucleoli (right) isolated from root apices of *Vicia faba* (phase-contrast; × *c.* 600)

On the other hand, DNA has been estimated chemically in nucleoli isolated from pea tissues[6,7] and rat liver[8,9]. In these cases, however, there has been some uncertainty as to how much of the DNA is truly nucleolar and how much is derived from accompanying chromosomal material such as the 'nucleolus-associated chromatin'[10]. In fact, it has been suggested that the nucleolus, together with any accompanying chromosomal components, should be considered as a discrete unit and called the 'nucleolar apparatus'[7]. It has never been clearly demonstrated, however, that the nucleolus still contains DNA after any accompanying materials, with the properties of chromosomal DNA, have been excluded. At least the nucleoli isolated by Rees *et al.*[9] may be free from high-polymer chromosomal DNA since they stain pink instead of blue-green with methyl-green-pyronin[11].

It is also significant that changes in the structure of both animal[12] and plant[13] nucleoli have been observed as the result of deoxyribonuclease (DNase) treatment.

The purpose of this article is to describe the results of chemical and cytochemical measurements which have led to the conclusion that nucleolar DNA may be of more widespread occurrence in plants than had hitherto been supposed. The measurements were made on nucleoli isolated from formaldehyde-fixed roots. There is reason to believe that such a fixation helps to prevent subsequent loss of both DNA and RNA[14].

The main experiments were carried out using the apical 2-mm segments of lateral roots of *Vicia faba* plants growing in vermiculite at 20° C. These root-tips were collected in ice-water and then fixed for 40 min in ice-cold 1·5 per cent formaldehyde in M/60 phosphate buffer at *p*H 7·0. They were then washed in 1 mM phosphate buffer at *p*H 7·0, to remove the formaldehyde, and crushed in a drop of the same buffer in a press[14]. The exudate, containing nuclei and cytoplasmic particles, was then diluted with more of the 1 mM buffer and clean samples of nuclei (Fig. 1) collected by three successive centrifugations, of 3 min each, at 500*g*. The nuclei were then resuspended in the 1 mM buffer and shaken for 15 min in a Mickle disintegrator (50 c/s). Glass beads were not used. This shaking disrupted the nuclei and dispersed the chromosomal material, but did not damage the nucleoli, which could be collected by centrifugation for 5 min at 1,500*g* (Fig. 1). All the operations were carried out in a cold room at 3° C. A small proportion of nuclei (0·5–2 per cent) was found to remain intact and could not be broken even with prolonged periods of shaking. Although it was possible to eliminate these contaminant nuclei by differential centrifugation, this led to some loss of the larger nucleoli and also increased the time taken for the whole isolation procedure. These nuclei were therefore allowed to remain and a small correction was applied to the final estimation of nucleic acid content in the nucleoli. The nuclei which resisted disruption did not appear to be derived from one type of cell in the roots since, when stained in Feulgen, microphotometry revealed a bimodal distribution and a mean stain value identical with that given by random samples of nuclei which had not been shaken.

The amounts of both DNA and RNA in the isolated nucleoli were determined by a modification[14] of the Schmidt–Thannhauser procedure[15]. The results of 15 separate determinations (Table 1) indicated that there is twice as much DNA as RNA. This is the same ratio as that found in rat liver nucleoli by Rees *et al.*[9]. In a second series of determinations, nucleoli were first washed in ice-cold 0·2 N perchloric acid and defatted before being incubated with RNase. Extraction of the RNA was then followed by extraction of the DNA by means of DNase. The quantities of the two nucleic acids determined in these two extracts were found to agree with those determined by the Schmidt–Thannhauser procedure (Table 1).

Approximately 17 per cent of the total DNA and 40 per cent of the total RNA of the nucleus is nucleolar. These figures are similar to those reported for *Pisum sativum*[7]. Estimations of nucleic acid concentration in nuclei and nucleoli have been based on measurements of total dry mass by means of interference microscopy (Table 2). These show that the concentration of RNA in the nucleoli is about three times higher than that in the rest of the nucleus, whereas the concentration of DNA in these two regions is about the same. Previous estimates of nucleic acid concentration in nucleoli have been lower than those found in *Vicia*. In a number of different species, concentrations of RNA of between 2 and 10 per cent have been reported[6,8,16–19]. Concentrations of DNA of 17 and 20 per cent[8,9] have been reported for rat liver nucleoli, but in the nucleoli of pea embryos, DNA is said to represent only 0·2 per cent of the total contents[6]. It is possible that more differences of this kind between species, and between tissues having different metabolic activities, will be found.

It should be mentioned here that, in the course of these experiments, some discrepancies were found which have

Table 1. AMOUNTS OF RNA AND DNA IN NUCLEOLI OF *Vicia faba*

Method*	No. of determinations	$g \times 10^{-11}$ Nucleolar RNA per nucleus	$g \times 10^{-11}$ Nucleolar DNA per nucleus	DNA/RNA
Schmidt–Thannhauser	15	0·51 ± 0·024	1·03 ± 0·079	2·05 ± 0·094
Enzymatic	4	0·54 ± 0·029	1·07 ± 0·046	2·01 ± 0·150

* Values derived from ultra-violet, phosphorus[27] and ribose[28] determinations for RNA; ultra-violet, phosphorus and deoxyribose[29] determinations for DNA.

Table 2. CONCENTRATIONS OF RNA AND DNA IN NUCLEI OF *Vicia faba*

	Dry mass per nucleus*	RNA Amount*	RNA % Nucleolar mass	RNA % Non-nucleolar mass	DNA Amount*	DNA % Nucleolar mass	DNA % Non-nucleolar mass
Nucleolar	2·84	0·53	18·7	—	1·05	36·9	—
Non-nucleolar (chromosomal)	12·46	0·81	—	6·5	4·98	—	39·9

* $g \times 10^{-11}$.

Table 3. Mean Numbers of Grains* after Tritiated Thymidine Labelling

Exp.	Nuclei	Nucleoli
1	21·6 ± 1·3	0·48 ± 0·01
2	32·9 ± 1·6	0·47 ± 0·01

* Each value represents the mean (± *S.E.*) of grain counts made on 5 slides.

Table 4. Specific Activities of DNA Fractions after ^{14}C-Thymidine Labelling

Methods of extraction	Counts/min/μg DNA Nuclear	Nucleolar
Schmidt–Thannhauser	110	12
Enzymatic	71	6

not yet been satisfactorily explained. On two occasions, using the Schmidt–Thannhauser procedure, a DNA : RNA ratio of exactly 1 : 1 was found in the nucleolar samples although the conditions of growth of the plants and the methods of isolation and extraction were apparently the same. In two other cases, samples of DNA extracted enzymatically from nucleoli gave a diphenylamine reaction of approximately twice the intensity expected from the ultra-violet absorption. Preliminary investigations suggest that this was not due to the presence of a contaminant.

Adenine, guanine, cytosine and thymine have been identified in the nucleolar DNA fractions by chromatographic analysis. The DNA, recovered either by the Schmidt–Thannhauser procedure or by enzymatic hydrolysis, was heated for 1 h in 0·1 N sulphuric acid at 100° C and the free purine bases precipitated as silver salts[20]. The purines, recovered by heating the precipitate in 1 ml. of N hydrochloric acid at 70° C for 15 min, were found to account for 50 per cent of the ultra-violet absorption of the original DNA solution. They were separated from one another on Whatman No. 1 filter paper using a descending solvent system consisting of methanol : ethanol : water : conc. hydrochloric acid (50 : 25 : 19 : 6)[21]. To recover the pyrimidine bases, further DNA samples from the isolated nucleoli were hydrolysed for 30 min in 5 per cent trichloroacetic acid at 75° C. The pyrimidine deoxyriboside phosphates were first precipitated as barium salts[20], and then freed from barium by dissolving them in water containing the NH_4^+ salt of 'Dowex 50'. The resin was then spun off and the supernatant dried down and hydrolysed with 98 per cent (w/v) formic acid for 2 h at 175° C. This hydrolysate was then evaporated to dryness, redissolved and the pyrimidine bases separated from one another on Whatman No. 1 filter paper using the descending solvent system consisting of ammonia in water-saturated *n*-butanol[22]. Each of the four bases was identified by its ultra-violet absorption spectrum after elution from the chromatogram in 0·01 N hydrochloric acid. Although not more than 30 per cent of the expected quantity of pyrimidines could be recovered by barium precipitation or other methods, it is difficult to believe that this is a true indication of the pyrimidine content; the results of all the other measurements, with the exception of the anomalous diphenylamine figures mentioned earlier, indicate that the nucleolar DNA is in a normal double-stranded form. However, the presence of single-stranded nucleolar DNA cannot be ruled out entirely and, with this possibility in mind, physical measurements and more intensive investigations of the base composition have now been started.

In order to see whether or not these nucleoli could have become contaminated by chromosomal DNA during the isolation procedure, the following tests were made. First, smears of isolated nucleoli were hydrolysed in N hydrochloric acid at 60° C for 12 min and immersed in Feulgen reagent for 2 h. In a few cases, small Feulgen-stained bodies (similar to those described as nucleolar organizers[23]) could be seen attached to the nucleoli. However, the dimensions and frequency of occurrence of these suggested that they would account for less than 0·1 per cent of the nucleolar DNA found. Apart from these bodies, the nucleoli were found to have no detectable stain whereas isolated nuclei treated in the same way had approximately the same amount of stain as nuclei in intact control roots which were formaldehyde-fixed, hydrolysed and stained concurrently. Since it could be argued that the absence of staining was due to leaching out of the DNA during the Feulgen procedure, another test was made. Isolated nucleoli were first subjected to the Feulgen procedure, then washed in ice-cold 0·2 N perchloric acid and finally hydrolysed in N perchloric acid at 75° for 30 min. Both the hydrochloric acid and the perchloric acid were then examined for the presence of DNA. In four experiments of this type it was found that between 25 and 50 per cent of the nucleolar DNA was being lost in the Feulgen procedure. In each case the rest could be recovered in the perchloric acid. It seems clear, therefore, that after the Feulgen procedure DNA is still present in the nucleoli. The fact that it is suggest that this was not due to the presence of a contaminant.

Two further tests for contamination were made. In the first, excised root-tips were immersed for 6 h in Knop's solution containing tritiated thymidine (1 μc./ml.). Nuclei and nucleoli were then isolated and smeared on separate slides. Autoradiographs were prepared and the average number of grains over nuclei compared with the average number over nucleoli. As can be seen from Table 3, the amount of nucleolar label (allowing for an average of 1·52 nucleoli per nucleus) is approximately 2·5 per cent of the nuclear. Had all the nucleolar DNA been derived from the chromosomes during the isolation procedure, a figure of about 17 per cent would have been expected. Unfortunately, sections could not be used for this comparison because the nucleoli, when centrifuged into pellets for the purpose of embedding, clumped and lost their separate identity. However, from interferometric measurements of optical path differences in media of known refractive index, the maximum thickness of nuclei and nucleoli in the autoradiographs was calculated to be 0·95μ and 0·33μ respectively. It is clear, therefore, that a correction for self-absorption can only accentuate the differences shown in Table 3.

In the final test for contamination, excised root-tips were again labelled for 6 h, this time using ^{14}C-thymidine. Nuclei and nucleoli were then isolated and DNA extracted from both. Known quantities of the two DNA fractions were then dried down on glass coverslips and the specific activities determined using a thin end-window counter. Again, had all the nucleolar DNA been derived from the chromosomes during the isolation procedure, the specific activities of the two fractions would have been the same. In fact, as Table 4 shows, the nucleolar fraction had approximately 10 per cent of the specific activity of the nuclear fraction. This is compatible with the autoradiographic results and suggests that at least 90 per cent of the DNA found in nucleoli cannot be accounted for by contamination unless, as seems unlikely, contamination by unlabelled chromosomal DNA occurs. The absence of Feulgen stain seems to rule this out. The relatively small amount of incorporation into nucleolar DNA, found in the above experiments, suggests that the material for this component might be drawn from a pool other than that involved in the synthesis of chromosomal DNA. Alternatively it could be derived from the chromosomal DNA itself at a time other than that covered by the period of labelling.

The technique described for the isolation of nucleoli from *V. faba* roots has been applied successfully in several other species of plants: *Pisum sativum*, *Phaseolus vulgaris*, *Allium cepa*, *A. ampeloprasum*, *A. babingtonii*, *Tradescantia ohioensis* and *Zea mays*. Nucleolar DNA has been found in all of them. Although comparatively few determinations of nucleic acid content in this material

have so far been made, it appears that the proportion of nuclear DNA which is in the nucleoli is always of the same order as that found in *V. faba*. The presence of such a fraction of DNA, which is Feulgen-negative, may seem to cast doubt on the validity of Feulgen microphotometry as a method for estimating total DNA content. However, if there proves to be little variation between species in the magnitude of the nucleolar DNA fraction, its presence need not be a serious source of error. The correlations which have been found between absolute amounts of DNA and the amounts of Feulgen stain[24,25] seem to provide confirmation of this.

If it is accepted that DNA is an integral part of nucleolar structure, the results of several previous experiments may be open to new interpretation. Perhaps the most relevant are those which have been concerned with the synthesis of nucleolar RNA.

The nucleolus is known to contain RNA with a high rate of turnover[26–28] and, furthermore, there is evidence to suggest that the synthesis of this RNA is DNA-dependent since it can be inhibited by both actinomycin *D*[29–31] and DNase[31]. Sirlin *et al.*[29] have suggested that nucleolar RNA synthesis is primed by 'messenger' RNA which is made on the chromosomal DNA and then moves to the nucleolus. But if the nucleolus itself contains DNA as well as RNA, possibly in a 2 : 1 relationship as the above results and those of Rees *et al.*[9] suggest, it would seem unnecessary to postulate the formation of an extrinsic 'messenger'. In any event, the autoradiographic evidence[32] for the movement of RNA from chromosomes to nucleolus has been seriously challenged[33] on the grounds that it fails to take into account the role of precursor pools as well as some fundamental differences between the nucleolus and the rest of the nucleus. These include differences in base composition[27,34,35], differences in concentration[6,8,16–19], and differences in the distribution of radioactivity according to the precursors used[33,36]. A continuation of such investigations into the chemical and physical nature of the components of nucleoli in different types of cells and environments is required before the true functions of these organelles can be elucidated.

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BINDING OF PROFLAVINE TO DEOXYRIBONUCLEIC ACID AND RIBONUCLEIC ACID AND ITS BIOLOGICAL SIGNIFICANCE

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IT has already been shown that proflavine inhibits DNA-dependent RNA synthesis in a way similar to actinomycin[1]. It binds to DNA[2] and leads to mutations[3,4]. Furthermore, it destroys the infectivity of several viruses in the presence of light[5] and interferes with virus multiplication[6]. Nevertheless, the suppression of RNA synthesis *in vivo* is not complete, and the RNA synthesized in the presence of proflavine shows some peculiarities in base composition and base sequence[7].

This article is concerned with the action of proflavine on DNA and RNA. We have shown that proflavine which has little effect on energy metabolism not only inhibits RNA synthesis but also prevents the production of protein. Proflavine binds not only to DNA, but also to RNA. Furthermore, it interferes with the multiplication of fowl plague virus as well as with Newcastle disease virus (NDV). This is in contrast to actinomycin, which binds only to DNA[8,9], does not affect protein synthesis immediately[10,11], and does not interfere with the multiplication of NDV[12,13]. The results suggest that proflavine inhibits multiplication of fowl plague virus in a similar way to actinomycin, while it inhibits NDV formation by reaction with an RNA template.

Chick fibroblast cultures, prepared according to Kraus and Schäfer[14], were used throughout. The changes in incorporation of isotopic precursors of RNA and protein synthesis, after transferring from growth to minimal medium, are described in refs. 7 and 11.

The 'Rostock' strain of fowl plague virus and the 'Italien' strain of NDV were used. The cell layers were infected with a multiplicity of about 100 as described elsewhere[15].

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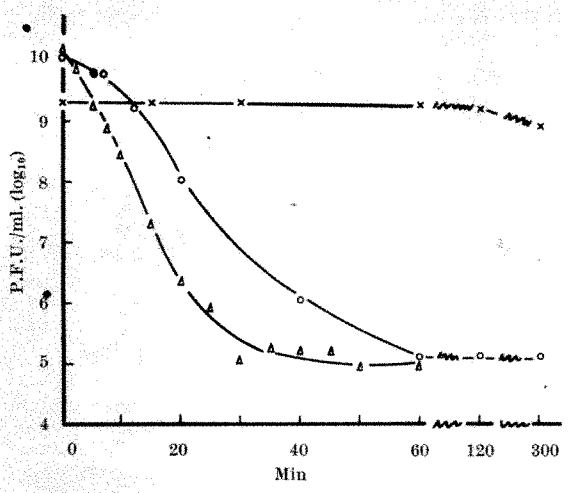


Fig. 1. Inactivation of NDV in the presence of proflavine with monochromatic light at different wave-lengths. NDV in sodium chloride-phosphate buffer *p*H 7·2 containing 50 μg/ml. proflavine was exposed for different lengths of time (abscissa) to monochromatic light of three different wave-lengths. As light source a Zeiss-spectrophotometer *PMQ* II with the fluorescence equipment *ZFM* 4 was used. The ratio of the energy at 365 mμ (○), 436 mμ (*Δ*) and 546 mμ (×) as measured by a thermo-element was 1/1/2·3; the ratio of the absorption at these wave-lengths was 0·11/1/0·006

The infectivity was determined by the plaque test[15]. The hæmagglutination titre was estimated in the usual manner (for fowl plague virus at room temperature, for NDV at 4° C). The microcomplement-fixation test[16] as modified by Hennessen[17] was used. *Q* is the ratio of the amount of complement causing 50 per cent hæmolysis with antiserum to that with normal serum. In the case of fowl plague virus only the synthesis of the RNP antigen (*s*-antigen) is measured by the complement-fixation test[18], while in the case of NDV, convalescent antiserum from hamsters, which reacts with all virus components, was used[19]. The neuraminidase activity was determined according to Aminoff[20], using *N*-acetylneuraminic acid as substrate.

The synthesis of RNA was measured by the incorporation of ^{14}C-uridine (1 mc./8·6 mg) into the acid-insoluble fraction, protein synthesis by the incorporation of ^{14}C-leucine (6·5 μc./μM L-leucine, dilutedby 1 μM/ml. ^{12}C-D,L-leucine). The procedures were described in detail in ref. 21.

Proflavine (free base) was a gift from the Bayerwerke, Leverkusen, Germany. Actinomycin *D* was a gift from Merck, Sharp and Dohme, New York. ^{14}C-Leucine was obtained from the Radiochemical Centre, Amersham, England. ^{14}C-Uridine was purchased from the New England Nuclear Corporation, Boston, U.S.A. The highly polymerized calf thymus DNA (type II) and the low-molecular-weight DNA from herring sperm (type IV) was obtained from Sigma Chemical Co., St. Louis, Missouri. The high-molecular-weight RNA was isolated from rat liver microsomes by phenol. The radioactivity was determined in a windowless flow counter model *FH* 516 (Frieseke and Hoepfner, Erlangen-Bruck, Germany).

(1) *Inactivation of fowl plague virus and NDV with proflavine in the presence of light.* It has been shown by Franklin[6] that proflavine inhibits the synthesis of fowl plague virus. Since we were unable to repeat his results in a consistent manner, we tested the influence of proflavine on the isolated virus. NDV and fowl plague virus were found to be inactivated by proflavine only in the presence of light. Both viruses were inactivated most rapidly with a wave-length close to the adsorption maximum of proflavine (436 mμ) (shown for NDV in Fig. 1). The hæmagglutination titre and the complement-fixing activity were not affected by this treatment. However, neither of the viruses was inactivated completely (Fig. 1). A second addition of proflavine at a time when the inactivation curve had levelled off had no further effect. Inoculation of the residual virus isolated from single plaques into embryonated eggs again led to the formation of proflavine-sensitive virus particles. Therefore there is no selection of a resistant mutant, and the formation of a proflavine-resistant fraction is not yet understood. Similar results were obtained with fowl plague virus, although the results are not so clear as those with NDV due to multiplicity reactivation[21].

(2) *Inhibition of fowl plague virus and NDV-multiplication by proflavine.* Since our results on the inhibition of fowl plague virus multiplication were not consistent when working in the presence of daylight, we compared the inhibition of both viruses by proflavine in red light and in normal light. Normal light means that the proflavine cultures received a light pulse of 15 min immediately after infection. Thereafter they were incubated in the dark. All further tests were performed in red light. Fig. 2 shows that the inhibition of fowl plague virus multiplication is much more pronounced if a light pulse is given immediately after infection. The same holds for NDV (not shown here). Nevertheless, there is a marked difference between the dose of proflavine needed to get a comparable inhibition of NDV and fowl plague (Fig. 3). Working in red light, considerably more proflavine was needed for the inhibition of the multiplication of NDV than for fowl plague virus.

In Fig. 2 it can be seen that, after a light pulse, the yields of the different virus components of fowl plague virus are affected to different extents by proflavine. The hæmagglutinin titre and neuraminidase activity do not go down in parallel with the infectivity, while the RNP antigen titre is unaffected by even relatively high doses of proflavine. Similar results have been obtained recently by the use of other inactivating agents on the isolated virus particles[21,22]. Therefore the best explanation for this phenomenon, and for Franklin's findings[6], is a partial inactivation of the virus genome of the fowl plague virus in the presence of proflavine and light. In the case of NDV, no differential effect on the production of the various virus components has been observed, except for a delay in the fall in the complement-fixing activity of little significance. Chemicals reacting specifically with the nucleic acids were

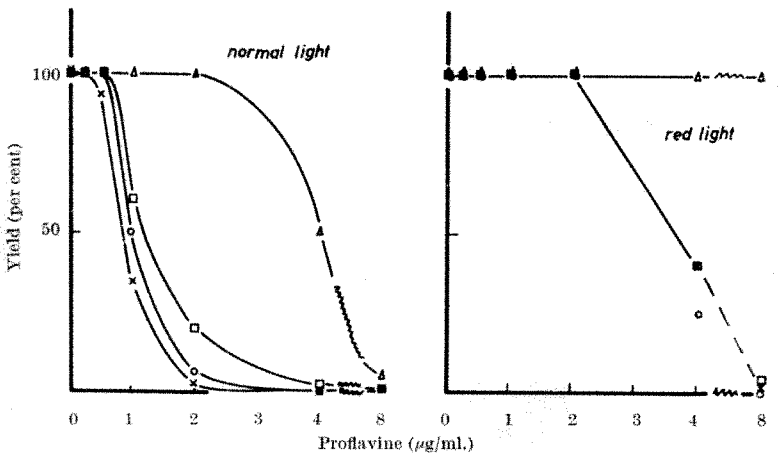


Fig. 2. Dose effect of proflavine on the formation of fowl plague components in tissue cultures after a light pulse (left) and working in red light (right). After infection and the addition of different doses of proflavine the cultures were exposed for 15 min to the light of three Philips lamps (*TL* 20 W, 34 D) at a distance of 30 cm or to red light, respectively. ×, plaque-forming units (P.F.U.), ○, hæmagglutination titre; □, neuraminidase activity, △, RNP antigen titre

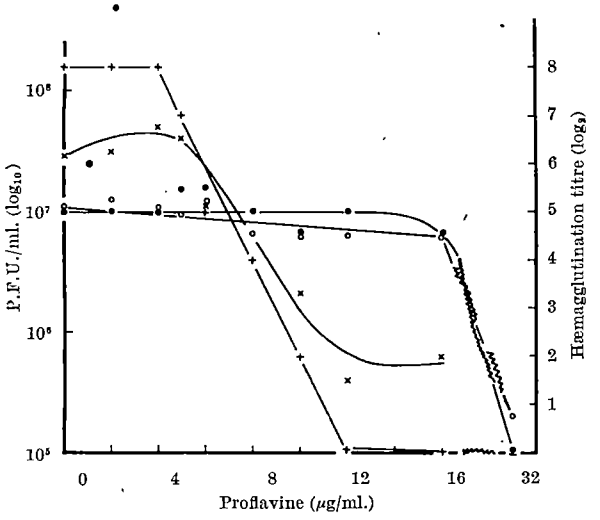


Fig. 3. Comparison of the effect of different doses of proflavine on the inhibition of fowl plague virus and NDV multiplication. Different doses of proflavine (abscissa) were added to NDV and fowl plague-infected tissue cultures in red light. The cells were gathered 8 h post-infection and the virus titre determined after the disruption of the cells. ×, P.F.U., +, hæmagglutinin, fowl plague; ○, P.F.U., ●, hæmagglutinin, NDV

also found to inhibit the ability to synthesize all the various components of NDV at the same rate[21].

When working in red light, no significant differential effect on the formation of the various virus components of fowl plague virus was observed, except for a greater resistance to proflavine of the RNP antigen formation (Fig. 2).

It has been shown that cellular RNA synthesis cannot be suppressed completely by proflavine[7]. Therefore a residual synthesis of viral RNA might proceed even in the presence of moderate doses of proflavine. This residual viral RNA might be enough to serve as template for RNP antigen protein synthesis, but not for that of other viral proteins. A similar effect has been observed when actinomycin, which inhibits the formation of fowl plague RNA, is added 2 h post-infection to fowl plague-infected tissue cultures. At this time only very low amounts of viral RNA are present in the infected cells, but this RNA gives rise, at a diminished rate, to normal yields of RNP antigen, without the formation of significant amounts of hæmagglutinin[23]. The RNP antigen could be labelled under these conditions with ^{14}C-leucine, but only very little with ^{14}C-uridine, suggesting that it contains little RNA (unpublished results). No RNP antigen was formed if actinomycin was added immediately after infection.

(3) *Binding of proflavine to DNA and RNA.* The binding of actinomycin to DNA is accompanied by a specific shift in the absorption spectrum of the chromophoric group of the antibiotic[8,24]. Since the absorption spectrum of proflavine also can be altered by nucleic acids (Häbich and Burberg, personal communication), this change has been selected as a quantitative test for the binding of proflavine by nucleic acids from different sources. At high concentrations of nucleic acid, the absorption maximim of proflavine shifts from 445 mμ to 460 mμ. Therefore the ratio of the absorption at 445 mμ to that at 460 mμ was determined as a function of the concentration of the nucleic acids. It can be seen in Fig. 4 that about three times more RNA or degraded DNA is needed than high molecular weight DNA, in order to get a comparable shift in the absorption spectrum of proflavine. This suggests that high-molecular-weight DNA (double stranded) binds proflavine about three times as well as single-stranded RNA, with a molecular weight of about one million. This is in contrast to actinomycin, which binds only to DNA. In order to get a comparable effect of RNA by actinomycin, at least 100 times more RNA is needed[24].

(4) *Inhibition of RNA and protein synthesis by proflavine.* Proflavine inhibits the incorporation of ^{14}C-uridine into the acid-insoluble fraction of the cell. But even with very high concentrations of this compound there is always a residual incorporation of the isotope of about 4 per cent[7].

The effect of proflavine on RNA synthesis was compared with that on protein synthesis. Fig. 5 shows that with low amounts of proflavine (2 μg/ml.) there is a small stimulation of the incorporation of ^{14}C-uridine, while the incorporation of ^{14}C-leucine is slightly depressed. Another difference has been observed with high concentrations of proflavine. While the incorporation of ^{14}C-uridine levels off at about 25–50 μg/ml. proflavine, that of ^{14}C-leucine drops further to an insignificant amount. But from the values between these extremes it can be seen that between two and three times more proflavine is needed for the inhibition of protein synthesis than for the inhibition of RNA synthesis. The inhibition of synthesis of both RNA and protein starts immediately after the addition of the drug (Fig. 6). The incorporation of precursors of RNA and protein in the presence of proflavine rises linearly with time. Therefore a destruction of the templates for synthesis of RNA and protein, respectively, during the experiment can be excluded.

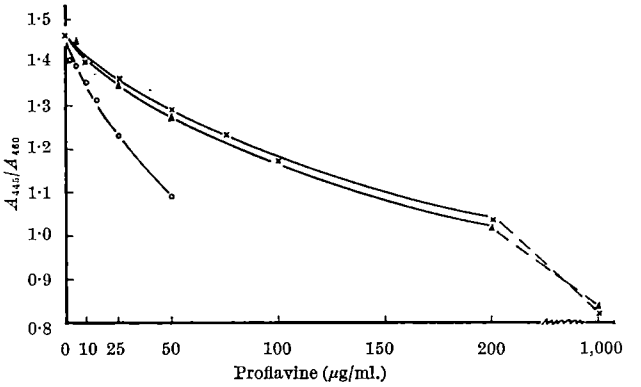


Fig. 4. Shift in the absorption maximum of proflavine by different doses of DNA and RNA. 10 μg/ml. proflavine in NaCl-phosphate buffer *p*H 7·2 was mixed 1 : 1 with solutions of different concentrations of nucleic acids and the absorption was measured at 445 mμ and 460 mμ. ×, rat liver microsomal RNA; ○, thymus DNA (high molecular weight); △, herring sperm DNA (low molecular weight)

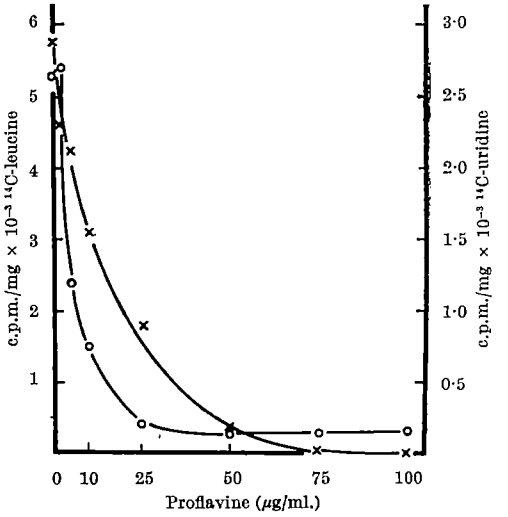


Fig. 5. Inhibition of synthesis of RNA and protein by proflavine. For each point one culture received 0·13 μc. ^{14}C-leucine or 0·2 μc. ^{14}C-uridine, respectively, and the corresponding dose of proflavine. The values are expressed as c.p.m./mg dry wt. ○, ^{14}C-leucine; ×, ^{14}C-uridine

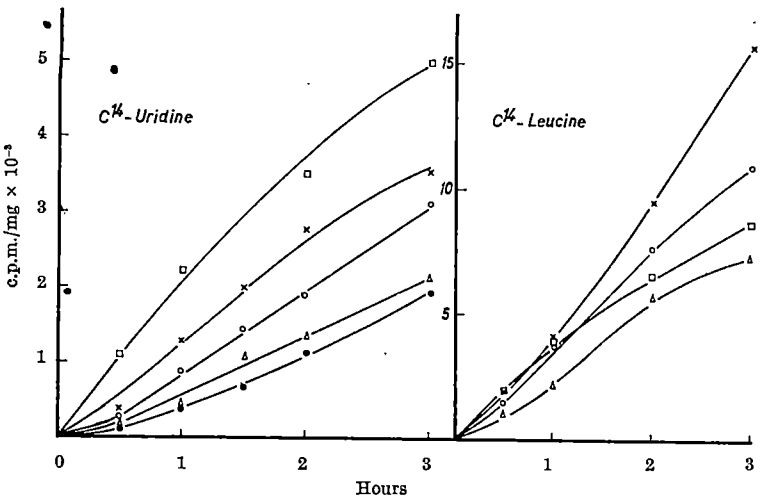


Fig. 6. Influence of a light pulse in the presence of proflavine on the synthesis of RNA and protein. For each point one culture received 25 μg/ml. proflavine and 0·13 μc. ^{14}C-leucine or 0·2 μc. ^{14}C-uridine, respectively. The cultures were exposed for different lengths of time to three Philips lamps (*TL* 20 W, 34 D) at a distance of 30 cm or kept in red light, respectively, before the isotope was added. Thereafter they were incubated in the dark for different lengths of time (abscissa). Left: ^{14}C-uridine: ×, red light; ○, 5 min light; △, 8 min light; ●, 11 min light; □, control without proflavine; the values for the controls have to be multiplied by 10. Right: ^{14}C-leucine: ×, red light; ○, 5 min light; △, 10 min light; □, control without proflavine; the values for the controls have to be multiplied by 3

All these experiments were performed with red light. Next we tested the influence of a pulse of normal light on the synthesis of RNA and protein in the presence of proflavine. In these experiments the tissue cultures were left in the light for different lengths of time immediately after addition of proflavine, then the isotope was added and the cells incubated in the dark. Fig. 6 shows the results of a series of experiments with different pulse-lengths. It can be seen that there is greater inhibition of RNA synthesis if the cultures received a light pulse in the presence of proflavine. But the effect of light on the RNA synthesis is much less pronounced than that on the production of infectious virus. Fig. 6 shows also that protein synthesis in the presence of proflavine can be influenced by a light pulse to about the same degree as RNA synthesis.

(5) *Action of proflavine on the energy metabolism of the cell.* The inhibition of RNA synthesis as well as protein synthesis by proflavine raises the question whether or not this inhibition is due to the breakdown of the energy production. Substances which interfere with energy metabolism also inhibit the production of the energy-rich phosphate compounds. Therefore the extent to which proflavine effects the formation of the 5′-uridine phosphates was tested. ^{14}C-Uridine was added to tissue cultures treated with different doses of proflavine. The results of one experiment are shown in Fig. 7. Proflavine has very little effect on the production of the 5′-uridine phosphates. Even as much as 75 μg/ml. proflavine did not inhibit the formation of UTP more than 30 per cent. Therefore a disturbance of the energy metabolism by proflavine can be excluded as an explanation for the inhibition of synthesis of RNA and protein.

The results presented here have demonstrated that synthesis of RNA as well as of protein can be inhibited by proflavine. To inhibit protein synthesis to the same extent as RNA synthesis, between two and three times as much proflavine is needed. This is in contrast to the action of actinomycin, which does not affect the protein synthesis immediately after its addition. The drop in the protein metabolism which is observed later is probably due to an exhaustion of the messenger RNA, rather than to a direct inhibition of protein synthesis by actinomycin.

Another difference between actinomycin and proflavine can be seen in the binding of these compounds to high-molecular-weight DNA and RNA. While actinomycin attaches only to DNA, proflavine binds to both DNA and RNA. But here again a factor of three is involved; three times more RNA is needed in order to get an effect comparable with that on DNA.

It is known that actinomycin inhibits the multiplication of the influenza viruses, of which fowl plague is a member, but not the production of NDV[12,13]. Two to three times more proflavine is needed for the inhibition of NDV multiplication than for the inhibition of the formation of fowl plague virus. Therefore it is suggested that proflavine prevents the formation of fowl plague virus by acting in the same way as actinomycin. In addition, proflavine inhibits the multiplication of NDV probably by binding to RNA and consequently preventing the synthesis of NDV proteins.

Since the production of the 5′-uridine phosphates, and consequently the energy metabolism, is not significantly affected by proflavine, we suggest that this compound acts on DNA as well as on RNA, preventing RNA and protein synthesis, respectively. Whether, or to what extent, binding of proflavine to soluble RNA is involved in the inhibition of protein synthesis is not yet known.

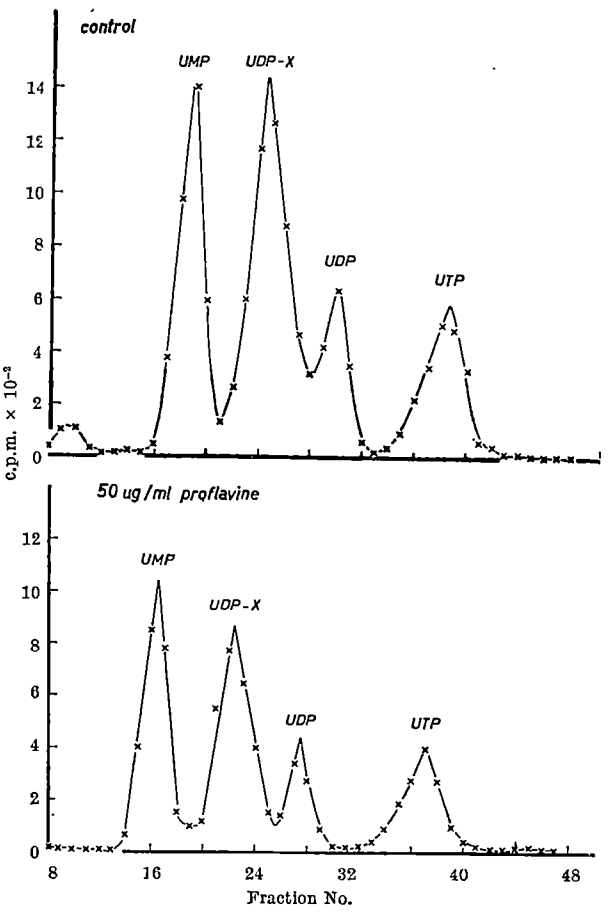


Fig. 7. Effect of proflavine on the formation of the 5′-uridine-phosphates. Five cultures, treated with 50 μg/ml. proflavine, and five controls received 1 μc. ^{14}C-uridine per culture in red light. 1 h later the cells were gathered and precipitated with cold perchloric acid (0·4 N final concentration). 1 mg 5′-UMP and 2 mg 5′-UTP (containing some 5′-UDP) were added to the supernatant. After neutralization with $KHCO_3$ and removal of the $KClO_4$ in the cold the supernatant was chromatographed on a 'Dowex 1 × 2'-column with ammonium formate according to Hurlbert *et al.* (ref. 25). UDP-*X*, 5′-uridine-diphosphate with a carbohydrate bound to the phosphate group

This work was supported by the Deutsche Forschungsgemeinschaft.

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EFFECTS OF NITROGEN, AND OF RESPIRATORY INHIBITORS, ON ETHYLENE PRODUCTION BY A SUB-CELLULAR FRACTION FROM TOMATOES

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NUMEROUS experiments with whole fruit and tissue slices have indicated the existence of a relationship between respiration and ethylene production[1]. This article deals with an investigation of the effects of an atmosphere of nitrogen, and of various inhibitors, on ethylene production by a sub-cellular system from ripening tomatoes.

Preparation of the sub-cellular particles (separated at 35,000 *g* for 15 min), their sonication; and the methods for collection, identification, and measurement of ethylene have been described previously[2,3]. When nitrogen was substituted for air, no consistent effect was observed on the initial evolution of ethylene (Table 1), possibly because of remaining traces of air, or the presence of preformed ethylene or ethylene precursors. Production thereafter was less in nitrogen than in air (runs 1, 2, 3*A* and *B*). However, if the samples under nitrogen were returned to air, ethylene production was greater than in the air controls (run 5). Addition of ATP to the samples in a nitrogen atmosphere also resulted in an ethylene production greater than that of the air controls (runs 3 and 4).

The results from these experiments with sub-cellular fractions from tomatoes are similar to those obtained with whole fruits by Hansen[4] and with apple tissue disks by Burg and Thimann[5]. The latter authors concluded that, in nitrogen, a precursor accumulated that could be rapidly oxidized on exposure to air, and then converted to ethylene. Our results indicate that, with the sub-cellular fraction from tomatoes, ATP may substitute for readmission of oxygen in the production of ethylene.

Several enzyme inhibitors were used to investigate the mode of ethylene production by the sub-cellular fraction from tomatoes. Effects on both the initial evolution of ethylene (during the first 3 h) and that produced after overnight ageing of the particles were observed, as the factors controlling production in these two cases appear to be different[6,7]. Results of the inhibitor experiments are presented in Table 2.

Cyanide at concentrations of 1 or 2×10^{-4} M did not inhibit ethylene evolution by the sub-cellular fraction (runs 1–4). When the cyanide was increased to 1×10^{-2} M, the initial production was not inhibited, but there was distinct reduction in ethylene evolved by aged, resonicated particles (runs 5 and 6). (No change in *p*H occurred in the particulate suspension.) These results are similar to those obtained by Hansen[4], with pears, and Gibson[8], with *Penicillium digitatum*, but Burg[9] found "no obvious effect on ethylene production" by apple disks treated with 10^{-2} M cyanide.

It should be noted that the sub-cellular particles were suspended in sucrose-phosphate buffer containing ATP, and the energy supply may not therefore have been limiting in the initial production of ethylene. Production of high-energy compounds may also have occurred through a cyanide-insensitive electron transport system known to be present in many plant tissues[10]. On the other hand, lack of inhibition by cyanide may reflect the presence of an excess of cytochrome oxidase, or the conversion of cyanide into other compounds[11].

Since cyanide in sufficient concentration did inhibit, to a considerable extent, the ethylene production of the aged and resonicated suspension, the possibility remains that metal-containing enzyme systems, or enzymes requiring carbonyl or disulphide groups for their activity, or cyanohydrin-forming metabolites such as pyruvate and oxaloacetate, are involved, directly or indirectly, in ethylene production. The lack of cyanide inhibition of the initial (1–2 h) ethylene production, even at 10^{-2} M potassium cyanide concentration, could be due to several factors in addition to those mentioned above: the ethylene precursor (or precursors), the new formation of which would be prevented by cyanide, initially may have been present in the preparation in such amounts that it was not the rate-limiting factor in ethylene production; alternatively, preformed ethylene may have been bound by some factor, and liberated on addition of cyanide. Liberation of preformed ethylene would mask inhibition of formation of new ethylene.

Malonate (10^{-2} M) had no pronounced effect on ethylene evolution (runs 7 and 8). Gibson[8] observed that the same concentration of malonate (10^{-2} M) produced a 13 per cent inhibition of ethylene production by *Penicillium digitatum*, but did not affect the respiration. The lack of effect of malonate on the tomato particles does not necessarily exclude a role for the Krebs tricarboxylic acid cycle in ethylene production. Malonate can be readily metabolized by plants and malonate inhibition in plant tissues is usually found only when the concentration of malonate is relatively high, and the medium is acid, and even then it may take some time to develop[10].

Monofluoracetate, like malonate, did not appreciably inhibit ethylene production (runs 9 and 10), but it is possible that higher concentrations of the inhibitor would have an effect[8,12].

Table 1. Effect of Nitrogen on Ethylene Production by a Sub-cellular Fraction from Tomatoes. C_2H_4, mμl.

Run No.	1		2		3		4			5	
Portions	*A*	*B*	*A*	*B*	*A*	*B*	*C*	*A*	*B*	*A*	*B*
Atmosphere	Air	Nitrogen	Air	Nitrogen	Air	Nitrogen	Nitrogen	Nitrogen	Nitrogen	Air	0–2 h : N_2 2–23 : air
Collection time (h)							ATP added				
0–1	248	428	180	137	227	225	197	290	261	197	188
1–2	—	—	15	6	*T*	*N*	*N*	17	—	26	34
									ATP added		
2–3	*N*	*N*	8	*N*	↓	↓	↓	25	341	*N*	*T*
3–17	*N*	*N*	6	32	51	23	*N*	↓	↓	17	64
17–18	*N*	28	*N*	*T*	*N*	*N*	*N*	*N*	*T*	*N*	*N*
18–19	> 984	876	568	444	388	242	406	203	> 2,070	727	1,231
19–20	3,870	1,035	424	386	422	191	517	478	1,372	738	938
20–22	> 3,960	1,890	—	—	388	268	405	333	1,350	900	938
Total	> 9,062	4,257	1,201	1,005	1,476	701	1,525	946	> 5,394	2,605	3,393

Reaction mixture: 0·05 M sucrose—0·125 M KH_2PO_4, *p*H 7. After sonication, the particles in the reaction mixture were divided into equal portions, *A*, *B* (and *C*) by volume. Particles were sonicated in an atmosphere of nitrogen at the beginning of the first, eighteenth, nineteenth and twentieth hour collections. In runs 1–4, the suspensions were evacuated and the vacuum released to nitrogen before the initial sonication. When ATP ($1{\cdot}98 \times 10^{-3}$ M) was used it was added after this sonication. Essentially the same relative activities were obtained when the samples were calculated on a nitrogen basis.
N, not detectable; *T*, trace.

Table 2. Effects of Respiratory Inhibitors on Ethylene Production by a Sub-cellular Fraction from Tomatoes. C_2H_4, mμl.

	A	*B*	*A*	*B*	*A*	*B*	*A*	*B*
Inhibitor	KCN (1×10^{-4} M)		KCN (2×10^{-4} M)		KCN (1×10^{-3} M)		Malonic acid (1×10^{-2} M)	
Run No.	1		3		5		7	
0–3 h	101	366	304	332	338	512	366	428
22–24	1,440	1,523	1,508	1,918	866	478	1,177	1,142
Run No.	2		4		6		8	
0–3 h	61	118	568	656	276	300	102	175
22–24	343	307	2,019	2,126	715	465	543	486
Inhibitor	Monofluoroacetate (1×10^{-4} M)		$NaAsO_2$ (1×10^{-1} M)		$NaAsO_2$ (1×10^{-4} M)		Iodoacetamide (1×10^{-2} M)	
Run No.	9		11		13		15	
0–3 h	192	209	229	301	171	405	225	169
22–24	471	479	2,317	122	658	624	1,052	383
Run No.	10		12		14		16	
0–3 h	102	249	96	287	61	75	164	237
22–24	543	400	2,199	333	343	259	675	388
Inhibitor	*p*-CMB (1×10^{-4} M)		*p*-CMB (1×10^{-4} M)		NaF (1×10^{-3} M)			
Run No.	17		19		21			
0–3 h	242	*T*	242	180*	248	399		
22–24	911	588	911	1,269	1,104	1,035		
Run No.	18		20		22			
0–3 h	310	180	310	39*	293	285		
22–24	1,061	754	1,061	1,233	1,322	1,350		

Reaction mixture as in Table 1. *A* and *B* are two halves of the same particulate preparation, *A* being the control and *B* the sample treated with inhibitor. ATP ($1{\cdot}98 \times 10^{-3}$ M) was added after the initial sonication. Sonications were performed and ethylene collections were made at the same time intervals as in Table 1, and the appropriate results added to give the above figures. When values were expressed on a nitrogen basis, about the same comparative activities were obtained. *T*, trace.
* Glutathione (1×10^{-2} M) added after 1 h.

Arsenite at a concentration of 10^{-1} M almost completely inhibited the production of ethylene by aged particles on sonication (runs 11 and 12). At 10^{-4} M, where it could be considered to act more selectively to inhibit oxidative decarboxylation of keto acids, it only slightly inhibited the production after overnight ageing (runs 13 and 14). At both concentrations, the initial production of the gas was greater in the presence of the inhibitor.

Treatment of the sub-cellular fraction from tomatoes with 1×10^{-2} M iodoacetamide resulted in pronounced inhibition of the ethylene production by the aged and resonicated fraction (runs 15 and 16). Similar results were reported by Burg and Thimann[12], with apple tissue disks, and Gibson[8], with *Penicillium digitatum*.

When *p*-chloromercuribenzoate (*p*-CMB, 1×10^{-4} M) was used with the sub-cellular particles, ethylene production was decreased (runs 17 and 18). Glutathione (1×10^{-2} M) reversed this effect (runs 19 and 20). The protective function of glutathione with respect to sulphydryl groups is well known. (Sonication of glutathione itself in the reaction mixture (without particles) did not result in detectable ethylene production. When glutathione was added to particles suspended in the usual reaction mixture, there was no increase in ethylene evolution over the controls.)

Sodium fluoride (1×10^{-2} M), in the presence of ATP, had little effect on ethylene production by the sub-cellular fraction from tomatoes (runs 21 and 22). This is similar to the results of Burg and Thimann[12], who found that fluoride inhibition of ethylene production by apple tissue disks was reversed by addition of ATP. Succinic acid dehydrogenase, phosphatases, pyrophosphatases and a number of glycolytic enzymes, are among those inhibited by fluoride. Negative results with inhibitors do not, of course, preclude participation of a reaction in the biosynthesis of a compound, for the product of the inhibited reaction may already be present in sufficient amounts in the enzyme preparation.

Although the results of experiments with any single inhibitor cannot be accepted as a conclusive test of the operation of a pathway, the summation of evidence from experiments with several inhibitors can be a useful guide. The first observation that may be made is that the effects of inhibitors on the initial ethylene evolution and on the evolution of aged and resonicated particles were often different, which indicated once more that different mechanisms might be involved. (It is for this reason that figures for total ethylene production over the 24-h period have not been included, their significance being doubtful.) Preformation and binding of the gas as factors in the initial production of ethylene appear possible, and participation of non-enzymatic reactions in this evolution has been suggested previously[6,7].

With respect to the aged and resonicated particles, operation of the complete TCA cycle would not appear to be a necessity for ethylene evolution. (Monofluoracetate (10^{-3} M), arsenite (10^{-4} M), malonate (10^{-2} M) and cyanide (2×10^{-4} M) did not significantly hinder ethylene production.) The fact that higher concentrations of cyanide (1×10^{-2} M) and arsenite (10^{-1} M) did inhibit evolution of the gas would not necessarily mean that the TCA cycle was involved, for at these concentrations many enzyme systems in addition to those directly concerned with the TCA cycle are likely to be affected[13]. Burg and

Thimann[5] concluded that the TCA cycle was not involved in ethylene production by apple tissue disks, and Gibson[8] found that pyruvate was a better ethylene precursor than fumarate or succinate, in *Penicillium digitatum*. On the other hand, Wang *et al.*[14], who used long collection periods for ethylene, concluded that there was an intimate connexion in *Penicillium digitatum* between production of the gas and the Krebs tricarboxylic acid cycle.

Involvement of essential sulphydryl groups in ethylene production by the sub-cellular particles was indicated by experiments with *p*-CMB and glutathione, as well as those with iodoacetamide. Thiol reagents can inhibit plant respiration by interfering with glycolysis, the hexose monophosphate pathway, and organic acid oxidation[15].

The inhibitors may exert their effects at any stage in the pathway of ethylene synthesis. The general pattern of inhibition of the production of the gas by aged, resonicated particles indicates that enzymatic reactions are involved. However, they may effect the production of a precursor of the gas, rather than the final evolution of ethylene itself.

This work was supported by a grant-in-aid from the National Research Council of Canada.

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INHIBITION OF PROTEIN SYNTHESIS BY ACRIDINE ORANGE

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THE interaction of acridine diamino derivatives with nucleic acids has been extensively investigated, particularly *in vitro*[1–5]. It is not yet known, however, how these substances affect the synthesis of protein.

We have examined this problem autoradiographically having chosen as an active substance acridine orange (2,8-*bis*dimethylaminoacridine) the effect on protein synthesis of which has been to some extent reported in the literature[6]. Preparations of 1–2 days monolayer cultures of amnion cells (strain *FL*) were placed for various times (3 min–48 h) in a medium with acridine orange (AO) in a 0·1–6·0 μg/ml. concentration. During the test, precautions were taken not to expose the cells to the action of light. After treatment with AO the excess dye was washed off from the slides, the latter being then placed in a fresh medium containing ^{35}S-methionine or ^{14}C-phenylalanine (3–5 μc./ml.), fixed in 70 per cent ethanol for 3–5 min and treated for 5 min with a 5 per cent solution of perchloric acid at 0°–2° C. The autoradiograms were prepared using *NJKFJ* dipping emulsions. Quantitative results characteristic of the intensity of the incorporation of the tracers were estimated by counting the number of silver grains per 1 cell or per field of standard size, fully covered by the cells.

The first series of experiments have shown the treatment of cells by relatively high doses of AO (6 μg/ml.) for 16–20 h to suppress fully the incorporation of labelled precursors. Subsequent experiments have led to the conclusion that the incorporation of tracers is partly inhibited when cells are treated with AO in a concentration of 0·5–1·0 μg/ml. AO practically inhibits protein synthesis already at a concentration of 1·5–2·0 μg/ml. (Table 1).

It has been further shown that the incorporation of a tracer is already markedly inhibited 10–15 min after the beginning of treatment of cells with AO (Table 2).

Some differences in the results of various runs seem to be due to the different conditions of cells.

Our experiments have thus shown that AO markedly affects protein synthesis and practically inhibits it several minutes after the beginning of its action. The activity of the compound is in this respect strong enough, being 1–2 μg/ml. In complete accordance with the inhibition of protein synthesis is the suppression of mitotic activity of cultures by AO. This suggests that the well-known antimitotic effect of AO and some other diaminoacridines[7,8] is due to their inhibiting action on protein synthesis.

It proved of interest to elucidate the ways in which AO influences protein synthesis. It seemed most reasonable that the effect of AO should be connected with the inhibition of the synthesis of the messenger-RNA. It was found, however, that actinomycin *D* (which completely hinders the synthesis of cell RNA, estimated by the incorporation of ^{3}H-uridine) even after 6–10 h of its action affect protein synthesis only by 60–70 per cent[9]), whereas treatment with AO suppresses the incorporation of radioactive tracer into the protein in 15 min after the beginning of the experiment. The supposition that the action of AO on protein synthesis is effected through the inhibition of RNA synthesis is also contradicted by the direct autoradiographic observation on the incorporation of ^{3}H-uridine and ^{3}H-thymidine into AO-treated cells (Table 3).

One can see from Table 3 that even after 48 h of AO treatment the rate of synthesis of RNA is reduced only to 75 per cent, this result being in agreement with the results obtained by Brown and Stewart[10], who showed that proflavine inhibited the synthesis of the protein part

Table 1. Effect of Different AO Concentrations during 20 h on the Incorporation of Labelled Amino-acids into the Cells

Concentration of AO (μg/ml.)	No. of silver grains in an equal space covered by cells, as percentage of the reference No. ^{35}S-methionine 1	2	3	^{14}C-phenylalanine 4	Average
0·0	100	100	100	100	100
0·1	90	95	69	64	78
0·5	51	70	43	33	49
1·0	21	61	40	28	37
1·5	—	26	25	10	20
3·0	8	29	14	—	17

Table 2. Effect of Different Times of Action of Acridine Orange (3 μg/ml.) on the Incorporation of Amino-acids

Time of action of AO	No. of silver grains in an equal space covered by cells (absolute figures) ^{35}S-methionine 1	2	3	^{14}C-phenyl-alanine 4
0	228	1,594	383	731
3 min	—	—	219	—
5 min	70	—	—	—
15 min	—	41	—	307
1 h	53·5	105	—	—
3 h	94	54	—	—

Table 3. Effect of Acridine Orange (3 μg/ml.) on the Incorporation of ^{3}H-Uridine and ^{3}H-Thymidine

Time of action of AO	No. of silver grains per nucleus ^{3}H-uridine		^{3}H-thymidine	
	1	2	1	2
0	82	79	13·6	44
5 min	—	—	—	35
1·5 h	53	25	13·0	29
4·5 h	—	32	—	28
24 h	—	53	—	25
48 h	—	53	3·3	—

of foot-and-mouth virus without any marked influence on the synthesis of its RNA.

It is necessary also to mention that AO does not affect primarily either the cell respiration[6,11] or oxidative phosphorylation. The latter fact follows from our experiments (Table 3) in which the possibility of normal undergoing of phosphorylation of ^{3}H-thymidine and ^{3}H-uridine molecules in AO-treated cells was shown.

It seems most probable that inhibition of the protein synthesis by AO takes place in the early stages of proteosynthesis and is connected with the inactivation of *s*-RNA by AO (ref. 5). On the other hand, AO (as follows from the data presented in Table 3) produces some inhibitory effect also on the synthesis of DNA and RNA. This effect takes place in a rather short period of time after the beginning of AO action and seems not to be connected with inhibition of protein synthesis but due to the direct interaction of AO with DNA, possibly by intercalation between its double helix molecule[3,4].

This article was translated into English by A. L. Pumpiansky, Moscow.

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MUTAGENICITY OF IONIC POLYMERS AND LEUKÆMOGENIC VIRUSES RELATIVE TO NUCLEIC ACIDS IN *Drosophila melanogaster*

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THE mutagenic activity of macromolecules was first discovered for deoxyribonucleic acid[1] (DNA), but was later ascertained for other cellular constituents[2]—such as ribonucleic acid (RNA), deoxyribonucleoproteins (DNP) and histones. These various molecules shared the property of being predominantly effective in the induction of small deletions resulting in the *Minute* phenotype, although their efficiency in this respect varied considerably. This indicated some degree of non-specificity as regards the molecular structure of the inducing agent and suggested that synthetic polymers might also be active, especially if they were somewhat imitative of the hereditary macromolecules. More interesting, from the point of view of the mechanism of carcinogenesis, is the question whether viruses—which might be looked on as infective nucleoproteins—also possess the macromolecular type of mutagenesis. In an attempt to probe these possibilities, mutagenic tests were undertaken with various synthetic polymers simulating natural macromolecules, as well as with cell-free extracts from normal and leukæmic mice (with Gross and Moloney viruses).

The synthetic polymers tested belonged to the following chemical series: (1) polyelectrolytes, represented by polymethacrylic acid (mol. wt. $\simeq 10^6$) used as the sodium salt; (2) polyamino-acids, represented by polyglutamic acid (mol. wt. $\simeq$40,000); (3) polynucleotides, polyadenylic acid (polyA) as a representative of a purine polymer and polyuridylic–cytidylic acid (poly-UC; 3U : 1C—kindly supplied by Prof. S. Ochoa, New York University Medical Centre), as a representative of pyrimidine polymers (both of low molecular weight; of the order of 20,000).

The cell-free extracts were derived from mouse tissues, which were either normal or leukæmic and were intended for the assessment of the change of mutagenicity in the presence of the leukæmogenic viruses (both of Gross and Moloney). In the Gross virus tests the extracts (kindly supplied by Dr. J. F. A. P. Miller, of this Institute) were prepared from thymus, spleen and lymph nodes (to be referred to collectively as lymphatic tissue) derived from $Ak \times T_6, F_1$ mice with leukæmia originally induced by the Gross virus. The non-viræmic control was an extract from the same tissues from healthy adult C_3H mice. Extraction was undertaken by the homogenization of the tissues in isotonic saline (20 per cent w/v) followed by the isolation of cells and their debris by centrifugation (1,400*g* for 15 min; 7,000*g* for 10 min) and then by filtration through a 'Selas 02' porosity candle under vacuum. The extracts were subjected to an amino-acid analysis, to obtain some idea of their constituent proteins. Although the amino-acids predominant in basic proteins were detected, their proportions were rather low; percentages by weight: lysine, 6·4; arginine, 3·3; isoleucine, 3·3; leucine, 9·1. These proportions were not considerably above those found in albumins and globulins of mouse serum, thus indicating that the presence of large quantities of basic proteins in the tested extract was rather improbable.

In the Moloney virus experiments, the material (kindly supplied by Dr. J. B. Moloney of the National Institutes of Health, Bethesda, U.S.A.) was normal or viræmic plasma from *BALB/c* mice. The virus was concentrated by differential centrifugation so that a corpuscle-free pellet derived from 10 ml. of whole blood was finally suspended in 1 ml. of 0·5 M sodium citrate. The leukæmogenic efficiency of this virus concentrate (assayed in Dr. Moloney's laboratory) was found to be ED_{50}/ml. $\times 10^{-4.5}$: 50 per cent of the *BALB/c* mice which received $10^{-4.5}$ dilution of the virus when newly born showed spleens which weighed in excess of 150 mg at 28 days post-inoculation. The material for the control genetic test was normal *BALB/c* mouse plasma, which was subjected to the same differential centrifugation procedures used in the preparation of the virus concentrate. The proteins in the tested material (normal and viræmic plasma) were also analysed for the constituent amino-acids. The proportions of these acids were on the whole comparable to those obtained for the proteins from the lymphatic tissue extract. The percentages by weight of the amino-acids (predominant in basic proteins) in the analysed samples were: lysine, 7·8; arginine, 3·9; isoleucine, 3·2; leucine, 9·9.

For the mutagenicity tests the various agents were administered in isotonic solutions by micro-injection into

Table 1. THE *Minutes* AND SEX-LINKED RECESSIVE VISIBLES IN THE F_1 MALES FROM ATTACHED-X EXPERIMENTS WITH VARIOUS POLYMERS AND CELL-FREE EXTRACTS

Mosaic mutants, included in the mutation rate, indicated in brackets

Agent	Conc. (%)	Progeny sampled		*Minutes*		Rec. visibles	
		Days after treatment	Males	No.	Per 10^3	No.	Per 10^3
Ionic polymers:							
Polymethacrylic acid	0·1	15	6,237	45 (1)	7·22 ± 1·07	5 (2)	0·48 ± 0·28
	1·0	6	7,906	42 (7)	5·31 ± 0·82	3 (2)	0·38 ± 0·22
Polyglutamic acid	1·0	15	9,070	46 (2)	5·07 ± 0·75	4	0·44 ± 0·22
Polyadenylic acid	0·2	21	12,945	79 (5)	6·10 ± 0·68	3	0·23 ± 0·13
	0·5	21	13,516	61 (4)	4·51 ± 0·58	2 (1)	0·15 ± 0·11
Polyuridylic-cytidylic acid	0·5	21	52,257	216 (8)	4·13 ± 0·28	11 (2)	0·21 ± 0·06
Cell extracts (mouse):	Approx. organic content						
Leukæmic lymphatic tissue (Gross virus)	0·2	22	82,051	292 (14)	3·56 ± 0·21	49 (4)	0·60 ± 0·09
Normal lymphatic tissue (control)	0·2	15	27,584	96 (5)	3·48 ± 0·35	7 (2)	0·25 ± 0·10
Leukæmic plasma (Moloney virus)	0·2	22	62,733	253 (5)	4·03 ± 0·25	18 (3)	0·29 ± 0·07
Normal plasma (control)	0·2	19	47,933	227 (6)	4·74 ± 0·31	14 (1)	0·29 ± 0·08

the hæmocœl of adult males (30 ± 5 h after eclosion) of the Oregon-*K* stock. The mutations scored in the progeny of the treated males were viable chromosome fragments and visibles (morphologically detectable changes)—both the sex-linked recessives and the autosomal dominants (especially *Minutes*). These mutations were detected by the attached-*X* technique with the markers: yellow (*y*), vermilion (*v*) and forked (*f*). Scoring for the visibles was restricted to the males only, since the slight *Minutes* (showing thin macrochætes) were difficult to detect in the females in conjunction with the forked marker which also affected the shape of the bristles. In all experiments the progeny of the treated males were fractionated into broods by repeated matings to an equal number of virgin females at 3-day intervals: the mutation rates in the successive matings were determined separately. Breeding was undertaken in mass cultures, with a maximum of ten pairs per half-pint bottle. For control purposes, males of the same stock and age were injected with isotonic saline and subjected to the foregoing attached-*X* technique. The spontaneous mutation rate detected in the male progeny over 7 broods (21 days after treatment) reached a mean value per thousand tested gametes of 0·95 ± 0·11 for the *Minutes*, and 0·15 ± 0·05 for the sex-linked recessive visibles[2]. No hyperploid females were detected among an observed sample of 80,000 progeny, which virtually excluded the spontaneous occurrence of large viable *X*-chromosome fragments.

The overall mutagenic activity of the agents investigated is summarized in Table 1. All were convincingly active as regards the induction of small chromosome deletions resulting in the *Minute* phenotype. The induced rates in the different experiments were very significantly above the control rate (by as much as 3–7-fold). Whenever more than one concentration of the same macromolecule has been tested, the recovered mutation rate was lower with the higher dose. This is entirely due to the rapid increase in toxicity with dose, both on the injected animals and on the germ cells. In the experiments with polymethacrylic acid, only 3 per cent of the injected males survived the higher dose (1 per cent) for 7 days and none gave any progeny after the sixth day. Even with the lower dose of this polymer (0·1 per cent) the survival rate at the end of the first week was still low (15·7 per cent) and their fertility was considerably lower than among the controls. This suggests that the mutations recovered in the mutagenicity tests with macromolecules are probably only a small proportion of those actually induced; the remainder being eliminated among the cells killed through cytotoxicity. The tests with the lower concentrations, therefore, are probably more representative of the actual mutagenic potency.

The synthetic polymers tested, like the natural macromolecules, were virtually ineffective in the induction of viable chromosome fragments. The hyperploid females induced by the various agents were as follows: polyglutamic acid, 1/8,863; polymethacrylic acid, 1/12,919; polyadenylic acid, 1/24,831; leukæmic (Gross virus) mouse lymphatic tissue extract, 2/82,051; leukæmic (Moloney virus) mouse plasma, 1/58,343. No fragments were recovered with polyuridylic–cytidylic acid, 0/48,916; normal mouse lymphatic tissue extract, 0/25,009; and normal mouse plasma, 0/43,560. These results add further support to the interpretation[1,2] that the induction of small chromosome deletions by macromolecules is the outcome of gene inactivation *in situ*, rather than the induction of chromosome breaks.

In contrast to the mutagenic efficiency of the tested agents in the induction of small deficiencies (*Minutes*), their activity as regards the point mutations (sex-linked recessive visibles) was not very dramatic. Although there was some rise in the rate of visible mutations in most tests, the induced frequency did not reach statistical significance except in the Gross virus series. The viræmic lymphatic tissue extract gave a rate of visible mutations considerably higher than the spontaneous rate (P = 0·00012) and also well above that induced by the non-viræmic extract of the same tissue (P = 0·0094). Unfortunately, however, the same mutagenicity did not occur with Moloney virus: the mutation rate (as regards the visibles) was the same with the normal and viræmic plasma, being insignificantly higher than the control level ($P \simeq$ 0·1). The difference in mutagenicity between the Gross and Moloney extracts is rather unexpected, since both viruses are of the RNA type, and are known to induce leukæmia in mice. This, however, does not exclude the possibility that some differences might occur in the biological activity of the two viruses in the fly's hæmocœl.

The significance of the cell stage during spermatogenesis on the activity of the tested agents was assessed on the basis of the variation in the mutation rate in different progeny fractions of the treated males. As with other macromolecules[2], this comparison was undertaken on the basis of the frequency of *Minutes* in the first 9 days progeny and that occurring in still later offspring (the 10th and later days after treatment). It should be recalled that mutations recovered in the earlier period were induced in the post-meiotic stages (sperm and spermatids), whereas those detected in the later interval must have occurred in germ-cells treated in the meiotic and premeiotic stages (spermatocytes and spermatogonia). The mutation rates induced in these two sectors of the germ line with the various agents are shown in Table 2. With polymethacrylic acid and poly-A there was conclusive evidence that the mutagenicity was significantly higher in the post-meiotic stages. In this respect, the mode of action of these agents simulates that ascertained for DNA. Polyglutamic acid was extremely toxic to the premeiotic germ-cells, thus not permitting the recovery of sufficient late progeny for a satisfactory determination of the mutation rate. Poly-UC, like RNA, was slightly more mutagenic in the early germ-cells. With the various tissue extracts (mainly heterologous proteins) there were no dramatic differences in activity in the various sectors of the germ

Table 2. The Mutation Rate (*Minutes*) in the Early (0–9 Days) as compared with the Late (Tenth and Later Days) Progeny, induced by Various Polymers and Cell-free Extracts

The limits of the mutation rates for the first two compounds are based on the Poisson expectation ($P = 0.025$)

Agent	Progeny sampled						Significance of difference P
	0–9 days Spermatozoa and spermatids			> 9 days Spermatocytes and spermatogonia			
	Males	*Minutes*		Males	*Minutes*		
		No.	Per 10^3		No.	Per 10^3	
Ionic polymers:							
Polymethacrylic acid	4,902	45 (1)	12·27 9·18 6·71	1,335	0	2·76 0·00 0·00	0·025
Polyglutamic acid	8,806	46 (2)	6·96 5·22 3·83	264	0	13·98 0·00 0·00	?
Polyadenylic acid	7,972	52 (3)	6·52 ± 0·90	4,973	27 (2)	5·43 ± 1·00	0·43 } 0·007
	7,934	46 (2)	5·80 ± 0·85	5,582	15 (2)	2·69 ± 0·69	0·004 }
Polyuridylic-cytidylic acid	31,211	114 (7)	3·65 ± 0·34	21,046	102 (1)	4·85 ± 0·48	0·04
Cell extracts (mouse):							
Leukæmic lymphatic tissue (Gross virus)	3,3084	135 (4)	4·08 ± 0·35	48,967	157 (10)	3·21 ± 0·26	0·04
Normal lymphatic tissue (control)	22,878	85 (5)	3·72 ± 0·40	4,706	11	2·34 ± 0·70	0·09
Leukæmic plasma (Moloney virus)	37,002	137 (4)	3·70 ± 0·32	25,731	116 (1)	4·51 ± 0·42	0·12
Normal plasma (control)	35,512	174 (5)	4,90 ± 0·37	12,421	53 (1)	4·27 ± 0·59	0·36

line. There was some indication, however, that the viræmic lymphatic tissue extract (Gross virus) might be slightly more active in the post-meiotic stages.

The mutagenicity of the various macromolecules relative to DNA is represented graphically in Fig. 1. This was measured as the ratio of the mutation rate induced by each agent to that occurring with DNA at the same concentration. Where more than one concentration was tested for the same agent, comparison was based on the mutagenicity results with the lowest dose, so as to minimize the complications due to cytotoxicity. The standard deviation for each mutability ratio (vertical lines in Fig. 1) was estimated on the basis of the binomial variances of the mutation rates entered in its calculation. The differences in mutagenicity between agents were fairly marked as regards the induction of chromosome deletions (*Minutes*). Polymethacrylic acid was most outstanding among the tested compounds, in so far as it was nearly four times as active as DNA. The mutagenicity of polyadenylic acid was 2·5 times that of DNA, which is somewhat lower than that of polymethacrylic acid, although not significantly so ($P = 0.12$). It is, therefore, probable that the activity of polynucleotides (especially those of purines) is not different from that of unnatural anionic polymers. The remaining agents fell clearly into two groups: one with a higher activity than DNA (by a factor of 1·5–2), while the other was somewhat less effective (giving 0·2–0·7 the mutagenicity of DNA). The more active agents were mainly heterologous proteins, whether basic (histones), acidic, or neutral (albumins and globulins in the extracts of Moloney and Gross virus series). The group of compounds of lower mutagenicity than DNA was very heterogeneous, including poly-UC, polyglutamic acid, DNP and RNA. There is no obvious common feature (as regards chemical structure) that might account for their comparative inactivity.

A comparison was also undertaken of the mutagenicity of the various agents, relative to DNA, as regards the induction of sex-linked recessive visibles (Fig. 1). This comparison proved to be more ambiguous than that based on the *Minutes*. The extreme rarity of the induction of visibles with the macro-molecules resulted in large sampling errors in the estimation of their relative mutability ratios, which made it difficult to establish significant differences between agents. On the whole, however, there was a considerable similarity in the order of activity of the different agents on the basis of the two classes of mutation; but with narrower differences for the point mutations. Nevertheless, deviations in the rank of activity for *Minutes* and visibles did occur with two agents, which might be indicative of some differences in their mutagenic mode of action. Foremost in this connexion is the position of the cell extract with Gross virus, which showed maximal activity as regards the visibles, but only an intermediate efficiency (equivalent to the non-viræmic extract) as regards the *Minutes*. This could mean that the virus was predominantly effective in the induction of the point mutations (visibles) whereas the tissue proteins (common to both the normal and viræmic extracts) were mainly active as regards the induction of deletions (*Minutes*). Another compound which showed rank deviation as regards the two classes of mutation was polyadenylic acid. It was more active than DNA as regards the induction of *Minutes*, but less effective in the production of visibles. This suggests that poly-A is preferentially effective in the induction of small chromosome deletions, rather than point mutations.

It has already been pointed out[1] that the mutagenicity of DNA in metazoan cells does not involve a specific exchange between the exogenous DNA and particular

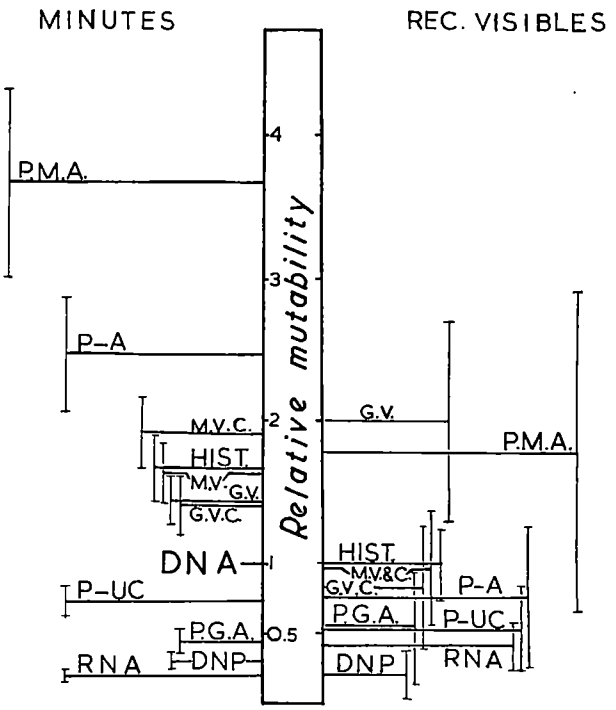


Fig. 1. Mutagenicity of natural and synthetic polymers relative to DNA: left half, *Minutes*; right half, sex-linked recessive visibles. Relative mutability was measured as the ratio of the mutation rate induced by each agent to that of DNA at the same injected concentration. DNA, deoxyribonucleic acid; DNP, deoxyribonucleoprotein; G.V., Gross virus (in mouse lymphatic tissue extract); G.V.C., Gross virus control (mouse lymphatic tissue extract); Hist., histones (calf thymus); M.V., Moloney virus (in mouse plasma); M.V.C., Moloney virus control (mouse plasma); P–A, polyadenylic acid; P.G.A., polyglutamic acid; P.M.A., polymethacrylic acid; P–UC, polyuridylic-cytidylic acid; RNA, ribonucleic acid

gene loci. Transformation at specific genes by the injection of DNA of the wild-type did not occur and mutagenicity was not restricted to the homologous material. Later investigations showed that the DNA type of mutagenicity also occurred with other cellular macromolecules, such as RNA, DNP and histones. It was accordingly suggested[2] that the mechanism involved was probably physical, involving the 'coverage' of some gene loci on the template chromosome, thus preventing their coding in the replica. The nature of the process of locus 'coverage' was unknown, but it was thought possible that it could be mediated by surface or electrical phenomena. The results summarized in Fig. 1, however, are incompatible with the above physical interpretation. There was no obvious correlation between the physical properties of the molecules tested (whether as regards size or charge) and their biological activity. Polynucleotides of the same charge (−ve) and roughly the same molecular weight showed considerable differences in mutagenic efficiency: poly-A was more active than DNA, while poly-UC was less effective. On the other hand, the activity of the positively charged histones was higher than that of DNA and approached that of poly-A.

The strongest evidence against the template coverage interpretation came from our recent work on the distribution of *Minutes* induced by different agents. A considerable specificity as to the site of genetic damage occurred with different macromolecules. With DNA (both homologous and heterologous), the majority of the induced deletions—about 80 per cent—involved the same segment on the fourth or 'dot' chromosome (most of segment 102 on the salivary chromosome map), whereas with radiation about 30 per cent of the deletions occurred in the same chromosome[3]. The proportion of fourth chromosome *Minutes* varied considerably with the other polymers[4] (both natural and synthetic), being highest with polyadenylic acid (about 55 per cent) and lowest with polymethacrylic acid, where it was of the same order as after irradiation. This differential activity of macromolecules on the genome immediately excluded any random physical mechanism and suggested some biochemical control, probably of an enzymatic nature. Support for this possibility came from the fact that ionic polymers, which were the most effective agents as regards the induction of *Minutes*, were also shown to be inhibitory to a number of biological systems[6], including enzymes, bacteria and viruses. The nature of the enzymatic disturbance that could lead to the induction of chromosome deletions has recently been indicated by the mutagenicity results with substituted nucleosides[5]—especially 5-fluorodeoxyuridine. This compound, which was shown to be a specific inhibition of thymidylate synthetase[7], was found to induce the macromolecular type of mutagenesis in *Drosophila*, in so far as it was predominantly active in the induction of *Minutes*. It is highly probable, therefore, that these mutations arise through the inhibition of cellular enzymes which, directly or indirectly, affect DNA synthesis: oxidases, phosphorylases, synthetases or polymerases. According to this interpretation, the variation in the induction of *Minutes* with the different polymers could arise as a consequence of differential enzymatic inhibition, coupled with the known variations in the time of replication of different segments of the chromosome complement[8,9].

The important question as to the mutagenic activity of tumour viruses still remains undecided. Experiments with cell-free extracts from mouse tissues, with and without Gross virus, suggested that the presence of the virus might have significantly enhanced the rate of point mutations (sex-linked recessive visibles). On the other hand, parallel tests with mouse plasma (from normal and leukæmic animals) did not reveal an increase in the rate of visibles in the presence of Moloney virus. The mutagenicity of the leukæmogenic viruses as regards the induction of chromosome deletions could not be determined, since the heterologous material (mainly soluble proteins of mouse tissues) was also active. To determine the activity of the pure virus, it will be essential to free it completely from the mouse proteins. Whether this proves feasible with leukæmogenic viruses still remains to be seen. On the other hand, it should be possible to isolate the RNA of the virus, at least from the plasma of leukæmic mice infected with Moloney virus. The investigation of the mutagenic efficiency of virus nucleic acids is now in progress.

We thank Profs. A. Haddow and J. A. V. Butler for their interest. We also thank Prof. S. Ochoa (New York University Medical Centre), Dr. J. B. Moloney (National Institutes of Health, Bethesda, U.S.A.), and our colleagues, Dr. P. Alexander and Dr. J. F. A. P. Miller, for supplying the material used in the work recorded here, and Miss P. Simpson and Miss D. Purkis for the amino-acid analyses.

This work was supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research: Royal Cancer Hospital) from the Medical Research Council and the British Empire Cancer Campaign for Research, and Public Health Service research grant *CA*-03188-08 from the National Cancer Institute, U.S. Public Health Service.

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INHIBITION OF INTERFERON BIOSYNTHESIS BY ACTINOMYCIN *D*

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VIRAL infection of vertebrate cells often results in the synthesis of interferon, a protein which inhibits multiplication of the same virus that induced its formation or a wide variety of unrelated viruses[1]. This observation is understandably of considerable interest as one possible mechanism of intrinsic cellular defence against viral infection. Definitive biological investigations of this phenomenon have been hampered by difficulties encountered in purifying interferon, but some progress has been made recently in characterizing its physicochemical properties[2] and mechanism of antiviral action[3]. Even less is known about the synthesis of interferon at the cellular level. In a recent analysis of this subject[1], three major alternatives, in increasing order of likelihood, were considered as possible pathways for synthesis of interferon: (1) that interferon synthesis is directed by nucleic acid of the infecting virus; (2) that interferon precursors normally present in the uninfected cell are activated by the infecting virus; (3) that interferon synthesis is a latent cellular function induced by viral infection (or other stimulus).

The suggestion was also made that insight into this problem could be gained by the use of selective antagonists of nucleic acid and protein synthesis.

The antagonist with the greatest potential value is actinomycin *D* because of its capacity to block cellular RNA synthesis without inhibiting synthesis of viral RNA[4]. The literature contains several reports[5] on the effectiveness of actinomycin in preventing interferon synthesis without inhibiting multiplication of RNA viruses used to induce its formation. In fact, in the presence of actinomycin the yields of infecting virus may be significantly greater than anticipated[5], presumably owing to suppression of endogenous interferon. The present experiments provide evidence for the cellular site of interferon synthesis based on this selective action of actinomycin.

The feasibility of these experiments depended on the availability of virus–cell systems in which interferon production would begin several hours after infection rather than days. The protocols also called for consistently high final yields of interferon in control-infected cultures. Two such systems satisfied these conditions. In one, primary monolayer cultures of chick embryo (CE) cells were infected with Chikungunya virus; in the other monolayers or suspensions of strain *L*-929 (L) cells of murine origin were infected with a vaccine strain of Newcastle disease virus (NDV). Chikungunya virus was cultivated in brains of new-born mice and prepared as a 10 per cent suspension of emulsified brain from moribund infected animals. The titre of the stock Chikungunya virus suspension determined by intracerebral titration in new-born mice was 10^9 LD_{50}/ml. The stock NDV suspension was prepared from allantoic fluid gathered from 12-day-old chick embryos 48 h after infection; the hæmagglutinin titre of this material was 512 when tested with 0·5 per cent chicken erythrocytes. Both viruses produced only indistinct plaques on CE cell monolayers and neither was cytopathogenic for L cells except at very high multiplicities.

As previously described[6], interferon activity was assayed by the plaque-inhibition method. Fluids removed from infected cell cultures were freed of residual virus by 3 cycles of centrifugation at 100,000*g* followed by dialysis for 24 h at *p*H 2. After neutralization by further dialysis at *p*H 7, the supernatant fluids were diluted serially in two-fold steps and titrated on cells of the same type as those in the original cultures. Confluent monolayers of CE cells in 100-mm plastic plates were drained of growth medium (lactalbumin hydrolysate, 4 per cent calf serum) and exposed for 1 h to 0·5 ml. of each interferon dilution. These cultures and appropriate controls were then infected with an estimated 100 plaque-forming units (P.F.U.) of Eastern equine encephalitis (EEE) virus and overlayered with medium containing 0·95 per cent agar and neutral red. Similar procedures were used for titrating interferon on L cells grown in 60-mm plastic plates in medium 199 with 10 per cent calf serum. In these latter assays monolayers of L cells were exposed for 1 h to 0·2 ml. of each interferon dilution, infected with approximately 100 P.F.U. of encephalomyocarditis (EMC) virus, and then covered at 40-h intervals with 2 agar overlays, only the second of which contained neutral red. Plaques were scored on both CE and L cells after incubation for 44–48 h in a humidified atmosphere of 5 per cent carbon dioxide. The titre of interferon was read as the reciprocal of the dilution at which the plaque numbers in duplicate plates were reduced by at least 50 per cent of the control counts. The method is sensitive and reproducible.

Interferon synthesis was first examined in monolayer cultures of CE cells infected with Chikungunya virus. In these experiments, a series of identical flasks contained approximately 2×10^7 cells and the virus input was 10^8 LD_{50}. After a period of 1 h for virus adsorption, the cell layers were washed and covered with 10 ml. of maintenance medium. At intervals after virus adsorption, the medium was completely withdrawn from each of 3 cultures for

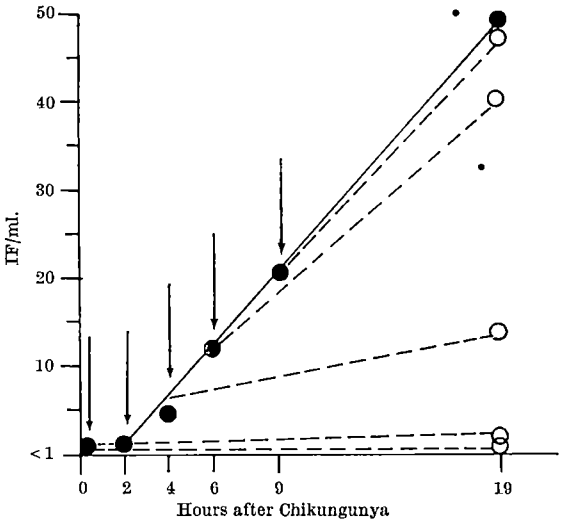


Fig. 1. Synthesis of interferon (IF) by CE cells infected with Chikungunya virus. The solid line and closed circles show the mean interferon content of control flasks at intervals after infection. The open circles and broken lines show mean interferon titres in three flasks after exposure of cells to actinomycin *D* (2 μg/ml.) at times indicated by arrows. As noted in the text, interferon synthesis was interrupted by actinomycin added to cultures by 3–4 h after infection but not by 6 h or later

interferon titration and replaced with an equal volume of fresh medium containing 2 μg/ml. of actinomycin. Finally, the actinomycin-containing fluids were gathered from all cultures at 19 h after infection and assayed individually for their interferon content. Actinomycin was removed from the media by the dialysis procedures used for processing the interferon samples.

Fig. 1 shows the rate of interferon synthesis in cultures of infected CE cells and the effect of actinomycin on final interferon yields. Interferon first appeared in the medium between 2 and 4 h after infection. Thereafter, as previously noted by Heller[5], interferon titres increased linearly to a maximum level at 19 h. When actinomycin was present in the medium from time 0 (the end of virus adsorption) or from 2 h after infection, interferon synthesis was completely inhibited. If exposure to actinomycin was delayed for 4 h, the time at which interferon was first

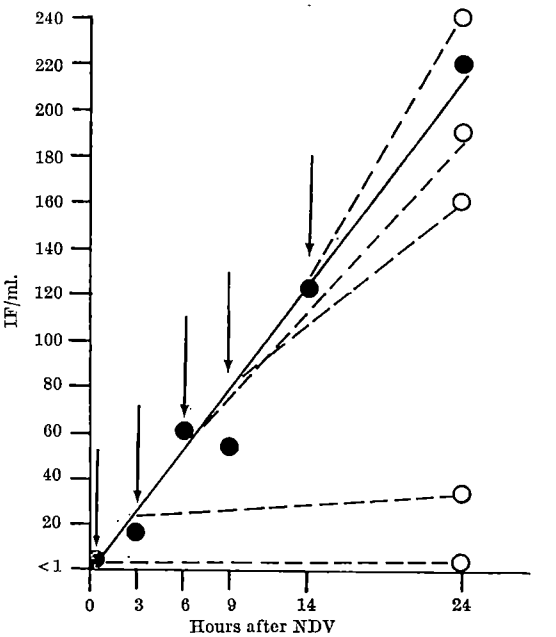


Fig. 2. Synthesis of interferon (IF) by L cells infected with Newcastle disease virus. The arrows indicate the times at which 2 μg/ml. of actinomycin were added to each of three culture flasks. See legend of Fig. 1 and text

detected, the titres at 19 h were considerably reduced, equivalent to the levels in control cultures at 6 h. The presence of actinomycin in the medium from 6 to 9 h after infection did not significantly influence the final yields of interferon.

Fig. 2 reveals that virtually identical results were obtained when monolayers of 6×10^6 L cells were infected with NDV at a multiplicity of about 5. In the absence of actinomycin the delay in appearance of interferon was at least 3 h. Once again, the ensuing rate of interferon synthesis was approximately linear; maximum titres were reached within 24 h after infection. Cells exposed to actinomycin (2 μg/ml.) at time 0 or 3 h were incapable of producing interferon, but by 6 h the interferon-producing capacity of the infected cells was not altered by actinomycin. Entirely comparable results were obtained in investigations with suspended L cells, although the final yields of interferon were generally lower than those from monolayer cultures.

The results strongly favour the hypothesis that interferon synthesis is under genetic control of cellular DNA rather than viral nucleic acid. The time-course of interferon production and the inhibitory effect of actinomycin appear to preclude the possibility that viruses activate a preformed precursor in the uninfected cell. The function of the infecting virus remains to be determined, but it seems likely that interferon synthesis is initiated in a manner similar to that of inducible enzymes. Another analogy can be found in the cell-directed synthesis of 'uncoating protein' induced by infection with pox virus[7]. The delay in onset of interferon synthesis can be attributed, in part, to the requirement for *de novo* production of messenger RNA from information encoded in cellular DNA. The failure of actinomycin to interrupt interferon synthesis late in infection suggests that the bulk of the messenger RNA is formed within 6 h and, once formed, is relatively stable.

This research was supported by grants from the U.S. Public Health Service and the National Science Foundation. I thank Miss Ruth M. Snyder for her assistance.

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FIRST RECEPTOR IN INTESTINAL SUGAR TRANSPORT: THE EFFECT OF PHLORIDZIN AND OF CETRIMIDE ON THE INTESTINAL ABSORPTION OF α-D-GLUCOSAMINE, L-GLUCOSE AND D-XYLOSE

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THE absorption of many nutrient substances by the intestine can proceed against higher concentration in the blood plasma. The term 'active transport' has been used to describe absorption against a concentration gradient, but some workers[1] use it to include all transport in which the cell plays an active part, even though the substance may not be absorbed against a gradient. To avoid confusion, we shall adopt Danielli's[2] term 'facilitated diffusion' for the latter. Using the criterion of absorption against a gradient for active transport, Wilson and Landau[3] examined a large number of carbohydrates and classified them accordingly. Thus, Crane[4] was able to specify the minimum structural requirements in the carbohydrate molecule for its active transport across the intestinal mucosa.

It has already been established that in the intestinal absorption of glucose two receptors are involved in series[5], receptors which are blocked totally by phloridzin and cetrimide respectively. This fact renders Crane's structural characterization without much effective value, for we now require the interaction of the two receptors with the different carbohydrates to be specified separately. We also know now that the inhibitors of the first and second receptor are not equivalent in effect, for of the two inhibitors, only cetrimide produced leakage of blood glucose into the intestinal lumen in rabbits, guinea-pigs, rats and mice, while phloridzin produced leakage only in mice (Nissim, unpublished observations). Substances closely related to D-glucose, such as D-glucosamine and L-glucose, or the pentose D-xylose, which were shown not to be transported against a gradient[3], have been assumed to be absorbed by simple diffusion. This assumption no longer seems justified for an absorption mechanism which involves two receptors, since failure of transport may occur at either one of the two receptors only. Furthermore, the original experiments of Wilson and Landau were carried out entirely on an *in vitro* intestinal sac preparation from hamsters. The sensitivity of the method could also have failed to demonstrate smaller degrees of active transport. Finally, the first receptor may in fact be concerned only with entry, so that investigations involving the criterion of transport against a gradient may not be sufficiently informative. These reasons made it clear that the absorption of carbohydrates, which have been considered to be transported by simple diffusion, must be investigated afresh with the foregoing points in mind.

The effects of phloridzin and cetrimide on the intestinal absorption of α-D-glucosamine hydrochloride, L-glucose and D-xylose were examined in pure strain male *C3H* mice and male albino rats. The effect of combinations of different concentrations of phloridzin and cetrimide was also examined in *C3H* mice. 0·2 per cent (w/v) of the carbohydrate—the glucosamine calculated as base—dissolved in 0·9 per cent sodium chloride was circulated through the small intestine of the anæsthetized animals by the intraluminal perfusion method[5]. In preliminary experiments with *C3H* mice, 25 ml. of fluid, containing 50 mg glucosamine, was circulated for 0·5 h. In all subsequent experiments, 10 ml. of fluid, containing 20 mg carbohydrate, was perfused in mice, and 20 ml. containing 40 mg was perfused in rats, for 1 h, to give higher percentage absorption. The sugars were estimated by a modification of the method of Haslewood and Strookman[6].

α-D-*Glucosamine*. Half-hour experiments. The same procedure was followed as previously for the absorption of D-glucose (34·6 ± 0·9 per cent) in *C3H* mice, so that the absorption of glucosamine could be directly compared with that of D-glucose. The mean percentage absorption of D-glucosamine in three control experiments was 2·31 ± 0·24, while the mean for three experiments with 10^{-3} phloridzin

Table 1. Effect of Drugs on the Absorption of α-D-Glucosamine in the Small Intestine of *C3H* Mice

Drugs	No. of animals	Percentage absorption (mean ± *S.E.*)	*P*
(Control)	10	22·0 ± 1·8	
Cetrimide, 10^{-3}	6	25·4 ± 2·1	< 0·3 > 0·2
Phloridzin, 10^{-4}	8	11·0 ± 1·2	< 0·001
Phloridzin, 10^{-4} + cetrimide, 10^{-3}	3	27·3 ± 2·4	< 0·2 > 0·1
Phloridzin, 10^{-4} + cetrimide, 10^{-4}	3	8·3 ± 0·5	
Phloridzin, 10^{-4} + cetrimide, 5×10^{-5}	12	7·4 ± 0·6	< 0·01 (compared with phloridzin 10^{-4})

was $-0{\cdot}38 \pm 0{\cdot}28$. Although the difference was small, it was highly significant with $P < 10^{-3}$.

One-h experiments. (*a*) *C3H* mice (Table 1). Even at a concentration of 10^{-4}, phloridzin reduced absorption to 50 per cent of the control value, while 10^{-3} cetrimide had no inhibitory effect. An interesting result was obtained with drug combinations, for while low concentrations of cetrimide potentiated the inhibitory action of 10^{-4} phloridzin ($P < 0{\cdot}01$), the higher concentration of 10^{-3} cetrimide completely nullified it.

Table 2. Effect of Drugs on the Absorption of α-D-Glucosamine, L-Glucose and D-Xylose in the Small Intestine of Albino Rats

Sugar	Drug	No. of animals	Percentage absorption (mean ± *S.E.*)	*P*
α-D-Glucosamine	(Control)	4	10·9 ± 1·2	
	Cetrimide, 10^{-3}	6	11·9 ± 0·6	
	Phloridzin, 10^{-3}	6	0·75 ± 0·7	⩽0·001
L-Glucose	(Control)	12	12·6 ± 1·1	
	Cetrimide, 10^{-3}	4	13·3 ± 1·3	
	Phloridzin, 10^{-3}	4	−3·7 ± 2·6	⩽0·001
D-Xylose	(Control)	12	22·0 ± 1·6	
	Cetrimide, 10^{-3}	12	20·3 ± 1·3	
	Phloridzin, 10^{-4}	8	13·2 ± 0·7	< 0·001
	Phloridzin, 10^{-3}	4	−0·5 ± 0·7	

(*b*) Albino rats (Table 2). 10^{-3} phloridzin produced practically total inhibition of D-glucosamine absorption. Cetrimide had no effect.

L-Glucose and D-xylose. Total block of L-glucose absorption was obtained with 10^{-3} phloridzin (Table 2). The negative value is attributed to error in the L-glucose determination. Cetrimide again had no effect. The absorption of D-xylose was practically halved by 10^{-4}, and completely blocked by 10^{-3}, phloridzin, while cetrimide had either a negligible or no effect.

The intestinal absorption of the three sugars, namely, D-glucosamine, L-glucose and D-xylose, amounted to no more than a fraction, 1/10–1/20, of the absorption of D-glucose in mice and rats. Theoretically, this absorption could be effected by simple diffusion, facilitated diffusion or active transport, alone or in combination. A striking result was the inhibition of the absorption of the three sugars by phloridzin. For all three sugars, inhibition was apparently complete at 10^{-3} phloridzin, so that no simple diffusion, apart from that through the portals of entry which are blocked by phloridzin, could occur. The phloridzin receptor must be associated with the portal of entry for these sugars in such a way that phloridzin blocks either the appropriate membrane pores through which simple diffusion of these substances occurs or an unknown process of facilitated diffusion. Facilitated diffusion may involve carrier transport across the membrane, or the walls of the pores themselves offer a pattern of forces which facilitates to some extent the passage of glucose, probably through hydrogen-bonding structures as suggested by Stein and Danielli[7]. It would be virtually impossible at this stage of knowledge to distinguish between simple diffusion through critical-size pores and facilitated diffusion inside the pores, for both mechanisms offer a basis of competition for transfer through the pores, and both are saturable. Facilitated diffusion, involving a mechanism of carrier transport across the membrane, will be later excluded deductively.

The next interesting result regarding the absorption mechanism of the three sugars was the absence of inhibition with cetrimide. This is in marked contrast to the effect of this substance on D-glucose absorption[5]. The only possible interpretation for the difference is that these three sugars do not share the second receptor for D-glucose, which is the receptor clearly involved in the active transport mechanism, as is evident from the D-glucose leakage caused by cetrimide. Our conclusion is in direct contradiction with that arrived at recently by Alvardo[8], who studied D-xylose transport by hamster small intestine *in vitro*, and found that it was inhibited by phloridzin, Na^+ lack, anaerobiosis and 2,4-dinitrophenol (DNP). These results led him to conclude that although this sugar is not absorbed against a gradient, it is nevertheless 'transported through the same pathway as the active sugars'. The 'apparent' lack of absorption against a gradient was attributed to the smaller affinity of this pentose to the receptor. Accepting this interpretation, we should expect cetrimide to be even more effective in displacing D-xylose from the receptor than D-glucose. It is therefore necessary to find other explanations for Alvardo's results. Inhibition by phloridzin need concern only the entry receptor and is energy independent. Alvardo argued that both anaerobiosis and DNP must inhibit a second reaction which is energy-dependent, and since D-glucose had been shown to block the absorption of D-xylose[9], he concluded that they must share the same active transport mechanism. Inhibition of D-xylose transport by anaerobiosis may not be considered established at present, for the results of Alvardo are contradicted by those of Salomon *et al.*[9], who obtained a slightly higher value for anaerobic D-xylose transport. Inhibition by DNP could be due to the inactivation—or merely alteration in the affinity constant for D-xylose—of a second receptor, distinct from that for D-glucose, and resistant to cetrimide. The block of D-xylose transport by D-glucose could occur at the first, phloridzin-sensitive, receptor. Reversal of D-xylose transport by D-glucose after equilibrium was established for the former, reported by Salomon *et al.*[9], may be accounted for by the build-up of an osmotic effect, on the serosal side of the intestinal sac, due to rapid D-glucose absorption; D-xylose would then have to flow back, particularly in the presence of an active mechanism for Na^+ transport as well.

Potentiation of the phloridzin inhibition of D-glucosamine absorption by 10^{-4} cetrimide, and the abolition of this inhibition by the larger concentration of 10^{-3}, was another noteworthy result. This may seem puzzling at first sight, particularly as cetrimide by itself had no activity. The effect of combinations of phloridzin and cetrimide on D-glucose absorption was analysed previously[5]. Synergism was in part primary, due to the blocking of the second receptor by cetrimide. A considerable part of the synergism, however, was auxiliary

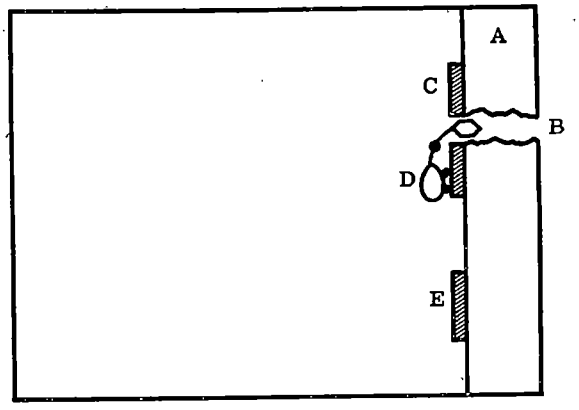


Fig. 1. Diagram representing mechanism of sugar absorption into an intestinal epithelial cell and its block by phloridzin. *A*, cell membrane; *B*, pore for entry of sugars; *C*, protein receptor for phloridzin (displaced by high concentrations of cetrimide); *D*, phloridzin molecule bound to protein, with glucose molecule in pore; *E*, silent receptor for phloridzin (displaced by low concentrations of cetrimide)

in type and caused by the conservation of phloridzin from 'silent' receptors. Evidently, the latter mechanism would still operate with sugars that are not blocked by cetrimide alone, and this would explain the present synergism between phloridzin and low doses of cetrimide on glucosamine absorption. On the other hand, the new finding of the abolition of phloridzin inhibition with higher concentrations of cetrimide must be due to displacement of phloridzin by cetrimide from the first receptor. Although cetrimide combines with the first receptor, it need not block the portal of entry if it has no D-glucose in the molecule. Even when the phloretin moiety of phloridzin is combined with other sugars, for example, galactose, it shows reduced activity[10].

Since cetrimide is an ionic surface-active agent with high affinity for protein, it is very likely that the phloridzin receptor is protein in nature. A previously established fact[5] which is of interest here is that the potentiation of phloridzin by cetrimide occurs after a relatively long interval, seemingly required for penetration of cetrimide into the cells. This would localize the protein phloridzin receptor on the inner aspect of the cell membrane. At sites where this protein receptor is adjacent to the pore, the glucose moiety of the molecule could fit into the pore, possibly with hydrogen bonding, and fail to be dislodged because of the firm phloretin attachment to the protein. A diagram representing the essential features of this model is shown in Fig. 1. It is now clear, from the new specifications of the phloridzin receptor, that it cannot be a mobile carrier ferrying sugar molecules across the cell membrane.

We thank the Medical Research Council for a personal grant to one of us (S.L.H.) and a grant for expenses.

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ISOLATION OF A MUCOPEPTIDE FROM THE SURFACE OF EHRLICH ASCITES TUMOUR CELLS

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DURING recent years considerable interest has been taken in the surface membranes of tumour cells. A relative increase in the electrophoretic mobility between tumour cells and their normal counterparts has been observed in a number of cases[1,2]. It has also been shown that a high cellular mobility can be correlated with rapid invasiveness of tumour cells[3,4]. Forrester *et al.*[5] and Ruhenstroth-Bauer *et al.*[6] have shown that the electrical mobility of various tumour cells is reduced after incubation with neuraminidase, showing the important contribution of sialic acid to the cell surface charge. Furthermore, Forrester *et al.*[5], when studying the increased mobility of hamster kidney fibroblasts transformed by polyoma virus relative to the normal cell, found that the mobilities of the two cell lines were identical after incubation with neuraminidase. In the light of such work an investigation was begun to study the mode of linkage of sialic acid in tumour cell surface membranes. The Ehrlich ascites carcinoma was chosen as a suitable system on which to begin the work, as it was readily obtainable in quantities large enough for the work envisaged.

Tumour cells were collected into saline or EDTA saline from *C*-mice, by peritoneal puncture, eight to nine days after transplantation. After four washes in saline the cells were incubated at 37° C with enzyme solutions for periods of up to three hours. A 1 per cent suspension of B.D.H. trypsin in Krebs-Ringer phosphate at *p*H 7·2 was routinely used. A preparation of crystalline ficin, activated as described by Lisowska[7], has also been tried. The viability of the cells was tested after incubation, with lissamine green, when not more than a small percentage were found to stain. The supernatant from the incubation mixture after centrifugation of the cells was deproteinized by the addition of trichloroacetic acid solution. The precipitate was removed and the clear liquid then dialysed against running tap-water overnight. The retentate was lyophilized. This crude preparation was a white powder having an absorption maximum at 260 mμ in aqueous solution. On analysis, after weak acid hydrolysis, by the thiobarbituric acid method of Warren[8] it was found to contain bound sialic acid. The quantities of sialic acid released by trypsin and ficin from the cells are recorded in Table 1. These values are also expressed as a percentage of the amount of sialic acid released by neuraminidase.

To find out if other sugars were present in the sialomucopeptide, paper chromatography in two different solvent systems was attempted after acid hydrolysis. This revealed the presence of galactosamine and the possible presence of its *N*-acetyl derivative in small amounts.

An attempt was made to purify the mucopeptide, using the technique of gel-filtration. This was performed with a column of 'Sephadex *G*-50'. The peak shown in Fig. 1 is the result of plotting the values of the optical density at 260 mμ of the eluate against the elution volume. The concentrations of both sialic acid and hexosamine were also determined on lyophilized fractions of the

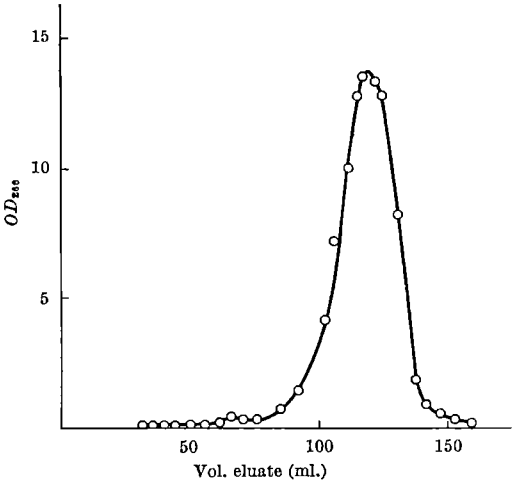


Fig. 1. Gel-filtration of the sialomucopeptide obtained by trypsin treatment of Ehrlich ascites tumour cells. The ultra-violet absorption at 260 mμ has been plotted against elution volume

eluate after hydrolysis with either 0·1 N sulphuric acid for 1 h at 80° C (for sialic acid) or 1 N hydrochloric acid in sealed 'Pyrex' tubes for 8 h at 105° C (for hexosamine). These results are shown in Fig. 2.

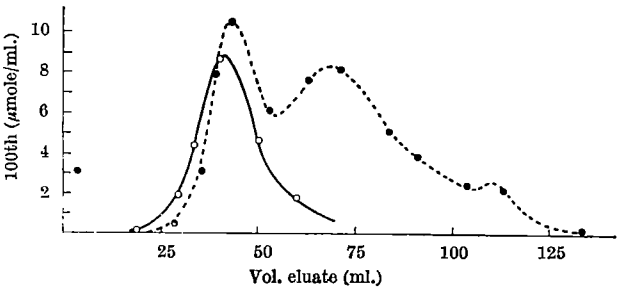


Fig. 2. Gel-filtration of the sialomucopeptide obtained by trypsin treatment of Ehrlich ascites tumour cells. The concentration of sialic acid, measured by the thiobarbituric acid assay[6] after acid hydrolysis, and the relative concentration of hexosamine in different fractions of the eluate, determined by Rondle and Morgan's modification[12] of the earlier described method of Elson and Morgan[13] and represented here by the optical density of the chromophore at 530 mμ, have been plotted against the elution volume. ●, $O.D._{530}$, Elson–Morgan reaction; ○, concentrated sialic acid

Gel filtration with 'Sephadex *G*-50' has shown the crude peptide to contain two major components, only one of which contains sialic acid. This latter component has a high molecular weight relative to the other. It is eluted in a volume approximately equal to the void volume of the column and consequently its molecular weight must be equal to or greater than the value of the exclusion limit of the gel. This value is approximately 8–10,000 for 'Sephadex *G*-50'. Another important result revealed by this technique is the coincidence of the hexosamine and sialic acid peaks in the eluate. Coupled with the evidence from paper chromatography that galactosamine is the only hexosamine present in the acid hydrolysate of the mucopeptide, and that this has probably arisen from its *N*-acetyl derivative, this indicates that both sialic acid and *N*-acetylgalactosamine are possibly linked together in the molecule. Evidence from enzymatic cleavage of sialic acid and weak acid hydrolysis of this somewhat purified material suggests that sialic acid may be terminally linked in the sugar side-chain of the molecule.

Cook[9], attempting to elucidate the mode of linkage of sialic acid in the erythrocyte membrane, prepared a sialomucopeptide by treating red blood cells with trypsin. His material contained sialic acid, *N*-acetyl galactosamine and eight amino-acids, two of which were present in trace quantities. He concluded that the saccharide chain was identical to that found in the submaxillary gland mucoproteins, namely the 6-α-D-sialyl-*N*-acetylgalactosaminoyl unit, and that it was joined through the γ-carboxyl group of glutamic acid to the peptide chain. Although the molecular weight of the sialomucopeptide prepared from the Ehrlich ascites tumour cells is very much larger than that of the erythrocyte preparation, it is interesting to note that the six major amino-acid components present in the latter are to be found in relatively large amounts in the former. The results of the amino-acid analysis of the fractions eluted from the 'Sephadex' column containing sialic acid are recorded in Table 2. These values were obtained using an EEL automatic amino-acid analyser, after the samples had been hydrolysed for 24 h with 6 N hydrochloric acid under nitrogen in sealed tubes.

The high proline, glutamic acid and glycine content of the peptide, together with the absence or very low content of aromatic side-chains, gives an amino-acid composition somewhat similar to collagen. Such an amino-acid composition will favour the stabilization of the peptide chain in an extended form[10] possibly in the collagen configuration[11]. Such a structure could lie compactly on the cell surface, which would not be possible with an α-helix. Experiments based on neuraminidase

Table 1. Released Sialic Acid

Enzyme	μ moles/ml. cells*	% Neuraminidase sensitive
Neuraminidase	$2{\cdot}60 \times 10^{-1}$	100·0
Trypsin	$2{\cdot}20 \times 10^{-1}$	84·5
Ficin	$2{\cdot}04 \times 10^{-1}$	78·5

* As determined in micro-hæmatocrit centrifuge.

Table 2. Amino-acid Composition of the Sialomucopeptide (g/100 g. Peptide)

Amino-acid	Sample 1	Sample 2
Aspartic acid	17·8	15·8
Glutamic acid	21·1	23·5
Glycine	5·7	5·6
Alanine	5·2	5·0
Valine	5·4	5·0
Leucine	5·6	5·1
Serine	5·0	5·0
Threonine	5·6	5·7
Phenylalanine	2·9	3·5
Tyrosine	1·1	1·2
Proline	9·5	8·2
Isoleucine	3·9	3·1
Methionine	1·1	1·0
Cystine	1·9	2·7
Lysine	5·4	4·2
Histidine	1·6	1·5
Arginine	2·3	1·9

treatment of intact viable cells, and of completely disrupted cells (produced by ultrasonic degradation), showed that there was very little detectable difference between the amount of sialic acid released from intact cells and the total neuraminidase labile sialic acid of the disrupted cell. This evidence confirms the suggestion that the sialomucopeptide has originated from the cell surface. The quantitative assay for sialic acid in the purified mucopeptide gives a value of approximately 2 per cent, on a weight basis. It may be possible to differentiate further components, thus obtaining a fraction richer in sialic acid, by a technique dependent on the electrical properties of the material, such as starch-gel electrophoresis. In such a technique the sialo substance, which is endowed with a high negative charge due to the free carboxyl groups of the sialic acid residues, would be easily separable from any basic, neutral, or weakly acid substances present.

One of the disadvantages of the tumour system used is the absence of a normal cell to use as a comparison. It is hoped, therefore, to be able to perform similar investigations to those described here on normal hamster kidney fibroblasts, grown in tissue culture and on such cells transformed *in vitro* by polyoma virus. Using such a system it may be possible to detect differences in the amino-acid and sugar composition of substances comprising the cell surface membranes of normal and tumour cells.

We thank Prof. A. Haddow for his advice and Mrs. D. Purkiss for her assistance. This work has been supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research: Royal Cancer Hospital) from the Medical Research Council and the British Empire Cancer Campaign for Research, and by Public Health Service research grant *CA*–03188–08 from the National Cancer Institute, U.S. Public Health Service.

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VARIATIONS IN THE RATE OF INDUCTION OF CHEMICAL CARCINOGENESIS ACCORDING TO DIFFERING PSYCHOLOGICAL STATES IN RATS

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ENVIRONMENT modifies endocrine responses; goalkeepers secrete adrenaline while the forwards secrete noradrenaline[1], and socially-induced stress responses in mice cause a significant enlargement of the adrenal glands and temporary eosinophilia[2].

If chemical carcinogenesis is the result of an antibody induced by a carcinogen–protein complex deleting marker sites on cell surfaces as Green[3] has suggested, different forms of stress, activating different aspects of the immune mechanism, may vary the rate of chemical induction of cancer.

Four groups of 12 Sheffield strain Wistar rats, six males and six females in each group, received 0·05 g 20-methylcholanthrene (subcutaneously) to the anterior abdominal wall.

Group 1 (*contented*). Daily stroking from the nose to tail on the lap for 5 min. Two females were in a cage with the male rats, and a sterilized male with the females.

Group 2 (*stressed*). Daily injection of adrenaline (0·05 ml. 1 : 1000 subcutaneously). This dosage was stressful and caused epilation at the site of the injection.

Group 3 (*alerted*). Kept permanently in tension cages. A 60-V light fixed to the top of the cage lights up for 5 min in every hour, and is followed by an electric shock of 32 μfarads delivered to the floor of the cage. For the first few days the light and the shock were synchronized to condition the animals to associate the light with the shock. The timer was then adjusted so that the light preceded the shock by 5 min, to alert the animals for 5 min in the hour.

Group 4 (*controls*). These animals received no further treatment other than the implant of 20-methylcholanthrene.

Towards the end of the second week most of the animals had nodules of 0·5 cm to 1 or 2 cm diameter. These nodules either resolved or progressed to ulcer formation, which were larger in the females and the two adrenaline-treated females sloughed the carcinogen. In this context it might be noted that Kitary[4] found that plasma corticosterone reached higher and more persistent levels in female than in male rats under ether stress, and the adrenals of female rats were found to be more sensitive to ACTH than those of male rats.

At 40 weeks, 4 out of 12 rats in the tension cage were without tumours whereas only the 2 adrenaline-treated females out of 36 other rats were without tumours. The result appears to be significant, the *S.E.* being 2·5, giving a chance probability of about 1 in 300.

Previous work has shown that either irradiation of the draining lymph nodes, or treating them with thorotrast, delays chemical carcinogenesis[5], apparently by temporary suppression of the local immune response.

Evidence has also been presented that there is a predominantly larger flare in the side of guinea-pigs which have received subcutaneous injections of autologous (chemically induced) fibrosarcoma cell suspension and autologous spleen cells (which had been sensitized by a piece of the autologous tumour in a 'Millipore' chamber placed intraperitoneally), than there was in the other (control) side of the guinea pig which received the same autologous tumour-sensitized spleen suspension and a suspension of autologous liver cells[6]. More recent work has also shown that the spleen of a tumour-bearing rat does not need to be sensitized by the charged 'Millipore' chamber. Eight out of nine guinea-pigs showed a larger flare in the side which had received autologous spleen and tumour cell suspension, than the control side which had received autologous spleen cell suspension and autologous liver. This is further evidence of an immune response playing a part in both tumour induction and growth.

Table 1

Group	Treatment	No. developing ulcers second week after carc. inj.		No. without tumour at 40 weeks	
		F	*M*	*F*	*M*
(1)	Stroked	2/6 small	2/6 small	0/6	0/6
(2)	Adrenaline	5/5 very large	2/6 small	2/5	0/6
(3)	Tension	6/6	0/6	3/6	1/6
(4)	Control	4/6	2/6	0/6	0/6

Everett[7] showed that some strains of rat go into pseudopregnancy on isolation. Guy[8] obtained heightened contact sensitivity in guinea-pigs after they had been kept in a tension box for a few days. Winoker[9], too, showed that stress (electric shock) reduced epilation in mice painted with methylcholanthrene, and Rashkis[10] also found that stress (swimming) delayed both ascites and chemically induced tumours. In this experiment apprehension gave some protection against carcinogenesis.

A clear distinction must be made between stroking, which is a soothing process, and handling, which is alarming. Weininger[11] found that gentling rats (by handling) caused them to withstand acute stress better than normal animals. Selye[12] has shown that cardiovascular damage is the expression of the liberation of ACTH under stress, so it is possible that a mild degree of chronic stress (gentling) may raise the target's threshold to ACTH. The reverse probably happens in severe shock[13].

It has been found, too, that guinea-pigs sensitized to an allergen have a lowered threshold after electric shock or loss of sleep[14]. Bloch and Nordin[15] showed that delayed hypersensitivity in a guinea-pig to the intradermal injection of bacteria is transferable by monocytes to normal guinea-pigs. If the bacteria were injected intradermally to unsensitized guinea-pigs, they responded with an immediate Arthus-type reaction, and a circulating antibody, instead of a cell-bound one as in the delayed hypersensitivity experiments. Nilzén[16] demonstrated a rise in skin reactivity in guinea-pigs following adrenalectomy, which he says is due to the importance of the adrenal cortex in topical defence mechanism.

Darrack[17] and Murphy[18] found that adrenal steroids suppressed antibody activity, while adrenalectomy caused an increase in lymphoid tissue and circulating lymphocytes. Cortisone, however, while preventing the lymphophoresis and number of circulating lymphocytes from rising after adrenalectomy, did not affect the antibody production or Arthus and anaphylactic reactions[19]. Nicol[20] has shown that diethylstilbœstrol greatly stimulates the phagocytic ability of the reticulo-endothelial system, and that stilbœstrol *per os* cuts down the effect of stress on the plasma corticoids.

After mental stress the capillary resistance has been found to be higher in the arms of students painted with corticosteroids than in the control arms[21]. Ischæmic shock, injections of ACTH or cortisone, have been found to inhibit mitosis of normal cells but not of cancer cells[22]. This suggests that malignant cells may lack endocrine-binding sites.

If antibody attack is the essential mechanism in the chemical induction of cancer, modifying the level of circulating corticosteroids would theoretically delay the onset of neoplasia. It has been shown[23] that several forms of stimulation caused rats to have higher levels of plasma

corticosteroids than undisturbed animals Breen[24] has suggested that in an immunological sense tolerance is more important than immunity. It is believed that environmental factors play a part in controlling the degree of tolerance or immunity that is achieved, and modifying the environment, and hence the psychological state, can vary the rate of induction of chemical carcinogenesis in rats.

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ANTIBODIES TO VIRUSES OF THE COLUMBIA *SK* GROUP IN SCIURIDAE IN BRITAIN

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THE antigenically related group of Columbia *SK* viruses appears to be universally distributed. Their host range is so wide as to make this group remarkable among RNA viruses. To the species already listed as being susceptible by Jungeblut[1] we may add an isolation from a laboratory colony of Orkney voles (*Microtus orcadensis*), and also transmission of the virus to the short-tailed vole (*Microtus agrestis*) and to the bank vole (*Clethrionomys*) (Vizoso and Sanders, unpublished results). Murnane *et al.*[2] isolated a virus of the Columbia *SK* group from a pig, and Craighead *et al.*[3] also showed that pigs were susceptible to infection by encephalomyocarditis (EMC). Recently Vizoso *et al.*[4] isolated a virus of this group, resembling EMC, from a British squirrel (*Sciurus vulgaris leucourus*); this apparently healthy animal was devoid of any measurable antibody when tested against EMC virus, and the virus will be referred to as *RS*3. It lacks one of the main characteristics of the group, namely, the ability to agglutinate sheep red cells. A new isolation has been made from a grey squirrel (*Sciurus carolinensis*) trapped near the laboratory, and this animal also did not have antibody against EMC when tested by the hæmagglutination inhibition (HAI) test. The relative ease of isolation, considering the small number of animals in which it was attempted (one red squirrel and five grey squirrels), suggests infection is common. Grey squirrels are susceptible to EMC and *RS*3 virus by intranasal inoculation and develop limb paralysis or die. Sub-lethal doses presumably induce antibody response; it was therefore decided to investigate the antibody level and frequency as determined by HAI tests on serum from animals obtained from several localities. The investigation recorded here was carried out mainly on grey squirrels from Surrey, Hampshire and Buckinghamshire; a few red squirrels from Suffolk were also used.

Antibody assays were made by the HAI test using sheep red cells with serum samples taken from grey and red squirrels which had been either trapped or shot.

Since the squirrel virus (*RS*3) does not agglutinate sheep red blood cells the HAI test was applied, using a strain of EMC virus originally isolated from a chimpanzee by Helwig and Schmidt[5] and adapted to Krebs mouse ascites tumour cells by Hoskins and Sanders[6].

A second virus, provisionally identified as also being of the Columbia *SK* group (*GS*8), was also isolated from the fæces of an apparently healthy grey squirrel (*Sciurus carolinensis*) trapped in the vicinity of this laboratory.

This animal, like that of the previous isolation, showed no detectable antibody against EMC. The isolation of the virus, the characterization, ether resistance, demonstration of infectious RNA, and final identification by neutralization test against a standard EMC antiserum in suckling mice, were made as described for *RS*3. The titre reached by this virus in suckling mice was $\geqslant 10^{8.5}$, on which the EMC antiserum neutralized $\geqslant 10^{5.0}$ LD_{50}.

Unlike the previous squirrel virus *RS*3, this virus *GS*8 has the ability to agglutinate sheep red cells. The agglutinin, nevertheless, appears to be less stable than that of the standard strain of EMC, and decreases rapidly on freezing and thawing. With this virus the HAI tests were made with samples frozen once only.

The strain *GS*8 grew rapidly in Krebs ascites cells and the virus used in this test was grown in a cell suspension of these cells, ampouled, and kept frozen at $-70°$ C.

Washed sheep red cells obtained commercially from Burroughs Wellcome were used throughout at a concentration of 0·1 per cent.

Diluent for the test was PBS[7] plus an equal volume of 4·5 per cent glucose in distilled water and 0·0025 per cent gelatine. The test was incubated at 4° C overnight before reading.

All serum samples had been inactivated in a water-bath at 56° C for 30 min. 1/20,000 sodium azide was added as preservative, and the samples were stored at 4° C until required. Two-fold dilutions of serum were challenged with 8 units of virus hæmagglutinin. The significant level of antibody was arbitrarily fixed at $\log_{10} 1{\cdot}8$.

Blood samples from a total of 61 grey squirrels and 8 red squirrels were tested. None of the red squirrel samples showed any detectable antibody against the standard EMC virus. Fourteen grey squirrel samples were positive (EMC antibody-level $\geqslant \log_{10} 1{\cdot}8$). Two squirrels released in the proximity of the laboratory, which showed no traces of antibody against EMC, were recaptured after a period of five months; one still had no detectable antibody, while the other showed a level of $\log_{10} 2{\cdot}8$.

It was noticed that the incidence of EMC antibody appeared to be higher among grey squirrel samples obtained in the vicinity of the laboratory. It was therefore decided to group the samples according to the type of locality from which they had been obtained. This gave an urban group comprising animals captured in back gardens or parkland with close proximity to human habitation, and a rural group well isolated from towns or villages. The urban group with 30 grey squirrels contained 14 with significant levels of antibody against EMC. The rural group of 31 squirrels revealed no antibody. One animal in the rural area (Bucks.) showed an HAI anti-

body-level of log 1·5, which is below the significant level. Forty-nine samples of the grey squirrel sera still available were then tested using the newly isolated strain *GS*8. Of these samples (24 urban and 25 rural), 12 were found to have a significant level of antibody.

It was found that although some of the samples found positive when tested with EMC virus were also positive when tested with *GS*8, there were also some samples which were positive only to EMC or to *GS*8.

Again, as in the case of EMC, the positive samples were found in sera from animals obtained from urban areas. Only one sample in the rural areas had an antibody index of 1·5 for *GS*8, and this sample was totally negative when challenged by HAI test with EMC virus. The recaptured squirrel with positive antibody for EMC also had antibody for *GS*8. Unfortunately, there was not enough serum left from the first sample to test against *GS*8.

The numbers of squirrels showing antibody against one or two of the viruses are shown in Table 1.

Table 1. ANTIBODIES IN GREY SQUIRRELS AGAINST EMC AND *GS*8 VIRUSES

Locality	No.	*GS*8 only	EMC only	EMC and *GS*8	Total with antibody
Urban	30	4/49	6	8	18
Rural	31	0	0	0	0
Total	61	4/49	6	8	18

The results described show a fairly high frequency of antibodies to viruses of the Columbia *SK* group in grey squirrel populations, especially in urban populations when two different viruses of this group, EMC and *GS*8, are considered together. High incidence of antibody to the Columbia *SK* group was found by Barski and Lamy[8] (from ref. 1) in pygmy populations in Africa, and Warren found antibodies in 43 per cent of the wild rats on the farm where the original isolation of EMC was made from a chimpanzee. However, the investigations of Warren *et al.*[9] showed that the percentage of antibodies in wild rats can vary in different localities from nil to as much as 87 per cent. In the work recorded here, and in spite of the small sample, there appears to be a correlation between the antibody-level of the squirrel population and the proximity of human habitation. No similar correlation can be made in the case of the British red squirrel. The only samples are from a rural area, and none of the eight animals examined showed antibody to EMC. Since from this area an isolation has been made (*RS*3) of another virus of this group, differing from EMC and *GS*8 in the lack of hæmagglutinin, it is possible that some antibody could be found by the use of another test. It is tempting to correlate the higher level of antibody incidence among urban grey squirrels with the presence of rats near human habitations, since rats excrete large amounts of virus in their urine and fæces. The high incidence in the rat could, in turn, possibly be linked to the proximity of man, domestic animals, or livestock. Antibodies have been found in cattle, swine and dogs[1,2]. Insects do not appear to play an important part in the transmission of the viruses of this group, and the method of transmission still remains obscure. Fifteen adult albino mice inoculated orally with 10^5 mouse LD_{50} virus failed to show any outward signs of infection, and when killed 15 days after inoculation none showed any measurable antibody against EMC. Since at certain times of the year squirrels spend a considerable time searching for buried food, presumably by scent, and also sniff at urine traces left by other squirrels, nasal absorption would seem to be a likely route of infection. Grey squirrels inoculated intranasally with at least 10^5 mouse LD_{50} of *RS*3 virus succumb to the infection.

It is also of interest that two viruses of the Columbia *SK* group could be separated by the HAI test, but not by plating in Krebs cells, or by neutralization test in mice (to be published). This is, to our knowledge, the first time that this has been reported, and does not apply to other strains of the same group of virus. The HAI test has by now been shown to be a reliable and convenient method for the detection of antibodies of the viruses of the Columbia *SK* group; however, in a situation with two viruses, as described in this work, it may give an incomplete picture or conflicting results between neutralization tests and HAI tests, such as have been described by Craighead *et al.*[10]. A situation such as that described may explain their findings. A strain like *RS*3 which does not agglutinate need not induce the formation of HAI antibody, so its results would not be detected by HAI tests using other hæmagglutinating viruses of this group as antigens.

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POTENTIATION OF THE NERVE-DEPRESSANT EFFECT OF LOCAL ANÆSTHETICS BY CARBON DIOXIDE

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AN analysis of the influence of carbon dioxide on the pharmacological activity of local anæsthetics on frog and rat peripheral nerves disclosed that the gas potentiates the depressant action of the drugs[1]. These investigations have been expanded and the relationship of carbon dioxide to the drug action further characterized.

Experiments were performed on rat sciatic-tibial trunks which were isolated from the animals and subjected to a pharmacological analysis of their excitability and conduction properties in a nerve chamber in which the gaseous and fluid environments of the nerves could be selectively modified.

The drugs examined were three prototype tertiary amine local anæsthetics : procaine, lidocaine and cocaine ; the concentrations of carbon dioxide used ranged from 0 to 20 per cent. All extracellular electrolyte solutions (Tyrode's solution) were continuously equilibrated with the appropriate concentration of carbon dioxide. This was accomplished by vigorously bubbling them with the gas mixture before they were applied to the nerves and by keeping them saturated during the experiment by maintaining a constant ambient carbon dioxide concentration. Since carbon dioxide affects *p*H, sodium bicarbonate was added to the carbon dioxide containing solutions to hold the *p*H at about 7·4 ; carbon dioxide-free Tyrode's solutions contained a 2 mM sodium phosphate buffer instead of the bicarbonate and had a *p*H of 7·2.

Briefly, the experimental procedure entailed the equilibration of the trunks for 30 min (at 35° ± 2° C) in one of the Tyrode's solutions containing a specified

carbon dioxide concentration. Then the drug, dissolved in the same tyrode solution, was applied to a 15-mm segment of trunk interposed between a pair of stimulating and a pair of recording electrodes and the spike potential of the $A\alpha$ fibres evoked by supra-maximal electrical stimulation was continuously monitored. The size of the spike recorded just outside the treated segment of nerve was used to determine the ability of the nerves to conduct impulses. Most of the experiments evaluated the effects of 0 and 5 per cent carbon dioxide, but intermediate and larger concentrations were also examined. The balance of the gas mixture was oxygen.

The results illustrated in Figs. 1, 2 and 3 show that a single concentration of any of the three drugs causes two distinct quantitative levels of spike depression depending on the concentration of exogenous carbon dioxide in contact with the nerves. When the Tyrode's solution and chamber atmosphere are free of exogenous carbon dioxide, the drugs produce only a minimum depression of the $A\alpha$ spike height; procaine, 2 mM in 4 trunks, causes only a 5 per cent loss of spike height; cocaine, 1 mM in 4 trunks, a 5 per cent loss; and lidocaine, 1 mM in 4 trunks, produces no depression at the end of a 30-min period. The spontaneous recovery of some nerves from the action of the drugs in carbon dioxide-free Tyrode's solution is a real phenomenon the significance of which remains to be evaluated.

In contrast to their minor effects in the absence of exogenous carbon dioxide, the drugs produce a significantly greater conduction block when a physiological concentration of carbon dioxide (5 per cent) is introduced into the extracellular medium. Then, they produce a depression of conduction in practically every fibre. The restoration of function to the nerves following either of the two experimental procedures was never impaired.

The lowest concentrations of carbon dioxide that unequivocally enhance the action of the anæsthetics are about 2·5–3 per cent. As the carbon dioxide concentration is increased to 5, 10 and 20 per cent, the depressant actions of procaine, which was studied more extensively than the other drugs, progressively increased. A quantitative estimate of the degree of enhancement of procaine by 5 per cent carbon dioxide is provided by the fact that nerves that are maintained in carbon dioxide-free Tyrode's solution require procaine concentrations in excess of 15 mM in order to be blocked to a level equivalent to that obtained by 2 mM procaine in 5 per cent carbon dioxide. This means that the absence of carbon dioxide reduces the procaine action by about seven-fold. Conversely, the procaine concentration in normal Tyrode's solution would have to be reduced to about 0·2 mM in order to match the minimum block produced by 2 mM procaine in carbon dioxide-free Tyrode. This indicates about a ten-fold increase in procaine action proffered by 5 per cent carbon dioxide. The evidence presented supports the conclusion that the relationship of carbon dioxide to the local anæsthetic drugs is one of pharmacological potentiation in which the gas enhances the action of, at least, procaine by a factor of 7–10.

The known depressant action of carbon dioxide on nerve may explain, in part, but not completely, the observed phenomenon. Excitability threshold of the nerves used in Figs. 1, 2 and 3 was consistently elevated by 5 per cent carbon dioxide, with an average increase of 23 per cent (range 4–40 per cent). If the potentiation of the drugs were caused solely by an additive effect on threshold, then the higher concentrations of carbon dioxide (10 and 20 per cent) should result in an increase in threshold beyond that effected by 5 per cent carbon dioxide, because these higher concentrations of carbon dioxide did enhance the action of procaine to a greater extent than did 5 per cent carbon dioxide. But the results from strength-duration investigations on trunks kept in environments of 10 and 20 per cent carbon dioxide

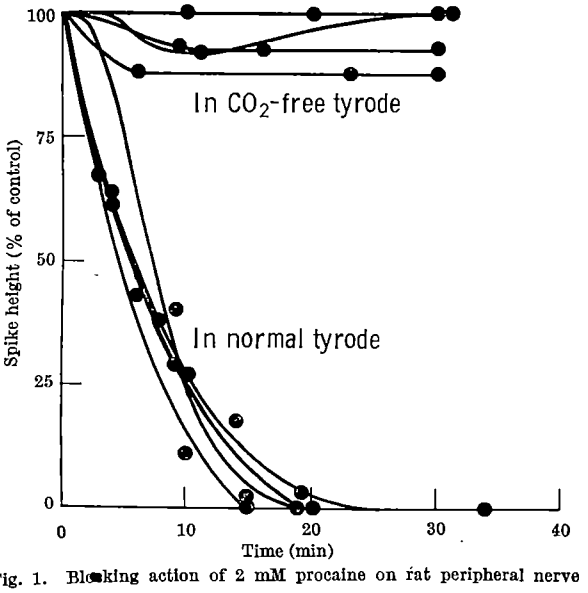


Fig. 1. Blocking action of 2 mM procaine on rat peripheral nerve

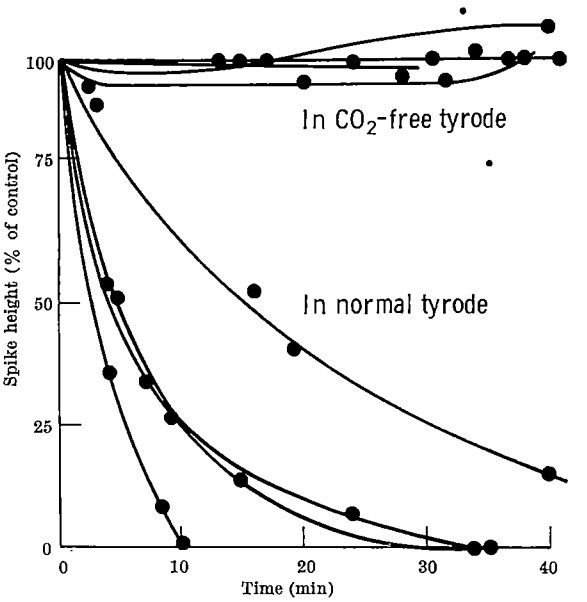


Fig. 2. Blocking action of 1 mM lidocaine on rat peripheral nerve

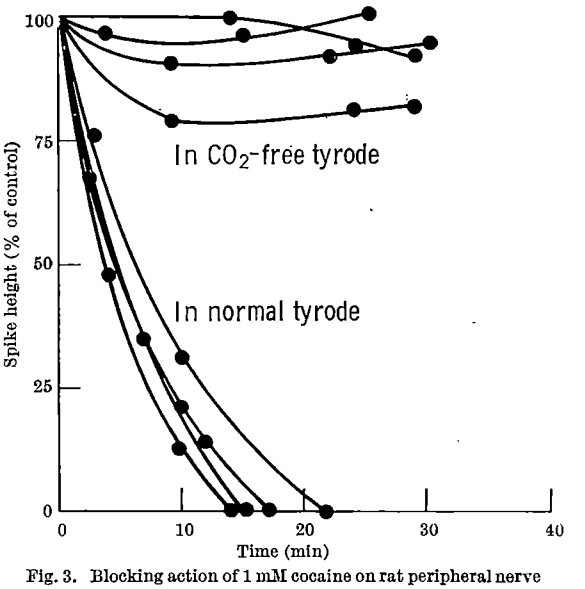


Fig. 3. Blocking action of 1 mM cocaine on rat peripheral nerve

failed to show any greater depression of excitability by the higher carbon dioxide concentrations than that already produced by the 5 per cent concentration. It seems, therefore, that some other action of carbon dioxide is involved. A change of extra-cellular *p*H by the carbon dioxide is also not the cause, because the *p*H was controlled and because, if the *p*H were lowered by carbon dioxide through carbonic acid formation, the activity of the drugs would be expected to be reduced rather than enhanced[2]. (The small difference in *p*H between carbon dioxide-free Tyrode (7·2) and 5 per cent carbon dioxide Tyrode (7·4) is not enough to account for the potentiation.) Of course, no conclusion can be made regarding the cellular *p*H change during these procedures. Another prominent action of carbon dioxide on nerve has to be considered. Lorente de Nó has shown that carbon dioxide increases the resting membrane potential of frog nerves[3]. Since the drugs used in the present investigation tend to stabilize the membrane, it is conceivable that the joint actions of the drugs and carbon dioxide on the membrane would augment each other. This would, of course, be partly reflected in an increase in excitability threshold. But this line of reasoning leads to a paradoxical situation, for, if the potentiation of the drugs by carbon dioxide were a result of an augmented and more stable membrane potential, an applied anodal current, which would raise the membrane potential, should also potentiate the action of the anæsthetics. But this is contrary to published evidence which clearly shows that, in frog nerves, anodal polarization relieves the conduction block by local anæsthetics[4,5]. An investigation of this interesting inconsistency is now in progress.

The finding that carbon dioxide can intensify the action of nerve depressants on isolated peripheral nerves raises the question whether carbon dioxide might also modify the pharmacological effects of other neurotropic drugs on other parts of the nervous system. Such a possibility is already established by several investigators[6], who have shown that carbon dioxide has an antagonistic action to the central nervous system convulsants such as pentylenetetrazol.

This work was supported in whole by U.S. Public Health Service research grant *NB*–01582-07, from the Institute of Neurological Diseases and Blindness.

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A MODEL OF THE INHERITANCE OF HANDEDNESS AND CEREBRAL DOMINANCE

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THERE are many puzzles about left handedness. Left handers are often less consistent in using the left hand than right handers in using the right ; the incidence of left handedness is raised in many pathological groups and yet left handers may be of high intelligence ; several different solutions have been offered to the problem of which cerebral hemisphere leads in speech functions in left handers ; left handers seem to be more likely to recover from aphasias than right handers. It is the purpose of this article to describe a model of the inheritance of handedness and cerebral dominance which, together with a hypothesis about the direction of shifts of dominance, might account for many puzzling facts.

The Model. (1) Handedness (of human beings in the literature referred to) is determined by two alleles, *D*, which manifests right handedness and *R*, which manifests left handedness. (2) *D* is usually dominant and *R* usually recessive, but there is partial penetrance of *R* in heterozygotes. The possibility that left handedness is caused by such an imperfectly recessive gene was suggested by Rife[1] and supported by Merrell[2]. Rife's suggestion that heterozygotes may become right, left or ambidextrous is, I consider, the key to the various problems discussed here.

(3) The dominant cerebral hemisphere, meaning the hemisphere which leads in language functions, is closely linked with handedness so that: (*a*) Dominant homozygotes (*DD*) are consistent right handers with speech more highly developed in the left hemisphere. (*b*) Recessive homozygotes (*RR*) are consistent left handers with speech mainly in the right hemisphere. (*c*) Heterozygotes (*DR*) may use either hand for skilled activities and develop speech in either hemisphere. The majority of heterozygotes will develop right handedness and left hemisphere dominance, but in some heterozygotes the recessive gene will produce tendencies to left handedness. The sinistrality may vary from slight to strong, but careful examination should probably reveal the presence of the dominant gene in some skill with the right hand. In the course of normal development the dominant gene probably increases control so that right handedness and mediation of speech by the left hemisphere are characteristic of heterozygous adults.

However, should there be any physical trauma either to the hemisphere or to the limbs, the heterozygote may develop the use of the alternate hemisphere and limb. It is being suggested that heterozygotes only can shift dominance to compensate for injuries. Homozygotes injured in the hemisphere predisposed to become dominant will be 'ambilevous'—or equally unskilful with either hand—and prone to disorders of speech.

Illustration of the Model. Assuming the proportions of *D* and *R* in a population to be $D=0{\cdot}8$ and $R=0{\cdot}2$, the proportions of the genotypes would be $DD=0{\cdot}64$, $DR=0{\cdot}32$ and $RR=0{\cdot}04$. In this population the lowest possible incidence of left handedness would be 4 per cent. The incidence could rise to 36 per cent if there were full expression of *R* in heterozygotes, but this would be extremely unlikely in a normal population. The degree of expression may be influenced by many factors including age, sex and defects of the central nervous system. Assuming a degree of expression of 25 per cent so that 1/4 of heterozygotes are called left handed, the expected proportions of left-handed offspring of the three possible matings are close to those observed in three studies of familial handedness[3]. These results could be interpreted as consistent with other models of the inheritance of handedness ; the special advantages of the present model can be appreciated through its application to the problems discussed here.

Direction of Shifts of Cerebral Dominance in Heterozygotes. Annett, Lee and Ounsted[4] suggested that children with unilateral electroencephalographic foci and mixed hand preferences tend to locate verbal functions in the hemisphere with the focus so that biologically more primary

skills of orientation in space can be developed in the opposite normal hemisphere. The possibility that, given one intact hemisphere and the alternative of using it for either speech or visuo-spatial functions, speech would be allowed to suffer, is strongly supported by the data of McFie[5] for children who suffered early unilateral brain injuries and afterwards underwent hemispherectomy. The majority of these children showed greater verbal than non-verbal impairments. It is also supported by Naidoo's finding cited in ref. 6 that apparently healthy children of mixed handedness were significantly inferior to matched consistent handers, both right and left, on tests of verbal intelligence. There are strong grounds for believing therefore that in some circumstances heterozygotes may shift cerebral functions at the expense of speech.

The identification of the factors which lead some heterozygotes to shift speech into the damaged hemisphere while some clearly do not awaits investigation. Annett *et al.*[4] suggested that only injuries sustained in early childhood would result in such shifts. There is a series of adults, however, with epileptogenic unilateral anomalies in whom paroxysmal dysphasia is associated with lesions of either hemisphere[7]. These patients, and those of Annett *et al.* and McFie, suffered chronic disruptions of cortical function. It is possible therefore that speech functions are transferred to the damaged hemisphere when the maintenance as well as the development of visuo-spatial skills is threatened by a persistent cortical malfunction. Acute injuries to otherwise healthy adult brains show no excess of left handers with aphasia[8].

Applications of the Model. (1) *Left handedness in normal populations.* There are several differences of opinion as to the nature of left handedness and the status of ambidexters[9,10]. The present model has three contributions to make : (*a*) It accounts for the finding that many people who regard themselves as left handed are in fact less consistent users of the left hand than the majority of right handers of the right[11]. But some left handers use the left consistently. The existence of two sorts of left hander is an essential feature of the present model, inconsistent ones being heterozygotes and consistent ones recessive homozygotes. (*b*) The well-documented decline in the incidence of left handedness with increasing age[12] is accounted for by supposing that the dominant gene increases its control during growth. (*c*) The cultural and environmental pressures usually said to induce left handers to use the right hand can be envisaged as operating in the context of a hereditary predisposition to develop the use of either hand. Without the concept of partial penetrance of the recessive gene in heterozygotes, Trankell[3] is forced to suppose that recessive homozygotes may develop right handedness in the absence of any genetic predisposition to the use of the right hand. If homozygotes can be induced to shift through cultural pressures, explanation must then be sought for the existence of a small group of intractable sinistrals which seems to have persisted, as Brain reminds us, at least since the army of the Children of Benjamin assembled (*Judges*, 20).

(2) *Left handedness in abnormal populations.* Attempts to explain the raised incidence of left handedness among the defective, the epileptic and those with langauge disorders are attended by many difficulties, not the least of these being the fact that the majority of all such cases are consistently right handed.

The present model supposes that consistent handers will suffer defects of development according to the site and severity of cerebral damage. Heterozygotes, however, have some ability to compensate for such damage by shifting dominance. The raised incidence of left handedness in these pathological groups is the result of such attempts to compensate. If it is the case, as hypothesized, that language functions tend to be shifted to the damaged side, there is no need to look for further explanation of the raised incidence of mixed and left handedness among the speech disordered or of the typically lower verbal than non-verbal intelligence quotients of the intellectually subnormal.

(3) *Probability of aphasia in left handed adults.* The facts and theories advanced to explain them are carefully analysed by Zangwill[6,13]. The present model suggests that the major differences of opinion are probably due to differences in the cases considered.

Hughlings Jackson's view that the leading hemisphere is usually contralateral to the preferred hand is probably true of consistent handers both right and left. But many people who regard themselves as left handed are in fact inconsistent. Zangwill[13] found only 15 cases in the literature of aphasia in sinistrals who are recorded as writing with the left hand. If the majority of left handers cited in the literature are in fact heterozygotes in whom a dominant gene has directed speech into the left hemisphere, it is not surprising that many have concluded that handedness and cerebral dominance are unrelated ; that the left hemisphere leads irrespective of handedness. But there are undoubted cases of aphasia resulting from lesions of the right hemisphere and in such cases it is almost always possible to find sinistral tendencies in the patient or his kin.

Regarding the probabilities that the left or the right hemisphere leads in sinistrals, Bingley's[9] analysis suggests that the chances are equal that speech will be placed in either hemisphere. If consistent and inconsistent left handers are differentiated it should be possible to improve this 50/50 prediction.

The arguments for the hypothesis of bilateral representation of speech in left handers seem to rest on cases which may have suffered early injuries or have injuries with chronic effects. The hypothesis that such cases shift speech into the damaged hemisphere is probably sufficient to account for the excess of left-handed aphasics with injuries on either side.

(4) *Probability of recovery from aphasia.* Finally, the present model explains the data on remission of aphasia[13]. It seems clear that the chances of recovery following acute lesions in adulthood are greater for sinistrals than dextrals ; the chances of recovery are also greater for dextrals with left-handed kin than dextrals without such kin. Left handers and right handers with sinistral relatives are more likely to be heterozygotes than dextrals of dextral stock. An important factor in the recovery from aphasia would seem to be the possession of the alternative gene which allows speech functions to be developed in the alternative hemisphere.

Tests of the Model. These will be readily made when there are three sets of information available for groups of patients. First, the presence of unilateral lesions differentiated according to probable age of occurrence and the relative transience or persistence of their effects ; second, the results of psychological tests of verbal and non-verbal abilities ; and third, the laterality of patients and their kin.

I thank Dr. J. A. Fraser Roberts and Dr. C. O. Carter for their advice. They do not necessarily accept the present formulation.

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LETTERS TO THE EDITOR

ASTROPHYSICS

A New Model of Solar Flares

THE extensive observational results and theoretical investigations of solar flares have recently been compiled in refs. 1 and 2. Most models of solar flares have assumed that the only reasonable mechanism for energy storage is that of magnetic field. Parker[3] has recently criticized these theories and shown, in particular, that they do not explain the rapidity (10^2 sec) of the flash phase.

There is a more general argument for rejecting all theories which assume that the stored energy is purely magnetic, being derived from sub-photospheric fields and propagating as the Poynting flux of slowly varying force-free configurations. Such theories require specific changes in the structure of the magnetic field at the photosphere, such as the twisting of flux tubes or the movements of sunspots towards each other. However, the phenomenon of homologous flares[4] indicates that a sequence of flares can occur when the magnetic field at the photosphere (which must provide the 'blueprint' for the flares) is unchanged.

If we may not invoke changes in the photospheric magnetic field to provide the stored energy released in a flare, it is necessary to find some other mechanism for extracting energy from within the photosphere and storing it above the photosphere. de Jager[5] has emphasized that one of the most striking characteristics of flares is the anomalously high density of the gas involved, and this suggests that one consider gravitational potential energy as an alternative or complementary form of storage of energy.

This chain of thought suggests that one visualize the pre-flare configuration as a dense layer of cool gas supported by and compressing a magnetic field. We might then explain homologous flares by proposing a mechanism for the slow build-up of such a configuration, which will at some stage become unstable. The instability, which will be identified as the flash phase of a flare, must be such as to allow the magnetic field to return to its unstressed state.

The build-up of the proposed pre-flare configuration must involve the circulation of gas in the chromosphere and perhaps involve circulation also in the corona. There is observational evidence that such circulation occurs in centres of activity[6] and that such circulation is closely related to flare activity[7]. It is possible that such a circulation of gas above the photosphere is the visible consequence of a primary circulation pattern below the photosphere. This interpretation would make it unnecessary to make further enquiry into the adequacy of the power supply.

There is, however, an alternative possibility. The growth of the pre-flare configuration can best be understood as the result of condensation of gas in the chromosphere. This implies that the source of power necessary to feed the flare storage system is one which heats the gas and, because of subsequent condensation, leads to circulation.

In order to show how this may come about, we should recall that, on magnetically 'quiet' parts of the Sun's surface, there is a flux of non-thermal energy, which we believe to be predominantly acoustic in form[8], which heats the chromosphere and corona above the photospheric temperature and thereby drives the solar wind. At a bipolar or complex spot group, gas which is heated by non-thermal means can rise only to heights permitted by the magnetic-field configuration and may afterwards cool by radiation. This heating-condensation sequence may lead to a circulation pattern in which gas is pumped up in one region and descends in another. The magnetic field of a sunspot should provide a channel for such downward flow, and one may argue[9] that the Evershed effect provides evidence that this is the case. The mass flow at a large sunspot is about 10^{19} g/day.

One would not expect that all the gas heated by non-thermal energy would circulate in a steady pattern. If the magnetic field lines anywhere form a 'trough', gas will tend to condense in this region and the consequent buckling and sagging of field lines will provide an even more effective 'cold trap'. As gas continually condenses, the magnetic field will be compressed so as to maintain a balance of forces. Provided the magnetic field has non-zero shear (and this must in general be the case), this process, which represents the storage of energy in both magnetic and gravitational forms, will continue until the plasma-field configuration becomes unstable. The relevant instability[10] is resistive and therefore allows the mass to fall to the photosphere and the magnetic field to rise into the corona.

The energy of a class 3+ flare is 10^{32} ergs, and it is known from observation of homologous sequences[4] that this is accumulated in about one day (10^5 sec). The observed area of such a flare is 10^{20} cm^2, but one might expect that the non-thermal energy is drawn from a somewhat larger area, say, 10^{21} cm^2. It is therefore necessary to convert into stored energy 10^8 ergs cm^{-2} sec^{-1}. Since Osterbrock[11] has estimated the total non-thermal energy flux into the chromosphere to be 10^7 ergs cm^{-2} sec^{-1} over quiet regions of the Sun's surface, and since the non-thermal energy flux is believed to be higher over magnetically active regions (as evidenced by the increased density and temperature of the chromosphere), we require that only a small fraction of this energy supply be stored in the pre-flare configuration.

If we further suppose that the mass condensed is comparable with that collected by a sunspot in one day, 10^{19} g, we are led to construct a field-plasma configuration, of area 10^{20} cm^2, with graduated density and magnetic field strength, the maximum values being $10^{11.3}$ cm^{-3} and $10^{2.5}$ gauss and, in consequence, the scale height being $10^{8.5}$ cm. The magnetic flux trapped in this way is about 10^{21} gauss cm^2, comparable with the flux of a sunspot.

The characteristic growth time of this instability[10] is the time of propagation of an Alfvén wave across the layer which, for the model quoted, is about 10^2 sec, in agreement with observation of the flash phase of a solar flare. If a large fraction of the stored energy is dissipated as Joule heating of the initially overlying mass of gas, the temperature rise will be a few thousand degrees, in agreement with observation. One would expect that a small upper layer, more tenuous than the bulk of the gas and overlying more magnetic flux, will be heated to considerably higher temperatures, perhaps 10^5 deg. or 10^6 deg. The conductivity would then be so high that the resistive decoupling of the motions of particles and field lines will fail to operate and the gas will be swept along with the magnetic field. It would not be visible in $H\alpha$ until, in a time of order 10^3 sec, it has cooled to a temperature of order 10^4 deg., by which time the magnetic field will have relaxed to its current-free state, forming arches extending into the corona. The visible manifestation of this stage of the flare process would be the appearance of a large mass of gas in the corona, which then streams down along the magnetic field lines. We offer this mechanism as an interpretation of the observations of Kleczek[12]. Kleczek's

estimate of the mass involved is 10^{16} g, which represents only 10^{-3} of the total mass involved in the pre-flare configuration.

Although the primary instability does not produce oscillatory electromagnetic fields, it is to be expected that the instability will generate turbulent magneto-hydrodynamic motion which can drive many types of plasma waves. Waves with frequency spectra covering the ion and electron gyrofrequencies will lead to stochastic acceleration as has been noted in a different context by Stix[13]. High-energy protons and electrons are known to be produced by solar flares, the latter causing certain types of radio bursts.

This work was supported by the U.S. Air Force Office of Scientific Research of the Office of Aerospace Research under contract *AF* 49(638)-1321.

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Spectrum and Relative Intensities of Interstellar OH Lines

The initial detection of the OH lines in the interstellar medium involved the two strongest lines at 1,665·402 Mc/s and 1,667·357 Mc/s of the four hyperfine lines making up the $^2\Pi_{3/2}$, $J=3/2$, Λ-doublet[1]. Subsequent observations resulted in the detection of the two weaker lines at 1,612·201 Mc/s and 1,720·559 Mc/s[2]. The strong OH absorption in the direction of the galactic centres, noted by the Australian workers[3], and the unexpected disagreement between the theoretical and observed intensity ratios of the lines[2], make it important to consider the detection possibilities of other radio lines of OH in the interstellar medium. A discussion of molecular energy-levels and the physical mechanism responsible for the Λ-doublet levels of OH has been prepared for publication elsewhere[4]; but here we wish to direct attention to 14 microwave lines of various energy-levels and isotopic species of OH, some of which appear to be capable of detection at the present time. The line frequencies, theoretical relative intensities, and transition probabilities are presented in Table 1. The frequencies have been computed by using the theory of Dousmanis, Sanders, and Townes[5], and the hyperfine coupling constants given by Radford[6]. The dipole moment for all isotopic species has been taken to be $(1{\cdot}60\pm0{\cdot}12)\times10^{-18}$ e.s.u., although this was determined only for $^{16}O^1H$ (ref. 7).

The lines given in Table 1 arise from the ground levels of $^{16}O^2H$, $^{18}O^1H$, and the ground and excited states of $^{16}O^1H$. Detection of the $^{16}O^2H$ lines does not appear to be very probable, because of the upper limit of 1/13,000 for the $^2H/^1H$ abundance ratio in the direction of Cassiopeia *A* (ref. 8), therefore only the strong $\Delta F=0$ lines are presented. The OH/H abundance ratio, however, varies

Table 1. Computed Frequencies of OH Λ-Doublet Transitions, including Hyperfine Structure, for Various States and Isotopic Species

Isotopic species	State	Transition $j\rightarrow i$	Frequency (Mc/s)	Relative intensity	$A_{i\rightarrow j}$ (sec^{-1})
$^{16}O^1H$	$^2\Pi_{3/2}$, $J=3/2$	$F=2\rightarrow1$	1,612·201 ± 0·017	1	$4{\cdot}50\times10^{-12}$
		$F=1\rightarrow1$	1,665·402 ± 0·007	5	$2{\cdot}47\times10^{-11}$
		$F=2\rightarrow2$	1,667·357 ± 0·007	9	$2{\cdot}66\times10^{-11}$
		$F=1\rightarrow2$	1,720·559 ± 0·024	1	$3{\cdot}24\times10^{-12}$
	$^2\Pi_{3/2}$, $J=5/2$	$F=3\rightarrow2$	6,017·2 ± 1·0	1	$4{\cdot}17\times10^{-11}$
		$F=2\rightarrow2$	6,031·2 ± 1·0	14	$5{\cdot}92\times10^{-10}$
		$F=3\rightarrow3$	6,035·2 ± 1·0	20	$6{\cdot}03\times10^{-10}$
		$F=2\rightarrow3$	6,049·1 ± 1·0	1	$3{\cdot}03\times10^{-11}$
	$^2\Pi_{1/2}$, $J=1/2$	$F=1\rightarrow0$	4,699 ± 4·0	1	$2{\cdot}56\times10^{-10}$
		$F=1\rightarrow1$	4,717 ± 4·0	2	$1{\cdot}73\times10^{-10}$
		$F=0\rightarrow1$	4,810 ± 4·0	1	$9{\cdot}10\times10^{-11}$
$^{16}O^2H$	$^2\Pi_{3/2}$, $J=3/2$	$F=5/2\rightarrow5/2$	310·24 ± 0·10	10	$1{\cdot}32\times10^{-13}$
		$F=3/2\rightarrow3/2$	310·06 ± 0·10	4·3	$8{\cdot}45\times10^{-14}$
		$F=1/2\rightarrow1/2$	309·97 ± 0·10	2·2	$8{\cdot}74\times10^{-14}$
$^{18}O^1H$	$^2\Pi_{3/2}$, $J=3/2$	$F=2\rightarrow1$	1,584·3 ± 0·2	1	$4{\cdot}30\times10^{-12}$
		$F=1\rightarrow1$	1,637·3 ± 0·2	5	$2{\cdot}36\times10^{-11}$
		$F=2\rightarrow2$	1,639·3 ± 0·2	9	$2{\cdot}54\times10^{-11}$
		$F=1\rightarrow2$	1,692·3 ± 0·2	1	$3{\cdot}10\times10^{-12}$

by a factor of ~1,000 between the directions of Cassiopeia *A* and the galactic centre[1,3], hence large spatial variation in the $^2H/^1H$ abundance ratio might also exist. The lines of $^{18}O^1H$ are particularly interesting. If the terrestrial $^{18}O/^{16}O$ abundance ratio of 1/490 holds throughout the galaxy, then an optical depth of $\sim2\times10^{-3}$ can be expected for the +40 km/s component of OH absorption in the galactic centre. This would give an absorption of ~0·5° K for the 210-ft. radio telescope at Parkes. A further consideration of the $^{18}O^1H$ lines is that their frequencies are sufficiently close to the $^{16}O^1H$ lines that new equipment is not needed. The frequencies of the first two excited states of $^{16}O^1H$ are also given in Table 1. Because of radiative decay to the ground-state, detection of these lines is less probable but the population of these levels could be drastically influenced either by the infrared radiation field or the method of production of OH in the interstellar medium.

An unexpected result of the astronomical OH observations is that the intensities of the four $^2\Pi_{3/2}$, $J=3/2$ lines are in the ratios 1 : 2·2 : 2·7 : 1, whereas the theoretical values are 1 : 5 : 9 : 1[2]. A possible explanation is the existence of many small condensations of large optical depth[2,3], but very special velocity and spatial distributions are needed to explain the entire observed profile. The OH observations have been interpreted by using the radiation theory developed by Milne[9] as modified by Wild[10] for the radio domain; but this theory may be inappropriate when the distance between absorbers is large compared with a wave-length and the radiation field is non-isotropic. Milne, and consequently Wild, considered isotropic radiation in the derivation of the optical depth and no account was taken of the possibility of atomic (or molecular) resonant coherent scattering[11]. If it is assumed that α is the fraction of the cross-section for resonant scattering out of the beam, then the optical depth τ and volume emissivity η can be written as:

$$\tau=\tau_0\left(1+2\alpha\frac{kT_s}{h\nu}\right),\qquad \eta=\eta_0\left(1+2\alpha\frac{kT_r}{h\nu}\right),$$

where τ_0 and η_0 are the values for the case of no scattering, and T_s and T_r are the excitation and radiation temperatures, respectively. The scattering parameter α is a function of the density of scatterers and the transition probability of the line and, therefore, will be different for each line. It follows that the ratio of intensities of the OH lines will be in disagreement with the ratios computed with scattering neglected. Furthermore, since α is a function of the position of the gas cloud relative to the continuum radio source, α may vary within the galaxy, and the line intensity ratios will vary accordingly. Note, also, that for T_s of the order of 1° K the optical depth may be as much as an order of magnitude greater than previously supposed. This may have a consequence for the problem of detecting weak radio lines, such as $^{18}O^1H$. To the extent that the radiation field is non-isotropic in inter-

galactic space, the scattering effect may force a reinterpretation of attempts to detect the intergalactic medium by a 21-cm absorption[12,13].

This work was supported in part by the U.S. Army, Navy, and Air Force under contract *DA*-36-039-*AMC*-03200(*E*), and in part by the National Aeronautics and Space Administration (grant *NsG*-419).

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RADIO ASTRONOMY

Rotation Rate of Venus measured by Radar Observations, 1964

During the June 19, 1964, close approach of Venus, radar observations at 410·25 Mc/s were made using the 250-ft. *Mark I* radio telescope at Jodrell Bank. Echoes were first obtained at full strength on June 5 and continued at intervals until July 26. The equipment[1] was basically that used in 1962: a C.W. radar system in which the quantities measured were the doppler shift and the frequency spectrum of the returned signal. Modifications to the receiver, including the use of a parametric amplifier, improved the signal-to-noise ratio to give a radio frequency signal to noise at closest approach of about 0·5. Again the spectral analysis was made with a multi-channel real-time integration system, having eleven channels in frequency each approximately 1 c/s wide spaced 1 c/s apart. As in 1962, the spectrum was very narrow, most of the energy being in one channel. Owing to the improved signal-to-noise ratio, however, significant rises were obtained in at least two channels on each side of the centre. Examples of the channel readings for June 17 and July 14 are shown in Fig. 1. These have been corrected for a 10 per cent bias by integrations of noise to determine correction factors.

The significant rises obtained in the channels on each side of the centre channel have enabled the methods previously described[1] to be applied to estimate the half-power width of the central feature of the spectrum. This was found to be a function of time, changing from below 1 c/s at closest approach to about 1·5 c/s 35 days later. This effect can be seen in Fig. 1 quite clearly. Measured values are plotted in Fig. 2.

It is not possible to calculate directly the apparent rotation rate of Venus from these observations, as the ratio of the width of the central feature measured to the total limb-to-limb width of the spectrum is unknown. However, the points may be normalized to unity at closest approach and the shape of the resultant curve compared with that expected for various values of the intrinsic rotation. This problem has been discussed by several workers[1–3]. The apparent rotation is made up of two components: (*a*) the intrinsic rotation relative to inertial space; (*b*) the apparent rotation rate caused by the relative motion of Venus with respect to the observer.

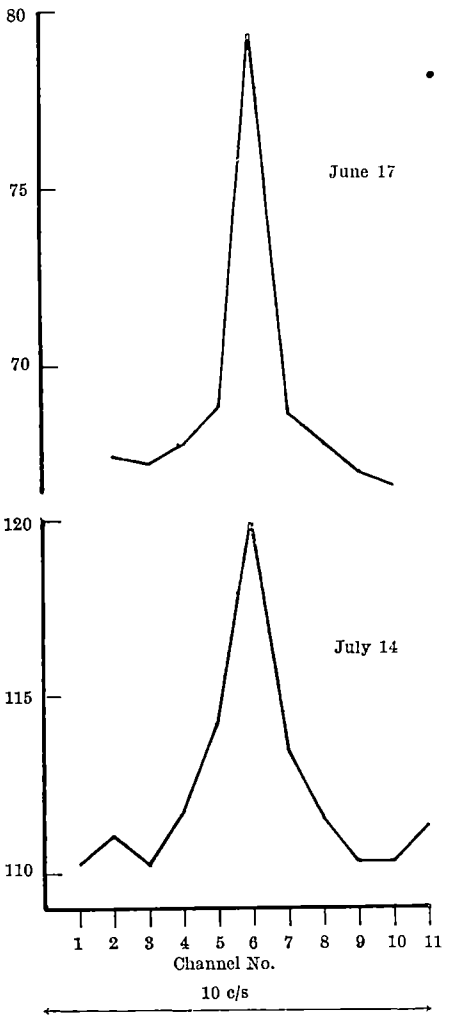


Fig. 1. Plots of the corrected integrator channel readings for June 17 (almost closest approach) and July 14 (25 days from closest approach). The increased width of the spectrum can be seen. The channels are spaced at 0·954 c/s so the abscissæ cover about 10 c/s. The ordinates are in arbitrary units from the integrating system. On June 17 the integration time was 162 min and on July 14 it was 214 min

This latter component is time variable, rising from zero at about ± 21 days to more than 10^{-7} rad/sec direct at closest approach. The intrinsic rotation, if the axis is perpendicular to the plane of the ecliptic, simply adds to this if direct, or subtracts from it if retrograde. Thus direct intrinsic rotation will give a maximum of apparent rotation at closest approach and retrograde intrinsic rotation (if greater than about 10^{-7} rad/sec) will give a minimum.

Clearly the measured points in Fig. 2 show a minimum rather than a maximum at closest approach, indicating retrograde rotation. This is in broad agreement with the results obtained in 1962 by Rzhiga[4] in the U.S.S.R. and by Carpenter[5] at the Jet Propulsion Laboratories in the United States. These workers, using a similar method, arrived at a period of 250–300 days retrograde.

The curves shown in Fig. 2 are calculated on the assumption of co-planar circular orbits and a rotation axis perpendicular to the orbital plane, and are normalized to unity at closest approach. The measured points do not fit any curve well, but a period of about 100 days retrograde is the minimum period they would reasonably allow. However, systematic effects such as the size of the discrete steps used in doppler removal (about 0·1 c/s) will

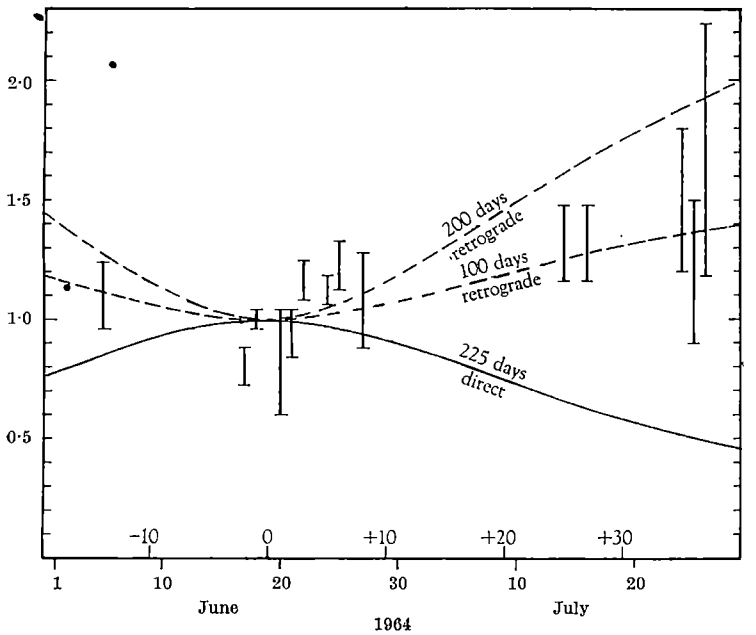


Fig. 2. Measured width to 3 db points of the central feature of the spectrum as a function of time. The lower abscissæ scale is date; upper abscissæ scale is days from closest approach. The ordinate is in units of channel width (1 unit = 0·954 c/s). Dotted curves are variations expected for 100 and 200 days retrograde rotation, normalized to unity at closest approach. The full curve shows similarly the variation expected for 225 days direct, corresponding to Venus always turning the same face towards the Sun

tend to broaden the spectrum and make the minimum of Fig. 2 more shallow. Thus any rotation rate derived by strict curve fitting is likely to be too small. If it is assumed that the central feature of the spectrum has been systematically broadened by 0·2 c/s, a rather similar fit is obtained with the 300-day curve. Hence our results confirm that the rotation is retrograde but can only indicate that the period is between 100 and 300 days. Similar curves can be calculated for various directions of the rotation axis, but the accuracy of the present work does not justify this.

It is hoped that further analysis by digital computer of the recorded returned signals will provide more accurate spectra; this may make further analysis profitable.

The data for the ephemeris were provided by the Royal Greenwich Observatory, Herstmonceux, and by the U.S. Ephemeris Office, and we thank G. Walker and R. A. Porter, who assisted with the observations.

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Inverse Compton Effect as a Possible Cause of the X-Ray Radiation of Solar Flares

Shklovsky[1] has suggested the inverse Compton effect as a possible cause of the solar flare-burst emission of hard X-ray photons. This process, as its name implies, involves a scattering interaction between a relativistic electron and a low-energy photon. The interaction produces an X-ray photon and the electron loses a corresponding amount of its energy. The X-ray photon production rate from this process is directly proportional to the density of ambient photons, the density of energetic electrons and the square of the electron energy. Using a radiation density which is twice the photospheric value, he estimates that it is possible to account for the observed flare-burst X-ray emission in the 1–100 keV region if the total energy of 10–100 MeV electrons in a flare is of the order of 10^{30} ergs.

Shklovsky also suggests that observations of the synchrotron emission from this hypothesized electron cloud in the millimetre and far infra-red would prove or disprove its existence. I wish to point out that observations are already in hand which indicate that this is not an important X-ray generating mechanism in the solar case. These are the existing observations of hard X-rays from solar flares.

We have computed the bremsstrahlung to be expected from the hypothesized cloud of strongly relativistic electrons which are required to produce the inverse Compton photons. The bremsstrahlung cross-section formulæ collected by Koch and Motz[2] were used (their equations $3BS[a]$, $3BS[c]$ and $3BS[d]$). We have assumed an atmosphere of neutral hydrogen, and these formulæ take account of nuclear screening by bound electrons. If the hydrogen were ionized the bremsstrahlung cross-section would be larger by less than a factor of two.

For electron energies, E_e, in the range 10–100 MeV in a neutral hydrogen gas and for emitted photon energies, k, such that:

$$k < 0{\cdot}1\, E_e \qquad (1)$$

the bremsstrahlung cross-section is, to a good approximation:

$$\mathrm{d}\sigma_k = 1{\cdot}6 \times 10^{-26} \frac{\mathrm{d}k}{k}\ \mathrm{cm}^2 \qquad (2)$$

The X-ray photon flux at the Earth in the spectral interval (k_0,k) from the radiating volume, V, is given by the expression:

$$F_{ph}(k_0,k) = \frac{N_H N_e^* V v_e}{4\pi\,(\text{A.U.})^2} \int_{k_0}^{k} \mathrm{d}\sigma_k \text{ photons cm}^{-2}\text{ sec}^{-1} \qquad (3)$$

N_e^* is the density of the relativistic electrons, N_H is the density of hydrogen atoms and/or ions, v_e is the velocity of the relativistic electrons (substantially equal to the velocity of light) and A.U. is the astronomical unit ($1{\cdot}5 \times 10^{13}$ cm). Evaluating the constants and performing the integration yields the expression for the photon flux at the top of the atmosphere:

$$F_{ph}(k_0,k) = 1{\cdot}7 \times 10^{-43} N_H N_e^* V(\ln k - \ln k_0) \qquad (4)$$

Using Shklovsky's values for N_H ($\approx 10^{11}$ cm^{-3}, this value is required to limit the duration of the burst by collisional losses), and $N_e^* V$ ($\approx 10^{34}$, that is, 10^{30} ergs of 50 MeV electrons) we compare the computed bremsstrahlung photon flux at the top of the atmosphere in the 20–50 keV and 50–150 keV spectral intervals with that reported by Anderson and Winckler[3] for the great solar flare of September 28, 1961.

	Bremsstrahlung (this communication)	Anderson and Winckler (peak of flare burst)
$F_{ph}(20, 50)$	190	≈ 100
$F_{ph}(50, 150)$	230	≈ 6

It is immediately clear that the flux of photons of $k > 50$ keV from the bremsstrahlung of the strongly relativistic electrons is proportionately much greater than

that observed by Anderson and Winckler. Even the very energetic solar X-ray event reported by Peterson and Winckler[4] appeared to fall off much more sharply beyond 500 keV than would have been possible if there had been a significant contribution from inverse Compton photons.

It may be argued that a careful choice of parameters would overcome the foregoing discrepancy. Our computations indicate, however, that any cloud of energetic electrons which can produce a measurable contribution of inverse Compton photons of the observed duration will always produce a greater flux of high-energy bremsstrahlung photons than has been observed.

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GEOPHYSICS

Model Studies of Elastic Wave Propagations in Crustal Blocks under Tectonic Stresses

ALTHOUGH the existence of tectonic stresses is known, so far no practical method has been discovered to locate and determine the magnitude of these stresses. In this communication a technique is discussed which may be utilized to detect the tectonic stresses.

Experimental evidence indicates that the velocity of elastic body waves in rocks is a function of the compressive stress along the direction of propagation[1–7]. If a crustal block is under tectonic forces, the velocity of elastic waves in such a block would be affected by these forces. Consequently, a change in velocity in a particular area as a function of direction could arise from an inhomogeneous stress field within that region. The blocks which are under gravitational forces alone have a relatively simple stress distribution. These are termed the quiet blocks, and those which are also under tectonic forces are called the active blocks.

Using the velocity pressure data of Birch[8] for P-waves and Simmon[9] for shear waves for Westerly granite, two distinct models are investigated adopting different values of Poisson's ratio. The first model is a quiet block. The ray path of elastic waves is determined for this model and the time–distance curves are computed, using the $B5000$ digital computer. In all cases, I find that the refracted energy propagates up to a critical distance X_c and the wavefront then swings back; the refracted energy from deeper horizons arrives at the surface at distances smaller than the critical distance X_c.

The second model is an active block of granite in equilibrium with some external tectonic forces. The travel–time curves for the active block are asymmetric. The circular symmetric travel times for the quiet block degenerate into an ellipse for a block which is under tectonic stresses.

Table 1

Velocity (km/sec)	Pressure (atm.) 0	10	500	1,000	2,000	4,000	6,000	10,000
P-waves	—	4·10	5·63	5·84	5·97	6·10	6·16	6·23
S-waves	2·77	—	3·27	3·36	3·44	3·51	3·54	3·58

In this investigation I used the velocity pressure data of Birch[8] and Simmon[9] for Westerly granite reproduced in Table 1. These velocity data are expressed empirically as:

$$V = V_0 + A_1\sigma + A_2\sigma^2 + A_3\sigma^3 + A_4\sigma^4 + \qquad (1)$$

where A_1's are coefficients and σ is the stress along the direction of propagation. If we assume that lateral deformation within the block is prevented, then the stress tensor for such an isotropic quiet block is described as:

$$S_q = \begin{bmatrix} \frac{\nu\sigma_z}{1-\nu} & 0 & 0 \\ 0 & \frac{\nu\sigma_z}{1-\nu} & 0 \\ 0 & 0 & \sigma_z \end{bmatrix} \qquad (2)$$

where the vertical stress σ_z is given by: $\sigma_z = \rho g z$; ρ, average density of the material; g, gravitational acceleration ; z, depth ; ν, Poisson's ratio. In general, the stress σ in any arbitrary direction in the xz plane may be described as:

$$\sigma = \sigma_x l^2 + \sigma_z(1 - l^2) + 2l(1 - l^2)^{1/2}\tau_{xz} \qquad (3)$$

where l is the direction cosine. In the case of the quiet block, $\tau_{xz} = 0$. Once σ is computed for an initial direction cosine l, the velocity V can be determined from equation (1). Using Snell's law, the refraction of elastic energy is determined numerically. The travel time curves for P-waves propagating along a ray in the xz plane are given in Fig. 1. It is interesting to note that for the first model the energy for P-waves travels to a critical distance, $X_c = 137{\cdot}92$ km, and then the wavefront literally swings back. For the S-waves the critical distance $X_c = 165{\cdot}26$ km.

The stress tensor for an active block (the second model) is assumed to be:

$$S_a = \begin{bmatrix} (az + b + cx) & 0 & -ax - cz \\ 0 & \frac{\nu\sigma_z}{1-\nu} & 0 \\ -cz - ax & 0 & az \end{bmatrix} \qquad (4)$$

where b, 100 kg/cm²; c, 0·1 kg/cm²/m; a, 0·27 kg/cm²/m.

The travel-time curves for this model are asymmetric (Fig. 2); the circular symmetric travel-time on the arc of a circle for a quiet block degenerates into ellipses.

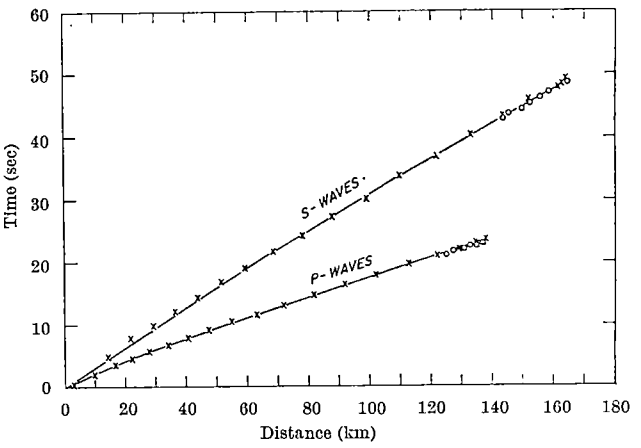


Fig. 1. Travel–time curves for elastic waves in model quiet block with Poisson's ratio = 0·35 (Birch and Simmon's data for Westerly granite). ×, Refractions from shallow horizons; ⊙, refractions from deeper horizons

The time difference involved for an active block was about 200 msec for the stations located over the arc of a circle with 5-km radius; the corresponding time difference for 6-km radius was about 300 msec (Fig. 2). Such a difference is easily observable in the field. In practice, however, the local geology and large-scale lithological variation within a block also give rise to variation of seismic wave velocity in different directions.

The idea deserves further theoretical investigation and extensive evaluation in the field. If successful, it will be a technique to determine the existence of tectonic stresses. By examining the time rate of change of velocities, it is conceivable that it may be possible to predict potential earthquake areas and thus prevent loss of life and property.

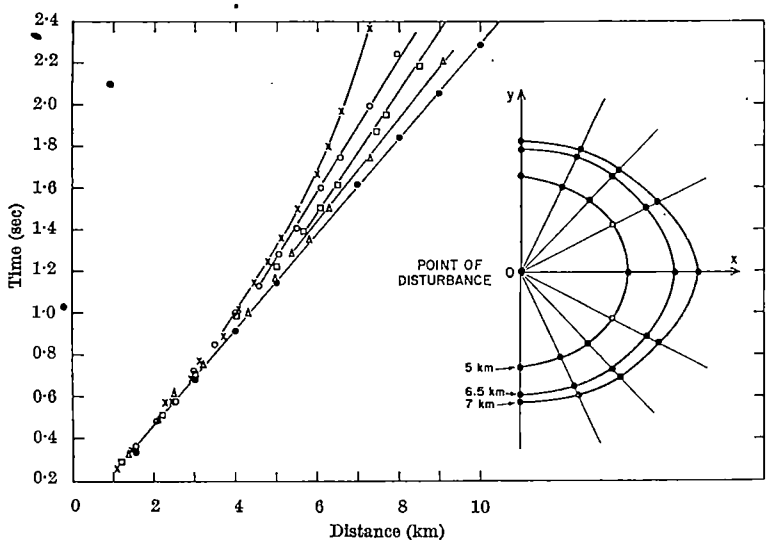


Fig. 2. Travel-time curves and distortion of these from circles to ellipses in an active block. ×, 0° x direction; ○, 30° from x axis; □, 45° from x axis; △, 60° from x axis; ●, 90° from x axis (y direction)

I thank Profs. J. L. Soske and B. M. Page of Stanford University, and Dr. R. B. Vaile, jun., and Mr. Warren Westphal of Stanford Research Institute for their advice.

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Palæomagnetic Results from Northern Canada suggesting a Tropical Proterozoic Climate

The Coppermine and September Mountains, in the north-central District of Mackenzie, Northwest Territories of Canada, are composed of a thick succession of tholeiite basalt flows. Directions of natural remanent magnetization were measured on 51 oriented samples from 24 sites (Fig. 1). The sites represent 24 of the more than 60 separate flows occurring in a vertical thickness of more than 5,000 ft. The flows dip from 0° to 5° north in the area sampled. Two potassium/argon whole rock age determinations on flow rocks, made in the Isotope and Nuclear Geology Section of the Geological Survey of Canada (Wanless, private communication), gave an average age of 1,150 m.y. with a probable error of ± 100 (calculated as two standard deviations from the mean). This places the age of extrusion of the flows in upper middle Proterozoic time. The lavas conformably overlie dolomite of the Hornby Bay Group, and in places thin sandstone bands are intercalated between the flows. A sample from a red sandstone intercalation gives a direction of $D = 236$, $I = +24$, which is similar to that of the flows.

Separate specimens from each sample were subjected to magnetic cleaning[1] and thermal cleaning[2] to reduce possible errors from secondary components. The precision parameter[3] of the natural remanent magnetization grouping ($k = 7$, $\alpha = 12$)° (Fig. 2a) was much improved after treatment in an alternating magnetic field of 75 œrsteds peak value ($k = 23$, $\alpha = 6$) (Fig. 2c). The direction after magnetic cleaning was 7° farther from the dipole field direction than that of the natural remanent magnetization. The scatter increased slightly ($k = 5$, $\alpha = 15$) in specimens heated to 480° C, and the resultant moved 13° away from the present field direction. This increase in scatter (Fig. 2b) may be due to the heating of about half the specimens above the Curie temperature of their dominant magnetic mineral. The differences between the mean directions of magnetization before treatment, after magnetic cleaning, and after thermal cleaning are not significant at $P = 0{\cdot}05$; but the fact that the cleaned directions are farther from the dipole field direction than before treatment suggests that a small viscous component in the direction of the dipole field has been removed.

Four specimens were heated to 625° C and cooled in a magnetic field of known direction and of intensity 1·3 œrsteds. The resulting thermoremanent magnetization direction was in each case within 2° of the direction of the field during cooling, showing that magnetic anisotropy does not affect the direction of thermoremanent magnetization in the specimens.

The laboratory tests support the idea that the natural remanent magnetization direction is that of the Earth's magnetic field at the time the lavas cooled. Furthermore, this direction is very close to that obtained in a preliminary investigation of gabbroic rocks of the nearby Muskox Intrusion (see Table 1), which has been assigned a radiogenic age of 1,150 m.y.[4] with a probable error of ±40 (calculated as two standard deviations from the mean).

The virtual geomagnetic pole position calculated from the mean direction of the flows (1° N., 176° W.), assuming that the dipole field hypothesis[5] is relevant to middle Proterozoic time, places the flows at a palæolatitude of 10°–15°, and suggests that the Hornby Bay Group with which they are associated was deposited under tropical

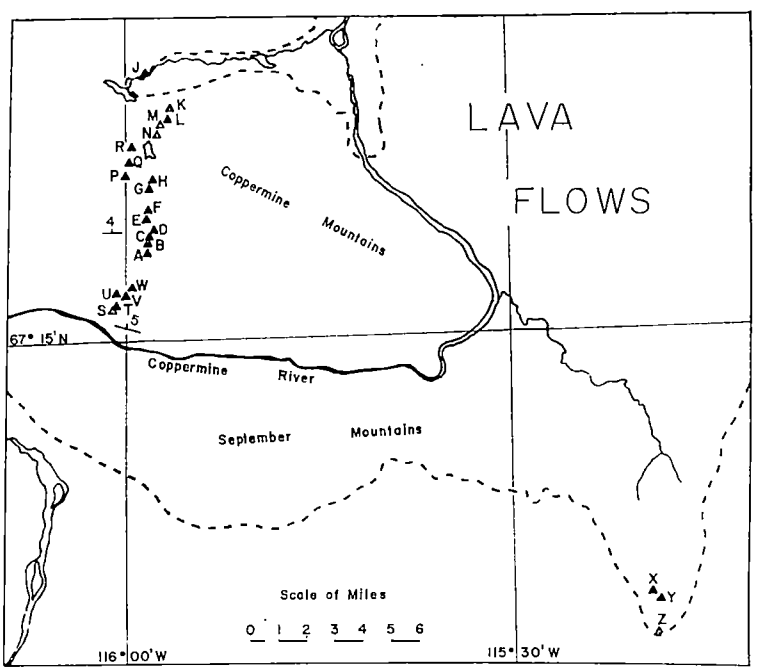


Fig. 1. A sketch map showing sampling sites

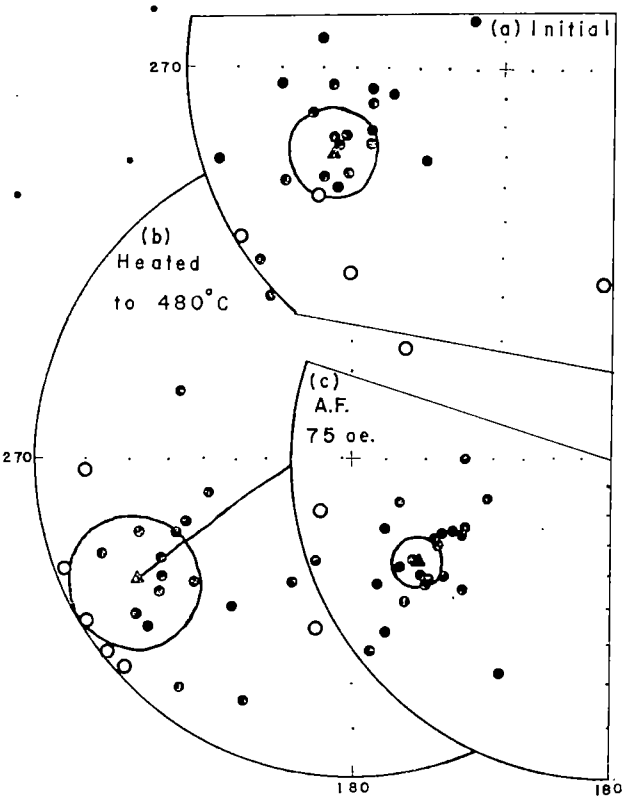


Fig. 2. Portions of stereographic nets showing mean site magnetization directions, (*a*) before treatment, (*b*) after heating to 480° C in a null magnetic field, (*c*) after treatment in an alternating magnetic field of 75 œrsteds peak value. North-seeking directions are plotted as open circles on the upper and dots on the lower hemisphere. Mean group directions, with the trace of the 95 per cent cone of confidence, are shown as triangles. The great circle between the heated mean and the dipole field direction is also shown

conditions. The occurrence of gypsum and anhydrite in the Minto Inlet Formation of Victoria Island, of late Pre-Cambrian age[6], is consistent with such a low latitude.

The close agreement of this pole position with those of comparable age from the Sudbury diabase dykes[7], the Duluth Gabbro[8], and the Muskox gabbroic rocks (Table 2), and the small polar errors ($d\chi = 7$, $d\psi = 4$) associated with the Coppermine lavas pole position, suggest that this virtual geomagnetic pole position may be used as an anchor for the North American polar wandering curve[8,9] for upper middle Proterozoic time.

Table 1. Coppermine Lava Flows and Muskox Gabbro Mean Directions and Statistics

Formation	Treatment	No. sites	No. samples	No. specimens	*D*	*I*	*R*	*k*	*α*
Coppermine lava flows	None	24	51	102	244	+29	20·88	7·4	12
,,	Alternating field of 75 œrsteds	24	48	48	242	+22	23·00	23	6
,,	Heated to 480° C	24	46	46	240	+15	19·11	4·7	15
Muskox gabbroic rocks	None	8	19	38	247	+33	7·48	13	16
,,	Alternating field of 75 œrsteds	8	19	19	242	+29	7·35	11	18

D, Declination east of true north; *I*, inclination positive downwards; *R*, resultant vector using sites as unit vectors; *k*, estimate of precision (ref. 3); *α*, half-angle of the cone of confidence at $P = 0{\cdot}05$ (ref. 3).

Table 2. Canadian Upper Middle Proterozoic Virtual Geomagnetic Pole Positions

Formation	Radiogenic age (m.y.)	Pole position Latitude	Pole position Longitude	Polar errors $d\psi$	Polar errors $d\chi$
Coppermine lavas	1,150 ± 100	1 N.	176 W.	[a] 4	7
				3	6
Muskox gabbroic rocks	[a] 1,150 ± 40 (ref. 4)	4 N.	175 W.	[a] 11	19
				7	12
Duluth Gabbro	[b] 1,120 (ref. 10)	24 N.	180 W.	6	10
Sudbury diabase dykes	[c] 1,200 (ref. 11)	8 S.	162 W.	6	14

a, Average of 4 potassium/argon determinations, 2 from biotite and 2 from whole rock; *b*, average of 2 potassium/argon and 2 rubidium/argon determinations, which range from 1,075 to 1,200; *c*, average of 4 potassium/argon determinations, 2 from biotite and 2 from whole rock; *d*, the larger polar errors for these groups were computed from site means; the remaining polar errors were computed from sample means.

The collection of samples was made possible by the support of the Upper Mantle Group of the Geological Survey of Canada, supervised by Dr. C. H. Smith, whom I thank. I thank Dr. A. Larochelle for his assistance with the laboratory work and Mr. G. Dallaire, who helped in the collection and measurement of samples. This investigation was supported by the Geological Survey of Canada where I held a National Research Council postdoctorate fellowship (1962–64).

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PHYSICS

Meta-stable Ions in Double-focusing Mass Spectrometers

'Meta-stable peaks' are normally observed due to ions which dissociate near the entrance slit of the magnetic analyser in the sector mass spectrometer. In addition 'meta-stable peaks' due to ions which dissociate within the electrostatic analyser of a double-focusing mass spectrometer can also be observed and their position can be adjusted by varying the voltage across the electrostatic analyser.

Ions which dissociate during their transit of a mass spectrometer analyser tube are said to be meta-stable[1]. They are noted on the mass scale of the spectrum at a position corresponding to m^*, where:

$$m^* = \frac{m_2^2}{m_1}\left\{1 + \frac{(m_1 - m_2)(V - V_1)}{m_2 V}\right\}$$

and m_1 is the mass of the ion before dissociation, m_2 is the mass of the charged fragment after dissociation, V is the accelerating voltage and V_1 is the potential difference through which the ion of mass m_1 falls before decomposing[2]. In general, dissociation can occur anywhere between the ion source and the collector. There may also be a release of internal energy during the transition, so that conditions may be somewhat more complex than indicated by the above equation and many of the ions will not be recorded. A very small internal energy release will discriminate strongly against ions formed at large values of $(V - V_1)$ and prevent their passing through the entrance slit of the spectrometer at the end of the accelerating region. Owing to the directional focusing properties of the sector magnetic field, the decomposing ions most likely to be recorded, in a single-focusing mass spectrometer of the Nier design[3], are those that undergo decomposition near the entrance slit. In this case :

$$m^* \approx m_2^2/m_1$$

as is well known.

In the case of a double-focusing mass spectrometer of the Nier–Roberts geometry[4] it has been shown that meta-stable transitions occurring before entry into the electrostatic analyser can be observed by altering the accelerating voltage sufficiently to deflect the main ion beam such that it is no longer transmitted by the analyser[5]. Under such conditions the daughter ion of mass m_2 can, however, sometimes be detected. In the work recorded here it is shown that ions which dissociate during their transit of the electrostatic analyser tube can also be distinguished from ions that dissociate either before or after their passage of this part of the spectrometer tube.

If E is the voltage across the plates of the electrostatic analyser, then the ratio E/V can only be varied between comparatively narrow limits if ions are to be transmitted through this analyser. In the instrument used in this investigation, an A.E.I. double-focusing mass spectrometer type *MS*-9, it is possible to vary E/V by 1·3 per cent and still permit ions to pass through the analyser.

It has been noted that where meta-stable transitions involve only a comparatively small mass change as, for example, in the case of the transition noted in the spectrum of toluene :

$$\underset{(m/e\ 92)}{C_7H_8^+} \rightarrow \underset{(m/e\ 91)}{C_7H_7^+} + H$$

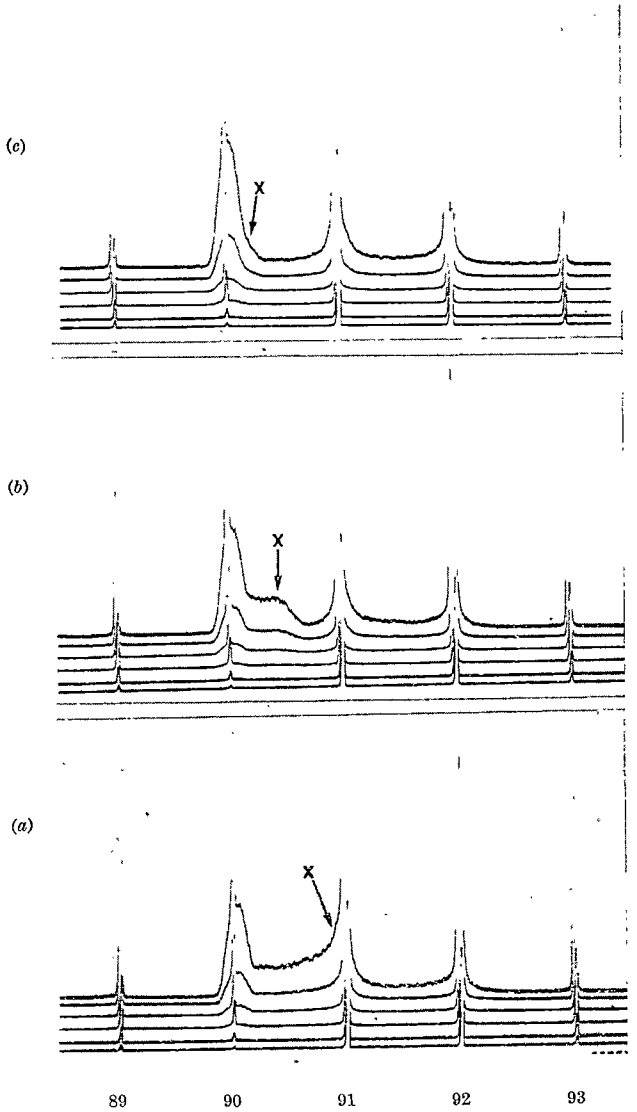


Fig. 1. Appearance of 'meta-stable peaks' in the spectrum of toluene, (*a*) $\Delta V/V = 0$, (*b*) $\Delta V/V$ = intermediate value, (*c*) $\Delta V/V \approx 1/91$

a peak can be observed due to transitions occurring within the electrostatic analyser, the position of which depends on the value of E/V. The 'meta-stable peak' corresponding to the above transition is shown in Fig. 1 at three different values of E/V. In each case it is possible to observe the 'normal meta-stable peak' at m/e 90·0 corresponding to a transition that occurs after the passage through the electrostatic analyser but before entering the magnetic analyser. In Fig. 1c it is seen that the peak corresponding to the electrostatic analyser transition, marked X in Fig. 1, merges into the peak from the 'normal' transition.

If the transition occurs at the entrance of the electrostatic analyser, the energy of an ion changes to

$$e\ V\ m_2/m_1 = e\ E\ r^1/2$$

where r^1 is the radius of curvature of the path of the ion of mass m_2

$$\therefore\ r^1 = 2\ V\ m_2/E\ m_1\ (< r)$$

where r is the radius of curvature of an ion passing centrally through the electrostatic analyser. To restore the ion of mass m_2 to its original path of radius of curvature r, the accelerating voltage V must be adjusted to $V + \Delta V$ (or E must be changed to $E - \Delta E$).

$$r = \frac{2(V + \Delta V)}{E} \cdot \frac{m_2}{m_1} = \frac{2\ V}{E}$$

from which it may be shown that :

$$(m_1 - m_2)\ V = m_2\ \Delta V$$

$$\text{or } \Delta V/V = m_1/m_2 - 1$$

If $m_1 \approx m_2$, as, for example, in the transition we have considered in toluene were $m_1 = 92$ and $m_2 = 91$, then :

$$\Delta V/V \approx \frac{1}{91}$$

Then depending on where the decomposition occurs :

$$0 < \Delta V/V < \frac{1}{91}$$

If the dissociation occurs after passage through the electrostatic analyser the peak occurs at mass 90 when $\Delta V/V = 0$. On the other hand, if the dissociation occurs before the ion enters the electrostatic analyser the peak occurs at mass 91 when $\Delta V/V = 1/91$.

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Low-background Beta Counters for Iodine-131 Sources

In a recent article[1] the use of two end-window Geiger counters (20th Century Geiger-Müller type *EW*2C) for scanning chromatograms containing iodine-131 was discussed. It was shown that using anti-coincidence techniques the background in the double-counter system was typically about 3·5 c.p.m. Furthermore, it was pointed out that significant fraction of this background arose from radioactive contaminants in the window material of the counters. The efficiency of detection for iodine-131 and Whatman No. 1 chromatography paper is about 50 per cent. Using the formula quoted in ref. 1 the total time

(T) required to determine the presence of 2 μμc. with an accuracy of 10 per cent is 375 min. By carefully selecting Geiger counters, backgrounds lower than the typical one can be obtained, although it is doubtful if it is possible to do better than about 2·8 c.p.m. The above value of T would then be reduced to 316 min.

By special request, 20th Century Electronics have provided a slit-window Geiger counter in which the window, confined to an area of approximately 2·5 cm × 0·8 cm, is set into a metal base-plate. The dimensions of the counter are otherwise the same as the standard *EW2C* counter. The window area was chosen to be of a convenient size for scanning chromatograms. A double-counter system, with an anti-coincidence screen, made from these counters has a background of as low as about 1·5 c.p.m. Under these conditions the value of T, for 2 μμc. and 10 per cent accuracy, is reduced to 204 min. Apart from this reduction in counting time, use of counters of this type has the advantage that the chromatogram can be pulled through the arrangement in a stepwise fashion without the necessity of cutting the chromatogram up into individual sources.

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Force associated with the Temperature Gradient on a Small Particle suspended in a Plasma

Mason and Chapman[1] have shown that the motion of small particles in a non-uniform un-ionized gas may be described through the Chapman–Enskog theory by treating the particles as large molecules. Their kinetic theory calculations agree with results obtained by previous investigators[2–4], who used free-molecule momentum transfer calculations, for all cases except when a temperature gradient is present.

For this case of a small particle in an un-ionized gas in a temperature gradient, it appears on experimental evidence[5] that the free-molecule momentum transfer calculations, which allow for inelastic collisions, provide a better description of the motion than the kinetic theory calculations[1] based on the elastically diffuse model.

It seems of value, then, to report the results of a free-molecule momentum transfer calculation of the force acting on a small suspended particle of a radius a in a plasma in which temperature gradients ∇T_i and a magnetic field of intensity $\vec{H}$ are present.

The assumption of free-molecule calculation is that the particle does not disturb the distribution functions of the surrounding fluid. Also in the calculation it is assumed that the radius of the suspended particle is much less than the radius of the Debye sphere; as a result of this assumption, collective effects of the plasma on the particle can be ignored[6]. A neutral, two-fluid model is assumed for the plasma; that is, the collisional coupling between the positive ions and electrons is considered unimportant.

The force acting on the particle is obtained by integrating the net momentum transferred over the particle surface S with normal $\vec{n}$.

$$\vec{F} = -\int_S \mathrm{d}S\vec{n} \cdot \sum_i \sum_{\pm} \int_{\pm} \vec{v}_i m_i \vec{v}_i f_i^{\pm} \, \mathrm{d}\vec{v}_i$$

and

$$\int_{\pm} \equiv \text{sign } (\vec{n} \cdot \vec{v}_i) \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \int_{0}^{\text{sign } (\vec{n} \cdot \vec{v}_i)\infty}$$

The distribution functions f_i^- for this physical system are the first-approximation functions given by Chapman and Cowling[7]. The functions f_i^+ are defined by the surface momentum accommodation coefficients, α_i:

$$f_i^+ = \alpha_i f_i^{(0)+} + (1 - \alpha_i) f_i^-$$

where the $f_i^{(0)+}$ are the equilibrium distribution functions for species i leaving the particle surface.

The following expression for the thermal force acting on the suspended particle is obtained:

$$\vec{F} = -\left(\frac{4}{5}\right)a^2 \sum_i (2\pi k/m_i T_i)^{\frac{1}{2}} (\lambda_i/C_{vi}(1+\omega_i^2\tau_i^2)) (\nabla T_i - \omega_i \tau_i (\vec{H}/|\vec{H}|) X \nabla T_i)$$

where

$$\omega_i \tau_i = 3q_i |\vec{H}| / 5m_i C_{vi} p_i$$

and for species i in the plasma q_i is the charge, m_i the mass, λ_i the thermal conductivity, C_{vi} the specific heat at constant volume, p_i the pressure, and T_i the temperature.

Thus, in addition to a force acting on the particle in the direction of decreasing temperature, there is an additional force in the transverse direction. In the calculation of the transverse thermal force, just as for the thermal force, the terms involving the accommodation coefficients of the plasma species on the particle sum to zero over the surface of the sphere.

Of interest would be an experimental confirmation of the transverse thermal force. Further calculations are planned out of the free-molecule régime for a charged particle and a more realistic plasma model with both electric and magnetic fields.

I thank the National Center for Atmospheric Research for an appointment as a 1964 summer visitor and Dr. G. Hidy for his advice.

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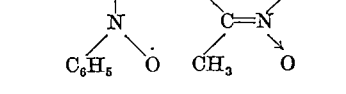
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CHEMISTRY

Electron Spin Resonance of β-Phenylhydroxylamino-β-methyl-pentane-δ-phenylimine Oxide

The compound formed by the oxidation of the condensation product of β-phenylhydroxylamine with acetone has been shown[1] to have the structure:



Electron spin resonance spectra of this compound have been obtained[2] and some[3] have shown a hyperfine structure consisting of 3 broad equal intensity lines 13 gauss apart, arising from the interaction of the unpaired electron with one of the ^{14}N nuclei. However, no hyperfine splitting due to the aromatic protons has been reported and it was decided to investigate any such structure. A

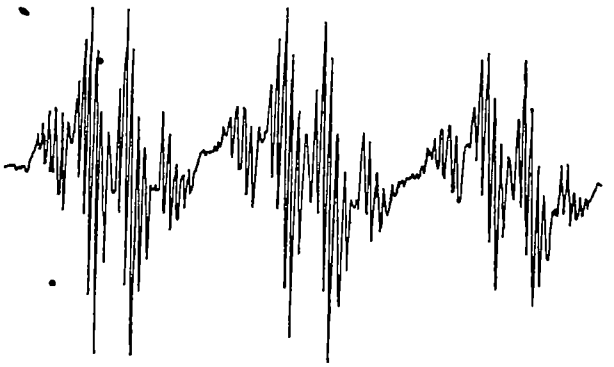


Fig. 1. Electron spin resonance of fully protonated compound

solution in tetrahydrofuran (about 0·003 M) was thoroughly deoxygenated and examined in a conventional 100 kc/s field-modulated X-band electron spin resonance spectrometer.

A well-resolved spectrum was obtained (Fig. 1) consisting of three similar groups of lines with a hyperfine coupling constant of 12·0 gauss, each group being split into four sub-groups with an intensity ratio of 1 : 3 : 3 : 1 and coupling constant of 2·36 gauss. Each of the 12 sub-groups is then further split into about 6 lines of variable width and non-binomial intensity ratios.

These data may be explained by assuming an equal (or near equal) spin density on the 2 *ortho*- and the 1 *para*-position protons.

The further splitting cannot be explained solely by coupling to the 2 *meta*-protons which would, of course, give only a 1 : 2 : 1 triplet, nor to any inequivalence of *ortho*- and *para*-proton coupling which would give a smaller number of lines in the wings compared with the more intense groups. Some degree of coupling evidently occurs with either the alkyl protons or the other aromatic group.

To resolve this ambiguity the compound was made with the alkyl protons replaced by deuterons, both the aromatic groups remaining protonated. The electron spin resonance spectrum (Fig. 2) of this partially deuterated radical is seen to resemble that of the fully protonated species in its gross hyperfine structure (a_N and $a_0 = a_p$ are the same), but to differ in its finer detail where the expected meta-1 : 2 : 1 triplets are the only features to be seen. This shows that some coupling to the alkyl chain does take place. The larger number of much more finely spaced 'deutero' lines have not as yet been resolved. The coupling constant for the *meta* aromatic protons can be determined from the deuterated spectrum and is 0·89 gauss.

Using these values it is possible to reconstruct the spectrum for the protonated radical by assuming a small coupling (a = 0·44 gauss) to one methyl group. This splits each line into four components with a 1 : 3 : 3 : 1 intensity distribution.

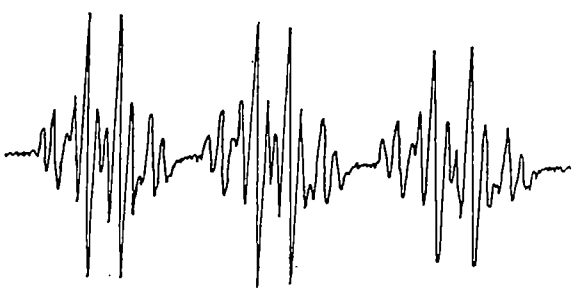


Fig. 2. Electron spin resonance of partially deuterated compound

By comparison with phenyl-*tert*-butyl nitric oxide[4], where the only proton hyperfine splitting arises from the phenyl group, it seems unlikely that coupling takes place with any of the eight nearest alkyl protons.

It appears, therefore, that the coupling is with the ε methyl group, which, sterically, may approach close to the aromatic system containing the unpaired electron. This is a case of hyperconjugation between remote parts of a molecule.

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Hydration of Calcium Sulphate Hemihydrate

The results of experiments carried out in this laboratory seem to indicate that the hydration of calcium sulphate hemihydrate is initiated by the heterogeneous nucleation of crystals of gypsum, and that thereafter fresh nucleation is not important, at least so far as the kinetics are concerned[1].

Hydration in pastes is strongly self-accelerating, with a marked induction period during which reaction is slight. This is followed by a rapidly accelerating reaction, and then a period of decay during which the rate of hydration falls away to zero. The acceleration of the rate has been accounted for as follows[1,2]. The rates of growth and dissolution of the two phases of calcium sulphate in the system may be expected to be proportional to their surface areas. It has been observed that the growing crystals of gypsum in mixtures of calcium sulphate hemihydrate and water are usually in the form of plates or needles, so their surface area will be approximately proportional to the mass of gypsum. Consequently:

$$d\alpha/dt = k\alpha$$

where α is the fraction of calcium sulphate hemihydrate transformed to gypsum, t is the time elapsed after the mixing of the reagents, and k is a constant that takes into account the rate of transfer of calcium sulphate from the dissolving grains of hemihydrate to the growing centres.

$$\therefore\ \alpha = Pe^{kt} \text{ for } \alpha < \alpha_1$$

where P is the effective fraction of gypsum present at the beginning of the reaction, and α_1 is the fraction transformed at the stage when depletion of reactants and mutual interference of the growing centres causes serious deviations from the conditions that lead to an exponential rate of growth. Experimental determinations show that α_1 can be as high as 0·55 (ref. 2).

A complete theory which takes into account the processes leading to the decay of the rate can be derived when it is considered that the rate of growth of gypsum depends on the rate of dissolution of calcium sulphate hemihydrate and that the grains of hemihydrate, unlike the crystals of gypsum, are usually more or less compact geometrically. Consequently the surface area of calcium sulphate hemihydrate will be approximately proportional to the two-thirds power of the mass. Therefore, the rate of hydration may be considered in terms of:

$$d\alpha/dt = k\alpha(1-\alpha)^{\frac{2}{3}}$$

which leads to:

$$F(\alpha) \equiv \tfrac{1}{2}\log\frac{\{1-(1-\alpha)^{\frac{1}{3}}\}^2}{1+(1-\alpha)^{\frac{1}{3}}+(1-\alpha)^{\frac{2}{3}}} - \sqrt{3}\tan^{-1}\left\{\frac{2(1-\alpha)^{\frac{1}{3}}+1}{\sqrt{3}}\right\} = kt + P \text{ for } \alpha_0 < \alpha < 1 \quad (1)$$

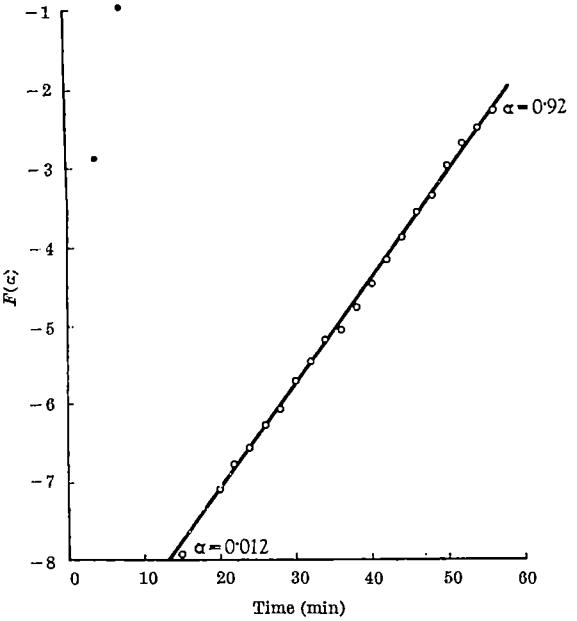


Fig. 1. $F(\alpha)$ plotted against time for 100 g calcium sulphate hemihydrate and 60 ml. water reacting at 20·5° C

Here, P is given by $F(\alpha_0)$, where α_0 is the effective fraction of gypsum at the initiation of hydration. In all these equations k characterizes the self-acceleration of hydration.

Fig. 1 shows $F(\alpha)$ plotted against time for 100 g of finely divided calcium sulphate and 60 ml. water maintained at 20·5° C. The extent of reaction was followed by chemical analysis[2]. It is seen that the kinetics conform very well with equation 1 for experimental results covering more than 90 per cent of the course of hydration.

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Stability of Indanetrione Hydrate Solutions

The maximum yield of the coloured complex formed by the reaction of indanetrione hydrate[1] and a compound containing an amino-group[2] may be obtained when a reducing substance is present in the reaction mixture. Stannous chloride[3], hydrindantin[4] or potassium cyanide[5] may be used as a suitable reducing substance in the reaction mixture, provided the reaction occurs at a controlled pH[3,6].

The presence of dissolved oxygen in the reaction mixture has been reported to produce an oxidation side-reaction[3]. Indanetrione hydrate containing stannous chloride is an efficient reagent mixture but is unstable and deteriorates on storage. A precipitate occurs and the production of this precipitate is hastened when the solution is in contact with atmospheric oxygen. In this laboratory the indanetrione hydrate for the quantitative determination of nitrogen[6] (as ammonia) or α-amino-acids[7] has been prepared in small quantities immediately before use; and consistent colour factors for the α-amino-acids and amino-sugars have been obtained.

Large quantities of the reagent are required for the continuous monitoring of eluates from the automatic amino-acid analysers now in common use. The reservoir for the indanetrione hydrate reagent in the Beckman Spinco amino-acid analyser (model 120) used in this laboratory is protected from atmospheric oxygen by aspirators which may be filled with oil or water. That arrangement has been found not to exclude atmospheric oxygen and a simple procedure has been adopted to overcome the absorption of atmospheric oxygen by the water in the aspirators and also by the indanetrione hydrate solution.

A modified Fieser[8] solution has been added to the water in the aspirators: and the latter are protected from the atmospheric oxygen by connexion to a guard bubbler tube containing the concentrated Fieser solution. Air which replaces the aqueous solution in the aspirator is made to bubble through the strong Fieser solution, while the pump with a two-way valve operates to force the indanetrione hydrate solution into the reaction coil. The concentrated modified Fieser solution contained 1 g sodium anthraquinone β sulphonate, 10 g sodium hydroxide and 10 g sodium hydrosulphite per 100 ml. of the solution. The 4 l. of diluted Fieser solution in the aspirators contained 200 ml. of the concentrated modified Fieser reagent and was red in colour when prepared. The colour of the solution changed slowly to brown as oxygen was absorbed; but the addition of 10 g of sodium hydrosulphate restored the red colour, and served to regenerate the 4 l. of diluted Fieser solution. This procedure may be repeated several times before it is necessary to discard the diluted Fieser solution in the aspirators.

The use of oil in the aspirator bottles will not protect the indanetrione hydrate from the atmospheric oxygen. Fieser solution offers considerable protection for the indanetrione hydrate if the latter is stored for any period.

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Some Double-layer Capacity Measurements on Platinum Electrodes

From a series of investigations of the rest potential on bright platinum electrodes[1] in oxygen-saturated acid solutions, it was suggested that the reversible oxygen potential of 1·229 V (ref. 2) is not ordinarily observed because platinum is not inert to such an electrolyte, and the system never comes to equilibrium. The Pt-O-acid system, then, is a polyelectrode[3], and the rest potential is a mixed potential[4]. It was further suggested that, if an electronically conducting surface which is inert to oxygen-saturated acid solution could be obtained, the reversible potential should be observed because, then, the system would be a simple electrode.

It was found[5] that a platinum surface could be passivated in concentrated nitric acid and that the reversible potential was observed for extended periods of time (more than 24 h). In this work, it was proposed that the nitric acid produced a complete layer of adsorbed oxygen which was an electronic conductor and did not build up with time. In order to throw light on the nature of this passivating film, the following double-layer capacity measurements were made.

A constant current single pulse technique[6] was used in which the differential capacity is determined from the relationship, $C = i/(\mathrm{d}E/\mathrm{d}t)$, where i is the current density and $\mathrm{d}E/\mathrm{d}t$ is the slope of the photographed trace obtained on the oscilloscope. Apparent current density values were used in the calculation of i. The measurements were

carried out in 'Teflon' cells[1] in 2 N sodium sulphate solution stirred with nitrogen to minimize contributions to the capacity from the pseudocapacity[7] of hydrogen or oxygen electrode processes. The preparation of the cell, solutions and electrodes was the same as that described elsewhere[1,5]. All experiments were carried out at a temperature of 25° ± 1° C and the potentials were recorded with respect to a Pt/H_2 electrode in the same solution.

In general, the procedure involved preparing the electrode under a given set of conditions, allowing the potential to come to a given potential under open-circuit conditions, stirring with purified nitrogen for 10 min, and finally pulsing the system from the resting potential. The capacity was determined from the trace obtained on the oscilloscope. It was found by monitoring the exit gas-flow from the cell with a Beckman oxygen analyser that the partial pressure of oxygen over an oxygen-saturated acid solution was reduced to a negligible quantity after bubbling nitrogen through the system for 10 min. The results are presented in Table 1 in which the pretreatment of the electrode, the potential at which the capacity was determined, and the capacity values are given. In all cases, the platinum electrodes remained smooth and mirror-bright.

Table 1. Double-layer Capacity Data on Platinum Electrodes

Experiment	Electrode pretreatment	Potential *vs.* Pt/H_2 (mV)	Double-layer capacity ($\mu F/cm^2$)
a	Clean Pt electrode in N_2 stirred 2N Na_2SO_4 solution	512	18·2
b	Bubbled O_2 over Pt electrode until potential was steady; bubbled N_2 for 10 min	995	29·9
c	Anodized Pt in separate cell; plunged Pt into O_2 saturated 2N Na_2SO_4 solution; bubbled N_2 for 10 min	942	40·8
d	Reduced anodized Pt with H_2 stirring; bubbled O_2 until potential was steady; bubbled N_2 for 10 min.	962	25·6
e	Passivated Pt in HNO_3; placed Pt in O_2 saturated 2N Na_2SO_4 solution; bubbled N_2 for 10 min	1,220	52·4
f	Bubbled H_2 over passivated Pt until H_2 potential was reached; bubbled O_2 until potential was steady; bubbled N_2 for 10 min	1,075	43·0

In experiment (*a*), the solution was stirred with hydrogen until the platinum electrode had reached the hydrogen potential (0 mV versus Pt/H_2), followed by oxygen stirring until the potential had reached 300 mV. Then, oxygen stirring was replaced with nitrogen stirring. After the potential had risen above 500 mV, the differential capacity was determined. This value of the capacity, 18 $\mu F/cm^2$, is considered to be the double-layer capacity of a platinum surface free of adsorbed hydrogen or oxygen, since the potential of 512 mV is above that point (400 mV) where hydrogen is no longer adsorbed[8,9] but below the point (800 mV) where oxygen is first adsorbed[8,10]. This capacity value is in agreement with that found by Wrotenbery *et al.*[11] in 2 N sulphuric acid, Banta and Hackerman[12] in 6 N sulphuric acid, and Laitinen and Enke[13] in 1 N perchloric acid in this potential range.

A platinum electrode free of adsorbed oxygen was stirred with oxygen until a steady rest potential was reached in experiment (*b*). After the oxygen stirring was replaced with nitrogen stirring for 10 min, the capacity was determined. Here the capacity value increased to 30 $\mu F/cm^2$.

Experiment (*c*) describes the case in which the capacity was determined on an anodized platinum electrode in a solution free of peroxide. This was accomplished by using two cells as described before[1,5]. After the electrode was prepared in one cell and the solution in the second, the electrode was removed from the first and plunged into the peroxide-free, oxygen-saturated solution in the second cell. As before, the capacity was determined after the system had been stirred with nitrogen for 10 min. A further increase in the capacity to 41 $\mu F/cm^2$ was found. When the anodized electrode was reduced with hydrogen stirring in experiment (*d*) and the conditions of experiment (*b*) repeated, the capacity was lowered again.

It appears that the differential capacity increases as the amount of oxygen adsorbed on the platinum surface increases. Although anodization does not build the adsorbed oxygen layer beyond a monolayer thick[8], it does convert some Pt-O sites to PtO_2 sites[14]. An explanation for the increase in capacity with an increase in the amount of adsorbed oxygen may include the possibility that Pt-O sites with a dielectric constant near that of a metal (the Pt-O layer[1] is an electronic conductor) replace $Pt\text{-}H_2O$ sites with a much lower dielectric constant, or that the effective thickness of the double layer is reduced by the presence of the Pt-O layer. These observations are opposite to those observed on gold[15] where the capacity decreases as oxygen adsorption increases. The oxygen layers on gold are not considered to be good electronic conductors[16], and an explanation which is the reverse of that for platinum may be considered.

A platinum electrode was passivated in concentrated nitric acid as described before[5] and placed in oxygen-saturated, peroxide-free 2 N sodium sulphate solution. After the system was stirred with nitrogen for 10 min, the capacity was determined in experiment (*e*). According to the previous experiments, the large capacity value of 52 $\mu F/cm^2$ would indicate a high coverage of the surface with oxygen, as suggested before[5]. The appearance of the reversible oxygen potential is in agreement with this contention.

When this electrode system was stirred with hydrogen until the hydrogen potential was reached, in experiment (*f*), neither the potential nor the capacity came to the same value as found in experiment (*b*) when the conditions of experiment (*b*) were used. It was found that both were higher. Repeated treatment with hydrogen stirring, and even with cathodic polarization, does not return to the nitric acid-treated platinum surface the properties of the untreated platinum surface. However, since the reversible potential is not observed, the oxygen layer in experiment (*f*) is probably no longer a complete layer. Apparently, the nitric acid treatment[5] produces a definite change in the nature of the platinum surface. By heating the platinum electrode in a hydrogen flame to white heat, it will then exhibit the properties of the untreated platinum surface.

A possible explanation may reside in the formation of an alloy of platinum and oxygen atoms by the action of the nitric acid. Kalish and Burshtein[17] have shown that massive platinum can dissolve oxygen. Such an alloy would most likely have the properties of a metal and would not easily be reduced by cathodic polarization or by reduction with hydrogen. However, heating the alloy to a white heat could drive out the oxygen. Supporting evidence is obtained from oxygen over-voltage measurements made on these electrodes (results to be published elsewhere). Because the formation of such an alloy would most likely require atomic and molecular rearrangements, it could explain the necessity for the platinum to be in contact with nitric acid for at least 72 h to form the passivating film[5].

The values of capacity found on the nitric acid-treated electrodes are near those found on anodized, untreated electrodes. This favours the idea that the passivating layer on the treated electrodes is not much thicker than a monolayer. Wrotenbery *et al.* found similar high values for the capacity of nitric acid-treated platinum electrodes in this potential region.

These results support the earlier contention[1,5] that the reversible oxygen potential may be observed on a platinum electrode only if it is covered by a complete, electronically conducting layer of adsorbed oxygen, and that, in agreement with the findings of Giner[18], the potential of a Pt/O_2 electrode becomes more noble with increasing amounts of adsorbed oxygen. The observed fact that the capacity increases with increasing amounts of adsorbed oxygen is

compatible with the concept of an electronically conducting layer of adsorbed oxygen on the platinum surface.

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BIOCHEMISTRY

Caffeine Metabolism

SOME plants contain organic compounds of potent physiological action on the animal organism; but as far as is known no biological function in the plants themselves. Are they waste end-products of metabolism? Are they the result of detoxification mechanisms? Are they produced to discourage attack by insects and animals? Or are they mere 'accidents' or by-products of faulty metabolism—or perhaps even the results of evolutionary biochemical experiments not yet discarded?

The use, in biological investigations, of carbon-14 produced in nuclear bomb testing has been discussed in earlier communications[1–3]. We have used this effect to examine the caffeine metabolism in the commercial tea plant (*Camellia thea*). Commercial tea is particularly suitable for such an investigation, since it is derived from buds, less than two weeks old, of a tree many years old.

A sample of commercial tea picked in Ceylon in September 1960 was separated into caffeine and water-extracted leaves. Both the samples were converted to carbon dioxide and counted in the New Zealand Institute of Nuclear Science low-level carbon-14 counter. The $^{12}C/^{13}C$ ratio of each sample was measured in a mass spectrometer so that the results could be corrected for any isotope effect in the metabolism of the tree or any possible fractionation in the laboratory procedures. The results are given in Table 1.

Table 1. PARTS PER THOUSAND $\Delta^{14}C$ VALUES FOR CAFFEINE AND EXTRACTED BUDS OF *Camellia thea*

	Caffeine	Extracted buds
Count rate	100·08 $\sigma = 0{\cdot}35$	104·05 $\sigma = 0{\cdot}36$
$\delta^{13}C$ with respect to P.D.B. standard (parts per thousand)	−26·6	−25·4
$\Delta^{14}C$ parts per thousand*	194·6 $\sigma = 4{\cdot}7$	239·0 $\sigma = 4{\cdot}7$

$\Delta^{14}C$ of atmosphere before nuclear bomb tests = −20 parts per thousand.
σ = standard deviation of counting.
* $\Delta^{14}C$ expressed as parts per thousand enrichment above 0·95 of National Bureau of Standards oxalic acid standard and normalized for isotopic fractionation to 0·975 of P.D.B. Chicago belemnite standard.

The caffeine activity is significantly lower than that of the leaves, showing that it was not synthesized in the leaves but that it, or its precursors, were transported into the young leaves.

Although the caffeine is lower in activity than the buds, it is much higher than the atmosphere before nuclear bomb testing ($\Delta^{14}C$ = −20 per cent). No results are available for the change in carbon-14 specific activity of the atmosphere as a function of time at the latitude of Ceylon, but it might be expected to lie between that of the southern[1] and northern[5] hemispheres, the rates of increase of which had become parallel about 1958. In 1960 the specific activity of the southern hemisphere atmosphere was rising at about 6 parts per thousand per month and reached $\Delta^{14}C$ = 195 parts per thousand early in 1960 (ref. 4).

It is concluded that the caffeine corresponds to the atmosphere approximately half a year before the growth of the buds. Since the *Camellia thea* plant is some years old (7–90 years) before commercial production, it follows that caffeine is not a metabolic end-product and that it has a metabolic half-life not longer than a few months. This suggests that caffeine is involved in active metabolism.

We thank the Institute of Nuclear Science for counting the samples, and the New Zealand University Grants Committee for financial support.

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Separation of Cerebroproteins of Human Brain

THE correlation of protein chemistry with complex functions of the brain has been hampered by the lack of basic information on the brain proteins. Extraction of brain proteins by techniques available so far has yielded usually less than 20 per cent of the total protein content of brain, and only up to 14 components have been demonstrable by electrophoresis[1]. Recent work in this laboratory has been directed to the definition of more quantitative methods for the extraction of brain proteins and for more adequate separation, physicochemical characterization, and histological localization by immunochemical methods[2]. We have observed that through a combination of mild buffered extraction, column chromatography on DEAE-cellulose and analysis of the carbohydrate content of each effluent tube, the presence of not less than 50 major chromatographic fractions can be distinguished (Fig. 1). Acrylamide gel-electrophoresis of each of these major fractions shows each to be heterogeneous. Accounting for overlap between chromatographic fractions, a minimum of 100 cerebroproteins from human grey matter have thus been observed. The total yield of protein in these experiments is 6·1 g/100 g wet wt. grey matter—a figure in range for previous figures for total unfractionated brain protein[3]. The previously known partially characterized protein fractions of brain[1], together with the contribution by serum proteins from blood remaining in small vessels of grey matter, are not likely to account for more than a small percentage of this total yield. Thus, the bulk of the proteins here demonstrated apparently have not been separated or examined previously.

In preliminary experiments 70 g of human grey matter, carefully dissected from autopsy specimens which had been stored at −20° C until extraction, are homogenized either in a Potter–Elvehjem homogenizer (5° C) or in a pre-cooled Waring Blender for 3 min in the cold room, with 100 c.c. of 0·005 M phosphate buffer, *p*H 7, then centrifuged at 80,000 *g* for 30 min in a Beckman model *L*-2 ultracentrifuge. The insoluble residue is rehomogenized with a further 100 c.c. of phosphate buffer and centrifuged, and the second soluble extract combined with the first. The soluble proteins thus obtained (extract *A*) account for 42 per cent of the total protein recovered.

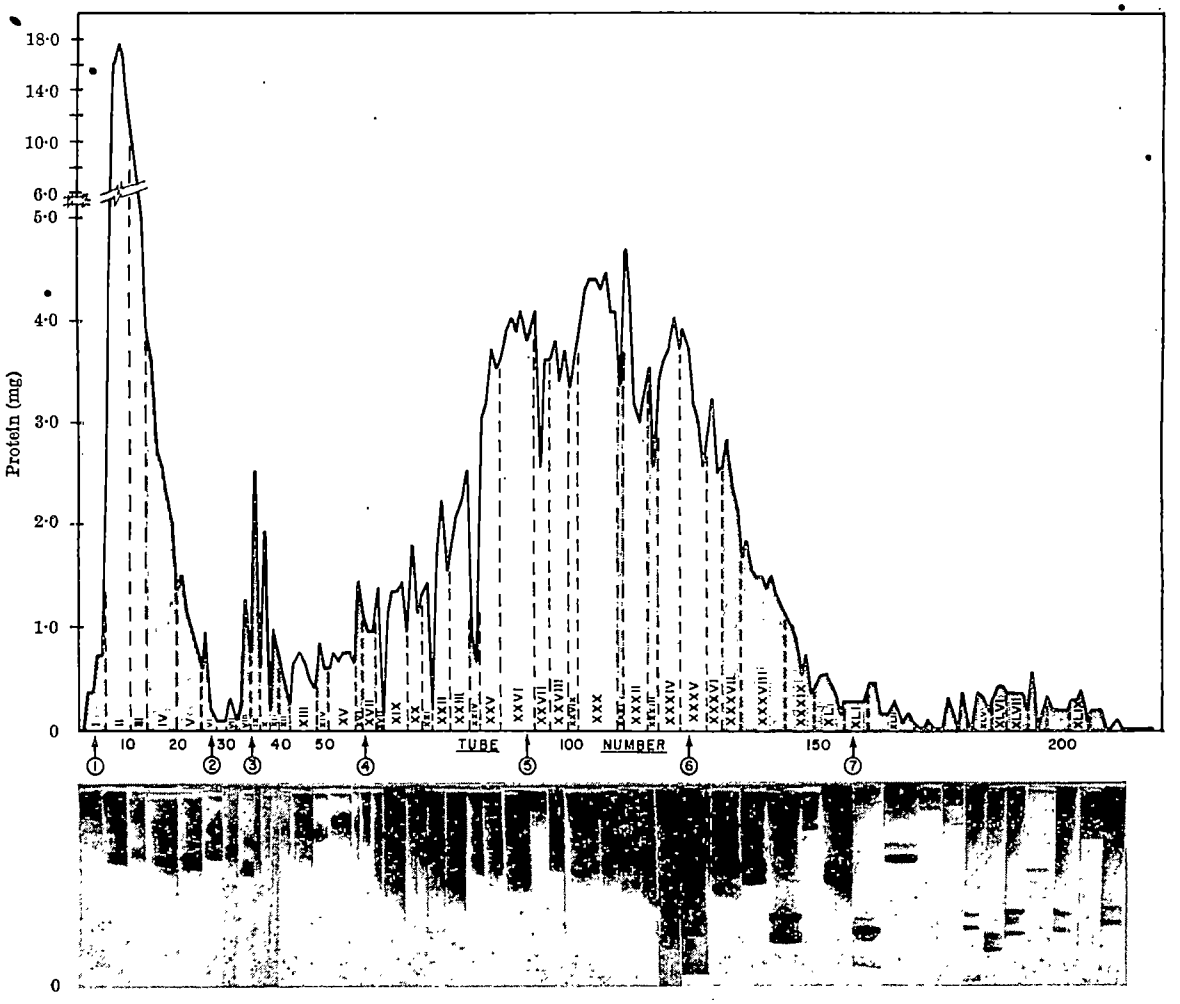


Fig. 1. Chromatography and electrophoresis of cerebroproteins of human brain. Cerebroprotein fractions are designated I–L. Shaded area represent cerebroprotein fractions with hexose content of 2·5 per cent or greater. Below each fraction is a photograph of the respective electrophoretic pattern. Numbered arrows on abscissa indicate points at which new solvents were introduced into automatic mixing chamber: (1) 75 c.c. of 0·005 M sodium phosphate buffer, *p*H 7; (2) mixture of 12·5 c.c. 0·005 M sodium phosphate buffer, *p*H 7, and 12·5 c.c. 0·05 M sodium phosphate buffer, *p*H 4·7; (3) 75 c.c. 0·05 M sodium phosphate buffer, *p*H 4·7; (4) mixture of 50 c.c. 0·05 M sodium phosphate buffer, *p*H 4·7, and 50 c.c. of 0·3 M sodium phosphate buffer, *p*H 4·3; (5) 100 c.c. 0·3 M sodium phosphate buffer, *p*H 4·3; (6) 100 c.c. 1·0 M sodium phosphate buffer, *p*H 4·1; (7) 100 c.c. 1·0 M sodium phosphate buffer, *p*H 1·0

Extraction of the residue with 0·5 M sodium chloride in 0·005 phosphate buffer, *p*H 7, yields another 6·9 per cent of the total protein recovered (extract *B*). The residue remaining after this extraction is then extracted with 0·3 per cent 'Triton *X*–100'[4] in 0·005 M phosphate buffer, *p*H 7, yielding only a further 15·8 per cent of the total recovered (extract *C*). The remaining residue has been separated into four distinct residues (residues 1–4) accounting for the remaining 35·9 per cent of the total recovered.

Extract *A* was dialysed for 4 h at 4° C against 4 l./0·005 M phosphate buffer with constant magnetic stirring of the dialysate, then concentrated approximately ten-fold against 'Carbowax' (20 M polyethylene glycol) for approximately 4 h in the cold to a final volume of dialysand between 15 and 20 c.c. This concentrate was then dialysed once more against 4 litres of phosphate buffer at 4° C with stirring. On completion, the volume of the dialysand was noted, an aliquot was taken for total protein analysis, and 15 c.c. was placed for fractionation in the cold room (4° C) on a DEAE-cellulose column 2·5 × 11·0 cm, which had been equilibrated with 0·005 M sodium phosphate buffer[5]. By the use of an automatic mixing chamber containing a constant 150 c.c. of solution, the solvent changes were made according to the schedule given in the legend for Fig. 1. Three c.c. aliquots of effluent were collected by means of an automatic fraction collector. Each tube was quantitatively analysed for protein[6], hexose[7], hexosamine[8], and neuraminic acid[9]. Where inadequate quantities were present in a single tube, 2–5 tubes were combined, lyophilized, made up to suitable volume and analysed for the carbohydrate constituents. Amino-acid analysis was performed by means of an automatic amino-acid analyser. Acrylamide disk gel-electrophoresis was performed at *p*H 9·4 (ref. 10).

Fig. 1 shows the chromatographic pattern obtained in terms of tube-to-tube protein content. The distribution suggests several relatively discrete peaks, but the presence of frequent sub-peaks makes it difficult to assess by this method alone how many protein fractions are actually represented. The tube-to-tube analysis for hexose, hexosamine and neuraminic acid of these protein fractions has here been used as one index of discreteness or non-discreteness of what appears to be a homogeneous peak on column chromatography, and sections or 'cuts' of the plotted protein values are made where gross changes in tube-to-tube carbohydrate content appear. On the basis of this combination of column chromatography and quantitative carbohydrate analysis, at least 50 discrete cerebroprotein fractions may be discerned, which are here denoted by Roman numerals I–L.

Recovery of protein from the column is 92–96 per cent. The peaks and the fractions are highly reproducible (within 2 tubes) from brain to brain, but show variation in relative amounts in different brains. The cerebroprotein

fractions in different brains also appear so far to be consistent in terms of high and low carbohydrate content respectively, as shown by the shaded and non-shaded areas respectively of Fig. 1, but the absolute amount of carbohydrate in each fraction varies. These variations may be due to age, pathological changes, post-mortem change, functional states at death, or individual variability, and these, as well as other possibilities, must be explored in future investigations.

Examination of each of the 50 cerebroprotein fractions by means of disk gel-electrophoresis reveals that each contains between two and eleven electrophoretically clearly separated protein zones or sub-fractions (Fig. 1). Allowing for overlap, a minimum of 100 cerebroproteins can be distinguished. Whether some of these differ only in molecular size, only in a few amino-acid residues, only in a few attached carbohydrate groups, or in some other minor structural way, cannot be ascertained at this point, but they differ sufficiently to be separable by means of electrophoresis.

It may be noted that the carbohydrate content frequently, although not invariably, tends to increase as one passes from the first to the last tube of a given chromatographic peak. For example, proceeding from cerebroprotein fraction II to VI, the hexose increases from 0·2 per cent to 6·6 per cent. Two areas in these first chromatographic fractions were analysed for constituent amino-acids in hydrolysed samples. The residue of amino-acids per thousand for almost all the amino-acids was similar or identical with two marked exceptions: serine and glutamic acid. Thus, comparison of the two cerebroprotein fractions showed one to have twice as many serine residues per thousand (123·3 compared with 62·9), and 50 per cent more glutamic acid residues per thousand (75·1 compared with 50·7) as the other. Since there is considerable evidence in other glycoproteins that these two amino-acids are involved in the linkage of carbohydrates to proteins, this variability in only carbohydrate content and a few amino-acid residues is of considerable interest and will be examined further.

In addition to specific isolation and characterization investigations of these human cerebroproteins, specific antisera are being prepared for immunological tests of homogeneity, and for the demonstration of the histological localization in cell types (neurones or glia), and in different architectonic layers of the cerebral cortex. The use of histochemical methods for the assessment of enzyme activities in each of these protein fractions has also begun, has been shown by us to be applicable to the esterases, and will be carried out with other enzymes as well.

These methods permit a more thorough examination of the biosynthesis of brain proteins[11], and offer an opportunity not previously available for the investigation of a broad range of neurologic and psychiatric disorders with regard to the possible implication of some of these proteins in pathological processes.

All the data in Fig. 1 pertain to extract *A* only. These discernible and separable proteins represent only 42 per cent of the total prepared by us from grey matter alone. It is clear that years of work lie ahead for many laboratories in terms of the further physical and chemical definition of species of proteins in human and other mammalian brain. The occurrence of such a multitude of individual proteins may not seem unreasonable when the complex functional requirements of the brain, such as those pertaining to the specificity of interneuronal transmission and the need for specificity in such functions as memory[12], are taken into account. The specificity of coding in the amino-acid chains of proteins has elsewhere been shown to be fundamental to specificity of function, as in the case of hormones, and in antigen–antibody reactions; and, as pointed out before[2], the type and sequence of carbohydrate units of the glycoproteins and amino-glycolipids of brain offer further opportunities for molecular specificity.

This work was supported by grants from the National Science Foundation, the National Institutes of Health, U.S. Public Health Service, and the Scottish Rite Freemasons of the Northern Jurisdiction, United States.

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Sialic Acid-deficient α_1-Acid Glycoprotein produced in Certain Pathological States

THE seromucoid fraction, one of the 'acute phase reactants', has been shown by many investigators to be increased significantly in the blood in a large number of unrelated disease states. This protein fraction[1] is known to contain as major component α_1-acid glycoprotein[2] or orosomucoid[3] and several minor components including haptoglobin, 3*S* α_1-glycoprotein and Zn- and Ba-α_2-glycoproteins[4,5]. The question has now been investigated whether α_1-acid glycoprotein derived from pathological plasma is identical to or different from that prepared from normal blood.

α_1-Acid glycoprotein was isolated by a two-step procedure from certain blood specimens. First, the sera were fractionated according to a modification of the Cohn method 10 (ref. 6). Second, the resulting fractions VI[2] were chromatographed on 'Amberlite *IRC*-50'[7] yielding α_1-acid glycoprotein preparations which appeared homogeneous on paper and starch-gel electrophoresis at *p*H 8·6. Sialic acid was determined by the techniques of Warren[8]. The seromucoid fraction, prepared and analysed according to Winzler[9], served as control to demonstrate the changes of the glycoproteins level of the plasma.

Two representative series of experiments are reported here.

An apparently healthy male, 66 years old, underwent an arterial graft operation. No post-operative complications were observed. As expected, the concentration of the seromucoid fraction rose rapidly and, thereafter, decreased gradually (Table 1). The amount of fraction VI the composition of which is similar to, but not identical with, that of the seromucoid fraction, and α_1-acid glycoprotein isolated from the three blood specimens followed a similar course. The sialic acid content of these three protein preparations, however, was constant and equal to that of normal α_1-acid glycoprotein.

Table 1. SIALIC ACID CONTENT OF α_1-ACID GLYCOPROTEIN DERIVED FROM SERUM OF A PATIENT WHO UNDERWENT AN ARTERIAL GRAFT OPERATION

Blood taken	α_1-Acid glycoprotein		Fraction VI (mg %)	Seromucoid (as Biuret) (mg %)
	Sialic acid content (%)	Blood level (mg %)		
1 day pre-op.	11	70	130	84
2 days post-op.	11	120	210	127
12 days post-op.	11	80	194	114
Normal values	11	45	110	61 ± 8

Sera from certain patients suffering from various chronic diseases revealed the anticipated increased seromucoid levels (Table 2). Again, the blood concentration of fraction VI and α_1-acid glycoprotein was shown to be elevated about equally. It was of interest to note, however, that the sialic acid content of many of these α_1-acid glycoprotein preparations was significantly lower than that of the normal glycoprotein. In certain cases values of approximately 50 per cent of the normal were observed.

Table 2. Sialic Acid Content of α_1-Acid Glycoprotein derived from Sera of Patients suffering from Certain Chronic Diseases

Diagnosis	α_1-Acid glycoprotein Sialic acid content (%)	α_1-Acid glycoprotein Blood level (mg %)	Fraction VI (mg %)	Seromucoid (as Biuret) (mg %)
Hodgkins' disease	8·3	119	182	121
Psochiaric arthritis	8·3	205	260	147
Diabetes	6·3	108	203	98

From these results it might be concluded that normal individuals subjected to an intense but temporary stress produce the expected augmented quantity of normal α_1-acid glycoprotein. In contrast, patients with certain chronic diseases who have probably produced α_1-acid glycoprotein in abnormally large amounts over a long period of time form an α_1-acid glycoprotein that lacks sialic acid to varying degrees.

One of us (M. D.-S.) thanks the Centre National de la Recherche Scientifique, Paris, for a fellowship. This work was supported by grants from the National Institutes of Health, U.S. Public Health Service (*GM*-10374 and *AI*-2392), and the National Science Foundation (*GB*-611).

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Lysozyme Content of Human Milk

Lysozyme has been detected in the milk of donkey, mare, bitch, sow and cow[1,2]. In order to investigate the possible biological significance of lysozyme an investigation is planned here to compare physical, chemical and physiological characteristics of the lysozymes isolated from bovine and human milk. Although several workers[1–4] have reported the presence of lysozyme in human milk and colostrum, not much work has been done to obtain information on the quantitative amounts of the enzyme in this milk. In the work recorded here lysozyme contents of a large number of bovine and human milk samples were determined. In an earlier publication[2] the lysozyme content of bovine milk was reported to vary between 0·0 and 260·0 μg with an average of 13 μg of the enzyme activity per 100 ml. of milk, using egg-white lysozyme (Difco) as the standard. This report deals with the lysozyme content of human milk and colostrum, and the effect of storage and heat on the enzyme.

The samples of milk and colostrum were collected from the maternity wards of the University Medical College and the local hospitals and held frozen until they could be assayed. The milk was assayed for lysozyme by the method of Smolelis and Hartsell[5], as modified and adapted to milk[2]. The modified procedure involved the dilution of the milk 1,000–3,000-fold with water, mixing it with an equal volume of a substrate consisting of a suspension of dead cells of *Micrococcus lysodeikticus* (Difco) and observing the extent of lysis spectrophotometrically. The details of the method are described in our earlier publication[2].

The lysozyme contents of 105 samples of human milk and 12 samples of colostrum, summarized in Table 1, indicated that human milk and human colostrum contain lysozyme in similar concentrations. This was also observed in the case of bovine milk and bovine colostrum in our previous investigation. However, the results did not bear out the possibility that colostrum might possess higher lysozyme titres than the post-colostrum milk, since it is known to contain natural immunological factors.

The lysozyme content in human milk ranged from 3 to 300 mg/100 ml. with an average of 39 mg/100 ml., or about 3,000 times higher than that of bovine milk. The results presented in Table 1 represent 117 samples and 105 subjects. Jollès and Jollès[3] reported the lysozyme content of human milk as 4 mg/100 ml. The numbers of samples and subjects in their investigation were not reported, however.

During the course of this work it was observed that the lysozyme activity of human milk was best preserved when the milk was held frozen. However, frequent thawing and refreezing of the milk inactivated the lysozyme activity considerably. It was further observed that human milk when not previously frozen could be held at room temperature or even at 30° C without any significant loss of activity up to 3 or 4 days or until the milk spoiled. In this regard it differed widely from bovine milk, which lost its lytic activity completely within 8 h at 30° or 37° C when the milk spoiled. These results are in accordance with the observations of Seleste[4], who has pointed out the remarkable keeping quality of the enzyme in human milk and of human milk itself. It is conceivable that the rapidly growing microflora contributing to the spoilage of cow's milk inactivate its lytic factor, and that the initial low bacterial content and higher *p*H of human milk do not permit a rapid bacterial multiplication and thereby provide the inherent high keeping quality of lysozyme in human milk and of human milk itself.

Table 1. Lysozyme Contents of Human Milk and Colostrum

Sample	No. of samples	Lysozyme (mg/100 ml. milk) Minimum	Maximum	Average
Human milk	105	3	300	39
Human colostrum	12	9	102	46

Table 2. Effect of Heat-treatments on Lysozyme Activity of Human Milk at its Natural *p*H

Sample No.	Lysozyme concentration (mg/100 ml.) Before heating	62° C for 30 min	After heating 71° C for 15 sec	71° C for 15 min	71° C for 30 min	82° C for 15 sec	82° C for 15 min	82° C for 30 min	Momentarily boiling	Autoclaving 15 lb./in.², 20 min
1	18·0	4·4	12·0	0·4	0·0	9·6	0·1	0·0	1·0	0·0
2	12·0	8·8	9·6	5·0	3·2	7·4	1·8	0·4	6·2	0·0
3	4·2	2·4	2·6	1·4	0·8	2·4	1·0	0·0	1·4	0·0
Average % destruction		47·0	30·0	74·0	88·0	43·0	87·0	99·0	68·0	100·0

In order to determine the heat stability of the lytic factor(s) in milk, three fresh human milk samples varying widely in lysozyme activity were heated at 62°, 71° or 82° for varying lengths of time, and the lytic activity of milk before and after these heat treatments was determined. The results showing the percentage destruction of the enzymatic activity by the heat treatments are presented in Table 2.

A fairly wide variation was observed in the heat stability of the lysozyme of the three samples of human milk. An average of 47 per cent of the lysozyme activity was lost when the milk was heated at 62° C (143° F) for 30 min, the conventional batch pasteurization process for milk, and 30 per cent was lost at 71° C (160° F) for 15 sec, the conventional high-temperature, short-time pasteurization procedure for milk. Heating at 82° C for 15 sec lowered the enzyme activity by 43 per cent, and momentary boiling caused 68 per cent loss of the enzymatic activity. When the milk was autoclaved at 15 lb./in.^{2} for 20 min, a complete inactivation of the lysozyme was observed. In regard to heat stability the human milk lysozyme closely resembled bovine milk lysozyme.

The human milk lysing factor was found to be more resistant to heat at acid *p*H than at higher *p*H levels, being most stable at *p*H 3 and 4. At *p*H levels 1, 2, 3, 4 and 5 complete inactivation of the enzyme resulted after boiling for 38, 92, 164, 142 and 60 min, respectively; at *p*H 6, 7 and 8 all the enzyme activity was destroyed by boiling only for 4, 1 and 0·1 min, respectively. No lysozyme activity was observed in human milk adjusted to *p*H 9, 10, 11 or 12.

The lysing factor of human milk appears to be a lysozyme since it lyses *Micrococcus lysodeikticus* cells and is fairly stable to heat, especially at acid *p*H, as is the egg-white lysozyme.

Lysozymes from both human and bovine milk have been isolated in a pure and homogeneous form using the method of Jollès and Jollès[5] with several modifications. The results of that investigation have been presented elsewhere[6].

This work was supported by a grant from the U.S. National Institutes of Health (*HD*–00858–01). We thank Drs. Morgan and Ebers for assistance in collecting human milk samples.

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A Reaction of Pyridoxal with C-3 Unsubstituted Indoles

The formation of a red pigment from indole and pyridoxal interferes in the enzymatic determination of L-tryptophan[1]. On the basis of the following evidence, it is proposed that the pigment has the structure II*a* shown in Fig. 1 and is therefore analogous to the roseindoles of Fischer[2].

Since C-3 is the most reactive position in the indole ring in electrophilic substitution[3] and since pigment formation is inhibited by dimedone[1], a condensation between the aldehyde group of pyridoxal and C-3 of indole was considered as a possible reaction in the production of the red pigment.

Aldehydes condense with C-3 unsubstituted indoles to give products analogous to the triphenylmethanes. Oxidation of the initially-formed leuco base (I) gives a yellow base with a system of conjugated double bonds (II). Salts of base II are intensely coloured, due to resonance; the positive charge is distributed between both nitrogen atoms in two identical mesomeric structures. The roseindoles, from the condensation of benzaldehyde with indoles (R_1 = phenyl)[2], are a classical example of this type of compound.

II*a*, II*b*, II*c* and II*d* have been prepared as the free bases and as the hydrochlorides. The hydrochlorides were synthesized by passing dry hydrogen chloride into a dry ethereal solution of the free base, whereupon the pure hydrochloride was immediately precipitated in quantitative yield. Under absolutely dry conditions, with protection from atmospheric moisture, the dihydrochlorides were formed. This confirms the presence of two basic nitrogen atoms in the molecule. In the atmosphere the dihydrochlorides rapidly lost one molecule of hydrochloric acid to become monohydrochlorides, which were recrystallized from ethanol/ether.

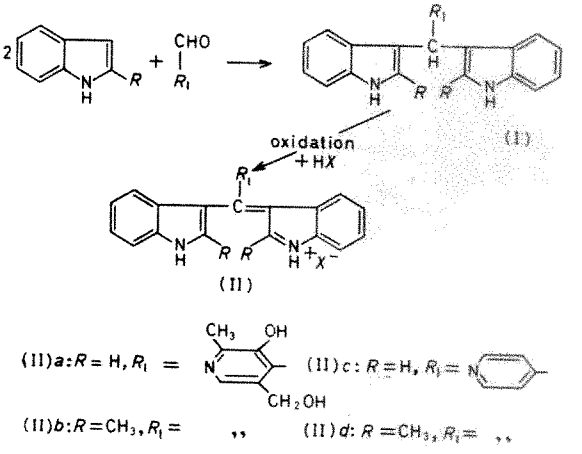


Fig. 1

Since the uroroseins (R_1 = H)[4] and the roseindoles (R_1 = phenyl)[2] form stable monohydrochlorides, the positive charge is probably shared equally between both pyrrole nitrogen atoms in the monohydrochlorides of the indolinylidene–indolyl–pyridylmethanes.

On recrystallization, the free bases stubbornly retained some solvent. When dried at 100°, the carbon values were 2–3 per cent too low. After drying at 150°, the combustion analyses gave values in agreement with those calculated.

Although compound II*a* is formed in the reaction of pyridoxal with indole in 2 N hydrochloric acid, the chief product is an unidentified yellow compound. In 25 per cent trichloroacetic acid, that is, the conditions under which II*a* was first observed, the yield of II*a* is about 70 per cent.

Satisfactory melting points of the pyridoxal compounds were not recorded. They decomposed at indefinite temperatures, depending on the rate of heating.

That all four compounds possess the same chromophore is confirmed by comparison of their visible and ultraviolet spectra. The free bases show a typical indole maximum in the region 250–292 mμ, which shows little change in acid. A shoulder in the region 330–360 mμ becomes a slight peak in acid. The absorption at 430–460 mμ, responsible for the yellow colour of the free bases, exhibits a marked red shift of about 100 mμ and an increase in intensity under acid conditions. Spectra were made by hand on a Unicam *SP*.500 spectrophotometer.

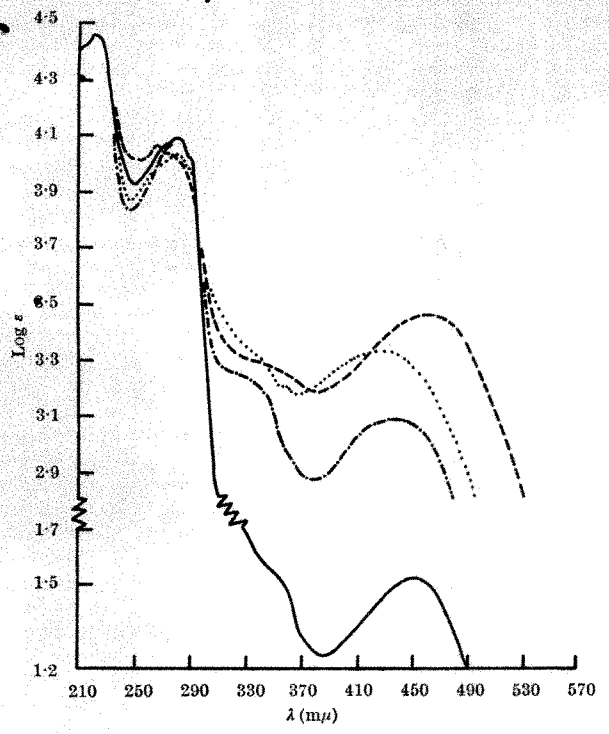


Fig. 1. Ultra-violet and visible absorption spectra of 3-indolinylidene-3-indolyl-4-pyridyl-methanes. Free bases in ethanol. —·—, IIa; ...., IIb; —, IIc; – – –, IId

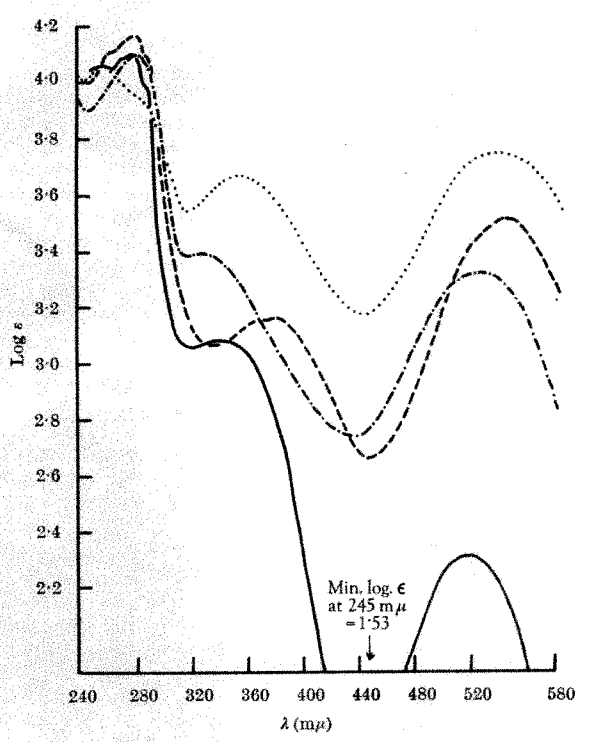


Fig. 2. Ultra-violet and visible absorption spectra of 3-indolinylidene-3-indolyl-4-pyridyl-methanes in 1 N hydrochloric acid. —·—, IIa; ...., IIb; —, IIc; – – –, IId

3-*Indolinylidene*-3-*indolyl*-4-(2-*methyl*-3-*hydroxy*-5-*hydroxymethylpyridyl*)*methane* (IIa): 33·4 mg of pyridoxal and 46·8 mg of indole were dissolved in 400 ml. of 2 N hydrochloric acid and allowed to stand two days at room temperature. The red solution was extracted with ether until the ether washings were negative for indole (*p*-dimethylaminocinnamaldehyde reagent[1]). The aqueous hydroxide solution was then made alkaline with conc. ammonium and the yellow pigment extracted with ether. After washing twice with water, the ether solution was dried over anhydrous sodium sulphate and gently blown to dryness in a stream of nitrogen. The remaining solid was recrystallized from ether/petrol ether (40°–60°). The yield was 20 mg (26 per cent). Free base: calc. for $C_{24}H_{19}O_2N_3$, C, 75·53; H, 4·98; N, 11·02 per cent; found, C, 75·41; H, 5·17; N, 10·98 per cent. Hydrochloride: calc. for $C_{24}H_{19}O_2N_3$.HCl, C, 68·98 ; H, 4·78 ; N, 10·06; Cl, 8·50 per cent; found, C, 68·90; H, 5·01; N, 9·93; Cl, 8·47 per cent.

3-(2-*methyl*-*indolinylidene*)-3-(2-*methyl*-*indolyl*)-4-(2-*methyl*-3-*hydroxy*-5-*hydroxymethyl pyridyl*)*methane* (IIb): 33·4 mg of pyridoxal and 52·4 mg of 2-methylindole were dissolved in 400 ml. of 2 N hydrochloric acid. The procedure was exactly as for IIa. The yield was 70 mg (81 per cent). Free base: calc. for $C_{26}H_{23}O_2N_3$, C, 76·47; H, 5·39; N, 10·29 per cent; found, C, 76·37; H, 5·70; N, 9·92 per cent. Hydrochloride: calc. for $C_{26}H_{23}O_2N_3$.HCl, C, 70·03; H, 5·38; N, 9·42; Cl. 7·96 per cent; found, C, 69·98; H, 5·40; N, 9·46; Cl, 7·96 per cent.

Table 1. Extinction Coefficients of 3-Indolinylidene-3-indolyl-4-pyridyl-methanes

	Free bases in ethanol			
	λ_{max} (mμ)	log ε	λ_{max} (mμ)	log ε
IIa	280	4·07	440	3·08
IIb	282	4·03	430	3·33
IIc	282	4·08	450	1·52
IId	280	4·03	460	3·47
	In 1 N hydrochloric acid			
IIa	282	4·10	530	3·32
IIb	260	4·06	540	3·74
IIc	282	4·10	540	2·32
IId	282	4·15	550	3·52

3-*Indolinylidene*-3-*indolyl*-4-*pyridylmethane* (IIc): 2·19 g of indole and 1·0 g pyridine-4-aldehyde were dissolved in 4 ml. of glacial acetic acid and allowed to stand two days at room temperature. After this time the mixture was deep purple. Water was added until precipitation just started and then ammonia until the mixture was just alkaline. The copious yellow precipitate was collected by filtration, washed with water and dried *in vacuo* over phosphorus pentoxide. The compound could be purified by precipitation from ether or ethyl acetate with petrol ether (40°–60°) to give an amorphous product, or by slow recrystallization (2 days) from ethanol. Both forms had the same melting-point: 155° (uncorr.). The yield was 2·7 g (90 per cent). Free base: calc. for $C_{22}H_{15}N_3$, C, 82·24; H, 4·65; N, 13·08 per cent; found, C, 82·10; H, 4·90; N, 13·00 per cent. Hydrochloride: melting point 205° (uncorr.): calc. for $C_{22}H_{15}N_3$.HCl, C, 73·56; H, 4·47; N, 11·74; Cl, 9·93 per cent; found, C, 73·20; H, 4·51; N, 11·76; Cl, 9·78 per cent.

3-(2-*Methyl*-*indolinylidene*)-3-(2-*methyl*-*indolyl*)-4-*pyridylmethane* (IId): 2·54 g of 2-methylindole and 1·0 g of pyridine-4-aldehyde were dissolved in 2 ml. of glacial acetic acid and allowed to stand 2 days at room temperature. The mixture became deep purple and there was a thick deposit of colourless crystals. The whole was diluted with two volumes of ethanol and filtered; the crystalline deposit remained undissolved. The coloured filtrate was neutralized with conc. ammonium; a yellow precipitate was formed, which was collected by filtration and recrystallized from ethanol/petrol ether (40°–60°). The yield was 0·5 g (18 per cent). Free base: melting point 160°–161° (uncorr.): calc. for $C_{24}H_{19}N_3$, C, 82·52; H, 5·44; N, 12·03 per cent; found, C, 82·20; H, 5·48; N, 11·89 per cent. Hydrochloride: melting point 215° (decomp.): calc. for $C_{24}H_{19}N_3$.HCl, C, 74·70; H, 5·18; N, 10·98; Cl, 9·20 per cent; found, C, 75·00; H, 5·25; N, 10·50; Cl, 9·13 per cent.

The combustion analysis of the colourless, crystalline substance from this preparation indicates that it is a leuco compound of type I, that is, 4-(3,3′-di-2-methylindolylmethyl)-pyridine. It was recrystallized from acetone.

Melting point 249°–250° (uncorr.). The yield was 2·4 g (74 per cent). Calc. for $C_{24}H_{21}N_3$, C, 82·05; H, 5·98, N, 11·96 per cent; found, C, 82·04; H, 6·01; N, 12·10 per cent. It was oxidized in almost quantitative yield to II*d* by ferric chloride in acetic acid, exactly as described by Fischer for the analogous phenyl compounds[2].

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Glycolysis and Respiration of Transformed *BHK*21 Cells

WARBURG *et al.*[1] observed that tumour tissues almost always had a higher rate of aerobic glycolysis than normal cells. Warburg attributed this to irreversible damage to the respiratory pathways. The theory was a source of contention from the beginning. It was abandoned by most workers when it was found that glycolysis very rapidly increased (in a few days) when tissues were explanted and maintained as tissue cultures[2–4]. Since then many factors, such as *p*H and oxygen tension, have been shown to influence both glycolysis and respiration in cultured cells[5–7]; the increase of glycolysis on explantation can easily be explained by these findings. Reduced respiration and increased glycolysis in tumours *in vivo* can be explained similarly, knowing that most tumours have an inadequate blood supply. For this reason, and because wide differences in behaviour are found among different cell lines[8], the significance of Warburg's findings has been questioned. Nevertheless, there is good evidence[9,10] that there is a fundamental difference in glycolysis of normal and tumour cells.

To test the question critically, systems are needed in which closely related normal and malignant cells can be compared in identical conditions. Ideally, the lines should be grown *in vitro*, should be derived from the same cell and should have diverged from it very recently. These requirements have been met by the isolation of a stable line of baby hamster kidney cells, designated *BHK*21, which can be transformed into cells of different morphology by the *SE* polyoma virus[11,12]. Cells transformed in this way have the properties of tumour cells, whereas the 'untransformed' cells used by us in this study have very low transplantability. Both have a similar growth rate and high cloning efficiency[13]. It is possible to derive equivalent sub-clones of either normal or transformed cells from within a single clonal population and to compare them directly. The lines used in the work recorded here were clones derived simultaneously from the same cell inoculum which was exposed to polyoma virus[14]. *A* and *C* were morphologically untransformed and clones *Y* and *Z* transformed. *A*, *C* and *Y* are predominantly diploid and *Z* near tetraploid. The cells were maintained in Eagle's medium with 10 per cent calf serum and 10 per cent tryptose phosphate broth.

Respiration was measured with the Cartesian diver by techniques which have been detailed elsewhere[15]. Glycolysis was measured by estimating the glucose used and lactate produced by 2×10^6 cells in 2 ml. medium in 17 h. Since these cells produce large amounts of lactic acid and *p*H has a profound effect on glycolysis[5] the medium was heavily buffered. The incubations were performed in 25 ml. conical flasks maintained in a shaking water-bath at 37° C. Table 1 shows comparative values for respiration; if anything it is higher in the transformed strains. Table 2 shows comparative values for glycolysis; it is clear that the transformed cells have

Table 1. RESPIRATION OF TRANSFORMED AND UNTRANSFORMED CELL LINES DERIVED FROM *BHK*21 AFTER TREATMENT WITH POLYOMA VIRUS

Respiration was measured at *p*H 7·4 in a salt solution in equilibrium with 5 per cent carbon dioxide in air

Exp.	Cell strain and type	Oxygen uptake μM/h/mgDNAP
1	*A* (untransformed)	509, 439
	Y (transformed)	451, 553
2	*A* (untransformed)	467, 505, 550
	Y (transformed)	598, 659, 534

Table 2. GLYCOLYSIS OFTRANSF ORMED AND UNTRANSFORMED CELLS

Glucose utilization was measured for 17 h at *p*H 7·4 in a medium in equilibrium with 5 per cent carbon dioxide in nitrogen. (10^6 cells: 0·8 mg DNAP)

Type of cell	Strain	No. of obs.	Glucose uptake μM/h/10^6 cells (mean ± *S.D.*)
Untransformed	*A*	4	336 ± 104
		4	418 ± 57
		4	317 ± 36
	C	3	190
			347 ± 37
Transformed	*Y*	3	667
		4	574 ± 43
		4	601 ± 56
	Z	3	978
		4	660 ± 80
		4	777 ± 80

greater glycolytic capacity than the untransformed. Enzymatic investigations have been undertaken to reveal the underlying nature of this difference and will be reported elsewhere.

While these findings provide no support for Warburg's view that tumour cells have an irreversible lesion of the respiratory pathways they very strongly support his contention that there is a marked difference in the glycolysis of normal and tumour cells. Before drawing a firm general conclusion to this effect it would be desirable to carry out similar studies on other systems, including some in which a malignant transformation was produced by agents other than viruses. Defendi[16] has described a spontaneously arising variant of *BHK*21 which (unlike the untransformed cells used in this work) is highly malignant though it has the appearance of an untransformed strain. Some untransformed lines maintained by us for a very long time are also malignant[12,13]. Another untransformed line which has been maintained continuously for very many passages has been found to exhibit a high rate of glycolysis. Its transplantability is being tested, having in mind the possibility of a correlation between transplantability and high glycolytic capacity.

While we feel it would be unwise to draw a general conclusion until we have more information, we consider that the results obtained in this work warrant a reappraisal of the role of glycolysis in carcinogenesis.

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Occurrence of Farnesene in the Natural Coating of Apples

Previous investigation of the cuticle oil of the 'Granny Smith' apple in this Laboratory indicated the presence of small amounts of a hydrocarbon with a conjugated diene absorption in the ultra-violet. In view of its possible connexion with the serious storage disorder 'superficial scald' it was isolated, and it has now been identified as the sesquiterpene farnesene (2,6-dimethyl-10-methylene-2,6, 11-dodecatriene)[1,2].

'Granny Smith' apples (700 kg), stored at 1° C for six months, were washed with cold ethyl ether. The recovered coating (ursolic acid, cuticle wax and oil), freed of ether, was extracted with cold light petroleum (b.pt. below 40° C), and the extract after concentration was treated with urea and methanol. The oily material not adducted by urea was chromatographed on 'Florisil'. Infra-red spectra of the material in the fractions eluted by light petroleum indicated a small saturated hydrocarbon fraction (80 mg) followed by fractions (750 mg) all showing the presence of unsaturated hydrocarbon. Gas chromatography on silicone and polyester columns of the unsaturated material showed one predominant peak and the compound responsible was recovered in a pure state from the silicone column. It has been positively identified as natural farnesene on the basis of the following evidence: (*a*) its mass spectrum showed a molecular ion of $m/e = 204$; (*b*) its infra-red spectrum was identical with that published for pure natural farnesene[3]; (*c*) hydrogenation on platinum in glacial acetic acid yielded farnesene (2,6,10-trimethyldodecane)[1], identified by its published infra-red spectrum[2] as well as by direct comparison of its infra-red and mass spectra and gas chromatographic retention times on the above columns with those of an authentic specimen prepared from pure farnesol[1].

Farnesene in the isolated fractions was observed to polymerize rapidly at room temperature in the presence of air, whereas it is stable in the whole extracted apple coating for about a year. Its labile nature and the fact that it constitutes at least 1 per cent of the 'Granny Smith' cuticle oil fraction has suggested to us that it may have a role in the development of superficial scald. Work is in progress to examine this hypothesis.

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Ubiquinone-40 and Vitamin K_2 (40) in *Chromatium vinosum*

Chromatium, an obligate photosynthetic anaerobe belonging to the purple sulphur bacteria (Thiorhodaceae), has been examined by several workers for the presence of lipophilic quinones. Thus, *Chromatium*, strain *D*, contains ubiquinone-35 (UQ_7; 2·7 μM/g, dry wt.) and a vitamin K (refs. 1 and 2); both these quinones were found in the chromatophores[2]. *Chromatium* 8379 was reported as having large amounts of ubiquinone-50 (UQ_{10}) and a vitamin K_2 (ref. 3). Recently we have had the opportunity of examining *C. vinosum* for the presence of ubiquinone and vitamin K and have established the chain-length of the quinones present.

C. vinosum used was obtained from Lake Beloye (near Moscow), and shown to be photoautotrophic, requiring only carbon dioxide and hydrogen sulphide for growth[4]. It was grown on the following medium (g/l. distilled water): NH_4Cl, 1; K_2HPO_4, 0·5; $MgCl_2$, 0·2; NaCl, 5; $Na_2S\ 9H_2O$, 2; $NaHCO_3$, 5 (*p*H 7·8–7·9). The medium was dispensed in 250 ml. stoppered 'Pyrex' bottles, inoculated from a previous liquid culture, and grown anaerobically at 28° with constant illumination from tungsten lamps.

After 7 days the cells were collected by high-speed centrifugation (2,230 ml. media ≡ 204 mg dry wt. of cells) and the wet cell mass extracted twice with hot methanol (75 ml.) followed once by a hot ether–methanol mixture (3:1, v/v; 75 ml.) The methanolic extracts were separated from the cell debris by centrifugation, the supernatants decanted into a separating funnel and the ether washed free from methanol with distilled water. The ethereal layer was dried over anhydrous sodium sulphate (1 h), filtered and taken to dryness under a stream of nitrogen. This yielded 64·3 mg of a dark red oil.

The lipid extract was chromatographed on 10 g Brockmann grade III acid-washed alumina ('Woelm'); 100-ml. fractions of light petroleum (40°–60°) containing increasing amounts (0·25, 1, 3, 5, 8, 12 and 20 per cent) of ether (peroxide free) were collected. The 1 per cent ether–light petroleum fraction eluted 1·1 mg of a pale yellow oil with selective absorption bands in the ultra-violet region at λ_{max} 243, 249, 251 and 260 ($E^{1\%}_{1\,cm}$ 120, 122, 111 and 106) in *cyclo*hexane characteristic of the vitamins K. The 5 per cent ether–light petroleum fraction eluted 1·5 mg of an orange-brown oil having a single absorption band at λ_{max} 275 mμ ($E^{1\%}_{1\,cm}$ 77) in ethanol; on treatment of this fraction with $NaBH_4$ the absorption at 275 mμ declined and a new peak appeared at λ_{max} 290 mμ; these changes are attributable to the presence of ubiquinone. In agreement with work carried out on *Chromatium* 8379 (ref. 3), tocopherols were not detected in any of the fractions. The levels of ubiquinone (from the spectroscopic changes on treatment with $NaBH_4$ using λ_{max} 275 mμ ($\varepsilon_{oxid} - \varepsilon_{red}$) = 12,250) and vitamin K (using λ_{max} 249 mμ ε = 19,000) in the fractions were estimated and the concentration per unit weight of cell material calculated as 2·5 μM UQ/g dry wt. and 3–3·5 μM vitamin K/g dry wt. Since the estimation of vitamin K in the original fraction was difficult owing to irrelevant end absorption, a higher value was obtained on the crude fraction and a lower one was obtained after further purification by thin-layer chromatography (see below).

In order to characterize the quinones the fractions were purified further by quantitative thin-layer chromatography on 'Kieselgel *G*' plates impregnated with rhodamine 6 *G*. The plates were developed in a saturation chamber[5] with benzene–chloroform (1:1, v/v) as developing solvent (vitamin K $R_F = 0{\cdot}68$, UQ $R_F = 0{\cdot}3$). After development the quinones, visible as dark bands in daylight (under ultra-violet light the bands are more intense), were scraped off into ether and filtered to remove the gel and rhodamine. The recovered quinones had ultra-violet spectra qualitatively identical to those of vitamin K and ubiquinone.

The ubiquinone recovered from thin-layer chromatograms was then chromatographed against standards in two ascending reversed-phase systems which allow the separation of the various homologues[6,7]. The quinones were visualized by spraying with leucomethylene blue[7]. In both systems the ubiquinone behaved as the −40 homologue (UQ_8).

The vitamin K sample, from its behaviour on thin-layer absorption chromatograms, was assigned to the K_2 series, since in the system described above it was resolved from vitamin K_1 and run with the vitamins K_2 (45) and (40). Accordingly, the sample was chromatographed against vitamin K_2 standards in two reversed phase systems[8,9] which will resolve the various isoprenologues. In both cases the menaquinone ran with and was not separated from vitamin K_2 (40). Additional evidence as to the

identity of the quinones was obtained from microhydrogenation (Adams catalyst) investigations. In both cases the hydrogenated samples of ubiquinone and vitamin K_2 from *Chromatium* when run on paper chromatograms[6,9] ran with the corresponding authentic perhydro derivatives of ubiquinone-40 and vitamin K_2 (40).

In agreement with the experiences of other workers[1-3] with different strains of *Chromatium* we have found the *C. vinosum* has a high level of ubiquinone and vitamin K_2; our value for the former quinone (2·5 μM UQ_8/g dry wt.) is similar to those reported for *Chromatium*, strain *D* (2·7 μM UQ_7/g dry wt.)[1,2]. It is of interest that both quinones in *C. vinosum* should have the same chain-length, suggesting a common biosynthetic sequence. Of systematic importance is the observation that of the three strains of *Chromatium* so far examined each contains a different ubiquinone homologue; unfortunately it is not possible at this time to make a similar comparison for the vitamins K.

We thank Dr. F. W. Hemming, Department of Biochemistry, University of Liverpool, for providing the ubiquinone and vitamin K_2 standards. This work was supported in part by the Department of Scientific and Industrial Research.

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Cellulase from Pea Epicotyls

Seedlings of *Pisum sativum* L. var. 'Alaska' were grown in darkness for 8 days and extracts of the apical 6 cm of epicotyl were examined for ability to catalyse the degradation of cellulose derivatives. After the tissue was washed in hypochlorite and weighed, 0·5 vol. of cold 0·1 M phosphate *p*H 7·0–0·01 per cent cysteine was added and a brei was prepared by blending at top speed with a 'VirTis' homogenizer. The brei was centrifuged at 37,000*g* for 20 min at 0° C and the precipitate was homogenized and extracted again. The combined supernatants were saturated with toluene and re-centrifuged just before use as crude extract. In order to remove contaminating reducing substances, 2 vol. of cold acetone were added and the precipitate was washed with 70 per cent acetone, dissolved in water, re-precipitated with ammonium sulphate at 60 per cent saturation, suspended in water and re-centrifuged. The final supernatant was used as purified extract.

Fig. 1 shows curves for the action of extract, and the supernatant from boiled extract, on the viscosity of carboxymethylcellulose solution (CMC, type 7*MSP*, Hercules Powder Co.). The substrate alone is stable for at least 10 days under the condition of the experiment but the addition of boiled extract causes a slow steady fall in viscosity, that is, a fall in the degree of polymerization of CMC. This non-enzymatic degradation has been observed before with heated extracts from cabbage tissue[1]. With active extract the viscosity is induced to fall at a rate

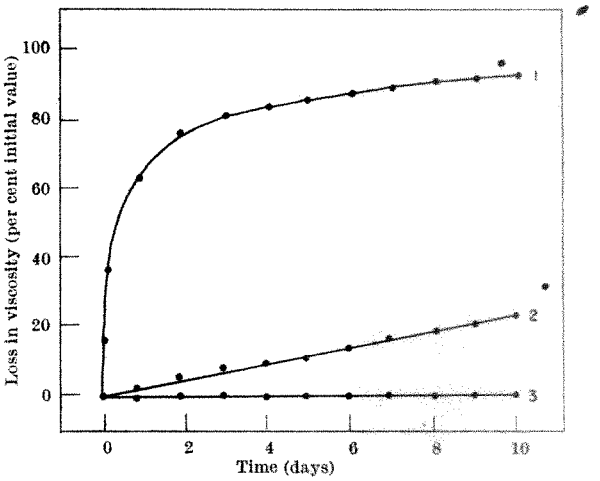


Fig. 1. Effect of pea epicotyl extract on viscosity of carboxymethylcellulose solution (CMC). Curve 1, 1·0 ml. purified extract added to 9·0 ml. 1·1 per cent CMC – 0·2 per cent NaF – 0·02 M phosphate *p*H 6·0 in an Ostwald–Cannon–Fenske viscometer incubated at 35° C; curve 2, boiled enzyme control; curve 3, water control. Viscosity calculated by the method of Nokrans and Hammarstrom (ref. 5)

which is proportional to the amount of extract added provided a boiled control is taken into account. After about one-third of the initial viscosity is lost, the rate declines and in time the solution approaches a stable viscosity near that of water.

The enzyme generates reducing power (Table 1), as determined by the copper method[5] for sugars, from both CMC and hydrocellulose (Whatman cellulose powder suspended briefly in 75 per cent sulphuric acid and washed exhaustively with water). The *p*H optimum is close to 6·0 for both reactions. Since less than 0·5 per cent of the potential reducing power in CMC is released by the time the solution loses 90 per cent of its viscosity (cf. Table 1 and Fig. 1), the polymer is apparently broken at points along the chain which are not terminal. In all these respects the enzyme acts as a typical cellulase.

Table 1. Enzyme Activity measured by Formation of Reducing Power

Time (days)	Reducing power increase over zero time (mg glucose equiv./ml. enzyme)	
	Carboxymethylcellulose solution*	Hydrocellulose suspension*
1	0·15	0·18
2	0·29	0·29
3	0·42	0·36
4	0·58	0·40
6	0·70	0·47
8	0·89	0·49

* Samples with 100 mg CMC as substrate per ml. enzyme were taken from experiment described in Fig. 1. With hydrocellulose, purified enzyme was passed through an ultrafine glass filter and 1·0 ml. pipetted aseptically to autoclaved tubes containing 90 mg substrate suspended in 4 ml. of 0·25 per cent sodium fluoride–0·025 M phosphate *p*H 6·0. Samples incubated with boiled enzyme formed 0·02 to 0·03 mg glucose equivalent in 8 days.

The relative amounts of cellulase activity which can be extracted from specific sections of the epicotyl are shown in Table 2. Expressed on a fresh-weight basis, extracts of sections from the apex or node yield much more activity than extracts from basal sections of the internode. The former group of sections delimits regions of cell division and potential growth (*PH* and *N*) and regions of active cell enlargement (1 and 2), while the latter contains regions of cells which differentiate but do not grow (4 and 5)[3]. Whether activities are re-calculated on a soluble protein basis or per unit dry weight of insoluble materials from which the extracts derived, all sections in the first group yield at least twice the activity of those in the second. The results appear to reflect real differences in enzyme level since we could find no evidence for cellulase inhibitors in any of the extracts, and tissue residues which are discarded during preparation of the enzyme yield no activity on further extraction with Na_2HPO_4, NaH_2PO_4, 20 per cent sodium chloride or sodium choleate.

Table 2. Cellulase Activity* in Extracts of Various Sections† of the Epicotyl

Section	Relative activity per unit fresh wt. Crude extract	Boiled extract	Difference (enzymatic)
PH	39·1	1·3	37·8
1	17·6	2·1	15·5
2	12·9	2·0	10·9
3	8·6	2·1	6·5
4	5·4	2·2	3·2
5	2·7	2·0	0·7
N	11·8	1·5	10·3

* Activity is expressed as percentage loss in viscosity of 1 per cent CMC solution caused by extract derived from 0·23 g fresh wt. tissue in 24 h; conditions as in Fig. 1.

† Sections used were the plumule plus hook (*PH*), 5 mm segments of the third internode (1–5 from apex) and a segment bearing the second node (*N*)[5].

There are obvious dangers in projecting the results of cellulase concentration investigations *in vitro* to effects on cellulose fibrils *in vivo*. However, there is more than usual justification for doing so here because the pea epicotyl is one of the few plant tissues for which there is physiological evidence of a metabolic turnover of cell wall materials. Those epicotyl sections which showed most active wall breakdown[3] are those which yield highest cellulase activity (Table 2). The association of both activities with growing or potentially growing tissue recalls Tracey's 1950 report[4] of a cellulase concentrated in the youngest parts of tobacco leaves and roots, and his recent suggestion[4] that the enzyme may "play a role in the remodelling of the primary cell wall during growth". Certainly a loosening effect on the cellulose framework is a real possibility if further investigation confirms that the enzyme breaks the hexose polymer internally. It may be that cellulase action permits growth by maintaining the cell walls in a plastic and extendable state, and that with time, loss of cellulase and continued cellulose deposition, the walls eventually become too rigid for cell expansion to take place.

This work was supported by the National Research Council of Canada.

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PHYSIOLOGY

Effect of Rhinencephalic Lesions on Cardiovascular Reactions to Noradrenaline

It had been observed in the course of previous investigations on pharmaco-dynamic dissociations between central and peripheral effects of catecholamines[1] that paradoxical cardiovascular responses to intravenously injected noradrenaline frequently occurred in rabbits with more or less important forebrain lesions. These observations suggested a series of experiments designed to assess eventual causal relations between both phenomena.

The experiments described here were carried out on 50 male adult rabbits carefully selected from the same source ('Fauves de Bourgogne'). The animals were anæsthetized lightly with ethyl-urethane. The head was fixed with the help of a special head-holder[2] in a standard Horsley-Clarke carriage. Blood pressure in the left femoral artery was measured with a mercury manometer throughout, and single-channel electrocardiographic tracings (*D*-3) were recorded prior to and after an intravenous administration of 2·5 μg of noradrenaline bitartrate, cerebral lesions were then made, and *D*-3 tracings again recorded before and after a second injection of the same dose of noradrenaline. The lesions were performed by coagulation through the tip of stereotaxically oriented electrodes, according to the (slightly modified) co-ordinates of Sawyer, Everett and Green[3]. The position of the electrodes was controlled by histological examination.

It is well known that the adrenergic transmitter engenders transient hypertensive effects, and that high concentrations of that substance occasionally initiate ectopic heart rhythms. We found that an immediate and definite deterioration of blood pressure and electrocardiographic reactions regularly occurred after a single injection of 2·5 μg of noradrenaline bitartrate whenever a bilateral symmetric lesion was made at the anterior basal part of the rhinencephalon (close to the anterior commissure at the level of the olfactory tubercle). Our preliminary experiments also showed that less-severe cardiac irregularities (bigeminal and trigeminal ventricular extrasystoles) may appear after a sequence of about ten intravenous injections of our small standard dose of noradrenaline at intervals of 5 min. But such aberrant results were only obtained in one case out of ten.

The experiment illustrated in Fig. 1 shows that a successfully operated animal responds to the transmitter with an abrupt, irregular and long-lasting hypotensive reaction. This sudden reversal is generally paralleled by progressive pathological alterations shown on the corresponding electrocardiogram. As can be seen from Fig. 2, the same dose of noradrenaline which leaves the ECG of the normal animal practically unchanged induces the appearance of abnormal components after the cerebral lesion (intervening between *A* and *B*). The tracing now shows an outburst of ventricular extrasystoles and becomes more and more anarchic, finally leading to a transformation of the S-T complex into the 'dome-waves' of Pardie (*D*), that is, into manifestations which remind one of the primary phase of human myocardial infarction.

Another experiment (Fig. 3) reveals a still more dramatic development of aberrant complexes. The heart rate of the rabbit changes from 220 (normal rate) to a supraventricular tachycardia of about 300, finally involving the presence of inverted *T*-waves (see *F*) such as generally characterize the second, ischæmic, phase of infarction. It seems particularly interesting that such far-reaching pathological effects can be caused by minute amounts of the adrenergic transmitter corresponding to 2·5 μg of noradrenaline bitartrate or to 1·2 μg of noradrenaline base, in an animal of the weight of 3 kg, that is, by a dose which might be considered as physiological.

A macroscopic exploration of the cardiac cavities of such an animal shows the presence of diffuse subendocardial hæmorrhages in the walls and valves of the left ventricle. Parietal petecchies are visible at the atria and

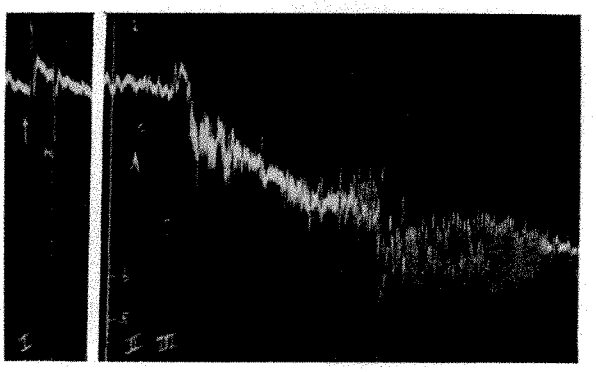


Fig. 1. Rabbit blood pressure : effects of intravenous injections of 2·5 μg of noradrenaline bitartrate ; between (I) and (II), at the arrow : symmetrical bilateral lesion at the base of the anterior rhinencephalon

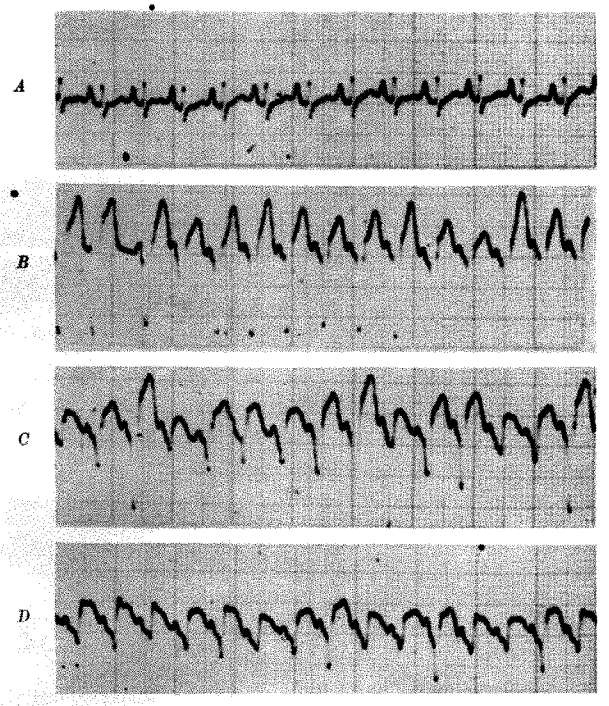


Fig. 2. Electrocardiographic tracings (*D*-3) of the same animal. *A*, normal reaction to 2·5 μg of noradrenaline bitartrate; between *A* and *B*, rhinencephalic lesion; at *B*, injection of the same dose of noradrenaline bitartrate; from *B* to *D*, progressive deterioration of the ECG

intra-atrial connexions. The rhinencephalic lesion apparently induces a facilitation of the action of noradrenaline on excitable tissue sites of the heart, and the resulting ruptures of intracardial vessels by the same agent may be the final cause of the observed functional disturbances.

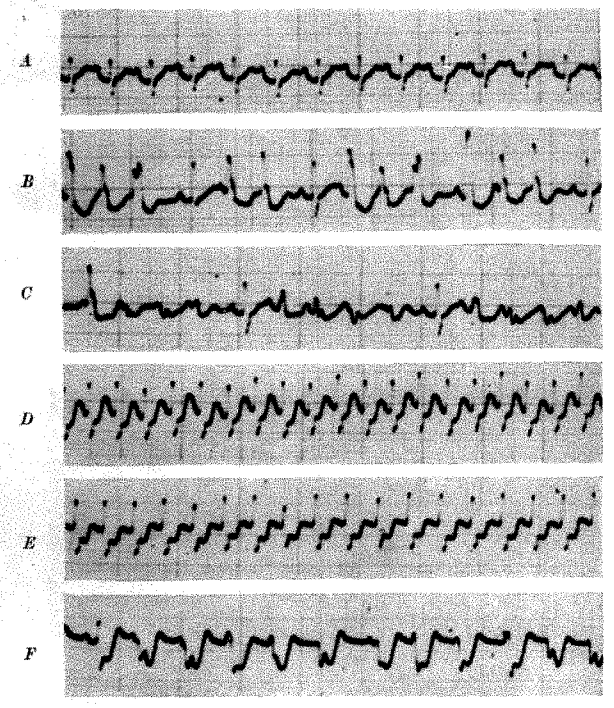


Fig. 3. Electrocardiogram of a rabbit. At *A*, normal response to the intravenous injection of 2·5 μg of noradrenaline bitartrate; between *A* and *B*, bilateral rhinencephalic lesion; from *B* to *F*, aberrant reactions to an intravenous injection of the same dose of noradrenaline made at *B*

Interpretation of these results, however, should take into account the fact that the same noradrenaline which accelerates the isolated heart engenders bradycardia when injected into the systemic circulation of animals. This latter reaction is probably due to the intervention of cholinergic regulation mechanisms. The lesion may thus eliminate, directly or through the interruption of afferent or efferent fibres, a centre that effectively controls the sympathetic supply of the heart. An impairment of the anterior basal part of the rhinencephalon may then lead to a parasympathetic disconnexion of that organ which becomes particularly sensitive to more or less excessive adrenergic impulses.

The rhinencephalon forms a physiological entity which includes not only the olfactory tracts and their main termination sites, but also such diverse structures as the hippocampus, cingulate gyrus, retrosplenial cortex, hypothalamus, parts of the thalamus, the habenular nucleus which is relayed to the pineal body, the fasciculus retroflexus, the amygdaloid nuclei and the mammillary bodies. This complex is involved in the cerebral control apparatus of psychosomatic correlations. Emotion as well as any other kind of mental stress is supposed to activate the adrenergic emergency reaction. It is tempting to put forward, as a working hypothesis, therefore, that the coincidence of the special rhinencephalic lesion and the observed sensitization of the heart to an adrenergic agent of the noradrenaline type, possibly released during a psychogenic shock, may create a condition similar to that which induces human myocardial infarction.

Recent (unpublished) findings in our laboratory indicate, on the other hand, that animals submitted to identical bilateral rhinencephalic lesions and showing the aforementioned aberrant cardiovascular responses to intravenous noradrenaline are still capable of reacting to the stimulation of a hypothalamic centre (located at the latero-medial part of the hypothalamus). When they are so stimulated, the systemic blood pressure rises, and this reaction, moreover, initiates a remarkable improvement of heart activity.

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Membrane Potential of Electrolyte-depleted Muscle Fibres

Koketsu and Kimura[1] have reported experiments in which the potassium concentration within frog muscle fibres $[K]_i$ was reduced by soaking sartorii in hypotonic sucrose solutions. They found that, although $[K]_i$ was reduced from 130 to about 48 m.equiv./l. fibre water by this procedure, the membrane potential of the fibres measured in normal Ringer's fluid by the micro-electrode technique was not decreased. It remained at about 93 mV, in spite of the fact that the potassium-equilibrium potential E_K was then only 80 mV. They concluded that the results did not favour the ionic theory of membrane potential[2–4]. Ling also quoted these experiments[5] as evidence against the membrane theory.

It seemed likely to us that the Cl^- distribution across the cell membrane might contribute significantly to the membrane potential here, particularly as the chloride equilibrium potential E_{Cl} might be expected to increase as the intracellular chloride concentration $[Cl]_i$ was reduced during immersion of the muscles in sucrose.

We soaked frog sartorii for 6 h in sucrose and then transferred to a normal[3] and then to a chloride-free Ringer's fluid for membrane potential measurement. In the latter the sodium chloride was replaced by sodium methylsulphate, and K^+ was present as 2·5 mM potassium bicarbonate. Following potassium analysis of the muscles E_K was calculated. The results are summarized in Table 1.

Table 1

$[K]_o$ (mM)	$[K]_i$	E_K (mV)	E_m (mV) With Cl	E_m (mV) Without Cl
2·5	38·8	69·0	98·1 ± 1·9	65·2 ± 1·3

In another series of experiments muscles were soaked in sucrose for 3 h until the mean intracellular concentration had been reduced from about 130 to 79 m.equiv./l. fibre water. These muscles were re-immersed in chloride-free fluid containing 2·5, 10 and 25 mM potassium bicarbonate and the mean membrane potential was measured by the micro-electrode. From the overall mean $[K]_i$ of the muscles, the theoretical E_K was calculated for the various $[K]_o$ used and was plotted as a straight line (Fig. 1). The potentials measured are also plotted in Fig. 1 against the log of the external potassium concentration. It can be seen that within the limits of experimental error there is reasonable agreement between the observed and calculated potentials over the range examined when Cl^- is absent from the external fluid.

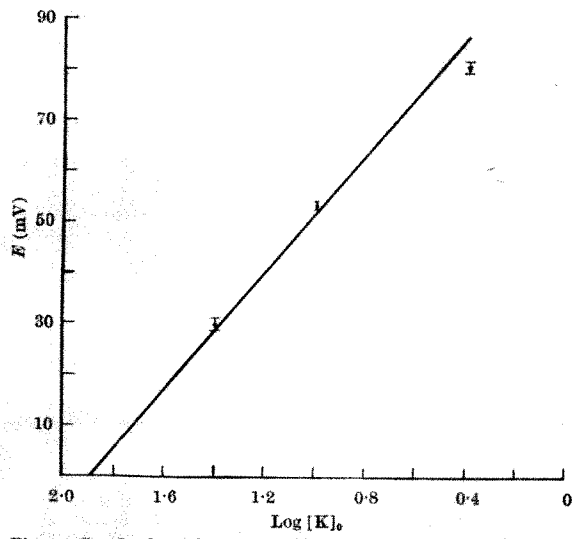


Fig. 1. Graph of membrane potential of muscle plotted against the log of the external potassium concentration. The line represents the calculated potassium-equilibrium potential for a mean internal potassium concentration of 79 m.equiv./l. fibre water. The points represent mean measured potentials and the standard errors are shown by barred lines. Potentials were measured in chloride-free Ringer with 2·5, 10 and 25 mM potassium respectively.

It appears then that Cl^- contributes to the membrane potential following depletion of intracellular K^+ and Cl^-, but that, in the absence of external Cl^-, the membrane potential is determined mainly by the concentrations of K^+ within the muscle fibres and in the external fluid. It is concluded that these findings are quite in keeping with the membrane theory.

I thank Prof. E. J. Conway for his advice, Miss Aileen Tangney for her assistance and the Medical Research Council of Ireland for a fellowship.

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Exocrine Secretion from the isolated Rabbit Pancreas

Study of the mechanisms of secretion of the digestive glands has been handicapped by an inability to isolate the glands from the influence of variable control mechanisms such as blood flow, nervous factors and humoral agents. In the work recorded here it was possible to maintain pancreatic secretion in an *in vitro* preparation of rabbit pancreas for 5–6 h and to demonstrate ion concentration-flow relationships similar to those found *in vivo*. Adequate oxygenation of the organ is possible because the pancreas of the rabbit is nearly as thin as the surrounding mesentery (about 0·2 mm). It is entirely located within the mesomentum of the first duodenal loop. The collecting duct enters the duodenum completely separated from the common bile duct, and thus is easily identified and isolated.

The pancreatic duct was cannulated *in situ* with polyethylene tubing (0·61 mm outer diam.). The entire mesomentum included in a duodenal loop extending from the gastroduodenal junction aborally 30–40 cm was removed as a unit[1]. The mesomental loop was then mounted on a 'Plexiglas' frame using stainless steel hooks attached to the duodenum, and the frame was placed in a 'Plexiglas' chamber with the catheter draining dependently to the outside (Fig. 1). The organ was bathed in a balanced salt solution (118·5 mM/l. NaCl; 4·75 mM/l. KCl; 2·54 mM/l. $CaCl_2$; 1·18 mM/l. KH_2PO_4; 1·18 mM/l. $MgCl_2$; 24·9 mM/l. $NaHCO_3$), maintained at 30° C and gassed continuously with 95 per cent oxygen and 5 per cent carbon dioxide. Mounting required 10–20 min. Chloride concentration was determined coulometrically, and total carbon dioxide concentration with the Van Slyke and Plazin micromanometric technique. Eighteen New Zealand white rabbits weighing 1·8–2·4 kg were used in this experiment.

Pancreatic secretion varied from 28 μl./h to 561 μl./h. The most rapid rates *in vitro* were comparable to *in situ* flows obtained by Baxter[2,3], as well as to *in situ* secretin-stimulated flows obtained in this laboratory. While flow varied with different preparations, it was relatively constant for a given preparation up to at least five hours after isolation.

Flow was inhibited when the oxygen–carbon dioxide gassing mixture was replaced with 95 per cent nitrogen

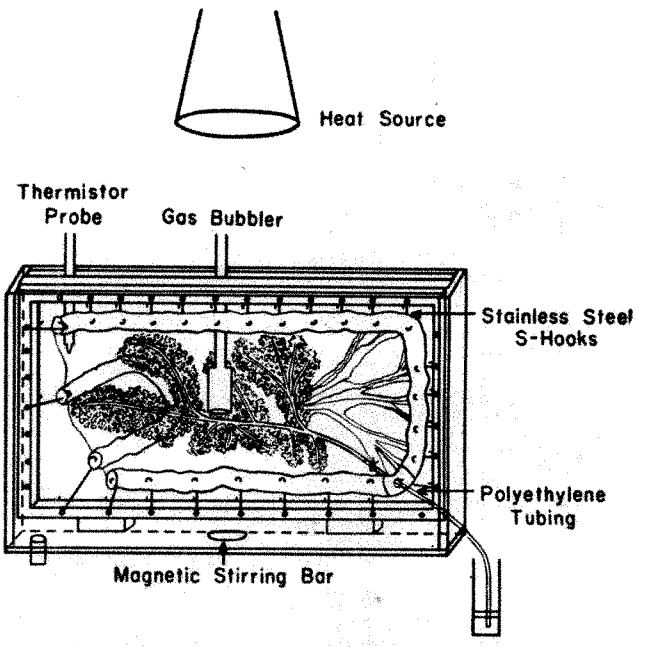


Fig. 1. Schematic diagram of rabbit pancreas and duodenum suspended in chamber (175 ml. vol.)

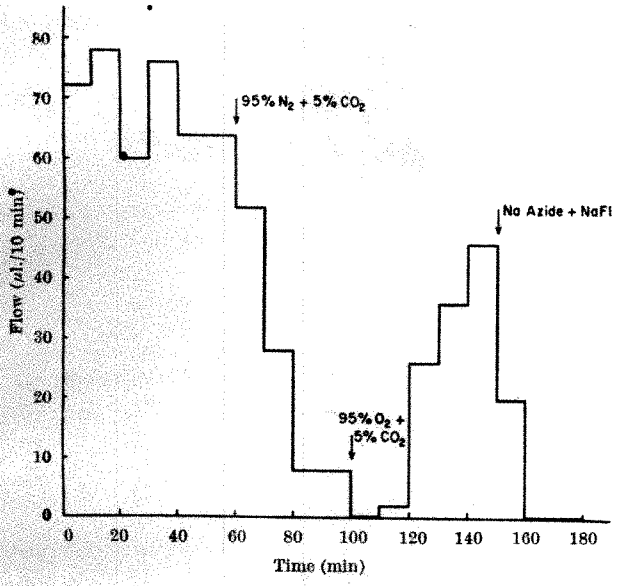


Fig. 2. Metabolic dependence of *in vitro* rabbit pancreatic secretion. Time zero represents 4 h after the gland started secreting *in vitro*. The mean flow for the preceding 4 h was 74·6 μl./10 min

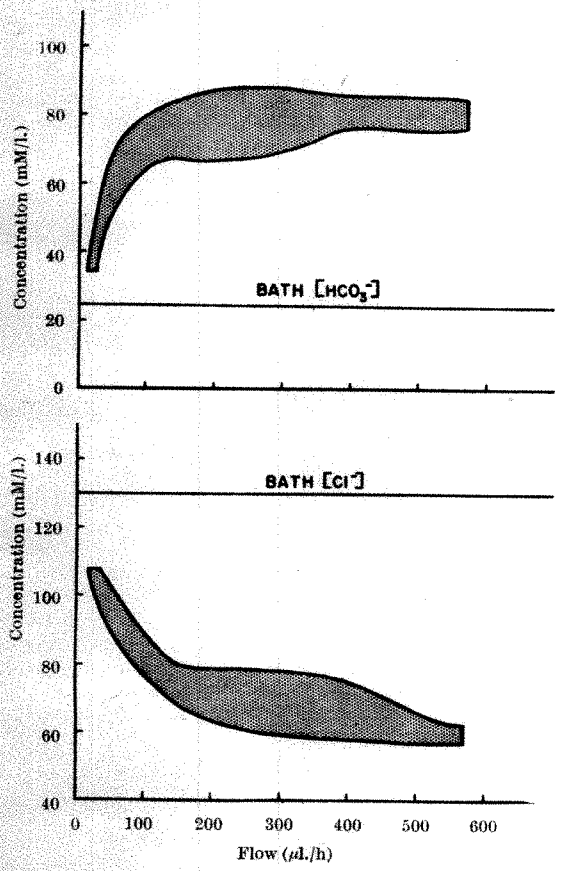


Fig. 3. Flow-concentration relations for total carbon dioxide (above) and chloride ion (below). Curves shown are the envelopes of the observed points

and 5 per cent carbon dioxide (Fig. 2). The addition of sodium fluoride and sodium azide to the fluid invariably produced complete cessation of flow within ten to twenty minutes.

Juice bicarbonate concentrations have been obtained *in vitro* up to four times the bath bicarbonate concentration. The flow-concentration curves are similar to those found in man and chronic fistula dogs, although the values of Fig. 3 apparently approach an asymptote at approximately 76–88 mM/l. for bicarbonate, whereas those for man and dog reach a plateau approximately at isotonicity with plasma, namely, 140 mM/l.[4,5]. *In situ* investigations with rabbits show a slightly higher plateau concentration than those obtained *in vitro* (90–100 mM/l.). The sum of the bicarbonate and chloride ion concentration was relatively constant with a mean of 146·1 mM/l. (range 132·0–164·6 mM/l.) as has been found in man and dog[4,5].

Davenport[6] has pointed out, on the basis of the large discrepancy between oxygen consumption and the possible potential energy supplied by the blood pressure, the relatively minor importance of perfusion pressure in supplying energy for secretion in this organ. The observation that in the absence of blood perfusing pressure as an energy source the pancreas can secrete a fluid of volume and ionic content comparable to that found *in situ* supports his conclusion. The mean 'basal' secretory rate *in vivo* in the fasted, anæsthetized rabbit over a 4-h period (excluding the 1st h after cannulation) was 59·4 μl./h, whereas the mean flow in the *in vitro* preparation taken from similarly fasted animals was 303·5 μl./h over the same period. The low flows obtained *in situ* may be due to a net inhibitory tone (nervous or hormonal) affecting the organ.

Apparently mammalian digestive glands, with the exception of the gastric glands, have only been studied *in vitro* with the aid of tissue-sectioning techniques. The present technique, therefore, may offer new opportunities for the study of peripheral control mechanisms of pancreatic secretion as well as for the study of cellular mechanisms of secretion from tubulo-alveolar secretory glands.

This work was supported in part by U.S. Public Health Service grant *ST*1-*DE*1-07 and the Pennsylvania Plan. I thank Drs. Frank P. Brooks and John G. Forte of this Department for their advice.

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PHARMACOLOGY

Mercury in Mouse Brain after Inhalation of Mercury Vapour and after Intravenous Injection of Mercury Salt

The question of whether or not exposure to mercury vapour is equivalent to parenteral administration of mercuric ions for tissue accumulation is of great importance when evaluating experimental results for the purpose of setting threshold limits for mercury in ambient air. Clarkson *et al.*[1] showed that mercury vapour is rapidly oxidized to mercury ions in the erythrocytes of the blood.

In experiments on rats, Hayes and Rothstein[2] found no difference in the proportional distribution of mercury after exposure to the vapour as compared with intravenous injection of mercury salt. However, they did not state the amount of mercury found in the brain. Clinical observers have reported[3] that after acute exposure to mercury vapour, the dominant toxic symptoms arise in the respiratory tract and the central nervous system, while renal damage is less conspicuous or not detectable.

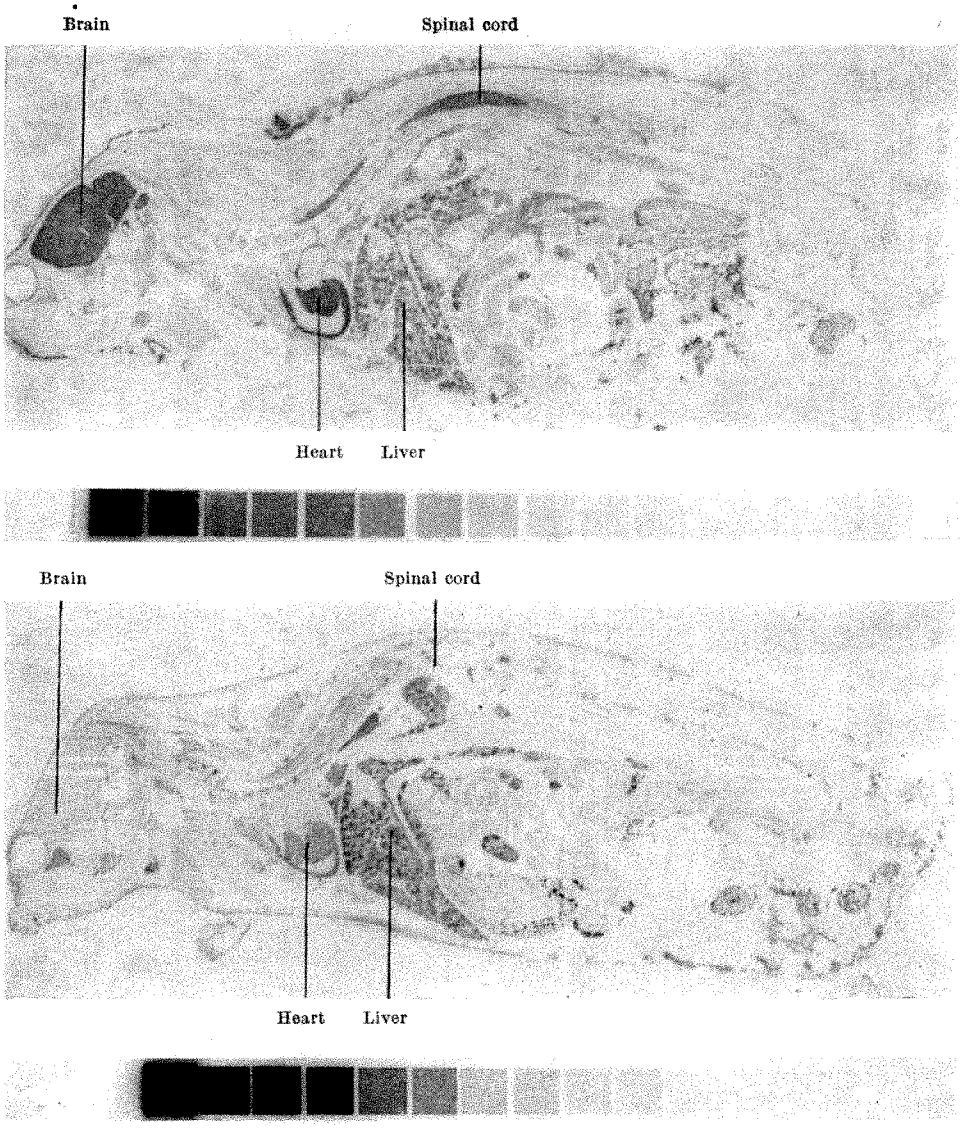


Fig. 1. Autoradiograms of distribution of mercury in whole-body sagittal sections of mice 8 days after inhalation of mercury vapour (upper), and after injection of mercury nitrate (lower). Below each section is the isotope standard 'staircase' autoradiographed on the same film

This is in contrast to the findings in acute sublimate intoxication, where renal damage is dominant.

To elucidate this question further we have exposed mice to radioactive mercury vapour, 10 mg/m³ of air, for 4 h. Immediately after exposure the amount of mercury absorbed was estimated in the living mouse by means of a body scintillation counter. A similar dose of radioactive mercury in the form of mercuric nitrate was then injected into the tail vein of another, similar mouse, so that for every animal exposed to the vapour there was one animal injected with the salt.

At predetermined intervals of 1 h–32 days after exposure, the paired animals were killed. 'Whole-body' autoradiography was performed on a half of each animal and the mercury concentration in the organs was determined by densitometric comparison with an isotopic standard 'staircase'[4]. From the other half of each animal the brain, liver, kidney and other organs were taken for mercury assay by scintillation count.

The autoradiograms revealed that the uptake of mercury in the brain was about ten times greater in the animal which had inhaled mercury vapour than in the animal which had received a similar dose intravenously. Fig. 1 shows the autoradiograms of sagittal sections of mice killed 8 days after inhalation of mercury vapour (upper) or injection of mercuric nitrate (lower). The increased amount of mercury in the brain of the vapour-exposed animal is well illustrated. The 'staircase' beneath the sections show the density of decreasing concentrations of isotope in a dilution series (1/1, 1/2, 1/4, 1/8, etc.). The findings were confirmed by determination of the mercury in the corresponding brain halves by scintillation count. Detailed reports of the studies referred to here will be published elsewhere.

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HÆMATOLOGY

Differential Plasma Turbidity as an Index of Platelet Count

RECENT work on the assay of platelet aggregation in platelet-rich plasma (P.R.P.) using a turbidimetric method[1,2] suggested the possibility that this simple technique might be adapted to give a direct measure of the platelet count of plasma.

Use of this principle was reported by Cruz[3]. His method involved the initial separation of the platelets, which were then washed and re-suspended in sodium citrate. The turbidity of this suspension was found to correlate well with the platelet volume as determined by the thrombocytocrit method of Van Allen[4]. The error of repeated turbidimetry on the same sample was about 6 per cent.

In order to simplify the procedure and to minimize the manipulation of the platelets, the technique used in this work was to measure the turbidity of serial dilutions of platelet-rich plasma against a blank of platelet-poor plasma (P.P.P.) from the same subject. P.P.P. was used as a diluent in preparing each concentration of P.R.P.

Venous blood samples were taken from 10 normal subjects (five male and five female) and transferred to a centrifuge tube containing 1/10 vol. of 3·8 per cent sodium citrate. A silicone technique was used throughout the entire procedure. After centrifugation at 1,000 r.p.m. for 10 min, the P.R.P. was removed. The remainder of the sample was centrifuged at 3,000 r.p.m. for 20 min to obtain P.P.P. Dilutions of P.R.P. were made with P.P.P. in 10 per cent steps from 100 per cent down to 10 per cent. However, at the two highest dilutions the error of the estimate was so high that they had to be discarded. The results are, therefore, given for the eight dilutions from 100 per cent to 30 per cent.

The turbidimetric measurements were made on a Unicam *S.P.* 600 spectrophotometer at a wave-length of 600 mμ using the 2-mm cells. Platelet counts were made in duplicate on samples from each dilution of P.R.P. and expressed as cells per mm³. The value log platelet count/turbidity × 10^5 was known as the turbidity index.

With this experimental design it was possible to test: (*a*) whether for any individual the differential turbidity between P.R.P. and P.P.P. was an accurate measure of the platelet count at all dilutions; (*b*) whether the turbidity index was tolerably constant from one individual to another.

Table 1 shows the analysis of the turbidity indices for each dilution in the whole group of 10 subjects. Analysis of variance showed homogeneity of the groups when separated in this way, that is, the dilution technique did not significantly affect the turbidity index. However, the variance of the individual results at any given dilution was found to be quite large. Thus the 95 per cent confidence limits for the mean value of 1·126 estimated for undiluted P.R.P. samples lie between 1·194 and 1·058, representing a range of ratios between 11·43 and 15·63. This is clearly too wide for the turbidity index to be regarded as an accurate assay of plasma platelet count in any group of individuals.

Table 1. MEAN VALUES OF LOG PLATELET COUNT/TURBIDITY × 10^5 FOR EACH DILUTION OF PLATELET-RICH PLASMA

Dilution (%)	100	90	80	70	60	50	40	30
Mean	1·126	1·096	1·082	1·087	1·114	1·147	1·161	1·197
S.D.	0·096	0·099	0·109	0·097	0·117	0·134	0·133	0·154
S.E.	0·030	0·031	0·034	0·031	0·032	0·042	0·042	0·049

Table 2. MEAN VALUES OF LOG PLATELET COUNT/TURBIDITY × 10^5 FOR EACH SUBJECT

Subject No.	1	2	3	4	5	6	7	8	9	10
Mean	1·204	1·356	1·131	1·009	1·109	1·034	1·173	1·006	1·192	1·047
S.D.	0·046	0·103	0·068	0·036	0·056	0·061	0·080	0·049	0·028	0·047
S.E.	0·016	0·036	0·024	0·012	0·020	0·022	0·028	0·017	0·009	0·017
Turbidity readings of P.P.P.	0·020	0·145	0·022	0·040	0·055	0·085	0·065	0·014	0·032	0·015

Another variable to be considered is the turbidity of the platelet-poor plasma used as a blank. The values for these as measured against distilled water are recorded in the bottom row of Table 2. It will be seen that the highest values for both the mean turbidity index and the P.P.P. turbidity are found in subject No. 2, and the lowest in subject No. 8. In the numbers used, the correlation between these two parameters was, however, quite insignificant. It is possible that a larger group with a wider range of P.P.P. turbidities would have shown such relationship, but this has not been tested.

Table 2 shows the results derived from each subject for the eight dilutions of P.R.P. It can be seen that the standard errors are small. Thus it appears that if the platelet count of the original specimen and its turbidity are known, subsequent turbidity measurements can be expected to give a reasonably accurate estimate of changes in the platelet count.

Analysis of variance reveals non-homogeneity of the data when grouped in this way ($F = 3·18$; P less than 0·01). This confirms the conclusion already reached from the analysis in Table 1 that the turbidity index is not sufficiently constant from one individual to another for the method to be of value as an alternative to direct counting techniques.

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Anthropological Observations of Blood Groups in Hiroshima

THE distribution of a number of human blood groups has now been established in many parts of the world[1].

Since the blood group antigen Di^a was found by Layrisse *et al.*[2] in Venezuela following a preliminary report of Levine *et al.*[3], we became interested in investigating the distribution of this factor in different populations. The Di^a antigen is found in 36 per cent of South American Indians and the factor is also present in Mongolians with a lower incidence. However, the presence of the Di^a antigen in Caucasians is presumably very rare.

The frequency of the Diego factor in Japanese in Japan has been reported by several authors[4–7], and the results indicate that the distribution of the Di^a antigen was between 2·3 and 4·1 per cent in the Tokyo area and 7·5 per cent in the southern part of Japan.

Recently, we investigated the frequency of the Diego (Di^a) factor together with the ABO, MN and Rh_0(D) blood antigens in three villages, Mukaihara, Yoshida and Miyoshi, situated in the northern part of Hiroshima Prefecture. These areas were selected particularly because of the isolated conditions under which the people in these villages were living. In addition to blood group distribution, the population offered other avenues of anthropological research.

The investigations were carried out with high-school students classified on genetic grounds into two groups. One group had been living in the area for more than three generations and had intermarried only with other members of the three villages, called non-mixed group; in the other group, either one or more people had entered the area

from another part of Hiroshima Prefecture within the past three generations, called mixed group.

In the work recorded here, the distribution of the blood groups in the two classes was compared for significant differences.

5 ml. of venous blood was collected in acid citrate dextrose (ACD) solution obtained from the Hiroshima Blood Bank. The ACD solution conformed to the minimum requirements of the N.I.H. of Japan. Red blood cells were washed three times with physiological saline and used as 2 per cent saline suspensions for agglutination.

ABO and MN blood typing sera, conforming to N.I.H. minimum requirements, were also obtained from the Hiroshima Blood Bank. Anti-Rh_0(D) was obtained from Knickerbocker Biologicals, Inc., New York.

ABO and MN typing was carried out at 25° C by direct saline agglutination tests; Rh_0(D) and Diego blood typing were performed at 37° C by the antiglobulin test using antiglobulin sera obtained from Hiroshima Blood Bank.

The results of the present investigation showed the frequency of group B to be higher in the areas under investigation as compared with the national population frequency (Table 1). The frequency of group AB was higher in the non-mixed group than in either the mixed group or the national population. On the other hand, group A and group O appeared to have a lower frequency in the two groups as opposed to the national figures. However, there was no discrepancy in the order of frequency of the two groups, or the national population: A > O > B > AB.

No differences in the distribution of the MN groups were noted in this work.

Table 1. Frequency of ABO, MN, Rh_0(D) and Diego (Di^a) Blood Groups in the Northern Part of Hiroshima

Classification	No. tested	Percentage observed			
		O	A	B	AB
Non-mixed group	1,125	28·53 ± 1·35	39·29 ± 1·46	21·51 ± 1·22	10·67 ± 0·92
Mixed group	508	31·10 ± 2·05	36·81 ± 2·14	23·23 ± 1·87	8·86 ± 1·26
		M	N		MN
Non-mixed group	1,125	33·24 ± 1·40	14·93 ± 1·06		51·82 ± 1·49
Mixed group	508	31·89 ± 2·07	17·13 ± 1·76		50·98 ± 2·22
		Rh_0 (+)		Rh_0 (−)	
Non-mixed group	1,125	99·82 ± 0·13		0·18 ± 0·13	
Mixed group	508	100·00		0·0	
		Di (a+)		Di (a−)	
Non-mixed group	309	8·09 ± 1·55		91·91 ± 1·55	

It is known that the frequency of Rh-negative individuals is quite low in Japan as compared with white populations, based on the investigations of Furuhata *et al.* The present investigations showed only 0·18 per cent in the non-mixed group and none in the mixed group.

Unfortunately the anti-Di^a serum was not suitable for testing all blood samples, and the tests, therefore, were only carried out on the non-mixed group. In this work, 8·09 per cent were found to be Diego positive.

The results of the ABO distribution suggested that a higher proportion of the B factor was found in the mixed group than in national distribution. On the other hand, the O factor and the A factor occurred with a lower frequency in the non-mixed group and in the mixed group respectively than in the others. In addition, the frequency of Rh_0(D) negative persons was slightly lower than in the report of Furuhata *et al.* The Diego factor was found to occur with a somewhat higher frequency but was in essential agreement with the findings of Yokoyama *et al.*, who have observed that the distribution of the Diego factor gradually increases from the northern to the southern part of Japan.

A higher incidence of group B, Rh_0(D) positive and Diego(Di^a) positive factors assures their position as Asiatic characters judged from an anthropological point of view.

We thank Dr. M. Layrisse, Caracas, Venezuela, for supplying the anti-Diego serum used in this work.

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Glucose-6-phosphate Dehydrogenase Deficiency in Indonesia

Glucose-6-phosphate dehydrogenase deficiency has been reported from Singapore[1], India[2], Malaya[3] and Thailand[4]. It has not been reported from Indonesia, although cases of hæmolytic anæmia due to the ingestion of drugs are known to occur.

It is therefore of interest to report the findings of a preliminary investigation of G-6-P dehydrogenase among Indonesians living in Djakarta, Indonesia.

The methods used were the dye test of Motulsky and Campbell[5] and the methæmoglobin reduction test of Brewer *et al.*[6]. A clearing time of more than 100 min was taken as abnormal for the test of Motulsky and Campbell. It was later realized that the clearing time was longer than 120 min in deficient persons. It was therefore considered necessary to follow up the test for at least 2 h in abnormal people. The clearing time was less than 90 min in normals and was usually 40–70 min. The Indonesians living in Djakarta originate from different parts of Indonesia but most of them are from west Java. Only males were examined, since the Motulsky test is not suitable for the detection of female carriers. 368 normal healthy Indonesians (laboratory workers, students and blood donors) and 78 Indonesian patients of the General Hospital medical wards were examined. 81 of the normal healthy persons were examined by the methæmoglobin reduction test; the rest were examined by the Motulsky and Campbell test. Results of the work are listed in Table 1.

Table 1

	No. examined	Deficient
Normals	368	5
Patients	78	0
Total	446	5 (=1·1%)

The frequency of 1·1 per cent is at the lower side as compared with the findings in other racial groups in Asia. A frequency of 2·5 per cent was found in normal healthy Chinese in Singapore and Malaya. In healthy Malays the frequency was 0·6 per cent for Singapore and 2 per cent for Malaya. The frequency in Indians has been found to range between 0·6 and 7 per cent[1–3,5,7]. In Malayan aborigines, high frequencies of more than 20 per cent were found in certain tribes. Higher frequency figures were also reported

from Thailand, where Kruatachue *et al.*[4] found a frequency of 14 per cent. Motulsky[5] found a frequency of 12·7 per cent in Philippinos. In the northern parts of Borneo frequencies of more than 6 per cent and up to 30 per cent in certain aboriginal tribes were found (Lie-Injo Luan Eng, unpublished).

The frequencies of G-6-P dehydrogenase in Djakarta, Singapore, Malaya and Thailand show a correlation with the frequencies of hæmoglobin E. The frequency of hæmoglobin E is low in Djakarta (2·5 per cent), high in Thailand and moderate in Singapore and Malaya (5 per cent). It is very high in the Malayan aborigines (30 per cent)[1,8]. This is higher than in many areas in Thailand. More or less parallel frequency figures have been found for G-6-P dehydrogenase. Similar positive correlations of frequencies for G-6-P dehydrogenase and hæmoglobin S or thalassæmia have been found in Africa and Sardinia[5,9]. Also a parallelism between G-6-P dehydrogenase frequencies and abnormal hæmoglobins has been demonstrated in Greece by Allison *et al.*[10]. It is suggested that this positive correlation would be expected if all the genes were favoured by malarial selection.

We thank all volunteers who have given their blood for examination and the Red Cross, Blood Transfusion Service, Djakarta, for the blood samples.

This work was supported by the U.S. Public Health Service grant *AI* 04189–02.

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IMMUNOLOGY

A Micro-slide Method permitting Autoradiography of Hæmolysin-producing Cells

THE immune response to a single injection of sheep erythrocytes has been widely used in investigations of the primary antibody response. Recently, Jerne *et al.*[1] have widened the potentialities of this experimental model by a method for identification and enumeration of hæmolysin-producing cells (the agar-plaque technique). Cells from a hæmolysin-producing lymphoid organ are mixed and incubated at 37° C with sheep erythrocytes in an agar medium. After addition of complement, hæmolysis occurs in zones around cells from which antibody has diffused into the medium. A similar method has been described by Ingraham[2]. The following modification of the agar-plague technique enables tracing of radioactive isotopes to hæmolysin-producing cells by autoradiography.

The agar used is rendered non-anticomplementary with di-ethyl-amino-ethyl-dextran[3] as follows. 2 mg of DEAE-dextran (made from dextran with mol. wt. around 2×10^6; nitrogen content 3·2 per cent; sulphate ash < 1 per cent (Pharmacia, Uppsala, Sweden)) are dissolved in 100 ml. of a doubly concentrated Eagle's medium[4]. At 60° C one part of this solution is mixed with an equal part of 5·6 per cent agar ('Noble-agar', Difco). The precipitate is eliminated by centrifugation for 15 min at 3,000 r.p.m. at 45° C. A stock of this 2·8 per cent agar is kept at −20° C.

Carefully rinsed micro-slides are first covered with a layer of blood-agar. Melted 2·8 per cent agar and a 16 per cent suspension of sheep erythrocytes in Eagle's medium are kept in a water-bath at 45° C. 11 ml. of the erythrocyte suspension are mixed with 6 ml. of the agar solution in a glass cuvette placed in the water-bath. Micro-slides are dipped into the blood–agar mixture, allowed to drip off on cheese cloth for a second, wiped off with the cloth on one side, cooled on a metal plate for a few seconds, and then placed horizontally in a cool moist chamber. The moist chamber is then kept in the refrigerator until the glasses are used within a few hours.

The lymphoid cells to be tested are prepared by cutting lymph nodes or spleen with scissors into small fragments which are then passed through a fine metal mesh into Eagle's medium. Throughout the procedure (from excision of the organ up to the final mixing with agar) the cell material is kept strictly at 0° C. The cells are washed twice with Eagle's medium by centrifugation at 1,500 r.p.m. for 10 min and finally resuspended in a volume of Eagle's medium that gives a concentration of 4×10^7 cells/ml. The cell–agar mixture is prepared as follows. A tube containing 0·5 ml. of the cell suspension is heated for a few seconds in the 45° C water-bath. From this tube, 0·3 ml. are transferred to a glass cuvette (in the water-bath) containing a mixture of 10 ml. Eagle's medium and 3·6 ml. 2·8 per cent agar. The cell–agar suspension is mixed gently. The blood–agar slides, which 15 min earlier have been preheated by transfer to a moist chamber at 37° C, are rapidly dipped in the cell–agar suspension, allowed to drip off on cheese cloth for a second, wiped off on the blood–agar free side, and immediately placed horizontally in a cool moist chamber. The chamber is kept in the refrigerator for 15 min.

The blood–agar- and cell–agar-covered slides are then transferred to a moist chamber at 37° C and incubated at this temperature for 70 min. About 0·15 ml. of a good guinea-pig complement diluted 1 : 12 with Eagle's medium is then poured on the agar layer and kept in place with a

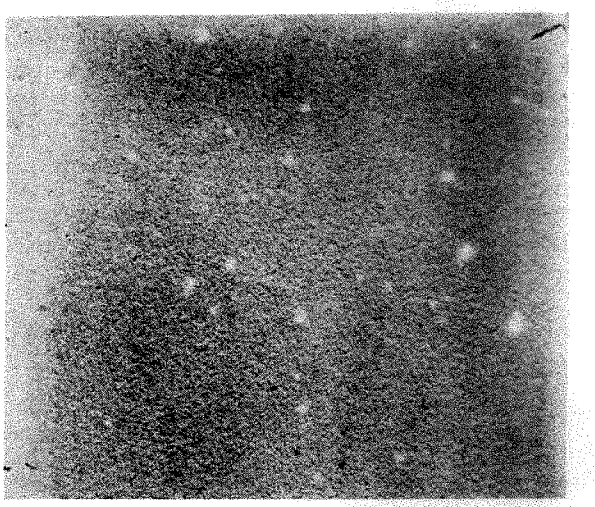


Fig. 1. Blood–agar-covered micro-slide with hæmolytic zones produced by mouse spleen cells (4 days after intravenous injection of sheep erythrocytes)

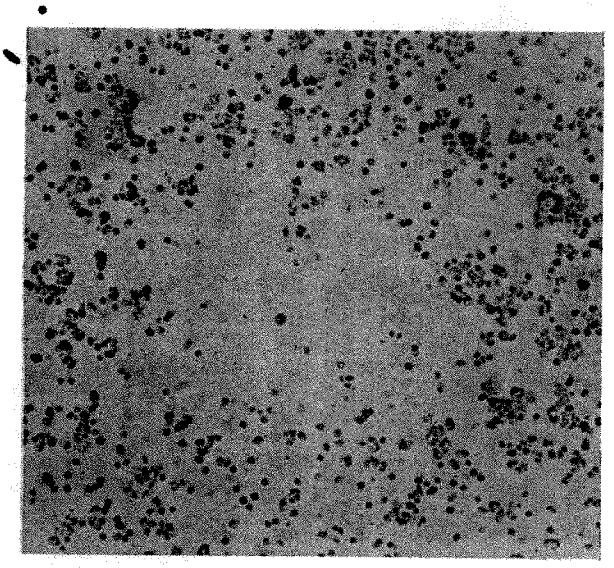


Fig. 2. Autoradiogram of a micro-slide preparation produced as indicated for Fig. 1. Low-power view of a hæmolytic zone with the central antibody-producing cell. The mouse had received tritiated thymidine intraperitoneally on the third post-immunization day

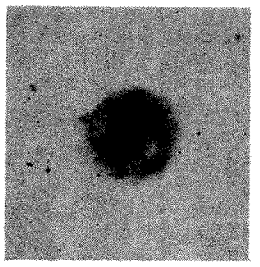


Fig. 3. High-power view of the central cell shown in Fig. 2. Five silver grains are located above the cell

cover-slip that covers the area containing lymphoid cells (about two-thirds of the slide). The slides are again incubated at 37° C for 40 min. The cover-slips are then gently slid off, and the complement is allowed to drip off for a few seconds. To protect the white cells during drying and fixation, the slides have to be washed by dripping about 0·2 ml. of a 1 : 6 dilution of serum (inactivated and absorbed with sheep erythrocytes) on the agar-covered area. As much as possible of the serum dilution is sucked off. The glasses are then dried by a cool fan for 1 h and finally fixed in methanol for 45 min. The hæmolytic zones are now easily detected by viewing the glasses through a low-power magnifier against a light source. It is advisable to encircle the zones on the back of the slide, using a diamond pencil. Slides incubated with inactivated complement may be used as controls. Figs. 1 and 2 show the macroscopical and microscopical appearance of the zones. The sheep-erythrocyte concentration used ensures fairly small but well-defined zones, which can be easily examined in the microscope. With more concentrated lymphoid-cell suspensions, more than one cell is often found in the centre of the zone and it is impossible to say which of them is the hæmolysin-producing cell.

The slides can be kept for a long time and used for histological investigation of the morphology of the antibody-producing cells. Sterzl and Mandel[5] have developed another modification of the agar-plaque technique, which also enables such investigations.

For autoradiographic tracing of tritiated thymidine to nuclei of hæmolysin-producing cells the methanol-fixed slides are first painted with egg-albumen–glycerol (1 : 1) on the margins of the agar layer and then coated with liquid autoradiographic emulsion (*K* 2 Ilford) by the method of Messier and Leblond[6].

After exposure, development and fixation, the slides are stained through the film with Giemsa by Gude's[7] technique.

This work was supported by a grant from the Swedish Medical Research Council.

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Nature of the Normal Lymphocyte Transfer Reaction

The normal lymphocyte transfer (NLT) reaction is aroused when lymphocytes from one normal adult guinea-pig[1] or human being[2] are injected intradermally into another normal adult guinea-pig or human being, and it makes itself apparent as an inflammation of delayed onset which reaches its peak about two days after injection. Reactions which are later to develop into strong reactions are already discernible as soon as 15 h after injection, and for this reason some immunologists have been hesitant to accept our interpretation that the inflammatory process is the outward sign of an immunological reaction of the lymphocytes against their recipient, that is, of a primary 'graft-versus-host' reaction. Our original case for this interpretation rested (*a*) on its firm theoretical foundation, and (*b*) on the fact that the intensities of the reactions aroused by lymphocytes from a guinea-pig *R* in a panel of potential homograft donors D_1, D_2, D_3 . . . were almost exactly correlated with the intensities of the reactions provoked by grafting skin from D_1, D_2, D_3 . . . on *R*. This is just as it should be if the NLT reaction is an immunological response of the *R* lymphocytes to the 'transplantation antigens' of the *D* guinea-pigs. We now give further and, as we believe, conclusive evidence under two headings that this interpretation is in fact correct.

(1) Guinea-pigs which have received 500–1,500r. whole-body irradiation on a single occasion at the rate of 80r./min from a cobalt-60 source are fully capable of giving expression to an NLT reaction excited by blood lymphocytes or lymph-node cells injected from 2 h to 3 days later. The reactions begin at ordinary levels of intensity and remain so for three days; but where in a normal guinea-pig the reactions would now progressively fade, the inflammatory response in irradiated guinea-pigs rises between the third and the fourth day and continues to rise until, by the sixth or seventh day, the sites of injection have become transformed into huge purple lesions lying outside the ordinary range of scoring. Thus, when any possible immunological contribution of the host is reduced or abolished by irradiation, the NLT reaction, so far from being weakened, is intensified and prolonged.

(2) By making use of two unrelated inbred strains of guinea-pigs—Wright Strain 13 and the Heston Strain, itself remotely derived from Wright Strain 2—we have been able to put into practice the now classical experimental design in which lymphocytes from members of the two inbred strains are injected into the first generation hybrids of a cross between them, and vice versa. If the lymphocytes are reacting against antigens in their recipients, then an NLT reaction will be aroused by parental cells in the hybrid but not by hybrid cells in the

Table 1. NORMAL LYMPHOCYTE TRANSFER REACTION

	Recipients			
Heston lymph node cells	Heston −, − −, −	Strain 13 + + +, + + ± + +, + +	Hybrid (*Hes* × 13) + +, + + + ±, + +	Hartley + + + ±, + + + + + + ±, + + + ±
Strain 13 lymph node cells	+ +, + + ± + +, + + ±	−, − −, −	+ + ±, + + + + ±, + ±	+ + +, + + ± + + +, + + +
(*Hes* × 13) lymph node cells	−, − −, −	−, − −, −	−, − −, −	+ ±, + + ±, +

Readings 48 h after the intradermal injection into normal adult Heston, Strain 13, hybrid (*Hes* × 13) and Hartley guinea-pigs of 10 million mesenteric lymph node cells from normal adult Heston, Strain 13, and hybrid (*Hes* × 13) guinea-pigs: showing that the 'graft-versus-host' component is the only outwardly discernible component of the NLT reaction at this stage.

parent. If on the other hand the NLT reaction is the consequence of the host's response to antigens carried by the lymphocytes, then the exact converse should obtain. These possibilities were tested by the intradermal injection of 10 million mesenteric lymph node cells, or 5 million blood lymphocytes, in the combinations set out in Table 1. (Lymph node cells were preferable because our yields of blood lymphocytes from Heston and Strain 13 donors were relatively low, and contamination by red cells therefore relatively high. Normally red cells create no problem, but in situations in which the lymphocytes arouse no inflammatory reaction the red cells are not removed, and the brown stain they leave at the injection site can be a source of confusion.) The results that can be read from Table 1 are fully representative: (1) lymphocytes of the same genotype as their recipients arouse no reaction; (2) Heston and Strain 13 lymphocytes excite reactions in F_1 (*Hes* × 13) hybrids; (3) F_1 (*Hes* × 13) lymphocytes arouse no reactions in Heston or Strain 13 guinea-pigs; (4) lymphocytes from F_1 hybrids give rise to smaller reactions in Hartley guinea-pigs than do lymphocytes of either of the parental strains: this must be so if the 'graft-versus-host' interpretation is the correct one. It may be added that parental lymphocytes excite strong and progressive reactions in irradiated (800r) F_1 hybrids, so eliminating the very remote possibility that the NLT reaction in this genetic context owes anything to a response of the hybrid host to powerful recessive antigens in the parental cells.

These further lines of evidence uphold the belief that the 'graft-versus-host' reaction is the only immunological component of the NLT reaction that is outwardly discernible in the first three days. It is this property which justifies the use of the NLT reaction in the selection of donors for tissue transplantation[1,2].

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Reagin-like Antibodies in Animals Immune to Helminth Parasites

RECENT investigations here have shown that rats, monkeys and sheep infected with helminth parasites produce an antibody with many of the properties of human reagin. Naturally occurring persistent antibodies of this type have not been demonstrated previously in animals, although in rats an antibody similar in its biological properties but transitory in appearance can be stimulated when antigen is injected with *Haemophilus pertussis* killed organisms as adjuvant[1,2]. Like human reagins[3], the antibodies stimulated by helminth infections become rapidly and apparently permanently attached to the skin of animals of the species in which they are stimulated, but they are incapable of sensitizing guinea-pig skin in passive cutaneous anaphylaxis.

Rats infected with their natural nematode parasite *Nippostrongylus brasiliensis* have been used as a model system in these experiments, and results obtained from this system applied to rats and monkeys infected with *Schistosoma mansoni* and to sheep infected with *Trichostrongylus colubriformis*. Reaginic antibodies are detected by utilizing their high affinity for cells in passive cutaneous anaphylaxis[4,5] with a 3-day interval between intradermal injection of antibody and intravenous injection of antigen. Serum from an infected animal is injected into the skin of a normal, worm-free animal of the same species as the animal donating the serum. Three days later, a mixture of antigen and Evans blue is injected intravenously. Within 20–30 min a discrete blue area, approximately 1·5 cm in diameter (but greater with strong reaginic antisera), appears at the site of injection of a serum containing the skin-sensitizing, reaginic antibodies. This test is the same in principle as the Prausnitz–Küstner test, which so far is the only reliable method available for measuring human reagins[3]. The reagin-like antibody titre of serum from helminth-infected animals is measured by serial dilution of the serum before intradermal injection. The test is positive for 7–10 days after intradermal injection of the antibody in rats, the maximum reaction being reached after 3 days. In this way, reaginic antibodies are distinguished from other antibodies which do not have an affinity for cells and which diffuse rapidly away from the site of injection[5]. With *N. brasiliensis* the best source of antigen for this passive cutaneous anaphylaxis is adult worms, although infective larvæ contain a lesser amount. Living adult worms collected from donors are broken up in a hand tissue grinder in phosphate buffered saline and the supernatant is used in the passive cutaneous anaphylaxis test, either fresh or after storage at −20° C.

In rats infected with *Nippostrongylus brasiliensis* (which becomes mature in the small intestine), reaginic antibody can be detected in the serum generally from day 25 after an initial infection and sometimes as early as day 19. In this species the production of reagin-like antibody is associated with sensitivity to anaphylactic shock[1,2]; and rats infected with *N. brasiliensis* can be shocked from the time that immunity first appears, that is, from the time egg counts fall at the end of an initial infection. As the gut is the 'shock organ' in rats[6], this behaviour suggests that the reaginic antibody is present on cells of the gut from the time the rat becomes immune. Unlike the transitory *H. pertussis*-induced reagin, the helminth-induced reaginic antibody in rats persists in the circulation for at least 7 months after a single infection, even in the absence of subsequent infections. Within 3 days of second and later infections, the antibody titre rises to high levels (1/200 to 1/1,600), remains high for a short period and then falls to a low level until a further infection.

Attempts to induce the reaginic antibody artificially by vaccinating rats with freshly homogenized adult worms or with the supernatant fraction only, with or without Freund's complete adjuvant, have been unsuccessful. No immunity to re-infection is stimulated by this method of vaccination, although precipitating antibodies (similar to those found occasionally in serum from multiply infected, highly immune rats[7]) are stimulated by it. On the other hand, the serum from vaccinated animals contains an

antibody which will inhibit the passive cutaneous anaphylaxis reaction if the antigen used in the reaction is incubated with the serum prior to injection; this blocking antibody may correspond to that found in the serum of allergic human patients after desensitization by injection of massive amounts of allergen[8]. The reaginic antibody found in serum from immune rats appears to be stimulated only by a living infection. A single normal infection of *N. brasiliensis*, a single infection with adult worms transferred into the duodenum, or 3 infections of larvæ irradiated to prevent development beyond the lung stage, will stimulate both immunity to re-infection and the production of the reagin-like antibody.

The antibody is stable at −20° C for at least 2 years, but it is destroyed by heating for 1 h at 56° C. Serum heated until reaginic activity disappears can no longer transfer immunity to re-infection passively. However, this does not provide definite proof of the role of reaginic antibody in immunity to infection with this parasite, as precipitating antibody from rats vaccinated with extracts of *N. brasiliensis* is also affected by such treatment so that it no longer precipitates in agar gel. This finding differs from previous work which indicated that hæmagglutinating rat antibody was unaffected by heat treatment[1].

In collaboration with Dr. S. R. Smithers, infections of *S. mansoni* have also been found to stimulate the production of reaginic antibodies in monkeys and rats. As with *N. brasiliensis*, the appearance of the antibody is correlated with the development of immunity to re-infection. Furthermore, when normal animals are infected by penetration of cercariæ through a skin area, saturated 3 days previously with reagin-containing serum, many cercariæ fail to develop to the adult stage.

There are many phenomena associated with helminth infections which may be explained by the presence of antibodies of the type described here, such as the self-cure reaction in sheep, immediate skin reactions in man, mast cell disruption and perh ps eosinophilia. Reagins cause disruption of mast cells when they react with homologous antigen[1,2]. Eosinophilia is closely associated with mast cell disruption, and is a characteristic feature both of helminth infections and of allergic reactions in man[10]. In mice infected with *Trichinella spiralis* degranulation of mast cells occurs when homologous antigen is injected[11], suggesting the presence of reaginic antibodies in *Trichinella* infections. Immediate skin reactions to worm extracts are used in diagnostic tests for helminth infections in man[9] and probably depend on the presence of reagins. It has been suggested that the self-cure reaction which is a characteristic feature of *Haemonchus contortus* infections in sheep may be explained by the presence of reagins[12]. Preliminary experiments jointly with Dr. D. L. H. Robinson have demonstrated an antibody with a high affinity for skin cells in sheep infected with *T. colubriformis*; but in this case the relationship between immunity to re-infection and the presence of this antibody is not yet clear.

So far, we have been able to stimulate reagins only by living infections, not by vaccinating animals with extracts of parasites. Nevertheless, these parasite extracts contain antigens which will react with reagins in passive cutaneous anaphylaxis. Stimulation of reagins must depend, therefore, on factors yet to be determined such as the route or rate of administration of the antigen, its physical or chemical state, and the ratio between the protective antigen and other components of the extracts used as antigen. We have shown that there is a close relationship between immunity to infection and the presence of reagins with the nematode *N. brasiliensis* and the trematode *S. mansoni*; it may well be that reagin-like antibodies are responsible for immunity to helminths in many species of animals. If reagins are necessary for immunity to re-infection, the problem of producing a vaccine for helminth parasites may be two-fold, first detection and production of the antigens responsible for protection, and secondly, injection of this antigen so that the protective antibody is stimulated.

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BIOLOGY

Effect of Damage and Fungal Infection on the Production of Ethylene by Carnations

Although it has been well established that ethylene is produced in floral metabolism it is only recently that precise measurements have been made[1]. The rate of production by cut flowers is sufficient under conditions of limited ventilation to raise the concentration to levels which can have harmful effects. We have observed concentrations as high as 0·06 p.p.m. in cardboard boxes close-packed in the hold of the R.M.S. *Scillonian* carrying daffodils from the Isles of Scilly to the mainland. Concentrations of this order have been shown in this laboratory to reduce the life of the flowers if they are subjected to them for a period of several hours at room temperature.

The extent to which mechanical damage during packing and transport contributes to this production of ethylene is not known. Several workers have shown that damage to plant tissues is instrumental in raising the rate of production of ethylene, and that fungal attack will stimulate ethylene production[2–5].

During a series of experiments in which the rate of production of ethylene by carnations was being determined by gas chromatography, using equipment described pre-

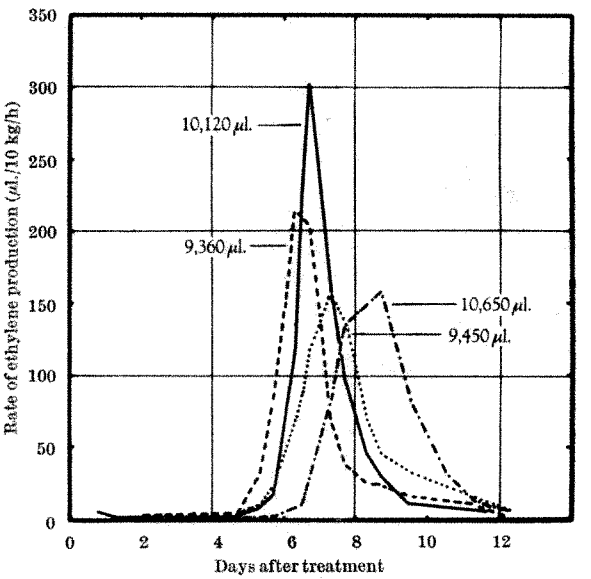


Fig. 1. Rates of production of ethylene from samples of 6 cut carnation flowers receiving treatments: untreated (·——·); inoculated only (.....); damaged only (—); damaged and inoculated (- - - -). Total volume of ethylene produced during the experiment from each sample is indicated

viously[6], a marked surge of ethylene production was observed with the onset of fungal attack. Further experiment gave comparison between the courses of production from undamaged flowers, flowers undamaged but inoculated with spores of *Botrytis* sp., flowers damaged by pricking the petals with a needle and flowers damaged and then inoculated. This showed that damage itself did not at once lead to a rise in ethylene production, that inoculation brought about an earlier rise in rate, while inoculation after damage resulted in the earliest rise in rate. The amounts of ethylene produced during the experiment by the samples were similar.

It seems likely that the ethylene is produced in the process of breakdown of the host tissues, since during growth of *Botrytis* sp. from carnation flowers on malt agar culture, no production of ethylene was observed.

These observations are of some practical significance. It is not unknown for flowers infected with fungal organisms to start to decay in transit. The magnitudes of ethylene production observed in these experiments suggest that if packages of infected blooms are included in a close-packed consignment they could constitute a hazard to the remainder.

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Reversal by Histidine of the Inhibition of *Prototheca* Growth due to 3-Amino-1,2,4-triazole

3-Amino-1,2,4-triazole (amitrole) is used as a systemic herbicide. It also inhibits the growth of a number of micro-organisms including several algae. Organic acids have long been known to inhibit the growth of algae by combining with iron[1]. Aaronson[2] concluded that amitrole was also working in this way as the inhibition of *Ochromonas danica* could be abolished by the supply of extra iron to cultures in the presence of the inhibitor. Later Wolf[3], examining *Chlorella pyrenoidosa*, and Boney[4] certain marine red algae, reported that the inhibition by amitrole could be reversed by adenine. In this the algae are similar to *Escherichia coli*[5]. Histidine is effective in releasing the inhibition of *Torula cremoris*[5] and of *Saccharomyces cerevisiae*[6]. Since the standard medium used by Aaronson contained histidine[7], an investigation of the reversal of amitrole toxicity towards *Prototheca zopfii* was carried out. This colourless alga is believed to be closely related to *Chlorella* and it will grow in a mineral medium supplemented with glucose and thiamine[8]. Aaronson and Scher[7] showed that there was no significant difference in the concentration of amitrole required to achieve inhibition of *Euglena* and *Ochromonas* growth in the presence of an organic substrate in the dark as compared with the light although chlorophyll synthesis was inhibited.

Pure cultures of *Prototheca zopfii* (Cambridge Collection No. 263/5) were grown in 100-ml. Erlenmeyer flasks containing 20 ml. of a mineral salts medium[9] with the addition of 1 per cent glucose and 10^{-5} M thiamine. Additions of amitrole, and of other substances being tested, were usually made prior to the autoclaving of the medium (10 lb. for 15 min). After the flasks had been inoculated under aseptic conditions they were shaken in darkness at 25° C. After 2 or 3 days the dry weight of each culture was determined by use of sintered glass filter crucibles. Amitrole was obtained from L. Light and Co., Ltd. Other chemicals were obtained from B.D.H., Ltd., and were of analytical reagent quality where possible.

Initial experiments suggested that the concentration of amitrole giving 50 per cent inhibition of growth after 2 days was in the region of 8 mg per cent. Control cultures reached their final dry weight in 2 days. If inhibited cultures were left for a further day the concentration of amitrole corresponding to 50 per cent inhibition of growth was increased to some 15 mg per cent. A similar effect was described by Hilton[6] in relation to the inhibition of yeast growth by amitrole.

The ability of histidine to reverse the inhibition of the growth of *Prototheca* during a period of 2 days is shown by the results of an experiment in which the amitrole concentration was 15 mg per cent (Table 1). Riboflavin supplied in a similar experiment was without effect. Castelfranco *et al.*[10] have recently reported that riboflavin is effective in overcoming the chlorosis of higher plants, shown in the presence of amitrole, because the amitrole is decomposed in the presence of riboflavin and light.

Table 1. Effect of Various Concentrations of Histidine and Riboflavin on the Growth of *Prototheca zopfii* in the Presence and Absence of 15 mg per cent Amitrole

Conc. of histidine or riboflavin	Dry wt. (mg) Histidine		Riboflavin	
	− Amitrole	+ Amitrole	− Amitrole	+ Amitrole
0	101	13	103	8
5 mg %	104	101	101	10
10 mg %	103	102	100	10
15 mg %	100	104	101	8
25 mg %	101	102	101	10
50 mg %	100	99	101	11

Each flask contained 20 ml. of mineral medium with the other additions indicated. After inoculation the flasks were shaken in the dark at 25° C for two days when the dry weight of *Prototheca* in each flask was determined. The dry weight of the inoculum was 1·5 mg.

In the medium used iron is supplied as $FeSO_4.7H_2O$ in the presence of sodium citrate. Increasing the amounts of these salts supplied in proportion to the standard medium concentrations did not alter the amount of growth in control flasks or the degree of amitrole inhibition even at ten times the normal concentration of iron. Supply of adenine did not reverse the inhibition although it was reported to be effective in reversing the inhibition of *Chlorella pyrenoidosa*[3] (Table 2). In the case of *Chlorella* the concentration of amitrole required for 50 per cent inhibition was only 0·5 mg per cent, and the adenine concentration completely reversing this was of the order of 1 mg per cent, during autotrophic growth over 3–4 days. Adenine sterilized by Seitz filtration was equally ineffective in the case of *Prototheca*.

Table 2. Effect of Adenine on the Growth of *Prototheca zopfii* in the Presence and Absence of 15 mg per cent Amitrole

Adenine concentration	Dry wt. (mg) − Amitrole 2 days	+ Amitrole 2 days	+ Amitrole 3 days
0	101	17	62
5 mg %	100	14	45
10 mg %	99	13	52
15 mg %	99	14	48
25 mg %	102	13	40

Conditions of experiment as given in Table 1. Inoculum was 1 mg dry wt.

The results show that histidine will release the inhibition of growth by amitrole in the case of at least one alga and are in agreement with the conclusion of Hilton *et al.*[11] that reductions of amitrole toxicity by purines are usually small in comparison with those of histidine. Weyter and Broquist[5] found that histidine would release the inhibition of *Torula cremoris*, for which adenine was ineffective, but not that of *E. coli* unless adenine was also supplied when the reversal of inhibition was more complete than in the presence of adenine alone. Interactions between histidine and purines are also known from nutritional[12] and genetical[13] investigations suggesting that the site, or sites, of action of amitrole in metabolism may soon be detected. Klopotowski and Hulanicka favour inhibition of histidine biosynthesis[14], probably at the level of imidazoleglycerolphosphate dehydrase, as imidazoleglycerolphosphate accumulated in *Saccharomyces cerevisiae* in the presence of amitrole.

This explanation could apply in all cases where inhibition is overcome by histidine alone including *Prototheca* and several yeasts[5,6]. Castelfranco and Brown[15] suggest that the inhibition involves one electron oxidation of the amitrole.

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Somatic Apospory and Polyembryony in *Minuria integerrima* (DC) Benth

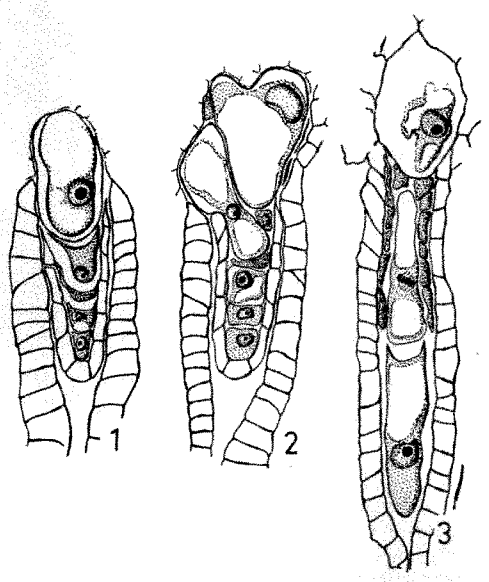
The genus *Minuria* (DC) is endemic to Australia and its six species are small undershrubs of the drier parts of the continent.

Material of *M. integerrima* (DC), Compositae, Astereae was examined from western New South Wales (Bourke, Condobolin, Brewarrina) and western Queensland (Dalby), all plants of which proved to be obligate apomicts with frequent polyembryony.

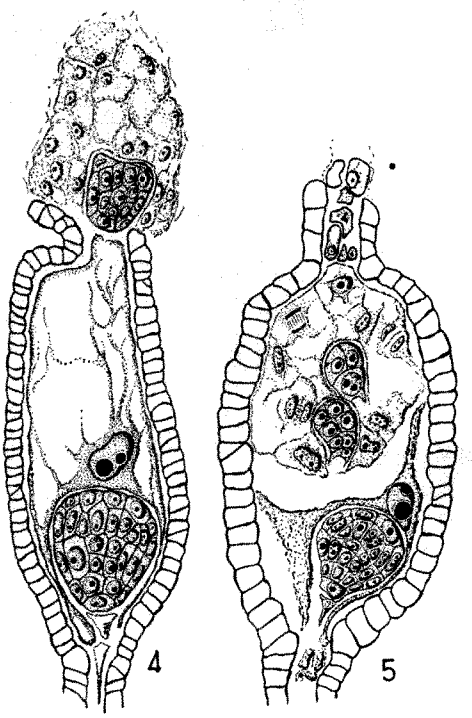
The single hypodermal archesporial cell functions directly as the megaspore mother cell and meiosis results in the formation of an apparently normal linear tetrad of megaspores which is enclosed by the nucellar epidermis.

During megasporogenesis, however, 1–3 chalazal nucellus cells develop large vacuoles and the closest one enters the nucellar lobe where it crushes, and finally replaces, the megaspores (Figs. 1 and 2). Breakdown of the nucellar epidermis then releases this aposporic cell into the micropylar chamber and after three successive nuclear divisions it becomes an 8-nucleate unreduced embryo sac of normal constitution. Fig. 3 shows two such aposporic cells developing within the endothelium, and a third one in the chalaza.

Multicellular apospory of this type has been reported in *Hieracium*[1], *Artemisia*[2] and *Crepis*[3], and in *M. integerrima* it is the basis of polyembryony, since each aposporic embryo sac commonly gives rise to an embryo by parthenogenetic development of the unreduced egg. A similar conclusion was reached by Rosenberg[4] in connexion with polyembryony in *Hieracium* subj. *Pilosella*.



Figs. 1–3. (Figs. 1 and 2, × 500; Fig. 3, × 400)



Figs. 4 and 5. (Fig. 4, × 150; Fig. 5, × 250)

In *M. integerrima*, when a single embryo sac develops, the polar nuclei unite to form the secondary nucleus which almost immediately divides into the first two endosperm nuclei. However, in every instance in which polyembryony occurred, the secondary nucleus of the micropylar embryo sac did not divide and consequently no endosperm was formed, although its embryo developed normally (Figs. 4, 5).

I thank Prof. N. C. W. Beadle, University of New England, and Mr. C. K. Ingram, Bathurst, for collecting the material on which this investigation was based.

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A Staining Technique for the Examination of Nematode-trapping Fungi

Janus green, a bluish green basic dye of the mono-azo group, was observed to react in an interesting and useful manner with both nematode-trapping fungi and their prey. The dye is used at a concentration of 0·01 per cent in 0·2 M sodium acetate–acetic acid buffer at *p*H 4·6, and added drop by drop to the test material. Excellent results were obtained when the staining solution was applied directly to surface cultures of *Arthrobotrys conoides*, *A. dactyloides* and *Dactylella ellipsospora*, on maize-meal extract agar and on 'Cellophane'[1]. These fungi capture nematodes by means of adhesive hyphal loops, constricting rings, and adhesive knobs, respectively. The nematode used was *Panagrellus redivivus*.

Fungal walls and particulates (mitochondria and nuclei ?) stain dark green to blue. Organelles of capture that trap nematodes by adhesion stain most intensively, suggesting that the mucilage with which they are coated is acidic. Nematodes, captured and killed as a result of fungal activity, were bright yellow in colour. This is of particular interest since heat-killed worms stain dark green and living worms do not stain at all. The latter, when immersed for 24 h in a solution of Janus green, remain active and penetration by the dye is limited to the mouth region.

Janus green can be reduced biologically to a leuco base[2]. It is possible that partial reduction of the dye occurs in fungus-infested nematode tissue, and that the product of this transformation imparts to the preparation a bright yellow colour. Further speculation regarding the biochemical basis of the difference in colour produced when heat-killed and fungus-killed nematodes are stained with Janus green is not justified at this time. It is necessary only to emphasize that in treated cultures the dark-green fungal filaments and organelles of capture contrast dramatically with bright-yellow nematode carcasses. Contrast is augmented by lactophenol, and addition of this solution serves also to reveal hyphæ located within dead worms. Janus green facilitates greatly the investigation of nematode-trapping fungi as they develop in pure culture or from a soil inoculum. It appears to be a useful adjunct to the agar-disk method described by Cooke[3] for the direct observation of nematode-trapping fungi in soil.

This work was supported in part by U.S. National Science Foundation research grants *G*9749, *G*15950 and *GB*548.

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Mechanism of Erection and Ejaculation in the Bull and Ram

It is generally considered that erection of the penis is produced by a combination of raised arterial pressure, restricted venous return, and relaxation of the walls of the cavernous spaces. In the bull and the ram, the process is extremely rapid, both in erection and relaxation, suggesting that a more positive mechanism exists.

A detailed investigation of this type of penis has been carried out using latex corrosion casts, gross dissection, and histological methods. The findings indicate a very different mechanism from that at present accepted, and certain anatomical similarities suggest that, in principle, it may apply to other mammals, including man.

The most significant feature of the two ruminant species investigated concerns the crura of the corpus cavernosum penis. These have become bulbous structures, situated between the ischial bones and the ischio-cavernosus muscles, so that contraction of the latter compresses the crura, forcing blood into the corpus cavernosum penis. This pumping action in the dog was noted by Henderson and Roepke[1]; but these authors were looking for a more obvious effect from the skeletal musculature and consequently attached little significance to the phenomenon.

The crura are covered externally by tunica albuginea, while internally there are large and small cavernous spaces. The larger ones receive blood from the deep artery of the penis (a. profunda penis), which is then passed to a large thick-walled vessel, which commences at the distal extremity of the crus. This joins the similar vessel from the other side and then, as a single vessel, traverses the dorsal part of the corpus cavernosum to the region of the sigmoid flexure, where it enters two large vessels situated at either side of the sulcus urethræ. These have their origin from the cavernous spaces at each end of the corpus cavernosum and their greatest diameter at the level of the flexure. They are intrinsic vessels of the corpus and their only connexion is to the dorsal vessel. The latter is generally described in anatomical texts as the continuation of the deep artery, while the ventral vessels are referred to as venæ profundæ. This nomenclature is obviously inapplicable in this case. From the ventral vessels arise side branches, which like the parent vessels have thick walls composed of elastic and smooth-muscle elements. The cavernous spaces are continuations of the side branches. Deysach[2] called them 'sluice channels' and postulated that they had a valvular action. The work recorded here does not support this finding, however, and probably the structure of the walls is concerned with maintaining a constant lumen under pressure. The cavernous spaces are lined by a single layer of endothelium and are surrounded by masses of fibro-elastic tissue which keep the spaces collapsed when the penis is quiescent. Pressure from the ventral vessels opens the spaces and compresses the fibro-elastic tissues, thus producing rigidity in the penis.

Increased pressure in the deep artery of the penis expands the larger spaces in the crura of the corpus cavernosum, causing them to swell. Under the stimulus of sexual excitement, the ischio-cavernosus muscles contract, compressing the crura and forcing blood into the vessels of the corpus cavernosum. Relaxation of the muscles allows blood from the deep artery to refill the spaces of the crura and this sequence is repeated until pressure in the corpus cavernosum equals that in the artery. Removal of the sexual stimulus stops this pumping action, and the retractor penis muscles actively pull the organ back into the prepuce thereby re-establishing the sigmoid flexure. This action displaces blood from the corpus cavernosum, which is passed back along the route by which it entered. Secondary drainage is carried out by relatively small veins which are connected to the smaller spaces of the crura.

In general this work has shown that the penis is adapted to attain erection with a minimum transfer of blood. The entire organ is enclosed in a thick, dense tunica albuginea which is virtually non-distensible, the corpus cavernosum is isolated except at the crura, and much of the internal volume has been reduced in terms of blood space by an increase in the amount of fibro-elastic tissue.

Forceful ejection from the urethral orifice is brought about by the bulbo-urethral muscle which encircles the bulbs of the urethra. The bulbs consist of large vascular spaces, filled by the artery of the bulb (a. bulbi urethræ). They are closed posteriorly but are continuous anteriorly with the sinuses surrounding the urethra. These commence around the pelvic urethra and have a continuous lumen to the glans penis where they connect with the deep dorsal vein. In the bull, the glans has very limited ability to distend. In the ram, however, there is a network of vessels in the galea glandis which is engorged by blood unable to escape by the deep dorsal vein. This is responsible for the enlargement of the glans in this penis. The vascular space of the tuberculum, situated behind the glans, is similar to that of the galea glandis in the method of engorgement.

The progressive contraction of the pelvic urethral muscle is continued by the bulbo-cavernosus muscle and forces blood from the spaces of the bulbs of the urethra into the sinuses. These, already distended and restricted by the corpus cavernosum penis and the tunica albuginea, are unable to expand externally, so that the pressure is transferred distally towards the glans, compressing the lumen of the urethra as it progresses. The contents of the latter are forced along the lumen ahead of this constriction and forcibly expelled at the external orifice.

Although veins have been described in relation to the bulbs of the urethra, they have not been observed in any of the specimens examined in this investigation. If they did exist their presence would detract from the efficiency of the system.

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Observations on Sexual Attraction and Copulation in the Nematode *Panagrolaimus rigidus* (Schneider)

When observing cultures of *Panagrolaimus rigidus* to examine their life-history, copulation seemed to depend simply on chance encounters between males and females. However, as males often touched males without attempting copulation, there was the suggestion that different sexes attracted each other and those of the same sex repelled one another. Experiments were therefore made to see whether the nematodes produce sex attractants or repellents. The act of copulation was also observed in detail.

Fourth stage larvæ from cultures of *P. rigidus* on Nigon's nutrient agar[1] were placed singly in separate culture chambers to develop into adults. Troughs (Fig. 1) were made from lengths of clear polyvinyl chloride tubing of two sizes. Pieces of the narrower tubing, with a 'Cellophane' sheet placed over the end, were fitted into pieces of the wider tubing to make a firm joint between different sections of the trough, which were separated from each other by 'Cellophane' barriers. The two centre sections were marked off in centimetres from the central 'Cellophane' barrier. The sections were anchored to thin strips of 'Perspex' with adhesive tape. The troughs were filled with hot 1·5 per cent water agar so that good contact was made between bubble-free agar and the 'Cellophane' barriers. When the agar had cooled, 50 nematodes were introduced at the mid-points of each of the two centre sections and the two end sections left blank. To prevent drying out, the troughs were placed in closed tubes and left in the dark for 17 h at room temperature. They were then removed from the tubes, cut into cm lengths and the nematodes in each length counted.

When nematodes of the same sex were separated by a 'Cellophane' barrier, they were randomly distributed over each section, but when the different sexes were separated, they collected at the barrier (Fig. 2). Few nematodes remained between 1 and 2 cm from the barrier.

These results suggest that the sexes are brought together by water-soluble chemical attractants of molecular weight small enough to pass through a 'Cellophane' barrier and to diffuse up to 2 cm in 17 h. Because both males and females congregated at the barrier, and because there was no attraction between like sexes, both sexes must produce and be attracted by different specific substances. There was no evidence that like sexes were repelled.

No visible response to the chemical stimulus was seen when observing cultures of nematodes or when tracking isolated pairs. The tactile response of the male which led to copulation occurred only when its posterior third came into contact with the female. The responsive region in the male is therefore probably spread over this part in which the spicules are situated. When a male touched a female there was an immediate ventral coiling of the male's tail to form a loop into which the spicules projected. On some occasions the female was trapped in the loop and the male tail then remained coiled until the end of copulation or until the female had escaped. The female continued to move about and feed during this process, and, in doing so, moved forwards and backwards through the loop. Thus the spicules sometimes, but not invariably, came into contact with the vulva and penetrated the opening. The spicules were then firmly held and the male was dragged about by the movements of the female. At the end of copulation, the male gradually uncoiled, but the strong grip was only broken with considerable effort. Copulation lasted from 15 sec to more than 30 min. Where the female moved out of the loop before the vulva was penetrated, the male made no attempt to

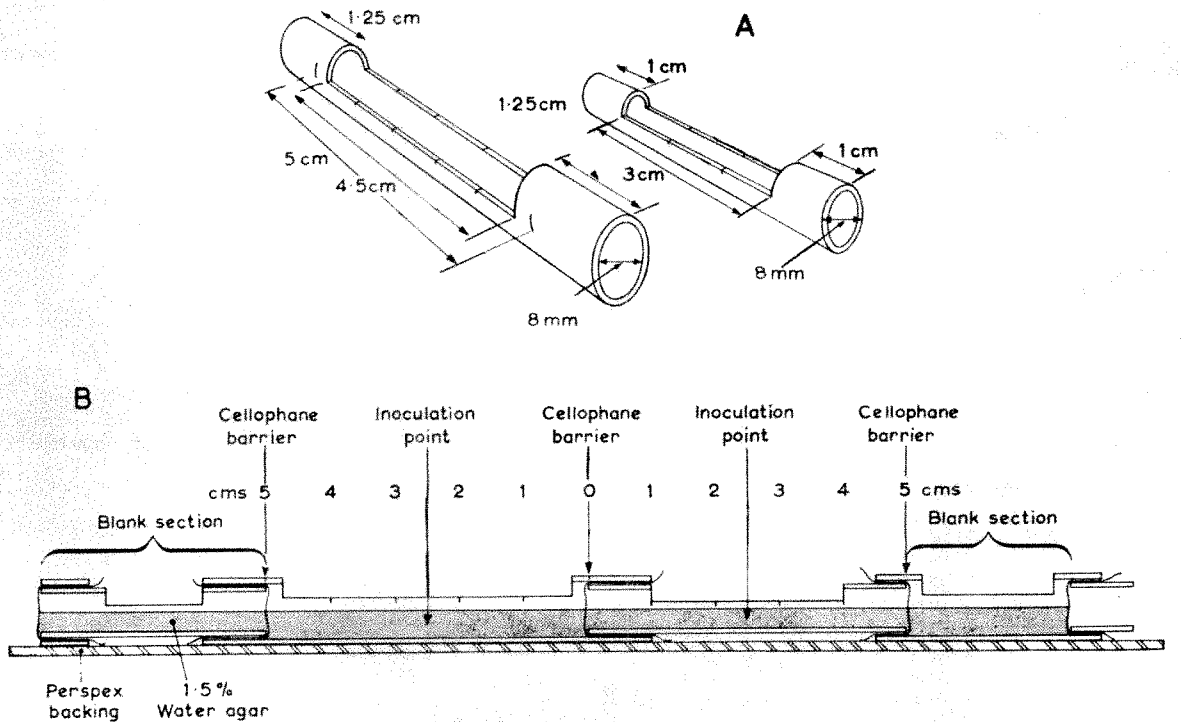


Fig. 1. A trough for testing for sex attractants. *A*, Sections cut from clear polyvinyl chloride tubing ('Tenatube'), with dimensions given; *B*, sectional view of assembled trough

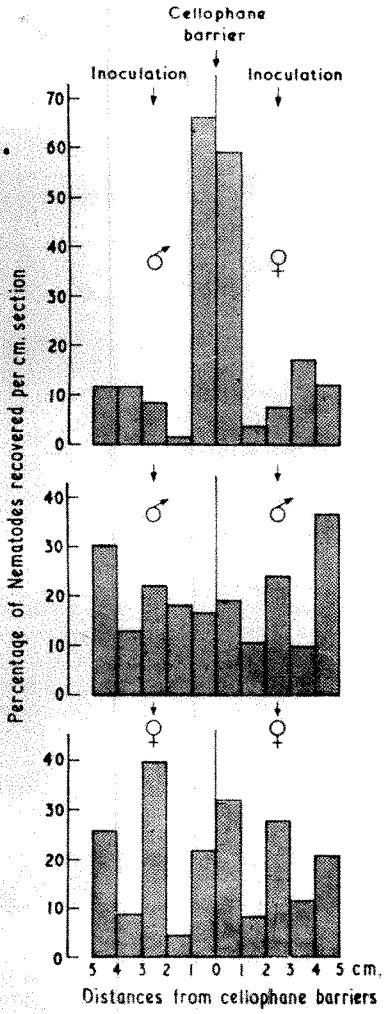


Fig. 2. Distribution of nematodes in troughs after 17 h at room temperature. The mid-line of each set of histograms represents the 'Cellophane' barrier between the two sections. Arrows indicate points of inoculation

re-engage unless their movements brought them into contact again. Copulation or attempted copulation by other males often occurred immediately after the female had been fertilized and females were often seen beset by several coiled males at the same time.

The attractant substances seem to serve only to bring the sexes together and their effects cannot normally be perceived in cultures. The act of copulation seems to be evoked only when the male tail makes contact with a female and so the apparently tactile stimulus may in fact contain an element of chemical stimulation which enables it to distinguish between male and female.

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MICROBIOLOGY

Structure of a Chromatophore Fraction of *Rhodopseudomonas spheroides*

Cohen-Bazire and Kunisawa[1] have reported that the chromatophores of *Rhodospirillum rubrum* can be separated into two fractions by centrifugation in a sucrose density gradient. From their relative positions in the gradient, they were designated 'heavy' and 'light' chromatophores.

We wish to describe the preparation of analogous fractions from *Rhodopseudomonas spheroides* and some of their properties, including X-ray diffraction investigations of the light fraction.

R. spheroides, strain 'Ga', was grown in a synthetic medium[2] at a light intensity of 600 ft.-candles. The cells were gathered by centrifugation, washed and resuspended in TSM buffer (*tris*-hydroxymethyl aminomethane, 0·02 M; NaCl, 0·08 M; $MgSO_4$, 0·01 M; *p*H adjusted to 7·8 with HCl). The cells were disrupted in a French pressure cell. DNase was added to reduce the viscosity, and the extract was centrifuged at 20,000 *g* for 25 min; the pellet was discarded. A solution of 0·1 M *tris* buffer (*p*H 7·8) containing $MgSO_4$ and RbCl was added to the supernatant to bring the final concentration to 0·05 M $MgSO_4$ and 27 per cent (w/w) RbCl. This mixture was centrifuged in a Spinco preparative centrifuge at 75,000 *g* for 4·25 h. The chromatophore fraction floated to the top of the tube, and the ribosomes formed a pellet which was discarded. The chromatophore layer was removed, diluted with TSM and centrifuged at 75,000 *g* for 1·5 h, after which all the pigmented material had formed a pellet. This was resuspended in TSM and layered on top of a linear density gradient of sucrose (35–47 per cent sucrose in TSM); this was centrifuged in the *SW* 25·1 rotor at 23,000 r.p.m. for 7 h. Essentially all the chromatophore material was present in two bands, the upper one of which was at the position in the gradient corresponding to a density of 1·14 g/ml.; this is the light fraction. The material in each band was removed through a hole in the bottom of the tube, diluted with TSM and centrifuged at 100,000 *g* for 1 h.

The chemical compositions of the two fractions are quite different (Table 1); this corroborates the observations of Cohen-Bazire and Kunisawa. It is significant that the ratio of bacteriochlorophyll to neurosporene is higher in the light fraction than in the heavy. This suggests that the two fractions are not entirely artefacts. The light fraction is monodisperse in the analytical centrifuge and has a sedimentation coefficient of 153*S* (corrected to infinite dilution in water at 20° C). The heavy fraction is polydisperse.

Table 1. Chemical Composition of Light and Heavy Chromatophore Fractions from *R. spheroides*

Fraction	Component (per cent dry weight)		
	Protein	Bacterio-chlorophyll	Neurosporens
Light	59·9	8·40	1·62
Heavy	63·2	5·08	1·24

The remainder of the dry weight is largely carbohydrate and lipid.

After two successive sedimentations at 100,000 *g* for 1 h of the material from the density gradient, resuspending each time in TSM, a sample of the final pellet was sucked into a quartz capillary, sealed, and used as the X-ray diffraction specimen.

Most diffraction photographs were taken with a low-angle short-slit camera using nickel-filtered copper $K\alpha$-radiation of 1·54 Å wave-length and Ilford 'Industrial *G*' X-ray film. The specimen-to-film distance was 15 cm and spacings could be resolved to about 400 Å. Other pictures were obtained with a quartz crystal focusing monochromator, which could only resolve spacings to about 100 Å, but which gave clearer patterns at higher angles.

The 'heavy' fraction gave no observable discrete diffraction maxima.

The 'light' fraction, however, gave a number of discrete maxima as shown in Fig. 1. The positions of these maxima can be interpreted quite precisely as the diffraction from a hollow sphere of mean shell radius 295 Å (see Table 2), and calculations show that the wall thickness could not be greater than about 100 Å. The best fit to the observed data is achieved with a uniform shell thickness of 60–80 Å. The data cannot be fitted to the diffraction predicted for a solid sphere.

Table 2. Analysis of X-ray Intensity Maxima from Light Chromatophore Fraction of *R. spheroides*

Peak	a	d (observed) (Å)	r (= ad) (Å)
1	0·715	?*	—
2	1·229	240	295
3	1·735	170	295
4	2·239	131	294

Five and beyond, unobserved; predicted to be very weak.
The equivalent radius r is calculated for a thin-walled hollow sphere from the equation $r = ad$ where a is a known constant and is tabulated in the second column above.
* Peak 1 should occur at $d = 412$ Å; present apparatus can just detect this peak, but it cannot be measured accurately.

There is also a diffuse reflexion in the neighbourhood of 50 Å (Fig. 2), suggesting a dominant spacing of this order in the structure. Calculations indicate that the intensity given by a uniform hollow sphere in this region would be much less than that observed, thus suggesting the presence of substructure of this order of size. Two explanations suggest themselves: First, if the shell were composed of a unit membrane, a diffuse reflexion might appear in this region, corresponding to the double lamellar structure. Secondly, since the chromatophore has a shell structure, the shell is very likely to be built up of sub-units in regular or quasi-regular array as are the spherical viruses[3], and the 50 Å reflexion could be a diffraction maximum corresponding to the distance between sub-units,

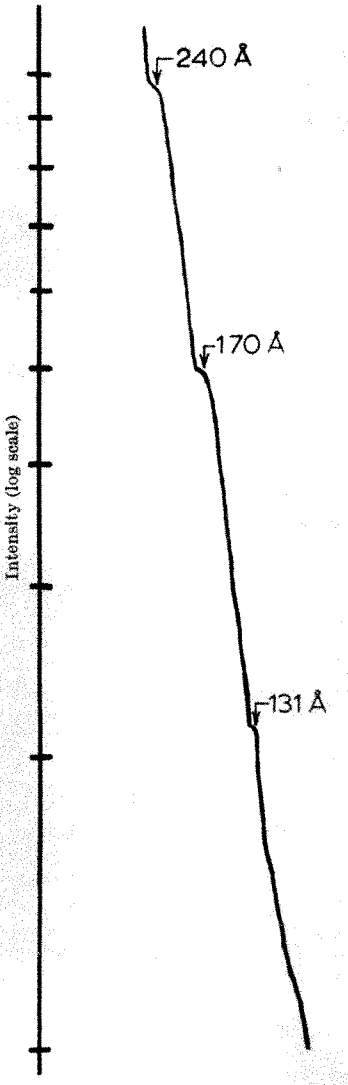


Fig. 1. The X-ray intensity distribution scattered by *R. spheroides* chromatophores (light fraction). This figure has been redrawn on an arbitrary logarithmic intensity scale from the original linear densitometer traces

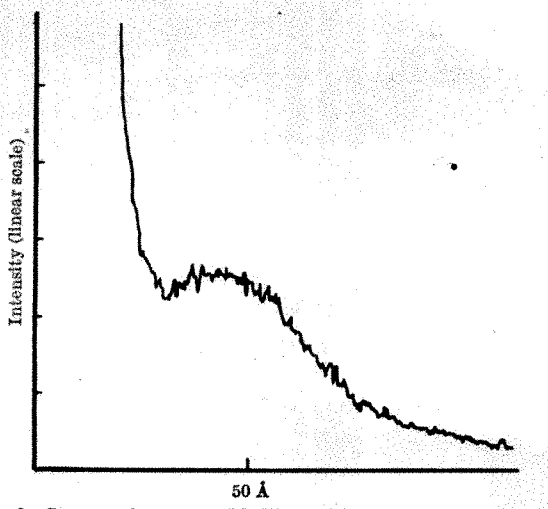


Fig. 2. Same specimen as used in Fig. 1, but wider angle data obtained with a different camera. Copied directly from the densitometer trace on an arbitrary linear intensity scale

or to the distance between holes in the shell (that is, the spaces between groups of sub-units, as observed in turnip yellow mosaic virus[4]). Since other chromatophores may have a shell structure (for example, *Chromatium*[5]), it is probable that similar construction principles apply. There is evidence for protein sub-units only in the case of *Chromatium*[5].

It may further be suggested that since the chromatophores of *R. rubrum* appear to be formed from the cytoplasmic membrane[6], then the membrane has a similar substructure. It is also likely that the structure of the lamellar photosynthetic apparatus of *R. molischianum* (which is also developed from the cytoplasmic membrane[7]) is based on similar principles.

It should be emphasized that these results on extracted chromatophore fractions do not necessarily indicate the intracellular structure of the photosynthetic apparatus. However, we feel that the structure and remarkable homogeneity of the light fraction are manifestations, under the conditions of isolation, of the high degree of order undoubtedly possessed by the photosynthetic apparatus within the cell.

This work has been supported in part by U.S. Public Health Service research grant *CA*-6570 to one of us (R. L.) and U.S. Public Health Service research grant *AI*-04896 and National Science Foundation grant *GI*4983 to another (W. R. S.).

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CYTOLOGY

Chromosomes of the Domestic Cat

THE recent advances in the visualization of diploid metaphase chromosomes of mammalian somatic cells have offered new opportunities to verify and extend previous techniques in which mitotic rates, DNA measurements, and various tracer techniques have been used to analyse cellular kinetics. Investigations of these latter types have been reported on human and dog bone marrow cells[1–3]. Some limited studies on replication of human chromosomes, particularly the sex chromosome, have also been reported[4].

The usefulness of chromosome studies for replication kinetics depends on the definite and reliable identification of chromosome pairs, or of small groups of similar chromosomes. Brief examination of the karyotype of the dog[5] suffices to show the inadequacy of this species in this respect. The human karyotype is superior to that of the dog, but it still leaves a great deal to be desired. Experimental limitations also demand an alternative species.

The cellular morphology of the bone marrow of the domestic cat is very similar to that of dog and man. The chromosomes are sufficiently varied to promise a reasonable degree of accuracy in reliably identifying a number of pairs (Fig. 1). Few investigations of cat chromosomes are available in the modern literature and these are not in agreement[6,7]. We are, therefore, presenting a karyotype which we feel offers a workable arrangement, particularly for replication kinetic investigations.

Bone marrow was obtained by aspiration from anæsthetized male cats. Suitable sites are the distal end of the femur, the proximal end of the humerus, the ribs, and the iliac crest. The aspirate was dispersed in 20-ml. heparinized saline (sodium heparin 1 : 10,000). The suspension was centrifuged at 800 r.p.m. for 10 min, the supernatant was discarded, and the cells were resuspended in serum–glucose–saline medium[8]. The cells were incubated for 1 h at 37° C after the addition of 1·0 μg/ml. 'Colcemid' (Ciba). The cells were again centrifuged and resuspended in 5·0 ml. of 0·95 per cent sodium citrate. After 10 min incubation at 37° C the suspension was transferred to a 15-ml. conical centrifuge tube and centrifuged again for 10 min at 800 r.p.m. The supernatant was discarded and the cells were fixed by the slow addition of 5 ml. of cold acetic acid/alcohol (1 : 3). After a minimum of 30 min the fixation process was repeated.

After a minimum of 12 h fixation at 4° C, the cells were resuspended in fresh fixative and the cells dispersed and fixed on a slide by 'blazing'[9].

The slides were stained for 30 min in 2 per cent Giemsa stain at *p*H 7·0 and photographs were made of best-quality chromosome preparations.

The karyotype presented here follows the general recommendations of the Denver conference on human chromosome karyotype[10]. Autosome pairs are grouped by size and by centromere location. Pairs within groups are ordered in respect to decreasing size. Metacentric and near-metacentric pairs are situated at the left and sub-telocentric and telocentric pairs at the right. In our karyotype (Fig. 2) chromosomes are grouped when they cannot be distinguished reliably as individual pairs.

The largest pair, 1, is sub-metacentric. Its arm ratio measures approximately 2 : 3. The metacentric pair 2 and the sub-telocentric pair 3 are appreciably smaller. The arm ratio for 3 is 1 : 4.

The medium-large group 4-7-*X* are metacentric or nearly so. Individual autosome pairs cannot be reliably identified and the *X* chromosome is generally indistinguishable within this group.

Pairs 8–10 are sub-telocentric with an arm ratio of 1 : 3. They cannot be individually identified as a rule although we occasionally can find one pair with a greater degree of telocentricity. Under the general scheme this pair would be assigned position 10.

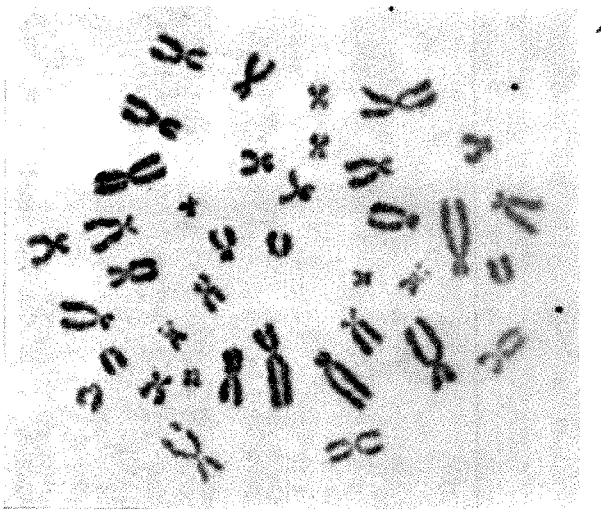


Fig. 1. Typical chromosome preparation from cat bone marrow

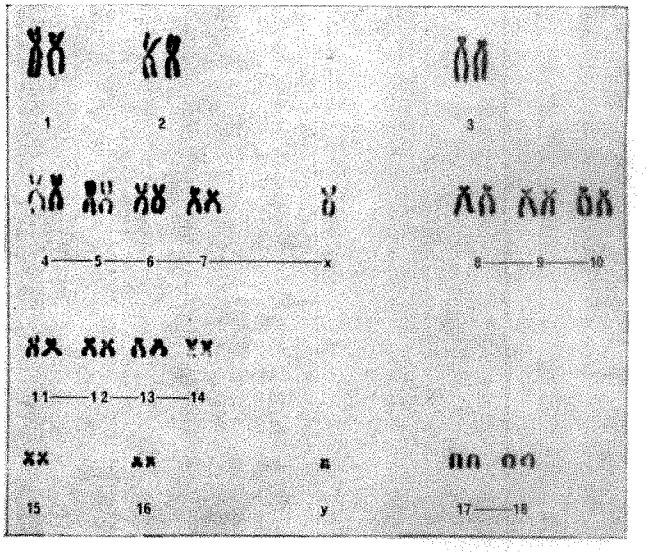


Fig. 2. Proposed karyotype for the domestic cat

The medium-small metacentric and nearly metacentric group 11–14 presents difficulties similar to those encountered in the 4–7 group. Pair 14 is distinguishable only when the satellites are well defined. We have observed this satellite phenomenon to be quite variable. Furthermore, it is common to find only 1 chromosome with a well-defined satellite.

The small pairs 15–18 present little difficulty. The metacentric pair 15 is larger than metacentric pair 16. Pairs 17 and 18 are easily identified as small telocentrics but are indistinguishable from one another.

The *Y* chromosome is the smallest metacentric and is sometimes confused with one of the autosomes of pair 16.

Of 203 chromosome preparations analysed by photographic methods, 186 contained 38 chromosomes. Three had 39, three had 40, and 11 had 34–37 chromosomes. A similar distribution of chromosome number has been reported for human bone marrow cells[11].

It should be mentioned that in all normal bone marrows which we have examined there is a constant small number (< 1 : 1,000) of polyploid metaphases. These may be megakaryocytic in origin, and our finding supports the observation of Garcia[12] that DNA contents of megakaryo-

cytic cells are often polyploid. Most of our polyploid metaphases are tetraploid (8 *n*), although there is a rare 16 *n*, and on one occasion a 32-*n* cell was seen.

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Unusual Action of Heparin on Ascites Tumour Cells during Freezing and Thawing

THERE is very little experimental evidence concerning the site or mechanism of cellular injury during the cooling, freezing and rewarming of living cells. Membranes of erythrocytes[1] and of mitochondria[2] have been suggested as cellular regions which are injured during freezing and thawing by increased salt concentrations. During investigations of freeze-thaw induced structural alterations of Littré ascites tumour cells, I decided to use heparin to minimize the coagulation of cell suspensions which frequently occurred soon after these cells were gathered from host mice. This use of heparin resulted in a dramatic fading of nuclei when the ascites cells were subjected to freezing and thawing. The purpose of this brief communication is to describe this action of heparin and to propose a tentative explanation of the phenomenon which provides evidence that the cell membrane is a site of freeze-thaw injury.

Ascites cells were obtained from Webster white Swiss mice, 12 days after intraperitoneal injection and incubation of 20 million cells per animal. Heparin (sodium, with 0·9 per cent benzyl alcohol in water; Vitarine Co., Inc., New York) was mixed with a portion of each sample in the amount of 0·025 c.c./c.c. of cell suspension, the equivalent of 250 heparin units/c.c. The remaining part of the sample served in comparison as the non-heparinized cell suspension. Phase microscope observations on unfixed, unstained cell preparations were made before freezing and after thawing in the presence and absence of heparin. One c.c. portions of cell suspensions were frozen rapidly in liquid nitrogen to −196° C in sealed glass ampoules at an average rate of 5° C/sec and thawed slowly in air (22° C) at 4·5° C/min. 'Live-dead' staining with eosin was used as an indicator of altered membrane permeability with each treatment.

Hæmocytometer measurements of total cell counts following 12-day incubation in hosts after transplantation were made in split-sample comparisons of control and experimental conditions. An increase in population served as an indicator of viability of transplanted cells.

Results revealed that in unfrozen control samples heparin did not alter either the structure of cells or their ability to divide. When cells were frozen and thawed, however, a marked difference in structure was noted between those suspensions with and without heparin (Fig. 1). Aside from the usual nuclear and cytoplasmic alterations (flattening of cells, blebbing, chromosome clumping, etc.) which occurred during these rates of freezing and thawing (details to appear elsewhere), nuclei of non-heparinized frozen-thawed cells showed distinct chromatin or chromosomal and nucleolar structures. In all but a few (< 0·5 per cent) frozen-thawed heparinized cells, however, the nuclei became as clear as an empty vacuole, as if devoid of nuclear contents. Only after careful scrutiny were evidences seen of faded nucleoli and hints of chromatin networks. Chromosomes in mitotic stages were never observed in the hundreds of these preparations examined, although they were numerous in non-heparinized frozen-thawed samples. There was no evidence of mitotic activity of the cell populations in the 12-day incubation period subsequent to transplantation of either frozen-thawed heparinized or frozen-thawed non-heparinized cells. Heparin action, therefore, seemed to provide a good index of survival. Ascites fluid in frozen-thawed heparinized samples also showed a marked gelation.

It appears that the intact plasma membrane acted as a barrier to the penetration of heparin since heparin was not observed to affect unfrozen cells. When this membrane was injured during freeze-thawing, however, and its integrity diminished, heparin readily entered the cell and acted on the nucleus. All but a very few cells became permeable to eosin after freeze-thawing, thus supporting the interpretation of such membrane alteration[1]. In addition, when heparin was added to frozen-thawed non-heparinized cells, the same fading of nuclei occurred as with cells frozen with heparin.

The belief that an intact plasma membrane is the barrier to heparin penetration was strengthened by results of additional experiments. Heparin (250 units/c.c.) was added to fresh nuclei isolated from chicken erythrocytes suspended in isosmotic sucrose prepared by Dr. Henderson of the Department of Biochemistry. The suspension

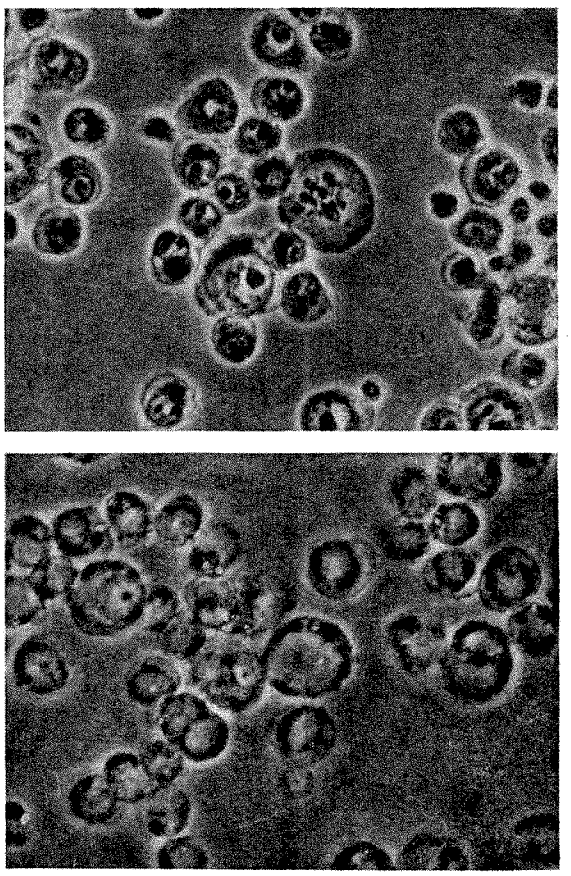


Fig. 1. Effect of heparin during freezing and thawing of ascites tumour cells. *a*, Untreated frozen-thawed cells; *b*, heparinized frozen-thawed cells. Note fading of nuclei. (Phase contrast, × *c.* 330)

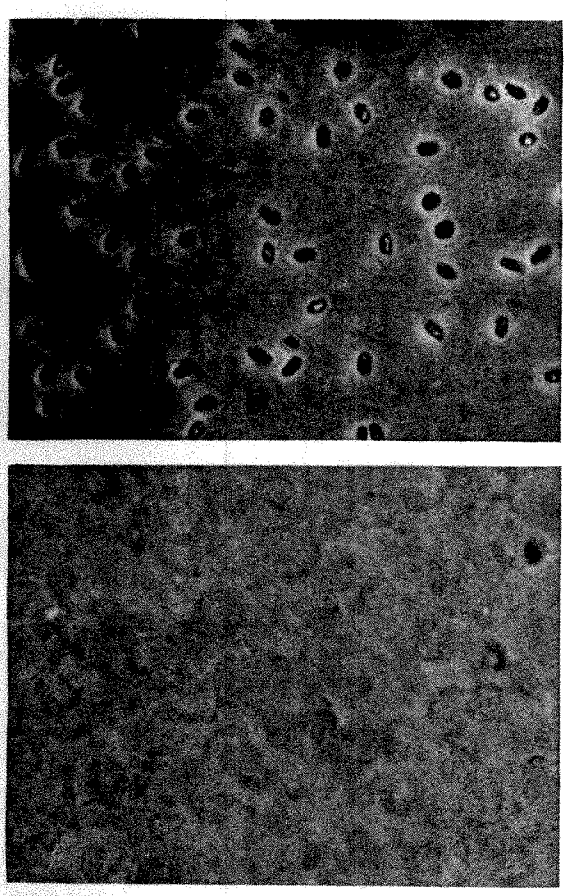


Fig. 2. Effect of heparin on isolated nuclei of chicken erythrocytes. *a*, Nuclei before addition of heparin; *b*, nuclei after addition of heparin. Note fading of nuclei and swelling of membranes. (Phase contrast, × *c.* 375)

immediately clarified macroscopically and became extremely viscous in the form of a continuous gel. Microscopically, the nuclei were observed to fade completely with their swollen membranes or ghosts representing the only nuclear structure visible (Fig. 2). Heparinized and non-heparinized chicken erythrocytes then were examined in the same manner as ascites cells. Heparin did not affect the structure of these nucleated cells before freezing. Subsequent to the hæmolysis induced during freezing and thawing with the coefficient membrane injury and the exposure of nuclei, however, heparin readily induce nuclear fading and increase the viscosity of the medium to almost the same extent as with preparations of isolated nuclei.

The increased gel-like viscosity of the medium which surrounded heparinized frozen-thawed preparations of chicken erythrocytes, or their nuclei, as well as ascites cells, was afterwards shown to be readily liquefied by the addition of DNase. It is strongly suggested, therefore, that nucleoprotein leaks from frozen-thawed ascites cells through the injured plasma membrane after the intracellular action of heparin. Preliminary experiments on sedimentation rate and ultra-violet absorption with nuclei of chicken erythrocytes were performed to evaluate possible heparin induced solubilization of nucleoproteins. Heparin was shown to cause, at the least, a dispersion of the usually insoluble nucleohistones in these nuclei. This and other aspects of chemical analyses are under investigation by Dr. Henderson and me.

Crystalline potassium heparin (250 units/c.c.) has been shown to duplicate effects described here, establishing heparin as the active agent in the Vitarine heparin solution. Closely related chondroitin sulphate *A* was found entirely without effect even in much higher concentrations. Results of parallel histochemical investigations on DNA and RNA have supported the concept of nucleoprotein dispersion. In addition, autoradiographic analyses with tritiated thymidine have given evidence for leakage of DNA from heparinized frozen-thawed ascites cells.

It is proposed that freezing and thawing at the rates described injure the plasma membrane of ascites cells, permitting the penetration of heparin which causes dispersion and leakage of nucleoprotein. A basis for a method of obtaining nucleoprotein from cells is suggested.

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GENETICS

Production of Maternal-type Plants through Crosses to Apomictic Species

The process of apomixis normally involves two complementary deviations from sexual behaviour. A functional egg cell with unreduced chromosome number must first be produced, and, secondly, it must develop into an embryo without being fertilized. When the first of these stages follows the apomictic pattern and the second follows a sexual pattern, triploid progeny are produced. In the opposite case, the resulting progeny will have the haploid chromosome number.

When apomictic triploid *Rubus canadensis* L. was crossed with sexually breeding tetraploid blackberries, Craig[1] obtained both triploid and pentaploid seedlings. The triploid seedlings were normal apomicts, whereas the pentaploids arose through unreduced eggs being fertilized by diploid sperm cells. When the reciprocal cross was made, he found diploid, triploid, tetraploid and pentaploid seedlings. The diploid seedlings arose through reduced eggs developing without fertilization, whereas the other seedlings were produced through reduced eggs being fertilized with haploid, diploid, or triploid sperm cells.

In the hope that triploid *R. canadensis* pollen might similarly induce haploidy in the diploid red raspberry, four red raspberry varieties were pollinated with *R. canadensis* pollen in 1961. Except for one triploid plant, none of the 26 seedlings obtained from the four crosses showed any evidence of blackberry parentage. Cytological investigations showed them all to be diploid.

A similar result was obtained in crossing cultivated strawberries with an apomictic species. When the runnerless ever-bearing diploid variety 'Baron Solemacher' was pollinated with 'Sparkle' pollen, only apomictic seedlings were produced. However, when six cultivated octoploid strawberry clones were pollinated with 'Baron Solemacher' pollen, all 36 seedlings produced were maternal-type octoploids.

The exact mode of origin of the maternal-type plants has not yet been determined. Three possible modes can be postulated: (1) apomixis; (2) chromosome doubling of haploids; (3) contaminated crosses. While some contaminants could have been included in the maternal-type raspberry seedlings, this possibility was virtually eliminated with the strawberry crosses. Morphological variations between seedlings would tend to rule out apomixis in the raspberry crosses; but with the possibility of contaminants one cannot be sure of this. There is, in fact, no reason to conclude that all the seedlings even in a given progeny were produced by the same mode of action.

The breeding behaviour of the plants concerned is being further examined. The crosses are being repeated on a larger scale and the raspberry seedlings are being tested for homozygosity. However, the technique for the pro-

duction of maternal-type seedlings using pollen of apomictic species should be of considerable value regardless of its mode of action. If the process is by apomixis, then it should be useful as a means of eliminating non-seedborne viruses. If it is by chromosome doubling of haploids, then the technique will be extremely useful as a ready means of producing homozygous lines for breeding purposes.

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$D_{Adelaide}$—a New Transferrin Variant in Man

Inherited variation of the serum protein transferrin was first detected in man by Smithies in 1957, using starch-gel electrophoresis[1]. At present 16 different human transferrin variants have been described. Fourteen of these are summarized by Parker and Bearn[2]. The fifteenth, B_{Lae}, was observed in a single pedigree in New Guinea[3], and the sixteenth, D_{Wigan}, has been reported recently in an English family[4].

Transferrin C is the commonest variant; it is found in all populations. Variants with an electrophoretic mobility faster than C have been given the notation B; slower variants, the notation D. Subscripts distinguish the different B and D variants.

The difference between C and any one of the variant types is probably determined by a single gene pair without dominance. In addition, the discovery by Beckman and Holmgren of a family in which B_2D_1, B_1D_1 and B_1B_2 individuals lacking a C band were found suggests that the genes for B_1, B_2 and D_1 are allelic[5]. Otherwise, little information is available concerning the relation between the genes which determine the different variants.

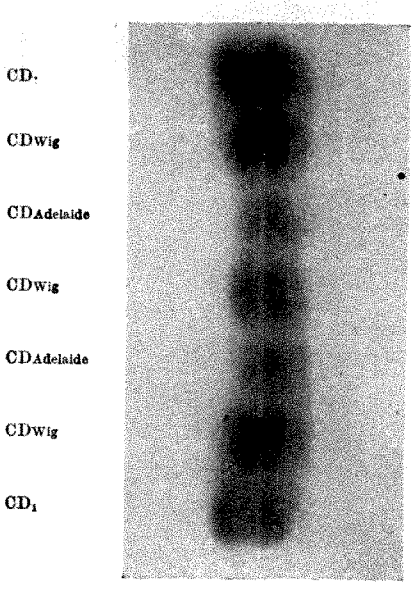


Fig. 1. Autoradiograph of a segment of starch gel comparing transferrin variants. Borate buffer *p*H 8·9, 7 V/cm for 16 h at 20° C

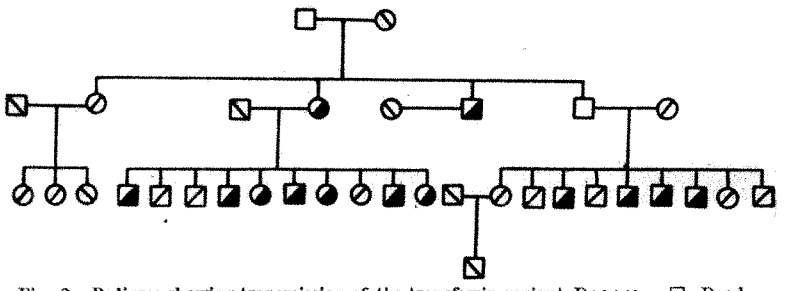


Fig. 2. Pedigree showing transmission of the transferrin variant $D_{Adelaide}$. □, Dead, not tested; ▣, alive, not tested; ◪, alive TFC; ◪, alive $CD_{Adelaide}$

We have recently found what would appear to be a new D variant in an Australian family of Irish–Italian origin. Fig. 1 shows an autoradiograph prepared after electrophoresis of serum from a member of this family alongside standard sera containing D_{Wigan} and D_1, using a vertical starch-gel system and a borate buffer at *p*H 8·9. ^{59}Fe-citrate was added to the serum to give an activity of 6 μc./ml. prior to electrophoresis.

Repeated tests have shown that the transferrin variant present in this family is faster than D_1, D_{Chi}, $D_{Montreal}$, D_{e-1} and D_{Wig}. Possibly it is identical with D_O, a variant first identified by Giblett *et al.* in an American Negro[6]. Unfortunately all supplies of the serum containing D_O have been exhausted, and the original donor is no longer available. For this reason no test of this possible identity can be carried out. However, the transferrin present in the Adelaide family is detectably faster than a transferrin found in the serum of a Thai from northern Thailand, provisionally identified as D_O by Kirk and Lai[7], in the absence of a comparison with standard D_O serum.

For the reasons cited here, we feel justified in establishing this transferrin as a new variant for which we propose the name $D_{Adelaide}$. It has the highest mobility of the known D variants for which serum is available for testing purposes.

The inheritance of $D_{Adelaide}$ is shown in the pedigree of Fig. 2. The hypothesis of a single gene pair without dominance is consistent with the data.

The pedigree was originally investigated because some members possessed a congenital non-spherocytic hæmolytic anæmia which was found to be associated with a deficiency of the enzyme, pyruvate kinase, in their red cells. This aspect will be considered elsewhere by one of us (H.L.) in association with others. There was, however, no evidence to suggest that the presence of this variant of transferrin was in any way connected with the possession of pyruvate kinase deficient erythrocytes in the family. Their loci appear to be independent.

The appearance of members of this family does not suggest an admixture of Australian Aboriginal blood, nor has the family been in Australia a sufficient number of generations for this to be undetectable had it occurred. It would appear, therefore, that the gene is European in origin. Its frequency, however, must be very low since it has not previously been detected in surveys of persons of European descent in various parts of the world, and in which serum from several thousand persons has been examined.

Part of this work was supported by a grant from the Nuffield Foundation to one of us (R.L.K.).

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PSYCHOLOGY

Machine Learning from Noisy Information

A METHOD of approach is described here in connexion with international telegraph (Morse) code, but not much change is necessary with other systems of substitution ciphers[1]. At this stage no reference is made to a vocabulary or to the syntactic and semantic context[2].

Let us start with zero noise-level and come later to the simulation of noise.

As is known, there are five different basic elements in the Morse code: dot, dash, intra-letter space, inter-letter space, and inter-word space. (For the sake of simplicity, we will now ignore punctuation marks. Their inclusion does not, however, represent any difficulty.) By using single-letter frequency charts for English and establishing the word-length distribution function with regard to the number of letters in a word (an analysis of 4,000 words, for example, has given an average word-length of 4·49 letters), we can show that the respective frequencies of the foregoing basic elements are $f_1 = 0{\cdot}308$, $f_2 = 0{\cdot}198$, $f_3 = 0{\cdot}297$, $f_4 = 0{\cdot}155$ and $f_5 = 0{\cdot}045$.

A computer can easily pick out the three basic elements that represent spaces since two identical of these can never be found in sequence. (We may note here that considerable research has been done in the related field of computer recognition and transcription of manual Morse, such as by Blair[3], Freimer *et al.*[4] and Gold[5].) When the information becomes noisy, as set out later, the machine looks for the elements with minimum number of repetitions in sequence. A comparison between the frequencies of occurrence of the elements so selected allows completion of the further classification (intra-letter, inter-letter and inter-word) due to the significant differences between f_3, f_4 and f_5. In a similar fashion dots and dashes can also be distinguished.

The next step is to apply the well-known statistical procedure used to fit different basic frequency distributions to an empirical one. It is necessary to minimize :

$$\chi^2 = \sum_{(\alpha)} \frac{(f_s^{(\alpha)} - f_p^{(\alpha)})^2}{f_p^{(\alpha)}} \qquad (1)$$

where the summation is extended over the whole alphabet; $f_p^{(\alpha)}$ is the frequency of occurrence of a particular letter (α) in the established chart (population value); $f_s^{(\alpha)}$ is the sample value of the same quantity for the letter assumed to be the same. The minimization is carried out by systematically changing the equivalence table (representations) of the Morse code. The starting set is usually very near to the optimum but, of course, it need not necessarily be the true set. A more reliable, but much more complicated, procedure would be the minimization of :

$$\chi^2 = w_1 \sum_{(\alpha)} \frac{(f_s^{(\alpha)} - f_p^{(\alpha)})^2}{f_p^{(\alpha)}} + w_2 \sum_{(\alpha,\beta)} \frac{(f_s^{(\alpha,\beta)} - f_p^{(\alpha,\beta)})^2}{f_p^{(\alpha,\beta)}} + w_3 \sum_{(\alpha,\beta,\gamma)} \frac{(f_s^{(\alpha,\beta,\gamma)} - f_p^{(\alpha,\beta,\gamma)})^2}{f_p^{(\alpha,\beta,\gamma)}} \qquad (2)$$

where also the frequencies of occurrence of digraphs and trigraphs, $f^{(\alpha,\beta)}$ and $f^{(\alpha,\beta,\gamma)}$, are involved[6]; w_1, w_2 and w_3 are certain weighting factors, undetermined at present, representing the varying importance of each term.

The noisiness of the information is simulated by over-writing a given percentage of basic elements by other (or sometimes the same) ones, at random. The aim of this procedure is to find the minimum redundancy necessary at different noise-levels so that it is still possible to decipher the Morse code. Fig. 1 shows the nature of an expected diagram. The curve represents the average relationship between noise-level (*NL*) and minimum information content (*MIC*) of messages. We note that it may well be idealized in the sense that even noiseless messages cannot always be recognized without errors, and there could exist a certain amount of 'irreducible' error.

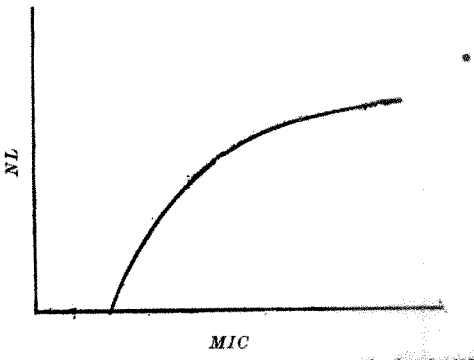


Fig. 1. Expected average relationship between noise-level (*NL*) and minimum information content (*MIC*) of messages

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Influence of Motivation, Perception and Attention on Age-related Changes in Short-term Memory

THE responses made by young normal adults to various methods of dichotic stimulation have been investigated and reported by Broadbent[1]. His observations have led him to postulate that both perceptual and storage mechanisms are necessary for the correct sequential recall of such simultaneous stimulation.

I have put forward the hypothesis that since increasing age in normal individuals also seems to affect learning capacity, and as such impairment may also depend on changes in some short-term storage process, it could be anticipated that responses to dichotic stimulation might also show associations with age.

Broadbent has suggested that the first half-set of digits recalled from each dichotic span does not involve the storage process. If age primarily affects storage then the reproduction of the first half-set recalled should not be affected by advancing years. If the second half-set recalled must pass through the storage process then the recall of these digits should be affected by age. Investigations by Inglis and Caird[2] and Mackay and Inglis[3] show that this appears to be the case.

The results of these two experiments, carried out on different subjects (total No. = 280) by different experimenters, proved to be in very close agreement. As age increases there is little or no significant impairment in the ability to recall the half-spans reproduced first. Progressively and significantly greater difficulty is, on the other hand, shown in the reproduction of the second half-spans as age advances. Furthermore, the longer the span

to be recalled the greater the difference, overall, between the first and second half-spans.

These findings make it seem quite unlikely that differences in motivation can explain such changes in short-term storage with age. It is not easy to conceive how overall changes in motivation as people grow older could make them respond accurately to one ear but not to the other.

It could, however, be maintained that the impairment shown by elderly subjects in the recall of dichotic digits might be due to a failure to hear, or a failure to attend to, the second half-spans on which they showed most errors of report. We have accordingly carried out a further experiment in order to evaluate these possibilities; a preliminary report of our results is offered here.

One hundred and twenty subjects between 11 and 20 years of age were tested. There were 20 people in each decade group: 10 male and 10 female. They were required to recall dichotic digits under three conditions; these being: (1) when the order of recall was left to the free choice of the subject; (2) when the ear order of recall was specified before; (3) after these digits had been delivered.

Condition (1) was used in order to provide a further repetition of the experiments carried out on similar groups by Inglis and Caird[2] and Mackay and Inglis[3]. It can be seen that no control was exercised by this condition over the laterality of the order of recall. The results of this part of the experiment were in accord with previous findings.

In condition (2) the side to be recalled first was indicated by means of a signal panel set in front of the subject simply bearing the printed words 'right' and 'left'; a red light was switched on above the appropriate word by the experimenter, in this case immediately before each span of the dichotic digits was delivered. This condition was used in the experiment recorded here to evaluate the notion that the changes with age which had previously been shown on this task might be due, for example, to an age-related hearing loss in one ear as compared with the other. If it were the case that as people grow older they tend to suffer such a differential loss in auditory acuity then the apparent deficit in the ear recalled second under conditions of free response might be explained by this kind of peripheral difficulty. If, however, an increased order effect were to appear with age regardless of ear-specification, then this hypothesis would not be supported.

The results obtained under this condition refute the notion that any unilateral decrease in auditory acuity could account for the appearance of the order effect noted under conditions of free recall. A marked age-related recall order effect appears which is relatively independent of ear laterality.

In condition (3) the side to be recalled first was again specified by means of the light signals, in this case immediately after the dichotic digits were delivered. This procedure provided a control for the possible effects of differences in attention between the different age groups. It might be argued, for example, that previous findings were due to the fact that as people grow older they will, in the dichotic digit experiment, only pay attention to the material coming into one ear. If, however, the subjects only know after the digits have been delivered which side they have to report first and, further, if they only attend to one side, then the ear order effect should vanish, or at least be greatly diminished, since they cannot know beforehand to which ear they should have paid most attention.

Under this condition the age-related order effect was again clearly shown; the main decline with age being in the half-sets recalled second. This means that the order effect cannot be entirely explained by the notion that older subjects only attend to the material presented to one ear.

In conclusion, therefore, it may be said that the results of this experiment confirm, once again, that the responses made to dichotic stimulation under conditions of free recall are affected by age. Since it is the order effect which is exaggerated in older subjects this finding lends support to the view that some short-term storage mechanism is affected by advancing years. This finding cannot be explained in terms of differences in motivation with age. The results obtained in this experiment when the order of recall was specified before delivery of dichotic digits are similar to the results from free recall. Since the age-related order effect still appears regardless of which side is specified for recall first, this finding is quite incompatible with the notion that changes in responses to dichotic listening with age might be due, for example, to some unilateral hearing loss which gets worse as people grow older. This finding cannot, therefore, be explained in terms of differences in sensation or perception. The results obtained when order of report was specified after the delivery of the digits are also very similar to the results obtained under conditions of free recall. This outcome runs completely counter to the view that the changes observed with age might be due, for example, to the fact that older individuals only attend to the material from a single channel under conditions of simultaneous stimulation. This finding, therefore, cannot be explained in terms of differences in attention.

All these findings are, however, compatible with the hypothesis that the age-related impairment shown in recall under the three experimental conditions is due to a decline in the efficiency of some short-term memory storage process.

The work described was supported by grant 25 of the Ontario Mental Health Foundation; the testing was carried out by Mrs. Mary Ankus.

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MISCELLANEOUS

Shape of Small Particles

I regret that the index 2 was omitted from one of the β's in the denominator of equation (19) in my article published in *Nature* under the above title[1]; this was due to an error in the analysis which escaped me and was later detected by Mr. J. Cartwright of the Safety in Mines Research Establishment when applying the method to coal dust. As a result, the figures given in Table 3 were in error and the Table should read as follows:

Table 3. Oblate Spheroids

Axial ratio β	$F(\beta) = \frac{\text{surface area}}{4\pi a^2}$	$1/\beta^{2/3}F(\beta)$	$2\sqrt{F(\beta)}$	$\beta^{1/3}\sqrt{F(\beta)}$
1·0	1·0	1·0	2·0	1·0
1·1	0·986	0·952	1·986	1·025
1·3	0·862	0·975	1·856	1·013
1·5	0·787	0·969	1·774	1·016
2·0	0·690	0·914	1·662	1·046
3·0	0·604	0·796	1·554	1·121
4·0	0·567	0·700	1·506	1·195
6·0	0·535	0·566	1·462	1·329
8·0	0·522	0·479	1·444	1·445
10·0	0·515	0·418	1·436	1·547
20·0	0·500	0·272	1·414	1·919
50·0	0·500	0·147	1·414	2·604
100·0	0·500	0·093	1·414	3·282
500·0	0·500	0·032	1·414	5·612
1,000·0	0·500	0·020	1·414	7·071

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, October 5

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, JOINT I.E.R.E.–I.E.E. MEDICAL ELECTRONICS GROUP (at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 2.30 p.m.—Discussion on "Transistorized Biological Amplifiers".

BRITISH SOCIETY FOR THE PHILOSOPHY OF SCIENCE (at University College, Gower Street, London, W.C.1), at 5.30 p.m.—Mr. R. Harre: "The Limits of Formalization".

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (at Shell Centre Theatre, Shell Centre, London, S.E.1), at 6.30 p.m.—Scientific Film Evening.

Tuesday, October 6

INSTITUTION OF THE RUBBER INDUSTRY, LONDON SECTION (at the Royal Society of Tropical Medicine, 26 Portland Place, London, W.1), at 5.30 p.m.—Mr. A. E. Moulton: "Rubber Springs"; 7 p.m.—Dr. H. J. Osterhof: "Isoprene and Polyisoprene Production at Goodyear—an Achievement of Bench-Scale Research".

INSTITUTION OF MECHANICAL ENGINEERS, AUTOMOBILE DIVISION (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Annual Meeting 6.30 p.m.—Mr. J. S. Clarke: "The Outlook of an Engineer on Social Problems" (Chairman's Address).

Wednesday, October 7

COLOUR GROUP (Great Britain) (in the Physics Department, Imperial College of Science and Technology, Prince Consort Road, London, S.W.7), at 3 p.m.—Dr. J. E. Pinney: "Computational Colour Photography". 3.45 p.m.—Miss M. Hayward, Miss A. M. Mitchell and Mr. D. A. Pavey: "Colour Teaching Methods".

GEOLOGICAL SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Mr. A. N. Burton: "Geological Mapping of the Province of Calabria, Southern Italy" (Demonstration); Dr. C. G. Adams: "The Foraminifera and Stratigraphy of the Melinau Limestone, Sarawak, and its Importance in Tertiary Correlation"; Dr. N. J. Snelling and Dr. R. B. McConnell: "Age-Determinations from British Guiana".

ROYAL SOCIETY OF MEDICINE, HISTORY OF MEDICINE SECTION (at 1 Wimpole Street, London, W.1), at 5.15 p.m.—Dr. Cuthbert Dukes and Mr. D. A. Brunning: "The Origin and Early History of British Cancer Research Institutions"; Dr. D. H. Trapnell: "Man's Understanding of the Lymphatic System".

INSTITUTE OF PETROLEUM (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. A. T. J. Hayward: "Compressibility of Hydraulic Oils".

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Dr. A. C. Copisarow: "The Contribution of the Engineer to the Economy".

INSTITUTION OF MECHANICAL ENGINEERS, INDUSTRIAL ADMINISTRATION AND ENGINEERING PRODUCTION AND STEAM PLANTS GROUPS (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Mr. J. A. Rogers and Mr. R. C. Brewer: "Faults in Cast Components for High-Press/High-Temperature Service".

SOCIETY OF ENVIRONMENTAL ENGINEERS (in the Mechanical Engineering Department, Imperial College of Science and Technology, London, S.W.7), at 6 p.m.—Introductory meeting of the Packaging Group.

SOCIETY FOR ANALYTICAL CHEMISTRY (at the School of Pharmacy, Brunswick Square, London, W.C.1), at 6.30 p.m.—Prof. H. J. Emeleus, F.R.S.: "Analysis in Modern Inorganic Chemistry".

BRITISH INTERPLANETARY SOCIETY (at 4 Hamilton Place, London, W.1), at 7 p.m.—Mr. F. W. Forbes: "Expandable Structures for Missile and Space Applications".

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (at the Royal Institution, 21 Albemarle Street, London, W.1), at 7 p.m.—Dr. M. F. Perutz, F.R.S.: "The Anatomy of Haemoglobin" (Third Humphry Davy Lecture).

Wednesday, October 7—Thursday, October 8

INSTITUTION OF MECHANICAL ENGINEERS, PROCESS ENGINEERING GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1)—Symposium on "Plastics and the Mechanical Engineer".

Thursday, October 8

ROYAL INSTITUTION OF NAVAL ARCHITECTS (in the Weir Lecture Hall, 10 Upper Belgrave Street, London, S.W.1), at 5 p.m.—Dr. R. Hurst: "Ship Research" (Fourth Amos Ayre Lecture).

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. O. W. Humphreys, C.B.E.: Inaugural Address as President.

Friday, October 9

UNIVERSITY OF LONDON (in the Main Lecture Theatre, London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. W. J. Kolff (Cleveland Clinic, Ohio): "Life Without Kidneys and Heart".*

Monday, October 12

INSTITUTION OF AGRICULTURAL ENGINEERS (at the Institution of Mechanical Engineers, 1 Birdcage Walk, London, S.W.1), at 10.15 a.m.—Mr. B. M. Camm: "Some Aspects of the Economic Utilization of Farm Machinery"; Mr. H. G. Pryor: "The Development and Application of High Power Units in Agriculture"; Mr. J. D. Savage: "Significant Features of Fuels and Lubricants for the Winter Use of Diesel-Engined Tractors".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. D. E. N. Davies: "Application of Electronic Vector Scanning Techniques Applied to Height-Finding Landing Systems"; Miss E. Killick: "Frequency Scanning Aerial Systems".

UNIVERSITY OF LONDON (in the Anatomy Lecture Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. John Szentágothai (Medical University of Budapest): "The Neuronal Circuits of the Cerebellar Cortex".*

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 6 p.m.—"The Living Pattern" (Colour Films).

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (joint meeting with the Ewell County Technical College Faraday Society, at the Ewell County Technical College, Reigate Road, Ewell, Surrey), at 7 p.m.—Mr. A. D. S. Tantrum: "Energy Production".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURER IN CONTROL ENGINEERING—The Registrar, University College of North Wales, Bangor, North Wales (October 9).

RESEARCH STUDENTS (with an honours degree, G.R.I.C., Dip. Tech.) IN THE DEPARTMENT OF CHEMISTRY, to investigate branching and cross-linking reactions in polymerization processes—Prof. G. M. Burnett, University of Aberdeen, Old Aberdeen, Scotland (October 10).

LECTURER IN MECHANICAL ENGINEERING (Applied Thermodynamics, Mechanics of Fluids); a PRINCIPAL LECTURER IN ELECTRICAL ENGINEERING (Circuit Theory); a SENIOR LECTURER IN ELECTRICAL ENGINEERING (Light Current Engineering); a LECTURER IN CIVIL ENGINEERING; a LECTURER IN INORGANIC CHEMISTRY; a LECTURER IN APPLIED PHYSICS; a SENIOR LECTURER IN MATHEMATICS (Numerical Analysis); and a LECTURER IN MATHEMATICS—The Principal, Lanchester College of Technology, Priory Street, Coventry (October 12).

RESEARCH ASSISTANTS (with a university degree or diploma in technology with first- or second-class honours) to work in the following fields: analytical chemistry, physico-organic chemistry, applied physics, light current electrical engineering, control or ultrasonics—The Principal, Lanchester College of Technology, Priory Street, Coventry (October 12).

TECHNICIAN IN THE DEPARTMENT OF PHYSICS, University of Natal, Pietermaritzburg—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, October 12).

SCIENTIFIC OFFICER (with a first- or good second-class honours degree in physics and an interest in the application of physical methods to biological problems) at the Meat Research Institute, to work in collaboration with the Anatomy Department on physical methods of determining the composition of live animals and carcases, in particular ultrasonics and photogrammetry—The Secretary, Low Temperature Research Station, Downing Street, Cambridge, quoting vacancy No. 64/4 (October 13).

ASSISTANT LECTURER (with a special interest in experimental psychology) IN PSYCHOLOGY—The Registrar, The University, Manchester, 13 (October 16).

LECTURERS (3) (with a sound academic record, well qualified in anatomy or related sciences, and preferably some experience in teaching at university level) IN ANATOMY at the University of Queensland, Brisbane, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, October 16).

SENIOR LECTURER (with experience as an applied geophysicist and preferably with a strong background in geology) IN GEOPHYSICS at Victoria University of Wellington, New Zealand—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, October 16).

SENIOR EXPERIMENTAL OFFICER (preferably with a degree or equivalent) IN THE DEPARTMENT OF MECHANICAL ENGINEERING to assist in the carrying out of research over a very wide field of interest—The Academic Registrar, The Queen's University, Belfast, Northern Ireland (October 17).

PROFESSOR OF CHEMISTRY and HEAD OF THE DEPARTMENT, University of Auckland, New Zealand—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, October 19).

M. C. SMITH LECTURER IN PATHOLOGY (Cancer Research)—The Assistant Registrar, The Medical School, The University, Birmingham, 15 (October 20).

GRADUATE (with an honours degree or equivalent in horticulture, agriculture or science) IN THE HORTICULTURE DIVISION, for mushroom research—The Director, An Foras Taluntais (The Agricultural Institute), 33 Merrion Road, Dublin 4, Republic of Ireland (October 21).

LECTURERS/ASSISTANT LECTURERS (2) (with a university degree in agricultural science, veterinary science or science) IN DAIRY HUSBANDRY/SHEEP HUSBANDRY at Massey University of Manawatu, Palmerston North, New Zealand—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, October 21).

DEMONSTRATORS (2) (with a degree in pharmacy, preferably with honours, and registered pharmacists) IN PHARMACY at the University of Queensland, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, October 23).

ASSISTANT (human geographer with a special interest in the urban field, preferably historical and morphological aspects) IN GEOGRAPHY—The Secretary of University Court, The University, Glasgow (October 24).

ASSISTANT LECTURER IN BIOCHEMISTRY—The Secretary, University of St. Andrews, College Gate, St. Andrews, Fife, Scotland (October 24).

PROFESSOR OF ORGANIC CHEMISTRY—The Registrar (Room 22, O.R.B.), The University, Reading (October 24).

LECTURER or ASSISTANT LECTURER IN BIOCHEMISTRY in the Faculty of Agriculture, Makerere University College, Uganda—The Secretary, University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (October 25).

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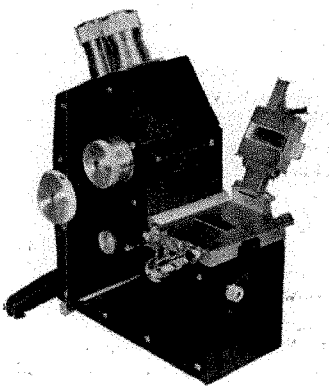
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PLANNING FOR THE DEVELOPMENT DECADE

LORD BRAIN, in his presidential address to the British Association at Southampton (*Nature*, 203, 943; 1964), referred to the growth of population as an outstanding example of our failure to predict and prepare for the consequences of scientific development. While it was fairly generally recognized that there was every prospect that the world population would have doubled from 3,000 million to 6,000 million by the year 2000, 10–15 per cent of that population were now living on a diet with insufficient calories, and 50 per cent were either undernourished or malnourished, or both. To raise this standard of living and to provide it for twice the present number was an inescapable challenge, which, as Lord Brain pointed out, has implications for water supply, roads, industry, education and, indeed, all that makes possible a full life. Yet Britain has no population policy and has given no thought to the implications of the present rate of growth.

Lord Brain instanced the position of medical education as one example of Britain's lack of foresight and her failure to use existing knowledge, but he made a percipient comment on the redistribution of leisure which would follow the expansion of automation. The population problem is, however, an international problem which can only be dealt with effectively at an international level, and in addressing the British Association on this point, Lord Brain reiterated what he had said in a debate in the House of Lords on world population and family planning on July 1. What is needed is an international body, sponsored by the United Nations Organization, to collect all available demographic information (and promote the search for it when necessary), to take into account present and prospective supplies of food and water and the economic and cultural needs of growing populations, and to review all present-day scientific work on population control. The United Nations is already doing something to meet these needs, through its Population Commission and the Population Branch of the Bureau of Social Affairs. The United Nations and six of its specialized agencies are sponsoring another World Population Conference in 1965. Nevertheless, Lord Brain thought that all this did not provide the essential combination of diagnosis and treatment, and he pleaded for greater initiative from the British Government, particularly in support of the recognition of the International Planned Parenthood Federation by the Economic and Social Council.

It is worth noting, in passing, that Lord Brain's remarks as to the implications of population growth in various fields found some support in an address which Mr. A. A. L. Caesar delivered in opening a symposium on "Regional Planning Problems in Great Britain" at the British Association meeting on August 28. In calling for more Government action in this matter, instead of leaving it largely to voluntary organizations, Lord Brain had welcomed the statement made earlier in the debate by the Earl of Dundee, that the Secretary for Technical Co-operation had informed some Commonwealth and foreign Governments, as well as some colonial Governments, that Britain was willing to apply to family planning exactly the same principles as govern her aid generally, and would seek to respond to requests for technical assistance made by overseas Governments. The Earl of Dundee added that Britain was prepared to consider sympathetically any proposals for assisting the developing countries, where this problem was pressing, and that the Government thought that technical assistance through the United Nations should be available to States desiring to spread the idea and practice of voluntary family planning.

The Earl of Dundee had outlined the Government's views early in the debate, which was opened by Lord McCorquodale of Newton. The latter had quoted extensively from a report presented by Dr. Prebisch to the United Nations Trade and Development Conference at Geneva in the summer, and referred also to the concern expressed in the last report from the Consultative Committee on the Colombo Plan that the rapid rate of increase in population might in some areas more than offset the development effort. A similar opinion had also been expressed by Mr. Eugene Black, until recently president of the World Bank. Lord McCorquodale stressed especially the importance of effective communication of knowledge of the problem: this was too large a task to be left to the undeveloped and poorer countries, and should be given high priority by the United Nations.

An early start should be made now to plan and build up, throughout the world, adequate maternal and child welfare services, of which education in family planning would be an integral part. The International Planned Parenthood Federation had sought to promote such a plan, but it needed the support of the United Nations and the World Health Organization—especially in the field of research, which Lord Brain later stressed. Research is required into the present facts of economic growth and effective methods of controlling fertility, suitable for different societies and religions, and for different levels of education. On the last-mentioned issue there was an immediate response from the Bishop of London, who agreed as to the importance of education, and suggested that, in the face of 87 million children in South and South-east Asia without facilities for elementary education, and the further 130 million children in these areas to be educated by 1980, it might be wise to consider a temporary lowering of Western educational standards, and channel our efforts to supply more teachers to those areas. He referred to the world appeal for literature which the World Council of Churches was sponsoring in this field and to the importance of the education of women, as well as to the need for a reconsideration of traditional moral theology.

As is usual on such subjects in the House of Lords, the debate maintained a high level of informed discussion and constructive criticism. The Earl of Listowel, who was somewhat critical of the Government's support in

the past, referred, for example, to the help which Sweden had given to Ceylon in implementing family planning. Lord Casey was concerned especially with India and Pakistan, and repeated the plea for more scientific research. He referred to the help given by the Rockefeller Foundation and the Ford Foundation, which during the past twelve years have spent some millions of dollars in support of research on human reproduction. It should be remembered here that the population problem and the conquest of hunger are now two of the five main objectives of the Rockefeller Foundation.

This debate was in some measure a sequel to an earlier debate in the House of Lords on June 24 when Lord Henderson directed attention to the vital importance of the United Nations Development Decade, and in particular to the United Nations International Conference on Trade and Development at Geneva, which had then just completed its three months session. In this debate, which perhaps sustained an even higher level than that on July 1, reference was freely made to the report of the Secretary-General of the United Nations, *The United Nations Development Decade: Proposals for Action**, submitted to the Economic and Social Council in consequence of a resolution of the General Assembly on December 19, 1961. That resolution designated the present decade as the United Nations Development Decade. In this period it was hoped that member States and their peoples would intensify their efforts to mobilize and sustain support for the measures required to accelerate progress towards self-sustaining growth of the economy of the individual nations, and towards their social advancement. For each under-developed country an objective was suggested of a minimum annual rate of growth of aggregate national income of 5 per cent by the end of the decade.

In the foreword to his proposals, the Secretary-General observes that development concerns not only man's material needs, but also the improvement of social conditions and of broad human aspirations: development is not just economic growth; it is growth plus change. Moreover, while it may be possible for the developing countries to compress the stages of growth through which the developed countries have passed, it may also be necessary to re-examine the methods by which the development may be attained. U Thant recognizes that we have now at our disposal such effective instruments as the International Bank and the International Monetary Fund, the International Development Association, the United Nations Special Fund, the Expanded Programme of Technical Assistance, and various special programmes such as the World Food Programme and the United Nations Children's Fund, the resources of which may require strengthening. However, he made it plain that he is equally prepared to adopt new instruments as new problems and new opportunities emerge.

The basic problem is to find ways in which the advanced countries can effectively help the developing countries, and new methods of technical co-operation which take full advantage of the new economic and technological possibilities are required. The main economic objective is to create conditions in which the material increases of the developing countries will be increasing by 5 per cent annually by 1970 and will continue to expand at this rate. If this can be done, even if the populations of these countries continues to rise by 2–2·5 per cent a year, personal living standards can be doubled within 25–30 years. However, if growth of population is more rapid this will take correspondingly longer.

U Thant believes that this objective for 1970 is within our reach, provided there is willingness to make the necessary efforts and sacrifices, and in justification of this optimism he refers to the increasing appreciation of the need for new approaches. These include the concept of national planning—for social as well as for economic development; greater insight into the importance of the human factor in development, and the urgent need to mobilize human resources; and acceptance of the principle of capital assistance to developing countries. Concerning the last-mentioned, he visualizes a level of 1 per cent of the national incomes of the advanced countries being made available during the decade. He also suggests that more progress would be possible if the main industrial countries directed as much attention to promoting trade as to dispensing aid. Hindrances to the entry of their products into industrial markets, and the expansion of the production of many primary products faster than demand, are largely responsible for the disappointing foreign trade record of the developing countries: here the related problem of stabilization of the international commodity markets also requires solution.

Increasing under-employment and unemployment is another serious problem and far-reaching action will be needed. Again, many developing countries lack any detailed knowledge of their resources and it is estimated that expenditure on pre-investment work must rise to about 1,000 million dollars a year, or, by 1970, about double the present rate, if the objectives of the decade are to be achieved. A crucial area is the surveying and development of natural resources, including water, minerals and power, while too little effort has been directed towards solving the social and technological problems of the developing countries, although many of them appear to present no insurmountable difficulties. Closely linked is the availability of skilled personnel: the shortage of highly skilled personnel, rather than a shortage of material resources or finance, may well be the greatest obstacle unless new steps are taken. Here U Thant stresses the need for foreign experts to participate in establishing institutions which will take over and continue their work when they leave.

This foreword well illustrates the scope of the Secretary-General's proposals, in which, incidentally, the briefest section is that concerned with technical co-operation. Here, however, U Thant stresses the vital importance of co-operation, and the fact that it must be based on the transfer and exchange of knowledge, with closer relations between scientists in the developing and the developed countries. He looks for new methods of linking the research problems of the developing countries and the research possibilities of the industrialized countries, and it is unfortunate that in the House of Lords no one took up this question of overseas research. Certainly this should not simply be left to the United Nations Educational, Scientific and Cultural Organization or to the Foundations and other institutions of the United States. On technical assistance generally, U Thant recognizes the need for effective co-ordination of effort, and the proposals indicate that the lessons of the previous decade have been learnt and are being turned to account.

Lord Henderson, in opening the debate on June 24, had quoted the secretary-general of the Conference, Dr.

* United Nations: Department of Economic and Social Affairs. *The United Nations Development Decade: Proposals for Action*—Report of the Secretary-General. Pp. xii+125. (New York: United Nations; London: H.M. Stationery Office, 1962.) 1.25 dollars; 9*s*. 6*d*.

Prebisch, who had stressed the danger of world-wide tension if the present situation was not matched by a vigorous policy of economic and social development in which international co-operation must play a decisive part. In some of the developing countries human rights were being set aside as a luxury which they could not afford, and as a hindrance to the essential plans for social and economic development. World poverty confronted us, therefore, with the challenge as to whether our Western standards, our values, and our democratic way of life had any meaning for the developing countries. It was a moral challenge, but it was also self-interest to make it one of the priorities of our policies to help the poorer countries to help themselves.

While being concerned with the lack of progress since the programme was launched in 1961, Lord Henderson insisted that the greatest single service that developed countries could render was to provide expanding markets for the products of the developing countries at stable and remunerative prices, thus enabling them increasingly to pay for their essential imports. Like U Thant, he maintained that aid through trade was the healthiest way to proceed, and he quoted ten key points which Mr. E. Heath had urged in his first speech at the conference on "Trade and Development" which should receive particular attention: (1) a standstill in new barriers to trade with less-developed countries in products identified as being of particular importance to them; (2) ending quantitative restrictions adversely affecting the trade of developing countries; (3) removal of duties on tropical products; (4) removal of duties on primary products from developing countries; (5) reduction of tariffs on semi-processed and processed products from developing countries; (6) ending internal taxes and revenue duties applying specifically to products wholly or mainly produced in developing countries; (7) granting preference to developing countries; (8) exchange of preferences among developing countries; (9) stabilization of commodity prices; (10) supplementary financial assistance. What was done on these points would, Lord Henderson suggested, provide a useful yardstick by which to judge the success and value of the Conference. A statement had already been made in both Houses on June 16, when the Conference ended, and in this it was stated that recommendations concerning aid for development included one, initiated by the British Government, inviting the International Bank for Reconstruction to examine the feasibility of a new scheme for multilateral aid, to be administered under the International Development Association, and designed to help countries the development programmes of which were threatened by adverse export trends which could not be adequately dealt with by short-term balance of payments support. The statement also indicated that, at a conference in the autumn, Britain would announce an increase in her contribution to the Special Fund and to the expanded programme of technical assistance for 1965; that, with other Western countries, she was willing to support an increase in the United Nations budget which would permit expansion of the activities of the Secretariat in industrial development; that she was willing to provide capital assistance to the African Development Bank and to examine sympathetically the possibility of assisting other regional development banks, including the Inter-American Development Bank.

Lord Drumalbyn, who followed Lord Henderson, explained that although it had been expected that the text of the resolutions of the Conference would be available for the debate on June 24, it would not, in fact, be available before June 29. The Government would present them to Parliament in a White Paper in due course. Annexed to the Final Act would be the observations and reservations made by individual delegations on certain recommendations, and the recommendations, after consideration by the Economic and Social Council in July, would be submitted to the Assembly of the United Nations meeting during November 1964–February 1965.

The Conference formulated a set of fourteen general principles governing international trade relations and trade policies conducive to development; however, on some of these Britain had important reservations. The new institutions established—including the Conference itself as a permanent organization of the General Assembly, a Trade and Development Board, and a special committee to be appointed by the Secretary-General—were charged with responsibility of producing a set of principles that would be fully acceptable internationally. There were also important discussions on shipping and insurance issues. Outstanding was the former, which led to the formulation of a "Common Measure of Understanding", stressing the value of liner conferences and suggesting the establishment of consultation machinery between shippers and such conferences. Priority was urged for port improvements, and the development was welcomed of merchant fleets in emergent countries where they had been established on an economic basis.

In the debate, a maiden speech from Lord Hawick of Glendale was notable for the way in which he drew on his own wide experience, and illustrated the possibility of developing secondary industries and assisting small farmers to farm scientifically. He also stressed the importance of discovering the appropriate technique in helping emergent countries. The Bishop of Chichester referred particularly to the place and importance of voluntary effort in technical aid, while in a penetrating speech Lord Lindsay of Birker emphasized the importance of technique and understanding, particularly the encouragement of a general scientific attitude and a readiness to try experiments in the under-developed countries. Lord Lindsay and Lord Hawick were largely supported by the Earl of Selkirk and Lord Walston, the latter stressing particularly the need for craftsmen and technicians as well as professional people. The Earl of Iddesleigh underlined the importance of voluntary service and Lord Sandys reminded the House that foresight and imagination were essential if aid and effort were not to be wasted: here he quoted Prof. L. Dudley Stamp's criticism concerning the fact that the Aswan high dam was already out of date because of the huge loss of water by evaporation: the water should have been brought by pipe-line from the mountains.

The question of a Ministry of Overseas Development was again raised, but here Baroness Gaitskell found little support, and although Lord Sandford, who stressed particularly the opportunity for voluntary service, referred to the need for a clear policy which was deliberately set forth, consistently applied and publicly supported, did not touch on organization at this level, Viscount Samuel had reservations and did not favour a Minister of Overseas Development, although he agreed that mass co-ordination of overseas activities was needed. The Earl of Listowel, however, supported Baroness Gaitskell on this point, but his main argument was that the wealthy countries should work out a common trade policy which

would give the poorer countries a larger share of world trade, and in replying on the debate, apart from pointing out that the magnitude of the British effort was often not appreciated, essentially endorsed most of the points that had been made about voluntary effort, trade and the supply of skill.

Almost simultaneously with Lord Brain's reference to this whole question of aid to the developing countries, the August number of the *OECD Observer* included an article on the role of trade with developing countries which explained the significance to members of the Organization for Economic Co-operation and Development of the United Nations Conference on Trade and Development. Even closer to Lord Brain's argument about the growth of population and its implications are two articles published in the summer 1964 issue of *Daedalus*. This issue is devoted to four disparate, though not entirely unrelated, themes: population, prediction, conflict and existentialism, and in the first of two articles on population, H. Hoagland discusses the mechanisms of population control, arguing that only by fundamental changes in our way of thinking can the population problem be solved. He is not pessimistic although he admits that family planning and limitation call for a critical level of education and prosperity which is not now found in the very poor countries.

Prof. Jean Mayer in his article "Food and Population: the Wrong Problem?" is more challenging and argues that it is unwise, if not dangerous, to link the population problem too closely with food. His argument runs very close to that of Lord Brain. He points out that there are other shortages which are likely to become critical, besides food supply, if we do not start urgently to face the situation, The whole weight of his article lies behind Lord Brain's assertion that our greatest need to-day is to acquire the power of looking ahead, forecasting and preparing for the consequences of the accelerating developments in science and technology.

Nevertheless, Prof. Mayer is emphatic that we need also to make effective use of the knowledge that we already have. This is not to deny that more knowledge is still needed in many fields or to disparage a further article in *Daedalus* in which Prof. D. Bell outlines twelve nodes of prediction. Knowledge may be wasted for lack of the right technique for its use. These articles and debates in sum, however, illustrate the timeliness of Lord Brain's observations and the urgency that the utilization of Britain's resources of materials and manpower should be tackled with all the insight and skill that existing knowledge permits, and also with the most effective tools and techniques that modern technology has placed at the service of the planner and the administrator. It is no longer a matter of whether to plan but how to plan most effectively.

SOVIET ANTARCTIC RESEARCH

Soviet Antarctic Expedition
Information Bulletin, Vol. 1: Pp. xv+404. 90*s*. Vol. 2: Pp. x+318. 80*s*. (Amsterdam, London and New York: Elsevier Publishing Company, 1964.)

SOME scientific reports of Antarctic expeditions take a notoriously long time to appear. Two scientific reports from pre-First World War expeditions have appeared within the past two years, and publication is not yet complete. Reports of inter-war and post-Second World War expeditions are similarly incomplete. (I have myself one such report on my conscience!)

In these circumstances, the shorter paper or brief preliminary report has much to commend it, both for the reader, who obtains a general picture of work carried out, and for the investigator, who establishes without much delay his claim to have contributed to knowledge at a particular time. This problem has been tackled in various ways. The Antarctic Research Programme of the U.S. National Science Foundation publishes a monthly *Antarctic Report* summarizing reports from the field. The British Antarctic Survey now publishes its *Bulletin* at irregular intervals, but this is mainly intended for shorter final reports. Annual reports of National Committees on Antarctic Research to SCAR (the Scientific Committee on Antarctic Research of the International Council of Scientific Unions) present useful summaries of present and proposed activities in the Antarctic, but these are not widely available.

The Soviet Antarctic Expedition, which has been in operation since 1955, has solved the problem in a useful manner by producing *Informatsionnyy Byulleten Sovetskoy Antarkticheskoy Ekspeditsii*, which has appeared at irregular intervals since 1958, the latest being No. 46. Issues average 50–60 pages in length, including illustrations, and include about ten short scientific articles many of which summarize work of considerable interest. There are also brief observers' notes on items of interest, summaries of radio messages from Soviet stations in the Antarctic and such items as recommendations of various Antarctic meetings, notes of work of other countries, reviews of books, maps, etc. An English index in each volume has served to direct those without a knowledge of Russian to articles for which they need a translation, but in spite of this aid it has been difficult for non-Russian scholars to gain a general appreciation of the scope and standards of Soviet researches in Antarctica.

The initiative of Dr. George P. Wollard and the University of Wisconsin, aided by a grant from the National Science Foundation, in translating the Soviet *Bulletin* has made this general preliminary survey accessible to English readers. Final editing has been done by Scripta Technica, Inc., and the first two volumes now published by Elsevier cover *Bulletins* Nos. 1–20 (1958–60).

A spot check shows that care has been taken in the translation, and the English language presentation is good. No errors of translation were noted; a couple of unimportant sentences were omitted. The system of transliteration is reasonably consistent though it differs from that of most map makers. Retransliteration of proper names is a fruitful source of errors, and a few minor errors of this type were noted, for example, *W*. Fuchs for *V*. Fuchs, Argentina Islands for Argentine Islands. Two errors, one of them unimportant, were found in five large tables.

The translation is not 'cover-to-cover', but deals with all the main scientific papers. The section called "By Radio from Antarctica" has rightly been omitted as ephemeral, while "Observers' Notes" have been translated only when they are of particular interest. The maps are photo-reproductions of the originals with new letterpress superimposed as necessary for the English language reader. Some are over-reduced, for example, I, p. 81, while half-tone reproductions are poor—but so are the originals in the Russian version.

A third volume in the series is due shortly, presumably covering *Information Bulletins* Nos. 21–30. It is to be hoped that the response will be sufficient to encourage the promoters to catch up on the backlog and to continue with a speedily available translation of future numbers. Close co-operation between the Russian editors and the translators and publishers could ensure that this interesting *Information Bulletin* receives the notice it warrants in other countries. If this can be arranged at the present reasonable price, a very useful service will be provided.

G. de Q. Robin

PHILOSOPHICAL AND SCIENTIFIC METHOD

Philosophy of Science

The Delaware Seminar, Vol. 2, 1962–1963. Edited by Bernard Baumrin. Pp. xviii+551. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, 1963.) 109*s*.

THIS is the second volume of the Delaware Seminars and it confirms the expectations aroused by the first, presenting a true image of philosophical activity, one of continual debate that constantly questions old assumptions and results (and, be it said, some not so old); a kind of dialogue between contending personalities, rather than an accomplished set of results. This volume is specifically devoted to problems in the field of the physical sciences—or rather, in physics; chemistry still does not seem to attract the philosophical minds of the present day. Part 2 discusses topics falling under the heading of space, time and relativity; Part 3 is on particles, fields, and quantum mechanics; Part 6 on cosmology. These parts are matched by others of a more general nature, going over some of the bread-and-butter ground of the academic philosophy of science.

Part 1 is perhaps the most interesting, with its attempts at the wholesale demolition of philosophical doctrines which had their heyday, it seems, only yesterday, such as 'logical empiricism', 'hypothetic-deductive theories', requirements for explanation in terms of precision and completeness, or the exclusion of probabilistic approaches from any final point of view. All these assumptions are questioned vigorously. P. K. Feyerabend's chapter in part goes over ground covered in some of this author's earlier writings, but it considerably clarifies his position, which is more clearly seen to have been motivated by an attempt to make the Anti-Copenhagen spirit of physicists like Bohm and Vigier methodologically respectable. The older logical empiricism made use of certain idealized models for theory construction, with certain implicit logical criteria, such as those of consistency and meaning invariance. The reality is (and, Feyerabend insists, certainly ought to be) different. One must not imagine that existing theories can be tested against a background of a certain set of given neutral observational 'facts'. Rather, theories must be tested against alternative theories, and these must first be formulated, so that some of the critical 'facts' (observed, or more often inferred) shall first be forthcoming. Ultimately, these alternative theories need not be mutually consistent, nor need the meanings of the terms which they contain (theoretical as well as empirical) all have fixed connotations.

Wilfrid Sellar's chapter goes into the problem of the reality of theoretical entities by way of the elegant method of a discussion of the nature of the correspondence rules of various kinds which co-ordinate the 'abstract' with the 'empirical' expressions of some given theory, or even the theoretical properties of two different theories, for example, physical and chemical, thus involving incidentally a clarification of the notion of 'reduction' of one to the other.

Part 4 contains the usual expected section on 'inductive inference', and an important paper on the theory of measurement by Brian Ellis. The former, by W. C. Salmon, makes another stab at the so-called 'pragmatic vindication' of induction. This it does in terms of Reichenbach's probabilistic approach, using the frequency concept of probability (sequence of a limit). It purports to prove that the 'rule of induction by enumeration' can be shown to give successful results, provided that the ratio of observed sample values to size of sample has a limit at all (a kind of 'uniformity of nature' maxim) and shows that any competing alternative rules would fail to give successful results, even if the maxim did hold, since they can be shown to sin against a number of necessary logical requirements for success. Of course, this is a vindication only if 'Nature is uniform', and if one limits oneself to a discussion of very simple numerical cases.

Of the specialist sections, I can only single out A. Gruenbaum's attempt to show, by means of an example from relativity, something of the importance of clear philosophical ideas when we try to solve certain questions in the history of science; Hilary Putnam in turn criticizes some of Gruenbaum's anti-conventionalist views on geometry. In Part 2, Hill, Pais and Suppes consider such matters as "the status and applicability of mathematics in physical theories, the plethora of elementary particles, and the non-standard character of the mathematical equipment of quantum mechanics".

In Part 6 N. R. Hanson and J. A. Wheeler contribute discussions on philosophical aspects of contemporary cosmologies; Wheeler's paper, despite a pretty clear beginning (straightforward almost to the point of being bizarre), turns out to be heavily technical.

The contributors to these Seminars, by and large, are leaders in their field, and though no one reader would be likely to follow each and every one of the discussions with equal interest, the standard is uniformly high, even though occasionally it seems as if old wine was being poured into new bottles. Certainly these colloquia try to keep alive the old dream of worth-while discussion between scientist and philosopher without the traditional British depreciation of the other man's interests, knowledge, and intellectual traditions.

G. BUCHDAHL

SPACE MEDLEY

Advances in Space Science and Technology

Vol. 5. Edited by Frederick I. Ordway, III. Pp. xvii+334. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1963.) 93*s*.

THIS book, like the first four volumes in the series, brings together articles on widely varied aspects of space science and technology, and it is pleasant to be able to report that this fifth volume maintains the high standard of its predecessors.

The book is divided into six chapters. The first, by R. G. Athay, is a useful short survey of what is not known about the Sun, with ideas for experiments. The second chapter, by R. P. Haviland, discusses techniques for communication satellites. The various possible systems are described, and the important technical problems are clearly stated; there is also a section on the world's desire to communicate and the best ways of assuaging it. The next chapter, by H. W. Ritchey and J. M. McDermott, is a fine review of solid-fuel rocket technology: all aspects of the subject, from detonation tests to the geometry of grain design, are covered in a readable and authoritative text. The fourth chapter, by R. E. Smith, is a thorough survey of the problems of keeping man alive and well during prolonged space flights: such possibilities as hibernation or transient hypothermic torpor are mentioned, and there is an illuminating discourse on the regenerative treatment of waste products. Writers on the subject of man-in-space should, however, beware of wrapping simple facts in layers of abstraction. The news that "very complex matrices of interlocking life systems . . . find their common denominators in terms of mutual requirements for energy . . ." may induce in the reader a "transient state of hypothermic torpor", from which he emerges with the suspicion that the author merely means "all living things need food and warmth".

The last two chapters of the book are the longest, and range widely. Chapter 5 deals with navigational and geodetic satellites, and their applications both on Earth and elsewhere in the Solar System. The historical survey of navigation and navigation-satellites, by J. D. Nicolaides, is particularly good, and brings to light many unfamiliar facts. The section on geodetic satellites, by J. D.

Nicolaides and M. M. Macomber, is satisfactory, though the examples quoted are not always the best. The contribution by W. M. Kaula, who discusses how satellites should be used to investigate the gravitational fields of the Moon and planets, is an excellent introduction to a difficult subject.

The final and longest chapter, of nearly 100 pages, is by K. A. Ehricke, on "Orbital Operations": this is not concerned with surgery under weightless conditions, but with the "controlled change of conditions of a technical system in space". Biological terms do appear, however, as is quite natural since space vehicles, like animals, can propel themselves and execute purposeful actions: thus we find discussion of "coupling, docking and mating". Dr. Ehricke's chapter is a masterly survey of what may well be going on in space in twenty years time when operations in cislunar and interplanetary space may be as routine as airline schedules are now. Occasionally Dr. Ehricke falters over points of detail, but this does very little to reduce the value of his survey.

The book is well printed and produced, with good indexes and only a sparse crop of misprints. The jacket strikes a slightly false note: a moonscape on the dust cover may be better than a dust cover on the Moon, but the lunar scene shown is too unrealistic for a soberly scientific book. This superficial blemish does not prevent the book being an admirable addition to the literature of lasting interest on space science and technology.

D. G. King-Hele

A SURVEY OF KRON'S THEORIES

Tensors in Electrical Engineering

By J. W. Lynn. Pp. x+216. (London : Edward Arnold (Publishers), Ltd., 1963.) 52*s*. 6*d*.

WITHIN the compass of about 200 pages, the author of *Tensors in Electrical Engineering* gives quite a detailed survey of Kron's application of tensors to electric networks and machines. He is firmly convinced that tensors are an essential feature of the unified theory of electric machines.

A brief first chapter summarizes the necessary groundwork of determinants and matrices, after which Dr. Lynn plunges at once into Kron's network analysis. Tensors are described and used early in the book without excessive use of geometry. From the development of a set of general equations describing the performance of a generalized machine, Lynn proceeds to the equations of actual types by derivation from the general equations.

A substantial proportion of the book is devoted to hunting, and the analysis of a two-machine system is given in full. It is pleasant to see that stability tests were carried out on a laboratory two-machine system, and the results compared with the theoretical calculations. Although the author's principal topic is electric machines, he includes in his final chapter a fairly comprehensive treatment of Kron's circuital models of the electromagnetic field equations.

This is by no means a book for beginners. It is aimed at postgraduate students of electric machines and research workers in this field. For such readers, it is probably not too difficult if they are already conversant with the subject. The book is obviously the culmination of many years of research and reflexion. The text is clear and concise. Lynn shares Kron's philosophy and follows his methods very closely with, however, less reference to geometry. He proceeds with ease and assurance through the more difficult parts of the mathematics.

It is necessary to pass now from praise to protest. The second chapter opens with a figure showing two simple static networks the power inputs of which are patently unequal. A transformation matrix which is singular (three rows and two columns) is also displayed. Yet the author opens his mathematical analysis of the two networks by equating the unequal power inputs. A few lines later, he uses the inverse of his transformation matrix which obviously has no inverse. There are other holes in the theory, but these two will suffice. The author is well aware of them and indeed discusses them: it is sobering to reflect that he can muster support for such avoidable subterfuges. The theory is bad and should be abandoned; Kron's technique is so impressive and so successful that it merits a rigorous theory.

W. J. Gibbs

ELIMINATION FROM THE BODY OF RADIOACTIVE ISOTOPES

Radioactive Metal Mobilization in Medicine

By Prof. Alexander Catsch, translated from German by Dr. Bergene Kawin. (Springfield, Illinois: Charles C. Thomas, 1964.) 7.50 dollars.

THE ever-increasing peace-time production and use of radioactive substances, including metals, together with the potential hazard of their extensive release in time of war, have brought a greater danger of accidental contamination to those handling these substances and otherwise exposed to them. The elimination of radioactive isotopes from the body, before they have had time to become irreversibly fixed to the tissues, calls for the use of every available means.

The author reviews, in *Radioactive Metal Mobilization in Medicine*, the present-day methods of removal of radioactive metallic isotopes, restricting his observations to a few of the metals likely to be encountered in the laboratory and to those which have so far been investigated. Attention is directed mainly to the use of chelating agents which the author regards as the most promising substances for this purpose. After a description of the chemical structure and probable mode of action of chelating agents, there follows a discussion of the results of their use under experimental conditions in animals. Although most of the present knowledge of this subject is based on animal experiment, it is probable that much of the information so gained is applicable to man, as the action of chelating agents depends very largely on physico-chemical forces. The author is, however, careful to point out that some of the experimental results may not apply to man.

The first attempts at heavy-metal elimination from the body date from the use in man, some 30 years ago, of calcium salts and parathormone in an attempt to remove lead incorporated in the skeleton. A similar competitive action using a stable isotope to dilute a radioactive isotope bound to body-tissues is still used, but has to a great extent been replaced by the use of complexing (chelating) agents. Of the chelating agents at present in use, the polyaminopolycarboxylic acid group offers the most promise. Much work still remains to be done to improve the complexing properties of chelating agents for individual isotopes, and the problem of toxicity of both chelator and its metal complex has prevented many active substances from becoming therapeutic realities.

The removal of radioactive isotopes from the body requires not only an understanding of the probable action and structure of complexing agents, but also a knowledge of how individual isotopes are stored in and eliminated from the tissues. The effectiveness of any chelating agent in binding a metal in any particular organ varies considerably and has to be established by experiment. Furthermore, a chelating agent used to complex with a given radioactive isotope may be found to complex with other metals, producing such serious effects as the removal of zinc from carbonic anhydrase and iron from succinic anhydrase.

Prof. Catsch also considers factors influencing the effectiveness of chelating agents, such as delay in their administration and the effects of competition with endo-

genous acceptor groups. Such factors may prove of great importance, because the rate of irreversible fixation of a radioactive isotope within individual body-tissues determines whether any form of treatment is likely to prove effective. The inability of some chelating agents to pass from one body compartment to another also seriously detracts from their efficiency; this has led to a search for improved complexing agents by chemical substitution of the side chains in existing substances.

The most probable routes of human contamination by radioactive metallic isotopes are thought to be by implantation into wounds and absorption through the respiratory tract, and experimental work bearing on the elimination of isotopes absorbed through these two routes is discussed.

Although most attention has been directed to chelating agents as the most promising means of eliminating radioactive isotopes from the body, alternative means, such as combining the metals to form insoluble products and their hormonal release from the skeleton, are also reviewed.

Finally, the author considers the still very meagre data concerning the removal of radioactive metallic isotopes from man, and concludes by showing how fragmentary is the present state of knowledge about the whole subject and how much still remains to be discovered.

This short work is a succinct and clear account of the present state of knowledge of radioactive metallic isotope elimination, and nothing of the clarity of the original account appears to have been lost in translation.

H. SPENCER

NUTRITION OF MAN AND HIS FARM ANIMALS

Comparative Nutrition of Man and Domestic Animals

By H. H. Mitchell. Vol. 1: Pp. xxi+701. 178*s*. 6*d*. Vol. 2: Pp. xxi+840. 200*s*. (New York and London: Academic Press, 1964.)

THIS substantial work by Dr. H. H. Mitchell, whose international reputation rests in several fields of research, but in none more than in the nutritional aspects of proteins and amino acids, is most timely. It correlates, in a quantitative fashion, the nutrient requirements of man and his domesticated livestock, and the factors which modify these. *Comparative Nutrition of Man and Domestic Animals* is a thoroughly critical and stimulating book, as are all Dr. Mitchell's reviews. The emphasis is on the physiology and biochemical aspects, rather than on the more practical.

As the author describes in his preface, this work arose in the preparation and revision throughout some thirty years of a postgraduate course in comparative nutrition, held by him at the University of Illinois. However, in parallel with the development of the formal course, an experimental research programme was pursued on the material and energy requirements of poultry, pigs, sheep, cattle, and, in later years also, of man himself.

The similarities and dissimilarities among the different species are pointed out, and Dr. Mitchell has provided background information, largely biochemical in nature, concerning the various nutrients, their functions and their participation in the energy transactions of the body. The courses which led to these two volumes were designed for students in nutrition, physiology, domestic science and animal science.

Net nutrient requirements, that is, the amounts requiring to be absorbed by a completely healthy animal, are expressed in terms of animal expenditures and storages, factored in their ultimate terms of maintenance, growth, activity, reproduction, etc. These net requirements are converted into dietary requirements, after due consideration of the wastages of dietary nutrients in the course of their assimilation in the body.

The actual preparation of these volumes took place on Dr. Mitchell's retirement from active service, and it is very heartening to know that the University of Illinois, in its wisdom, provided facilities, including office space.

The first volume deals with the nutrient requirements of the animal body, in terms of energy, protein, water, minerals, and with the nutrient requirements for muscular work, growth and senescence, storage, reproduction, lactation and egg production. The second volume deals with vitamin requirements in terms of dietary equivalents, the wastage of dietary nutrients during assimilation and net nutrient values.

In combining the assessed nutrient expenditures or storages involved—in growth, activity, reproduction, lactation, etc.—to obtain a total nutrient requirement of an animal of given species, age, sex and physiological status, Mitchell warns against assuming that the whole is the sum of the parts, because the imposition of one function on another may result in stimulation or inhibition of one, or both, functions. Thus, pregnancy stimulates basal metabolism, while inhibiting lactation.

Mitchell defines a balanced diet as one containing amounts and proportions of each of the essential nutrients large enough to promote maximum performance of those animal functions with respect to which the diet is balanced, but not so large as to impair, in any way, the well-being of the animal consuming it or of any of its tissues, or to depress the net energy content of the diet. He defines the carbohydrate and fat content as one sufficient in amount to permit the full use of the essential nutrients to perform their specific functions in the body. The utilization of the metabolizable energy of a completely balanced diet is maximal, and the same for all such diets for each animal function, but varies from species to species. The corollary of this is that the nutritive value of feeds is specific and characteristic for the various feeds only with respect to their content of nutrients and their digestibility. The degree to which the absorbed nutrients are utilized in the body is dependent on the way in which feeds are combined and the animal functions they support.

The more of such a balanced ration that is consumed, the better nourished the animal will be, with reference to which the ration is balanced up to the point of repletion of its requirements, but, in agriculture, the law of diminishing returns has generally to be taken into account and, at some stage, an input/output relation has to be struck in the light of present-day economic circumstances.

Superimposed on the apparent fixity of nutritional behaviour among different species, there are secondary and specific modifications that reflect adaptations to different environmental conditions. These are the modifications that distinguish one species from another. Within any one species, adaptations to changes in environment and in food supply may occur in relatively short spaces of time. These necessitate the conception of a nutrient requirement as a range of values, symmetrically including a mean measure, rather than the mean itself. Nevertheless, this conception does not hold for net requirements because these are what have to be absorbed.

Because of lack of necessary nutritional data, little consideration can be given to some domestic species, such as the cat and dog. The author makes a strong case for the profitability of the investigation of farm animals in furthering the investigation of human nutrition.

To end the second volume there is a strong philosophical note, and this is what we would expect from a life-study of the resultant of satisfying the integration of factored requirements.

Each chapter has its own extensive bibliography and there is a complete author and subject index. The author and his publishers are to be congratulated on a magnum opus which will long remain a standard reference book, as well as being full of dynamic thought and criticism.

D. P. CUTHBERTSON

Matrix and Tensor Analysis in Electrical Network Theory

By S. Austen Stigant. Pp. 505. (London: Macdonald and Co. (Publishers), Ltd., 1964.) 80*s*. net.

THIS book presents the subject of network theory in a manner which was originally largely due to Gabriel Kron.

The volume is divided into three parts; theory, applications and appendixes. The first part, approximately one-third of the book, comprises introductory chapters on determinants and matrices, followed by three chapters on diagonalization of matrices. The three final chapters of this part develop tensors in relation to electrical networks.

The second part, again approximately one-third of the book, is taken up with applications in power distribution and engineering. A chapter on simple applications of determinants is followed by three on matrix applications, the examples being largely connected with three-phase systems. Examples on untransposed and transposed conductor systems, with and without faults, are given in later chapters. The application of tensors to multi-phase networks, multiple-winding transformers and automatic voltage control by series capacitor is considered in the concluding chapters of this second part.

The third part contains appendixes on material needed in the course of development of the second part, but unconnected with the main theme of the book. The treatment used in these appendixes is largely classical.

The book is well written, easy to follow and is directed at engineers rather than applied mathematicians. The latter would be rather unimpressed with the introductory chapters on matrices and tensors. The book should prove very useful to engineers who are interested in the modern treatment of power distribution systems and, possibly, to honours graduates studying heavy current electrical engineering in departments having an enlightened approach to the subject. The large number of practical examples included in this volume should make it more attractive to the less-academically inclined than some of the earlier treatises on matrices and tensors in network theory.

Handbuch der Physik

Herausgegeben von S. Flügge. Band 27: Spektroskopie 1. Pp. vi + 507. (Berlin, Göttingen und Heidelberg: Springer-Verlag, 1963.) 130 D.M.

THIS volume, the first of the two on spectroscopy in the *Handbuch der Physik*, contains three articles. The titles (which are in the languages used), authors and lengths of these are as follows: "Line Widths", R. G. Breene, jun., of Centerville, Ohio (pp. 79); "Atomic Spectra", Bengt Edlén of Lund (pp. 140); "Spectroscopic électronique moleculaire", Boris Rosen of Liège (pp. 253).

R. G. Breene is the author of the well-known book *The Shift and Shape of Spectral Lines* published in 1961. As would be expected from this, his article, which covers molecules as well as atoms, contains a clear account of many of the relevant mathematical researches. The more recent work does not, however, receive as much attention as it merits; thus, of the 95 references cited, 62 are dated 1939 or earlier, 15 are in the period 1940–49, 18 are in the period 1950–59 and none are later than this.

Bengt Edlén himself points out that "Atomic Spectra" is less comprehensive than the title suggests. It is indeed concerned mainly with the undisturbed gross structure of the energy-level systems of free atoms and ions. A considerable part of the material, especially that concerning isoelectronic sequences, is original. The article will be consulted for many years.

In "Spectroscopic électronique moleculaire", Rosen provides a much-needed outline of the principal theoretical and experimental investigations of diatomic and polyatomic molecules. The article is a valuable addition to the literature of the subject. A defect is that the bibliography is not so full and as carefully compiled as might perhaps be wished. There are useful appendixes by W. B. Price on ionization potentials, by K. Wieland on the spectra of radicles and by F. W. Dalby on absolute transition probabilities.

The book concludes with German-English and English-German subject indexes of all three articles, and a French subject index of the last article. The production is of a high standard. In particular, praise must be given to the anonymous artists responsible for the numerous beautifully drawn figures. D. R. Bates

Theorie et Technique de la Radiocristallographie

Third edition. By Prof. A. Guinier. Pp. xvi + 740. (Paris: Dunod, 1964.) 124 Fr.

THE first edition of this admirable text-book appeared in a paper-covered form in 1945. An English translation was published in 1952, but was soon superseded by a much enlarged and almost entirely rewritten second edition in French. The third edition, just published eight years later, is not enlarged but has been brought up to date by the addition of new references and some changes in text and tables. The binding and paper are good, but unfortunately many of the photographs are still very badly reproduced and some minor drafting inaccuracies remain in line drawings. In spite of these perennial criticisms, however, there is no doubt that this book is a classic, and that the author's French is so beautifully clear that it is a pleasure to read.

The five main parts of the book deal with the general properties and practical production of X-rays; elementary crystallography and diffraction theory; experimental diffraction techniques for powders and single crystals; applications to various practical and theoretical problems; and, finally, diffraction by imperfect crystals and amorphous bodies, including small-angle scattering. Problems of crystal structure determination are confined to about eight pages. For systematic treatment in that field there are many good text-books; but there is no other text-book having the range and the quality of *Theorie et Technique de la Radiocristallographie*. K. Lonsdale

The Waterfowl of the World, Vol. 4

By Jean Delacour. With contributions by Hildegarde Howard, Milton W. Weller, Philip S. Humphrey and George A. Clark, jun. Pp. 364+15 plates. (London: Country Life, Ltd., 1964.) 126*s*. net.

THE first three volumes of this work, published between 1954 and 1959, gave a systematic account of the Anatidae, species by species (*Nature*, 185, 567; 1960). This final volume reviews the group as a whole, thus complementing the extensive presentation of facts by a valuable synthesis. Here Jean Delacour plays a mainly editorial part, although himself writing chapters on aviculture and on domestic waterfowl. The major contributor is Milton W. Weller, who provides the review of the biology of the family in four chapters dealing with general habits, the reproductive cycle, ecology, and distribution and species relationships. The same writer is also responsible for chapters on fowling and on conservation and management, while P. S. Humphrey and G. A. Clark deal with the anatomy of waterfowl and Hildegarde Howard with fossil Anseriformes. Four of the colour plates by Peter Scott portray domesticated forms derived from the greylag goose, the swan goose, the Muscovy duck, and the mallard; the other—a somewhat unusual feature—pictorially corrects errors in the artist's illustrations of wild species in the earlier volumes. The procedure of adding a full general review to the systematic treatment is much to be commended, and the work as a whole forms an admirable monograph of a large and cosmopolitan family of birds.

Landsborough Thomson

A NEW INTERPRETATION OF THE GEOLOGY OF BRITISH GUIANA

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PROVISION from the Colonial Development and Welfare Fund enabled the Geological Survey of British Guiana to carry out an intensive mapping campaign during the years 1957–61 (ref. 1) with the object of reporting on the advisability of further mineral search by airborne and other modern prospecting techniques. A new geological map, based on 1 : 125,000 geological cover, was ready in 1961 (now published in colour)[2], and formed the basis of a successful application to the United Nations Special Fund for assistance in an airborne prospecting campaign which began in 1962. The object of this article is to present briefly, pending the publication of detailed descriptions[3,11], the results of general interest regarding the structure and radiometric dating of the Guiana Shield and its similarities with West Africa.

British Guiana covers an important central segment of the Guiana (Spanish: *Guayana*) Shield (Fig. 1), the basement of north-eastern South America, divided from the Brazilian Shield only by the early Palæozoic downwarp of the Amazon River basin. Geological exploration was begun by the British Government in 1868 (ref. 4) and a great deal of information was accumulated during the growth of the Geological Survey previous to 1957, and described in various publications[5–7]. Mapping, following the re-organization of the Geological Survey, was based on strictly objective lines adapted to Precambrian terranes, and full use was made of the available photogeological cover supplied by the Directorate of Overseas Surveys. Representative specimens were carefully selected for radiometric dating which has since confirmed and extended the interpretations adopted and fixed the time-range of the major geological events as Lower Proterozoic or Archæan. Except for one age supplied by the Atomic Energy Division, Geological Survey of Great Britain[8], all the radiometric dates quoted are based on the work of the Age Determination Unit, Overseas Geological Surveys in the laboratory of the Department of Geology and Mineralogy, University of Oxford[9].

British Guiana (Fig. 2) can be divided essentially into three geological and geomorphic units as follows: (1) a narrow coastal strip of marine sediments of Cretaceous to Recent age; (2) the Pakaraima Mountains in the west, which are formed by the eastern margin of the tabular, detritic, Roraima Formation covering a large area in neighbouring Brazil and Venezuela; (3) a vast extent of low pediplains with occasional plateaux and mountains rising to 1,000 ft. and, rarely, 2–3,000 ft., covered, except for a strip of savannah in the south-west, with Amazonian rain-forest. This area is underlain by Precambrian metasedimentary and metavolcanic rocks, gneisses and granites with a sporadic cover of Tertiary to Recent sediments.

Geological and structural considerations indicate that the latter unit can again be divided into two geological provinces as defined by M. E. Wilson[10]. These northern and southern provinces are separated just south of latitude 4° N. by an east–west rift valley about 100 miles long and 30 miles wide which magnetometer observations suggest may contain up to 20,000 ft. of (?) Cretaceous–Tertiary sediments. East of longitude 58° 30′ the younger sediments disappear and granites and gneisses occur, but the relations of the major units are obscured by bodies of younger granite.

Table 1 shows the major geological formations in British Guiana in their stratigraphical succession as determined both by geological and structural considerations and by radiometric dating. The dates given are approximate minimum ages based on potassium/argon, rubidium/strontium and uranium/thorium/lead on a variety of minerals: a detailed discussion of the radiometric methods used and the geological significance of the various results is in the press[11].

The most widespread of the Precambrian formations of northern and central British Guiana is the Barama–Mazaruni Assemblage which is now shown to be essentially a eugeosynclinal accumulation of mudstones, greywackes and conglomerates, with fine-grained igneous rocks chiefly of the spilite–keratophyre suite, metamorphosed throughout in the greenschist facies, with local zones of higher grade[3,12]. Pillow lavas have been found in Venezuela[13] within the extension of these rocks, and occasional graded bedding throughout the succession has served to disentangle the stratigraphy and indicate a structural style of open folding, with axial directions mainly west-north-west–east-north-east, and a well-developed, steeply dipping foliation sometimes discordant to the fold trend. A field trip to the upper Cuyuni and Yuruari River basins, kindly arranged by the Venezuelan Government, enabled

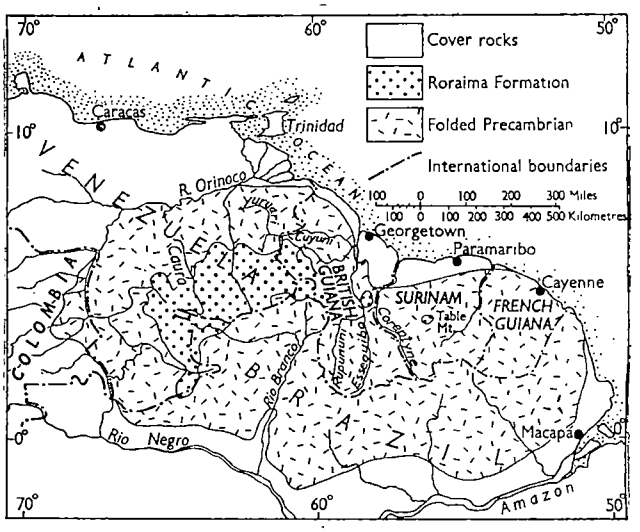


Fig. 1. Outline geological map of the Guiana Shield, South America, showing outcrop of folded Precambrian rocks and outliers of tabular Roraima Formation of Proterozoic age

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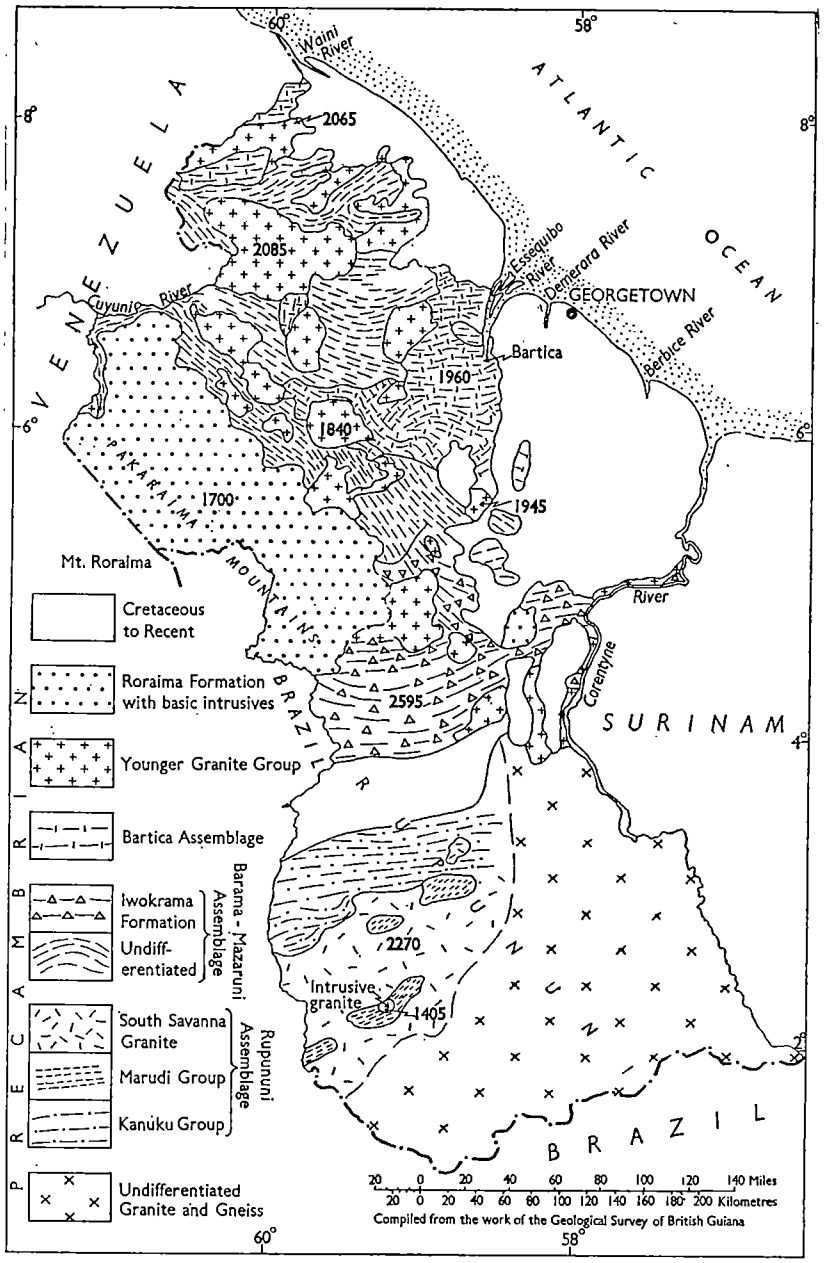


Fig. 2. Generalized geological map of British Guiana showing trendlines and minimum radiometric ages (million years) now accepted as significant for particular groups of rocks

two of us[14] to affirm the extension of the geosynclinal régime to the west-north-west, and this has now been established. In the opposite direction, to the east and east-south-east, similar formations have been described in Surinam[15] and, in some detail, in French Guiana[16], where radiometric dating supports this provisional correlation. A minimum age of about 2,500 million years[11] for the final filling of this geosyncline is tentatively suggested by a firm potassium/argon determination (2,595 ± 125 m.y.) on riebeckite from a granite[18] cutting the Iwokrama Formation.

The margin of this approximately east–west geosynclinal basin containing the Barama–Mazaruni Assemblage, the gneisses and granites of the Bartica Assemblage and Younger Granite Group is unknown to the north, towards the Atlantic, but to the south it is marked by a change to more orthoquartzitic sediments (Muruwa Formation)[17] overlain by a great thickness of rhyolitic and dacitic volcanic rocks (Iwokrama Formation)[18]. These marginal formations appear to follow a wide arc through Brazil to the Rio Caura area (7° N.; 65° W.) in Venezuela where they have now been recognized[19,20]. Thus the existence of an older land mass, now represented by the Rupununi Assemblage, which was postulated on other geological and structural grounds[21], is now supported by information on the sedimentological zoning of the Barama–Mazaruni Assemblage.

The Archæan Barama–Mazaruni eugeosyncline, therefore, forms a belt about 350 km wide along the Atlantic coast of the Guiana Shield, and appears to be cut approximately lengthwise by the present coastline.

Emplaced in the eugeosynclinal prism are bodies of crystalline rocks consisting chiefly of biotite and hornblende-biotite gneisses with amphibolites which have been grouped as the Bartica Assemblage[22], so named from the type area where the rocks have been examined in more detail. These gneisses were originally mapped as an ancient basement; but geological, structural and sedimentological arguments, later supported by radiometric evidence, have shown that they are probably derived by

Table 1. STRATIGRAPHICAL TABLE FOR BRITISH GUIANA

With significant minimum radiometric ages

RECENT TO CRETACEOUS		Superficial		Sands, clays and laterite
		Corentyne and Rose Hall groups		Marine clays and sands
		Takutu Formation		Red and grey shales and sandstones, in Rupununi District only
			GREAT	UNCONFORMITY
PALAEOZOIC		Minor Intrusives		Dolerites, etc. 450 m.y. in Rupununi
			GREAT	UNCONFORMITY
		Younger Basic Intrusive Group		Dolerites and quartz dolerites in thick dykes and sill, about 1,700 m.y.
		Roraima Formation		Continental orthoquartzitic succession with shales and jasper
			GREAT	UNCONFORMITY
	Northern Province			*Southern Province*
PRECAMBRIAN		Younger Granite Group	Granite, grandiorite and adamellite complexes, about 2000 m.y.	
	BARTICA ASSEMBLAGE	Muscovite granite	Muscovite granite passing to muscovite biotite gneiss, about 2,000 m.y.	RUPUNUNI ASSEMBLAGE: South Savanna Group — Biotite granite with enclaves, about 2,300 m.y.
		Banded gneiss	Banded biotite and biotite-hornblende gneiss; amphibolites	Marudi Group — Metaquartzites, biotite schist and gneiss
				Kanuku Group — Hypersthene and biotite-garnet granulites; charnockitic gneiss and granite
	MAZARUNI GROUP	Haimaraka Formation	Mudstones and greywackes; volcanic rocks	
		Cuyuni Formation	Sandstones, pebbly sandstones and conglomerates of greywackes suite; volcanic rocks	UNCORRELATED: Kuyuwini Group — Rhyolitic and andesitic volcanic rocks in Southern Rupununi (age very uncertain)
		Iwokrama Formation *	Rhyolitic and dacitic volcanics, > 2,500 m.y.	Gneiss and granite — Widespread biotite and biotite-hornblende gneisses and granites in partially explored southern British Guiana
		Muruwa Formation *	Orthoquartzitic sandstones and acid volcanic rocks	
	BARAMA GROUP	Various Formations	Mudstones, quartzites, argillites; lavas and pyroclastics; manganiferous phyllites; mica schists and gneisses	

* Formations of continental margin, roughly equivalent in age to the eugeosynclinal Haimaraka and Cuyuni formations.

metamorphic–metasomatic processes from the metasediments and metavolcanics of the Barama–Mazaruni Assemblage during a period of tectonism. They may be regarded, following Wegmann[23], as an *Unterbau* of which the supercrustal Barama–Mazaruni rocks are the *Oberbau*. Granites, adamellites and granodiorites of the Younger Granite Group[24], mostly foliated, both conformable and cross-cutting, have also been emplaced in the eugeosynclinal succession and radiometric dating[9,11] relates them in time to the diastrophism reflected in the Bartica Assemblage.

The apparent ages of these gneisses and granites have been disturbed by later events, either diastrophic or thermal (for example, widespread intrusion of dolerites at about 1,700 m.y.), which have reconstituted certain minerals causing them to give ages younger than the age of original emplacement. We consider, however, that reliable potassium/argon determinations on pyroxene, hornblende and muscovite indicate a minimum age of about 2,000 m.y. for the close of this pre-Roraima period of metamorphism and granitization. Gneisses and granites generally regarded as occupying a similar stratigraphical position in French Guiana have been dated 2,200–1,900 m.y.[16].

The Rupununi Assemblage, forming the southern margin of the Archæan geosyncline, consists chiefly of the Kanuku Group of banded biotite, biotite–garnet and charnockitic gneisses, mostly in the amphibolite and, to a lesser extent, the granulite facies of metamorphism, the Marudi Group of mica schists and metaquartzites in the greenschist facies, and a large batholith of biotite granite, the South Savanna Granite, which has intrusive contacts with both groups[25,26]. Radiometric evidence based on uranium/thorium/lead ratios in monazite indicates a probable age of about 2,300 m.y. for the granite, thus tentatively fixing a minimum age for the assemblage. The rocks of the Marudi and Kanuku Groups have been migmatized by this granite, but the finely banded granulites of the Kanuku Group have suffered an earlier metamorphism and on structural and geological grounds are regarded as the oldest shield rocks known in British Guiana. Here again, the potassium/argon and rubidium/strontium isotope relationships in minerals of the granite have been disturbed by later events, probably including the emplacement of a completely mobilized granite dated about 1,400 m.y.[27], and tectonic stresses resulting in brittle faulting indicated by mylonite belts dated about 1,300–1,100 m.y.[25]. These difficulties in radiometric dating will be further discussed in the communication in preparation[11].

The quartzitic sandstones, quartzites and shales of the Roraima Formation[28] form a tabular succession, remarkably undisturbed, about 7,000 ft. thick[29], which, judging from its outliers, appears to have once covered some 1,200,000 km² in the centre of the Guiana Shield[30]. The age of this formation has been variously estimated as Cretaceous, Triassic, Palæozoic and late Precambrian. It is clearly younger than about 2,000 m.y. as it transgresses the Younger Granites, and older than the thick dolerite sills and dykes of the Younger Basic Intrusive Group[31]. Radiometric work on the latter, including a sill from high in the succession, and on mica from their contact hornfelses, has now given a radiometric age of *c.* 1,700 m.y.[32], based on a large number of potassium/argon and rubidium/strontium determinations, thus placing the Roraima Formation in the Lower Proterozoic of the Canadian classification. An earlier age[33] has been postulated but is not accepted by us. Radiometric work on dolerites from the southern province has given a middle Ordovician age (450 ± m.y.)[11].

Attention has already been directed to the lithological similarities between the manganiferous rocks of British Guiana and the Birrimian of West Africa[34]. The descriptions of Junner[35] show the recognition of graded bedding and pillow lavas, and other indications of a eugeosynclinal origin for the Birrimian. A close comparison, both lithological and structural, can also be made between the post-Birrimian granites and gneisses and the crystalline rocks of the Bartica Assemblage and the Younger Granite Group. The age of the deposition and folding of the Birrimian is not precisely fixed, but must be placed between that of the post-Birrimian granites, which have yielded many apparent ages in the 2,100–1,850-m.y. range[37–39], and the Sula Mountain schists of Sierra Leone which are dated approximately 3,000 m.y.[37]. Thus the Barama–Mazaruni and Birrimian eugeosynclines, both cut across by the present Atlantic coastline, could have been in continuity before a postulated Atlantic spreading. Ages of 2,600–2,550 m.y. have also been published[38,40] for a formation in Mauretania the relationship of which with the Birrimian is unknown. Lithologically the Roraima Formation, with its basic intrusives, though unfolded and unmetamor-

phosed, is similar to the Tarkwaian of Ghana and belongs to the same time-range. Comparison between the tabular marine Palæozoic of the Amazon basin and Brazilian Shield, and similar rocks in the Taoudeni and other West African basins, could also be fruitful.

We thank Prof. J. Sutton for his advice and the Directors of Overseas Geological Surveys, London, and the Geological Survey of British Guiana for their support and for permission to publish.

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ORGANIZED ELEMENT DISTRIBUTION IN RELATION TO SIZE IN THE ORGUEIL METEORITE

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IN 1961 Claus and Nagy reported that 1 mg of the Orgueil and Ivuna carbonaceous chondrites contained approximately 1,700 organized elements, respectively, which they thought to be microfossils of possible extra-terrestrial origin. This report was augmented by them[1] with similar observations conducted on the Alais and Tonk carbonaceous meteorites. The number of the organized elements in these meteorites was also reported to be about 1,350/mg. Briggs and Kitto[2] also found 1,000–1,700 stainable microstructures per mg of the Mokoia meteorite. Claus *et al.*[3], re-investigating the problem, came to the conclusion that approximately 90 per cent of the organized elements in any of the aforementioned meteorites is comprised of the most simple forms which they designated type 1 and type 2 (ref. 4). The greatest controversy, after their original report, centred round two points: (1) whether the seemingly very high number of the organized elements found by them could be substantiated, and (2) whether these simple forms, which they classified as type 1 or 2, have characteristics such that biogenic origin could be assumed for them. In the present report we will deal with these two problems.

Carefully weighed amounts of approximately 1 mg of the Orgueil meteorite were placed on precleaned microscope slides coated with fresh egg albumin or with a drop of glycerine. The sample on the albumin-coated slide was crushed and more or less evenly distributed on it by rubbing between the original slide and another also coated with albumin. After these slides were dried in an oven at 80° C, balsam was applied to them and they were covered with 18 × 18 mm precleaned cover slips. Each of these preparations thus contained about 0·5 mg of meteorite powder. The weighed samples in the glycerine drop were crushed with a glass rod and also cover-slipped. For counting the organized elements the glycerine preparations were used in which the amounts of the sample were exactly known. Counting was done with a Leitz 'Ortholux' microscope using a times 93 oil-immersion objective and a pair of times 25 wide field oculars. Each observational field in this apparatus measured 8·464 μ^2; the entire preparation thus corresponded to 325.153.024 μ^2 or 38.416 observational fields. The content of three slides was counted, one of them entirely, and half the other two. Counting was started at the lower left corner of the preparation and, by moving the stage towards the north, a row of fields was counted. After having reached the upper left corner of the preparation, the slide was moved by one observational field towards east, and starting from here, again a row of fields was counted by moving the stage towards south. When the south edge of the preparation was reached the same procedure was applied again, and thus field by field the entire slide was counted. The numbers obtained for the organized elements in the three different preparations were averaged and corrected so as to correspond to 1 mg of meteorite sample.

One of the slides coated with albumin was used for the measuring of organized elements. The microscopic set-up differed from that previously described only in the point of using times 12·5 oculars instead of the times 25 used previously. One of the oculars contained a calibrated measuring disk. A Leitz 'Orthomat' automatic photographic attachment was added to the microscope. The preparation was scanned somewhat more randomly than those used for counting, but care was taken that the same field never should be seen twice. An independent observer was trained to recognize the types 1 and 2 organized elements, and those which were selected by this observer were measured to the first tenth of a micron and were

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photographed. In this way 303 organized elements were randomly selected from a single preparation containing about 0·5 mg of meteorite. It was thought that this population is large enough and can be accepted as a representative sample of the meteoritic microstructures.

The experiment resulted in the recognition of 1,534 organized elements belonging to types 1 and 2 in mg of the Orgueil meteorite. The same high number reported earlier for a similar experiment[4] was challenged on two different grounds. Anders and Fitch[5] and Fitch and Anders[6] stated that such a high number, even if it contains all types of organized elements, is an over-estimation. Other workers, like Staplin[7] and Ross[8], were of the opinion that the number of the genuine organized elements—those which can possibly be accepted as microfossils—is very small and that a micropalæontologist would not consider forms like type 1 or 2 as representing fossil forms of life.

A simple division based on the previously enumerated facts shows that approximately every twenty-fifth microscopic field contains one organized element which certainly does not seem to be an over-estimation.

The second point to be discussed is more complex. Fitch and Anders[6] write: " 'Organized elements' . . . are a heterogenous group of particles which, in our opinion, are best classified into two types: those that have a highly structured morphology and those that have a much simpler appearance". They think that the forms belonging to their first category represent terrestrial contaminants, while the second group is composed of simple mineral grains. The electron-probe microanalyses conducted on different types of organized elements by Nagy *et al.*[9] clearly demonstrated that the particles are mineralized with iron and some chlorine; thus they are indigenous to the meteorites. This fact is even accepted by the aforementioned authors; however, Anders[10] is of the opinion that: "The case for their biological origin now rests entirely on their featureless morphology". They certainly dispute our results and those of others, demonstrating the presence of an acid-insoluble pellicle even in the types 1 and 2 organized elements (Engles—cited by Urey[11], Nagy *et al.*[1], Claus *et al.*[12], Palik[13], Van Landingham[14]). They also point out that the biological stains used by us[3]—widely accepted histochemical reactions used to demonstrate certain cellular components—when applied to the organized elements give ambiguous results. The investigations of Nagy *et al.*[15], on the ultra-violet absorption spectra of single demineralized type 1 organized elements, either in crushed preparations or in petrographic thin sections, indicating the presence of possible protein and nucleic acid-like substances in their interior is simply not referred to in the usual criticism.

At the conference of the New York Academy of Sciences held on May 1, 1962, during the panel discussion, Urey[16] suggested the compilation of dimensional histograms for the most simple forms of organized elements. In the systematics of lower life forms, especially among the algae, such histograms often proved to be useful in distinguishing between closely related but different species or among a single species and its varieties[17,18]. One of us[3], commenting on the remarks of Urey, pointed out that: "Morphological variations, as well as size distribution of organisms, follow a Gaussian distribution curve. 'Organized elements' follow the same pattern". (At the time of the cited conference only some preliminary results concerning a rather well-circumscribed organized element named *Apollinarisphaera meteoricola* Claus and Nagy[19] were known.) The idea of Urey has, however, been pursued and in Table 1 the numbers and measurements of the previously mentioned 303 randomly selected types 1 and 2 organized elements are given. Fig. 1 is a graphical representation of these numerical results. From Fig. 1 it is clearly evident that the types 1 and 2 organized elements according to their measurements fall into three distinct groups (three peaks of the curve). Their measurement distribution shows certain regularities, which should not be the case if they were simply to represent mineral grains. If terrestrial algal species were to be represented in the same manner as was done with the organized elements one might conclude that three possibly closely related but still distinct species are present. It is interesting to point out that according to the investigations of Palik[13], peak 1 would coincide with a microfossil she found in and described from the Orgueil meteorite and named *Chlorococcites minima* (the same species was found also by Van Landingham[14] in both the Alais and Orgueil meteorite), peak 2 would correspond to our species *Apollinarisphaera meteoricola* (*loc. cit.*), while peak 3 represents again a new species named by Palik[13] as *Apollinarisphaera ellipsoidea*. The other small peaks occurring on the graph represent obviously rarer and other somewhat more distinct forms. Here one has again to emphasize that the types of Claus and Nagy[4] do not represent systematically homogeneous populations. At the time of their publication the forms observed in the Orgueil and Ivuna meteorites were divided into five types only for the sake of convenience.

Table 1. Size Distribution of 303 Randomly Selected Types 1 and 2 Organized Elements

Group	Diameter (μ)	No.
1	2·4	2
2	2·7	7
3	3·0	17
4	3·3	14
5	3·6	10
6	3·9	14
7	4·2	23
8	4·5	32
9	4·8	26
10	5·1	24
11	5·4	18
12	5·7	16
13	6·0	13
14	6·3	11
15	6·6	9
16	6·9	7
17	7·2	6
18	7·5	4
19	7·8	3
20	8·1	2
21	8·4	4
22	8·7	5
23	9·0	11
24	9·3	7
25	9·6	4
26	9·9	2
27	10·2	1
28	10·8	6
29	12·0	1
30	12·3	2
31	13·5	1
32	15·0	1

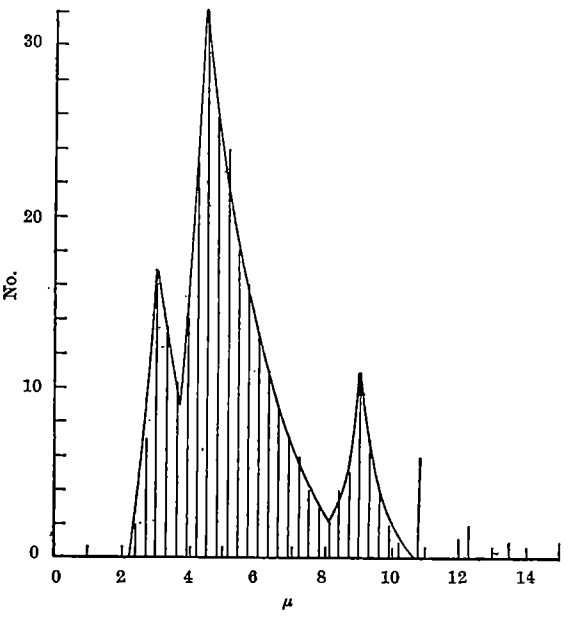


Fig. 1. Size distribution of 303 randomly selected types 1 and 2 organized elements

The dimensional histogram obtained through the careful measurements of 303 types 1 and 2 organized elements would possibly suggest that even these most simple microstructures found in Wiik's[20] four, type 1 carbonaceous chondrites do not represent just simple mineral grains as suggested by Fitch and Anders[6] or Mueller[21], but may be the remains of some kind of primitive life indigenous to the meteorites.

We thank Prof. Harold C. Urey, University of California, La Jolla, San Diego, for suggesting this work, and Dr. Urey and Dr. Bartholomew Nagy, of the same University, for their advice. We also thank Dr. A. Cavaille, Montauban, for providing the Orgueil meteorite sample.

This work was supported by the National Aeronautics and Space Administration (contract *NsG*–341).

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NEARLY DIURNAL NUTATION OF THE EARTH

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ASTRONOMICAL investigations of the theory of the rotation of the Earth round its centre of mass have always considered the theory of precession and nutation. They have approached this problem considering the Earth as a rigid body, and the last published investigations were made by Woolard[1]. The investigations of Woolard deal with the nutation in two parts, one referred to as nutation and the other as diurnal nutation. The separation of the nutation into these two parts is made because of the different axes adopted in the development of the theory.

It is well known that in the theory of the rotation of the Earth we have to consider the axes of rotation, figure and angular momentum. Woolard mentions that the positions and motions of these axes are changed by geophysical effects.

The investigations, referring to the observations and computation of the diurnal nutation, have been revived recently, and that is the reason we are dealing with it, describing its evolution in the past few years.

The theories concerned with the bodily tide of the Earth have, on the contrary, considered models of the Earth as near as possible to what we knew about the Earth's constitution, at the time they were formulated. They were not interested in the motions of precession and nutation, which are also produced by the external forces due to the actions of the Sun and Moon.

The investigations referring to the tides of the solid Earth and, on the other hand, to the shifting of the Earth's axis, originating the nutations, can be considered as a whole problem, where we consider at the same time the elastic deformations caused by the external forces (Sun and Moon) and the effects these forces have on the position of the Earth's axis of rotation.

Better knowledge about the internal constitution of the Earth, that has been gathered during the past decades, and also the known discrepancy between the observed and computed values of the constant of nutation, led to investigations which attempted to explain the discrepancy in terms of the Earth's constitution.

One of us (H. J.)[2–4] has tried to explain the difficulties encountered in the theory of nutation, using a method originally used by Poincaré[5], and taking into consideration some of the elastic properties of the shell and core of the Earth. These investigations, as has been done in the past, consider separately the case when there are no external forces (free nutations) from the case when there are external forces (forced nutations).

The earlier investigations, where the analysis is carried out in terms of displacements instead of angular velocities, show one of the features of the problem of the nutations, that is, the existence of solutions grouped in pairs near certain values. This feature of the problem was first noticed by Poincaré, and the solutions are grouped near zero and $-\omega$, where ω is the Earth's mean angular velocity with a period of a sidereal day.

The investigations, not only of the nutation but also of the bodily tide of the Earth, should be linked because both of them are different aspects of the actions of the external forces (Sun and Moon) on the Earth.

Examination of the free nutations, when there are no external forces acting on the Earth, shows a solution with a speed exactly equal to ω and another solution, near to it, but differing from it by quantities which depend on the fact that the Earth and all the layers that constitute it have a certain ellipticity. The ellipticity of the core is one of the quantities which appear in all these calculations.

We have developed a theory[6,7] that deals with both aspects of the actions of the Sun and Moon on the Earth. We adopted two Earth models, both with the same shell constitution, one called the central particle and the other called the Roche model, corresponding to two extreme cases for the constitution of the core.

We are here concerned with the free nutations, and especially nutations with periods of the order of a day. The solutions obtained for the free speeds are grouped in pairs, near zero and $-\omega$, as was expected.

The central particle model described in the paper[6] gives two pairs of solutions: 0 and $0{\cdot}00255\omega$, $-\omega$ and $-(1 + 0{\cdot}00224)\omega$. The solution zero corresponds to a statical displacement of the core without moving the shell. The solution $0{\cdot}00255\omega$ is the variation of latitude and gives the period of the Eulerian nutation. The value $-\omega$ corresponds to a constant displacement of the axis in space and, hence, relative to the Earth, in the opposite direction

to the Earth's rotation, with a period $\left(\frac{\omega}{\gamma} = 1\right)$ of a sidereal day, and is similar to the value found for a rigid body. The solution $-(1 + 0{\cdot}00224)\omega$ has a period $\frac{\omega}{\gamma} = \frac{1}{-1{\cdot}00224}$ = −23 h 56 min 47 sec sidereal time and corresponds to a nearly diurnal nutation in the opposite direction to the Earth's rotation. This last solution is not written in a convenient way in equation (22), page 159, of the aforementioned paper. But the article which one of us (R.O.V.)[8] wrote later on shows the correct solution as indicated here.

The Roche model, described in ref. 7, gives three pairs of solutions: 0 and $0{\cdot}00253\omega$, $-\omega$ and $-(1 + 0{\cdot}00403)\omega$, $-\omega$ and $-(1 - 0{\cdot}00679)\omega$. The first two pairs of solutions correspond to the central particle model, but now the solution $-(1 + 0{\cdot}00403)\omega$ has a period −23 h 54 min 13 sec sidereal time, that is, a nearly diurnal nutation with a period about 2 min shorter than the central particle model. The last pair of solutions appears because of the density structure, in the core, adopted for this model, and we have now a nearly diurnal nutation with a period −24 h 9 min 50 sec, slightly longer than a sidereal day. It is the existence of these two periods that has led to uncertainties in the calculation of the effects corresponding to semi-annual tides.

The possible explanation for all these different solutions is shown to be the motions in the core. The results obtained permit us to say that the existence of a nearly diurnal nutation is deduced from Earth models which take account of the internal constitution of the Earth, but the value of its period depends on the Earth model adopted. Because we adopted two Earth models that correspond to extreme cases of the possible behaviour of the core, we can say that the period will be between 23 h 54 min and 23 h 56 min.

The problem of the computations of the motions of the Earth's axis, with periods of the order of a day, have also been treated with more detail in a paper written by one of us (R. O. V.)[9]. It is shown that a nearly diurnal free nutation appears for Earth models with a liquid core, and this nutation differs from a sidereal day by a quantity of the order of magnitude of the ellipticity of the core.

The solutions, corresponding to the nearly diurnal nutations, have not been emphasized in the papers mentioned, because of the well-known difficulties in the detection of nutations with periods of the order of a day.

Molodensky[10], in a paper published later than the investigations already mentioned here, also solves the problem of the nutations and the bodily tides of the Earth, using two different Earth models and finding out, by numerical methods, a certain type of solution for a system of partial differential equations. The values found, for the nearly diurnal nutation, are about 3 min shorter than a sidereal day, and the difference between the two models adopted is approximately 2 sec.

Comparing Molodensky's results with the values mentioned before, and taking into consideration the different approaches used by the two theories, we can say that there are no striking disagreements in the computed values.

We wish to point out that all the solutions mentioned before are approximate, in the sense that they do not take account of all the geophysical properties of the Earth's interior, in order to be able to arrive at a mathematical solution of the equations of motion.

Several astronomical observatories have tried, in the past, to observe and compute the diurnal nutation. The observations are difficult to make because of several astronomical and geophysical causes associated with diurnal phenomena. Some of these reasons are: (*a*) Astronomical—the very small amplitudes of this nutation; variations of the refraction and aberration during 24 h, and the fact that the values of the constants of refraction and aberration are not very well known; the existence of errors in the tabulated star places that will affect observations made during a number of years. (*b*) Geophysical—the diurnal oceanic tides; motions in the Earth's atmosphere with periods of a day; the bodily tides of the Earth; deflexions of the vertical.

The detection of the diurnal nutation, from astronomical observations, is also made more difficult because what we observe is the actual shifting of the Earth's axis under the actions of the Sun and Moon, and so it includes the diurnal variations which are caused by the lunisolar component. But the values we determined before refer to the free nutation, that is, the motion of the Earth under no external forces.

It was N. Pariisky who, recently, again directed the attention of astronomers to the possibility of observing the diurnal nutation. The researches of Popov[11,12] claim to have detected a nearly diurnal nutation, based on the period of 23 h 56 min 54 sec indicated by Molodensky. Thomas[13], analysing observations made with the photographic zenith tube at Greenwich Observatory, also produces some evidence about a nearly diurnal nutation. But it points out the smallness of the amplitudes found, the large probable errors and the possibility that the observations could be well represented without taking into consideration the diurnal nutation.

The considerations made here show the complexity of the diurnal nutation. The theoretical computations, taking into account the internal constitution of the Earth, lead to the forecast of nearly diurnal nutations because of the form and structure of the core. The possibility of observing these nutations requires the utmost care in the methods adopted and the selection of the stars. The reduction of observation also needs close scrutiny.

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AFTER PESTICIDES—WHAT ?*

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WHEN a man accidentally touches a hot stove, he jerks his hand away instantly and without waiting to consider whether this is the proper thing to do. In the same way, his eyes blink automatically and involuntarily in response to a brilliant flash or an approaching blow. Physiologically, there is a nervous short-cut, or reflex arc, between the sensors that perceive the danger signal and the motor nerves that start the protective response. The central nervous system is largely by-passed.

It seems likely that in an insect—and particularly in a small one—limitations of space and weight will have made most of the activities reflexes of this sort: with very

* Substance of the opening address at a meeting of the Entomological Society of Ontario on September 2, 1964.

little involvement of brain and nothing that depends on what we would recognize as an intelligence. If the observed reflex behaviour seems to be intelligently and purposefully directed, this is because natural selection has long ago eliminated the inappropriate responses. The automatic nature of the reflexes becomes evident when we present the stimulus under some artificial condition so that the response is inappropriate or absurd but is nevertheless persisted in. For example, I have seen photographs of insects the abdomens of which have been cut off and whose heads continue to devour unlimited quantities of syrup which passes out through the severed end of the gut as fast as the mouth-parts take it in.

Many of these reflex activities are stimulated by chemical signals which, when they are air-borne, can be regarded as being odours or scents. There are scents by which insects locate their mates and recognize them, and other scents to make the mates receptive. Still other scents identify food supplies, oviposition sites, and, with crawling insects, the pathways to them. The nature of the olfactory process is such that a very few scent molecules can initiate a nerve impulse that sets off an amount of activity out of all proportion to the size of the original stimulus. Let me make a comparison.

The largest hydrogen bomb releases energy equivalent to 100 megatons or 10^8 tons of TNT. I do not know exactly how much chemical explosive is needed to trigger the bomb, but let us suppose it is 200 lb., or 0·1 tons of TNT. Evidently the magnification factor between the trigger and the full force of the bomb is 10^9 or 1,000 million.

Now consider the sex attractant of the American cockroach. If the reports are correct[1], 30 molecules of the pure chemical are enough to excite a male cockroach. The kinds of energy involved are difficult to determine, but let us consider the ordinary kinetic energies. Thirty molecules of the pure stuff weigh 10^{-20} g, and at room temperature their kinetic energy of translation is about 10^{-11} ergs. If the male insect weighs 2 g and is induced by the scent to run with a speed of 4 cm/sec, his kinetic energy is something more than 10^4 or ten thousand ergs. Thus the magnification factor—the disparity in energy between cause and effect—is 10^{15}, which is a million times greater than the disparity between the energy of the biggest hydrogen bomb and the trigger which sets it off.

This is why insect sex-attractant chemicals are indeed and truly the most potent biological materials known.

It is only recently that the true importance of these substances has been recognized; and their exploitation as pest control agents has still to come. One reason for this is the fact that so far they have been thought of primarily as attractants or lures. The Mediterranean fruit fly outbreak in Florida in 1956 was mapped, and control measures were monitored by means of traps baited with an artificial sex attractant[2]. Sex attractants have also been used with some success to lure insects into contact with poisons—which is obviously more economical than trying to send the poisons to the insects[3]. More recently, there have been suggestions that the sterile male technique of pest control might be effected by combining a sex attractant with a chemi-sterilant[4].

All these uses exploit one kind of property only: the ability of the chemical to draw certain insects to certain places.

The first suggestion that there might be more to the matter than this was made by M. Beroza in 1960, when he ended an article on insect attractants (in which he reviewed all their obvious applications) with one vitally important extra sentence:

> "One interesting prospect for control without the use of toxicant is the spraying of the natural sex attractant over a wide area in order to confuse males in their attempts to locate females of their species." (Ref. 5.)

What this amounts to is a suggestion that instead of using the chemical to draw an insect from *A* to *B*, the chemical should be used to prevent it from being drawn from *A* (where he is) to *B* (where she is). However, coming as it did at the end of a rather routine review article, this suggestion did not attract the attention it deserved, although it transpired later that an attempt was made in 1961 to control gypsy moths in this way[6]. Unfortunately it was a failure, apparently because the manufacturer supplied an inactive preparation the inactivity of which was not discovered until after it was all over. This summer, however, the U.S. Department of Agriculture has been repeating the experiment (with an active preparation) under the name 'Operation Confusion', but the results are not yet known[7].

No matter how that experiment turns out, I think that some, and probably many, insect species will eventually be prevented from breeding in this way. The literature contains the plainest possible hints. For example, Kettlewell writes of *Biston betularia*, the peppered moth:

> ". . . individual males can only spend a comparatively short period of time on any occasion carrying out active searching. If unsuccessful they fly off. We have tested this both in the laboratory and the field and it appears to be due to a state of fatigue which is produced by constant stimulation by scent particles." (Ref. 8.)

Again, Ignoffo says of the cabbage looper:

> "The duration of response, while the moths were continuously exposed to the strips, ranged from 2 to 5 minutes. The ability of the male to detect the attractant is lost after exposure and regained only after a 'rest' of several hours." (Ref. 9.)

Working with the same insect, Shorey described a bioassay procedure which could detect a concentration of the sex attractant equivalent to "1×10^{-4} of a female". However, he reports that there is:

> ". . . a major potential pitfall in this type of bioassay: attenuation of male responsiveness after prolonged, frequent and high-level exposure to the sex pheromone." (Ref. 10.)

The sex attractant chemicals are the most biologically potent and spectacular of the behaviour-control chemicals, but they are not the only ones. There are oviposition attractants, food scents, and, in the social insects, nest odours, all of which can in principle be used to modify the normal behaviour to the detriment of the insect and the benefit of man. Nor are the behaviour modifiers necessarily chemical.

The attraction of the moth to the flame is proverbial and taken pretty much for granted. Actually, how much do we really know about it? For example, what would the moth have done if the flame had not been there? What is the normal behaviour, and at what point did the normal behaviour change because the light was there? What was the nature of the change? Movement toward the source of the light was one effect, but there must have been other effects, and if so, what were they and how can they be turned to account? Once started, it is easy to ask the questions and to project the most interest-experiments with infra-red 'snooper-scopes' and other modern apparatus.

What I am really saying is that insect behaviour needs to be investigated from a new point of view. Behaviour has been pretty much taken to mean 'life-history' and has been investigated largely for the sake of determining the best time to apply conventional poisons or to find out whether parasites are at work, or to locate intermediate hosts the elimination of which might break the normal life-cycle. What we need to know more about now are the external agencies that tell a particular kind of insect to do a particular thing at a particular time.

Then we can give it the signal at the wrong time or place, or repeat the signal so often and so strongly that it becomes too fatigued to respond any more.

Since my main concern at the moment is with chemical agents for behaviour control, and especially with sex-attractant chemicals, I should like to look particularly at some of the problems which must be solved before they can be exploited as fully as I think they could be.

Recently, there have been some spectacular achievements in the preparation of sex attractants for several well-known insects. In Germany, Butenandt *et al.* have isolated, identified and synthesized the sex-attractant chemical of the female silkworm moth[11]. This was an enormously difficult and really excellent technical achievement, equalled only by the brilliant work of Jacobson *et al.* at the U.S. Department of Agriculture in identifying the natural sex attractant of the gypsy moth and then preparing an even more potent analogue which they call 'Gyplure'[12].

At the same time, Beroza *et al.* have been systematically exploiting another pathway to these substances and have produced highly effective lures for male medflies and a few other insects[13]. They begin by testing a wide selection of organic chemicals for signs of attractiveness. When they uncover even a hint of biological effect they have sometimes been able to build up the potency to a high level by synthesizing and testing analogues and structural variants of the active substance.

The highest possible technical virtuosity has been displayed in both approaches, and I intend no disparagement when I say that the brilliance of the work has tended to divert attention from an essential weakness. In military terms, what has been achieved is a deep penetration on a very narrow front. We now have very effective sex-attractant lures for about half a dozen insects; but we know nothing about how they act on the sensory receptors and therefore we do not know why they are effective for only one species at a time.

What is more important, the success of these workers in preparing single chemical substances which carry the whole biological effect has created an impression that in every case what must be sought is *the* sex-attractant compound. Recent writers on the subject have apparently taken it for granted that female insects can attract their mates by secreting some one, unique, attractive chemical, or just possibly a family of related chemicals each of which can be attractive all by itself.

Something that was taken for granted fifteen years ago has been largely forgotten. Thus Dethier wrote in 1947:

> "More attractive than either compound alone is a mixture of geraniol and eugenol 10 to 1 parts by volume respectively. This follows one of the most important principles relating to attractants—that mixtures are superior to any one of the component parts. The reason for this lies in the fact that attractants are odors. . . ." (Ref. 14.)

This comment is vitally important, and I should like to show why.

In the first place, it carries the implication that the mechanism by which insects perceive odours is not basically different from the mechanism by which vertebrates perceive them. From this it follows that the same underlying principles can be expected to apply in both cases. Of these principles, probably the most important for my immediate purpose is the 'information theory' developed by telephone engineers and first applied to the phenomena of olfaction by R. M. Hainer *et al.*[15].

It has been shown by Neuhaus[16], Kalmus[17], and others that each human being probably has a unique body odour which a well-trained dog can recognize. More recently, H. M. Bruce has found evidence that a female mouse can recognize the scent of her own specific mate[18], and the ability of ewes and seals to find their own offspring among thousands of others has been a source of wonder for centuries. It follows that in the vertebrates the number of discriminable smells must be virtually unlimited—a conclusion that is supported by the assertion of more than one professional perfumer that there are millions of recognizably different smells.

Now it is impossible to believe that the nose carries a specific type of receptor for each smell, and there seems to be no reason why it should. What it needs is the ability to detect a limited number of primary odours and to register mentally the differing patterns which appear when different combinations of primaries are stimulated at the same time. Information theory enables the number of separate primaries to be approximately estimated in the following way:

An odour retains its characteristic quality even when very faint, from which it follows that the sensory apparatus probably uses a binary system for coding the various primary impulses into a distinctive pattern[19]. With only one primary, and with binary coding, there can be only two 'patterns': 'there' and 'not there', or + and −. With two primaries there can be 4 patterns: + +, + −, − +, and − −; and with three there can be 8. In general:

No. of primaries:	1	2	3	4	5	....	N
No. of patterns:	2	4	8	16	32	....	2^N

If N is 20, more than a million 'on-off' patterns can be produced. There is some anatomical evidence that in the rabbit N may be 24, in which case the animal should be capable of discriminating more than 16 million odours[15]. In the vertebrates, therefore, the sense of smell has more in common with hearing than with vision. Colour perception depends on only three primaries, whereas the piano, which plays only semi-tones, has 88 keys and still reaches neither bottom nor top of the scale of audio-frequencies.

A single molecule will usually have the ability to stimulate several primary sensations depending on which primary receptor organ it is in contact with, so that the smells of compounds like vanillin or camphor are not necessarily primary. At the present time, we do not know which common odours are the primary ones because we do not know what the molecular quality is that causes a particular primary sensation to be triggered. What information theory tells us is simply this: that there is some molecular property, which we will call X, which can exist in variant forms corresponding to each of the primary odour sensations. We can call these various primary qualities X_1, X_2, X_3, X_4, X_5 . . . X_N, where N may be as high as 25 or 30.

While a dog with its highly developed olfactory sense may be able to perceive 30 primaries, in an insect it seems likely that the system is greatly simplified by reducing the number of primaries which any one species is equipped to register. The ultimate in simplification will be reached in an insect which can register only one of the primaries, say X_5. In that case, the system will have very little discrimination because it can be expected to respond to any sufficiently volatile substance the molecules of which happen to have the X_5 quality. If the number of required primaries is increased to 2, the response is a little more discriminating, and if it is as large as 5 the response will probably be quite specific with respect to a single chemical substance because it is rather unlikely that a randomly selected compound will have precisely the right combination of 5 primary olfactory qualities to activate all 5 types of receptor. It will usually be easier for a mixture of substances to do so because some of the needed qualities can be carried by one lot of molecules and the rest by another different lot.

On this basis, Dethier's comment that ". . . one of the most important principles relating to attractants [is] that mixtures are superior to any one of the component parts" makes excellent sense. It also explains both the successes and the failures of much recent work. Beroza has been

successful in producing artificial attractants for the Mediterranean fruit fly presumably because it is an insect the sensory equipment of which responds to a rather small number of primary stimuli—perhaps only 2 or 3. On the other hand, Beroza's technique of mass screening has been singularly unsuccessful in producing a gypsy moth attractant, probably because the male gypsy moth needs a very special combination of primaries to evoke a response. It so happens that this combination is embodied in a single compound which is the natural lure, but I suggest that this is unusual. Most insects probably generate an attractive scent which requires the simultaneous presence of several different molecules.

If this is so, we can see at once why the technique of mass screening has been so conspicuously successful in a small number of cases, and yet so unsuccessful generally. We can see something else too. Many people have extracted the bodies of female insects with solvents and produced crude preparations which were highly attractive to the male insects so long as they were impure, but the activity of which disappeared when an attempt was made to purify the material and isolate the attractive component from the mixture. This has commonly been taken as showing how very unstable these attractive materials are. What I suggest is that in at least some of these cases the real explanation was that several kinds of keys were needed to turn several kinds of locks simultaneously before the door would open. The idea of sex attractants as very labile substances scarcely seems to be consistent with their natural function, which is to remain intact while being carried along in the air and exposed to the destructive effects of oxygen, sunlight, water vapour, dust, and a host of minor atmospheric contaminants of the most varied kind.

It might seem, from what I have been saying, that if an attractive preparation can be made by mixing chemicals which by themselves are inactive, then we ought to be able to produce a 'universal attractant' by mixing almost any large number of substances. A mixture of 10,000 organic compounds would be expected to include all the possible combinations of primaries, and why, therefore, does it not show a general, indiscriminate attraction for all kinds of insects?

To answer this question, we must look at all the things that must happen before a smell sensation can be perceived. First, the odorous molecules must evaporate and be carried by the air into the vicinity of the sensory surface, whether it is the olfactory epithelium of a vertebrate or the antennal setæ of an insect. Secondly, the molecules must be adsorbed on to the sensory surface. Thirdly, the adsorbed molecules must interact with the sensory surface in some special and specific way so that a nerve impulse is transmitted into the interior of the animal.

Davies and Taylor[20] have derived a mathematical picture of the second and third of these stages, which suggests that the process by which the molecules become attached to the sensitive surface is quite independent of the process by which they 'puncture' it and initiate the nervous signal. In other words, it is quite possible for the sensory surface to become covered with an adsorbed layer of molecules which are not able to trigger an olfactory response themselves, and the presence of which on the surface interferes with the attachment of an odorous molecule that could trigger one. Thus a mixture which is too complex can be as biologically inactive as one that is not complex enough—though for a quite different reason.

We can, perhaps, see this actually happening in the attempts of Casida *et al.* to isolate the sex attractant of the introduced pine sawfly[21]. They made solvent extracts of female insects which in some cases showed very little biological activity until they had been given a partial purification by column chromatography. The considerable activity of their partially purified extracts usually declined on further purification. This kind of behaviour is just about what one would expect if the attractive scent is due to a mixture of substances which must all be present, and which must compete with indifferent molecules for places on the sensory surface.

I know that what I have been stating will be rather discouraging to those who had hoped that for each pest insect a specific sex attractant or other behaviour control chemical could be found and used to control its activities. If what is needed in most cases is a mixture of chemicals to provide the correct combination of primary olfactory signals, then the mass screening approach becomes extremely difficult, and the method based on isolation + identification + synthesis which is already very difficult becomes many times more so.

Nevertheless, I think we can hope to move forward in this field if we know what we are looking for. If we look for a single substance which leads the male codling moth to the female, we may be asking the right question, but more probably we are not. If we suppose that the carrot-rust fly lays its eggs alongside the carrot plant because of some single chemical emanation from the plant, we may be making a quite unwarranted assumption without even realizing it.

Karlson and Luscher have coined the word 'pheromone' for materials such as sex attractants and recognition odours which they define in this way:

> "Pheromones are defined as substances which are secreted to the outside by an individual and received by a second individual of the same species, in which they release a specific reaction, for example, a definite behaviour or a developmental process." (Ref. 22.)

This definition carries the implication to a chemist that each pheromone is a specific chemical substance. I think I have shown that this implication is likely to be incorrect, at least in so far as the pheromone is something that releases a definite behaviour in the animal that receives it.

I have chosen as my title, "After Pesticides—What?" because I believe that in the long run the use of behaviour control agents offers a better, safer, more effective, and cheaper means of pest control than is likely to be achieved by pesticides and chemi-sterilants or other inherently noxious substances. For this reason I think that research in this field needs to be greatly expanded—but it must also be properly directed. I think I have shown that the obvious things to do are not necessarily the best things to do. For example, we could waste a very large amount of money by rushing into a gigantic scheme for mass screening thousands of organic chemicals in all parts of the country or all over the World. We might do a little better by taking a smaller number of compounds and testing them in paired combinations, but even that would be a huge undertaking because 500 compounds taken at two at a time will give 250,000 pairs.

We could also spend years collecting and fractionating and bioassaying extracts and emanations from various insects in a vain attempt to isolate specific sex-attractant pheromones or other behaviour control chemicals. We would do a little better if every experiment in fractionation was followed by a bioassay of the recombined fractions; but this makes an already difficult and tedious process even more so.

In short, while the potential importance of sex attractants in practical pest control is incalculably great, there are some formidable problems to be solved before they can come into general use. My purpose has been to see what some of these problems are and to show that the obvious ways of attacking them are not necessarily the best. To attack the problem successfully and go forward quickly there is a need for new approaches to the problem and the highest type of creative thinking. Much of what I have said is speculative, but how else are we to decide what question to ask?

Long ago Darwin wrote (in a letter to A. R. Wallace):

"I am a firm believer that, without speculation there is no good or original observation."

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STRUCTURE OF FILAMENTS PRODUCED BY RE-AGGREGATION OF *Salmonella* FLAGELLIN

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FLAGELLA are the contractile organelles responsible for locomotion in bacteria. They exist as long filaments (up to about 10μ) of a uniform width (diameter 100–200 Å depending on the species) and helical shape which in electron micrographs is distorted into a sinusoidal wave form. Our experiments were performed with *Salmonella typhimurium*, where the wave-length of the flagella is about 2·5μ (ref. 1). Work from a number of laboratories has so far led to the identification of only one kind of protein (flagellin) in these structures; the molecular weight of the flagellin of *S. typhimurium* has been estimated from physical measurements as 40,000 (McDonough, unpublished results), in agreement with Weibull[2] for *Proteus vulgaris* and by Abram and Koffler[3] for *Bacillus pumilus* flagellins.

Recent investigations with the electron microscope using negatively stained preparations of several species of bacteria have shown that flagella, still attached to the cell body, can exist in two structural forms[4,5]. Structure *A* consists of regularly spaced globules which appear alike and are roughly spherical in shape (Fig. 1*a*). The diameter of these globular units as estimated by their spacing is about 50 Å and this is consistent with the view that each is a molecule of flagellin. The molecules are aligned in longitudinal rows and alternate with each other in adjacent rows (Fig. 1*a* and *b*). They are helically arranged so that the number per turn of each helix equals the number of longitudinal rows. In the model proposed[4,5] (Fig. 1*b*) this number is 8 for *S. typhimurium*, the pitch of the helix is about 200 Å, and there are about 4,000 molecules per wave-length (2·5μ) of the flagellum.

Structure *B* does not show globules or helices but an alternating sequence of long regions of continuous lines and short regions where the continuity of the lines is interrupted; the number of lines is the same as the number of rows of globules in the *A* structure[4,5]. In *Pseudomonas rhodos* both structures *A* and *B* can be seen in different parts of the same flagellum[5]. Only the *A* structure has so far been observed in *S. typhimurium* strain *SW* 1061 (ref. 5), a mutant of strain *LT* 2 which is monophasic for the phase-2 antigen 1,2 (ref. 6). Here we describe the structures seen when molecules of flagellin prepared from the flagella of this organism are allowed to aggregate *in vitro*. As reported by Ada, Nossal, Pye and Abbot[7] for *Salmonella adelaide* flagellin and by Abram and Koffler[3] for *Bacillus pumilus* flagellin, such re-aggregation may result in the appearance of long, sinusoidal filaments with

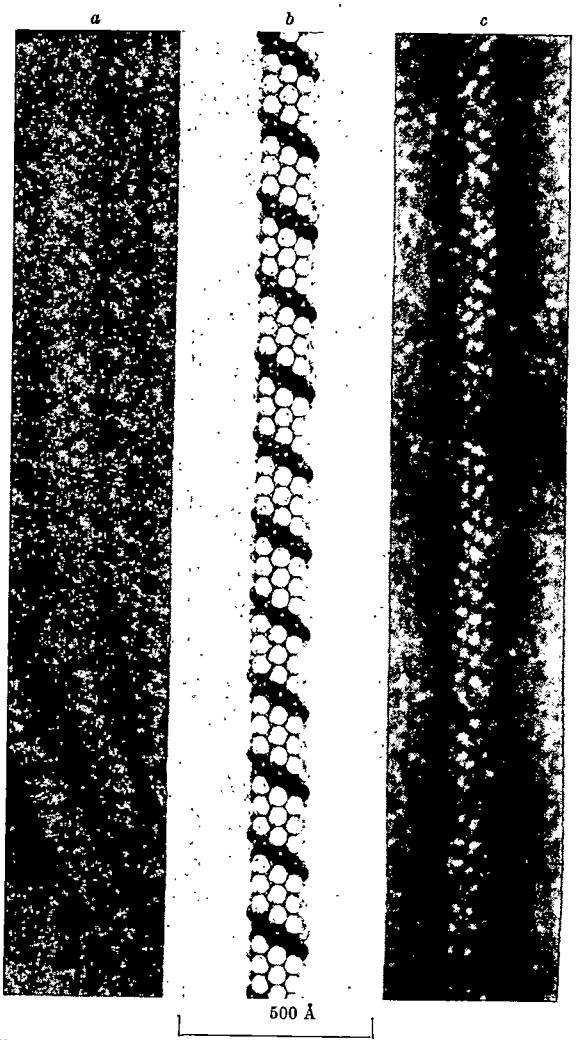


Fig. 1. *a*, *Salmonella typhimurium*, electron micrograph of flagella showing *A* structure (negatively stained with uranyl acetate); *b*, model showing possible arrangement of the molecules in the *A* structure (the spheres represent molecules and their helical organization has been indicated by showing some of them in black); *c*, electron micrograph of part of a filament prepared from flagellin of *S. typhimurium* (negatively stained with uranyl acetate)

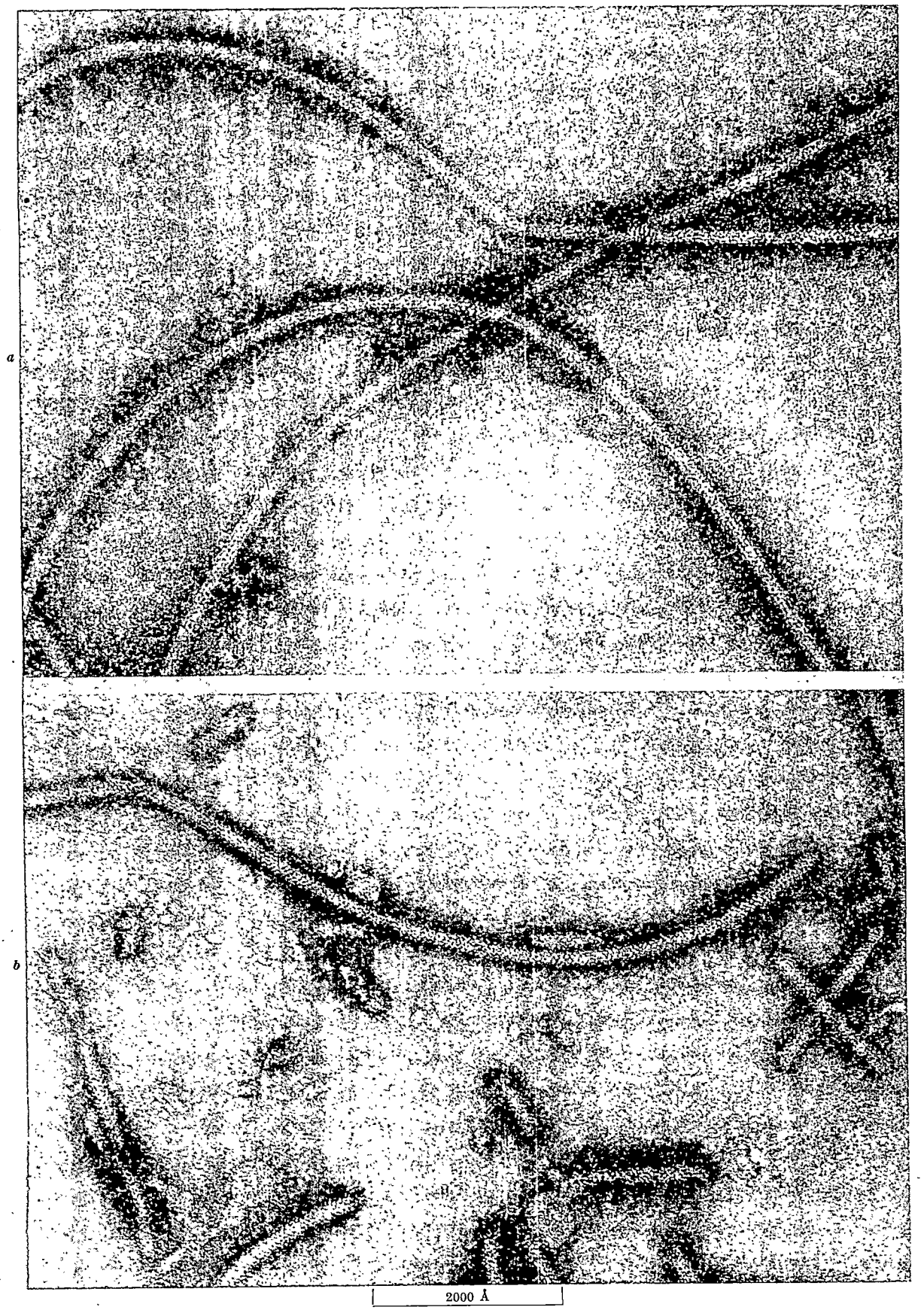


Fig. 2. *a*, *Salmonella typhimurium*, electron micrograph of flagella showing *A* structure (negatively stained with uranyl acetate); *b*, electron micrograph showing filaments prepared from flagellin of this organism and stained by the same method

a width comparable to that of the native flagella. The fine structure of the filaments was not described.

Flagellin was prepared as follows. The bacteria were deflagellated in a blender, and the cells removed by centrifugation. The flagella which remained in the supernatant were concentrated and purified by alternate fast (105,000*g* for 1 h) and slow (20,000*g* for 5 min) centrifugation, and then dissociated into monomeric flagellin by adjusting the *p*H to 2·0 with N hydrochloric acid[2]. Insoluble material was removed by centrifugation at 105,000*g* for 1 h. The remaining material in the supernatant which contained the flagellin was adjusted to *p*H 7·0 with 0·1 N sodium hydroxide and then brought to 70 per cent saturation with ammonium sulphate. The flocculent white precipitate was spun down, suspended in distilled water and then dialysed against several changes of distilled water at 4° C. The suspension, which was now opalescent and somewhat viscous, was centrifuged at 105,000*g* for 1 h and a large, clear, gelatinous pellet was obtained.

Small portions of this material were suspended in distilled water and prepared for examination in the electron microscope by the negative contrast method of Huxley and Zubay[8]. The microscope was a Philips 200 (80 kV, double condenser, 20μ objective aperture).

Much of the flagellin material thus prepared consisted of short structures of filamentous form up to about 2000 Å long; many curved filaments (several thousand Å in length) were also present (Fig. 2*b*). Occasionally filaments several microns in length and looking like sine waves were seen. All the re-aggregated structures show regularly arranged globules which are lined up both longitudinally and obliquely with respect to the long axis (Fig. 1*c*). Either 4 or 5 longitudinal rows can be seen (Fig. 2*b*) and their lateral separation was found to be about 41 Å (20 measurements, ranging from 38 Å to 46 Å). The axial separation of the globules was about 50 Å (26 measurements, ranging from 46 Å to 54 Å). In all these respects the structure of the flagellin filaments very closely resembles that of *A* structure flagella attached to the bacterium (cf. Fig. 1*a* and *c*, Fig. 2*a* and *b*), where the corresponding results were 37 Å (17 measurements, ranging from 29 Å to 40 Å) and 51 Å (38 measurements, ranging from 48 Å to 55 Å). Furthermore, as in negatively stained preparations of *A* structure flagella[4,5], there was no indication of a hollow centre in the flagellin filaments.

We conclude that flagellin molecules can 'self assemble' into curved filaments, presumably helical, which have a fine structure indistinguishable in the electron microscope from that of the original flagella. Perhaps the most interesting problem now is to find whether flagellin filaments can be made to contract or if, as in muscle, this process requires the presence of two protein components.

We thank Sir John Randall and Prof. B. A. D. Stocker for their encouragement and provision of facilities, and Mr. Z. Gabor for assistance with the photography. This work was supported in part by a research grant (*AM* 06166-01) from the Institute of Arthritis and Metabolic Diseases, U.S. Public Health Service.

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NEWS and VIEWS

Director of the Royal Aircraft Establishment: Sir Robert Cockburn, K.B.E., C.B.

Sir Robert Cockburn, chief scientist of the Ministry of Aviation, has been appointed director of the Royal Aircraft Establishment, Farnborough, in succession to Dr. M. J. Lighthill, who is taking up a Royal Society research professorship (*Nature*, 202, 134; 1964). Sir Robert Cockburn took up his new appointment on October 1, 1964. Sir Robert has been chief scientist of the Ministry of Aviation since its formation five years ago (*Nature*, 184, 938; 1959). He was born at Portsmouth in 1909 and educated at the Portsmouth Secondary School and Portsmouth Municipal College. He entered the Scientific Civil Service in 1937 and served at the Royal Aircraft Establishment, at the Air Ministry Research Establishment at Worth Matravers and later at the Telecommunications Research Establishment (now Royal Radar Establishment), Malvern. On promotion to senior principal scientific officer in January 1946 he was appointed deputy head of the Nuclear Physics Division at the Atomic Energy Research Establishment, Harwell. He was seconded to the Air Ministry as a scientific adviser, with the grade of chief scientific officer, in September 1948, and on return to this department in January 1954 was appointed principal director of scientific research (guided weapons and electronics). In June 1955 he was appointed deputy controller of electronics and in October 1956 became controller of guided weapons and electronics and three years later was appointed chief scientist.

Physics in the University of Warwick : Prof. A. J. Forty

Dr. A. J. Forty, whose appointment to the chair of physics in the University of Warwick has recently been announced, was educated at the University of Bristol. He took his B.Sc. there, and stayed to work for his Ph.D. under Prof. F. C. Frank. His work was on the mechanism of crystal growth, and the paper by Forty and Frank on the growth of cadmium iodide crystals, showing spiral growth steps associated with screw dislocations, is now a classic. After service in the Royal Air Force, Forty joined the research laboratories of Tube Investments, where he worked for several years. He then returned to Bristol, where he has been directing an active research group investigating the mechanical properties of solids. This research has been on several fronts; notably the direct examination of the atomic processes of damage to crystals by electron bombardment in an electron microscope; the investigation of the physical processes governing stress corrosion; examination of the mechanism of cleavage and fracture of crystals; and the use of ultra-violet microscopy. An interesting by-product of the damage investigations, which may have practical application, has been the observation that lead iodide at high temperatures is capable of forming a photographic image of high definition. Dr. Forty combines a flair for applying fundamental physics to problems of technological interest with an ability to organize fruitful research programmes. His appointment to a new university in a highly industrial region is most fitting.

Physical Chemistry in the University of Strathclyde: Prof. Manfred Gordon

Dr. Manfred Gordon, who has been appointed to the chair of physical chemistry, recently created in the University of Strathclyde, Glasgow (*Nature*, 203, 125; 1964), graduated in the University of London in 1940, and afterwards was awarded the degrees of M.Sc., Ph.D. and D.Sc. of that University. He has had a wide and varied experience in departments of (pure) chemistry, technical chemistry and textile chemistry in the three

institutions which have now been designated as 'SISTERS'. His previous posts include those of senior physical chemist in the Dunlop Rubber Organization, lecturer in high-polymer chemistry at the Royal College of Science and Technology, Glasgow (now the University of Strathclyde), Courtauld Fellow at the Manchester College of Science and Technology, assistant director at the Arthur D. Little Research Institute, Inveresk, and finally reader in physical chemistry at the Imperial College of Science and Technology. He is the author of more than fifty publications, largely in the physical and statistical aspects of polymer science, a subject in which he has deservedly earned an international reputation, and is the author of a text-book on high polymers. His long and varied experience in the industrial sphere, together with his keen interest in bridging the gap between basic science and technology, will be an invaluable asset to the University.

Medical Research Council: New Research Units and Groups

The Medical Research Council is to establish the following two new research units and three new research groups: *The Microbial Systematics Research Unit* will be set up at the University of Leicester under the direction of Dr. P. H. A. Sneath. The Unit will be engaged in research on the classification of micro-organisms, with special reference to numerical taxonomy and computer methods. *The Mineral Metabolism Research Unit*. On the retirement of Prof. L. N. Pyrah as director of the Metabolic Disturbances in Surgery Research Unit in the University of Leeds, Dr. B. E. C. Nordin will become director of the Unit, which will be renamed. The Unit will be mainly engaged in research into the causes of renal stone, the dietary requirement of calcium and the metabolism of bone-seeking isotopes. *The Research Group in Applied Neurobiology* will be established at the Institute of Neurology, University of London, under the honorary direction of Dr. J. B. Cavanagh. The Group will be concerned with investigations of cellular relationships in the peripheral and central nervous system. *The Research Group in Clinical Respiratory Physiology* is being set up at the Postgraduate Medical School, University of London, under the honorary direction of Dr. J. B. West. The Group will investigate the physiological mechanisms controlling the distribution of air and blood in the lungs in health and disease. *The Research Group in Medical Demography* is to be established in the Department of Medical Statistics and Epidemiology at the London School of Hygiene and Tropical Medicine, under the honorary direction of Mr. W. Brass. The Group will undertake research into the effects of changes in population size and structure on the occurrence of disease and, conversely, of the effects of developments in medicine on population characteristics.

The Metabolic Reactions Research Unit

The Council's plans to establish a Metabolic Reactions Research Unit under the honorary direction of Prof. E. B. Chain were announced in June 1963 (*Nature*, 198, 1252; 1963). The Unit, which now has been set up in the Department of Biochemistry at the Imperial College of Science and Technology, London, will undertake research into the problems of metabolic reactions in the tissues of higher animals, including man, and in micro-organisms.

U.S. National Academy of Sciences and the National Research Council

Organization and Members 1963–64 includes full lists of members of the U.S. National Academy of Sciences and of the National Research Council (Pp. 143. Washington, D.C.: National Academy of Sciences–National Research Council, 1964). Details are also given of the membership of the various committees and boards and of the divisions and offices with their officers and committees. There is an introductory note outlining the organization and objectives of the Academy and the Research Council, and the functions of the various committees are shown under the appropriate headings. There is an index of names.

Development and Research in the Furniture Industry

The fifteenth annual report of the Furniture Development Council, covering the year ended December 31, 1963, notes a steady increase in the demand for method study consultancy services, and the scope of the activities of this department has widened. The Council's Product Costing System has been installed or revised by the Council's cost consultants at 20 different firms during the year, and a report, *Production Control in the Furniture Industry*, is being published shortly, while one on *Furniture Factory Planning and Layout* is in preparation. A list of papers issued by the Council and the Furniture Industry Research Association, 1949–63, is appended. On July 6, 1964, the Furniture Industry Development Council (Amendment No. 2) Order, 1964, was approved in the House of Lords. The research activities of the Furniture Development Council were taken over by the Furniture Industry Research Association on its establishment in 1961, and the Association receives most of its industrial income through the Council from the levy authorized by the Furniture Industry Development Council (Amendment) Order, 1958. As Lord Drumalbyn explained in moving the approval of the Order, the Council itself provides many services to the industry, including method study and costing, statistical and economic services, and the study of design and marketing, some of which are self-supporting, but the purpose of the order is to provide additional funds to enable the Research Association to expand and maintain its programme of research. The Order raises the amount of the levy which may be imposed on the industry by the Furniture Development Council to a maximum of £75,000 a year, and the proposed increase is supported by the British Furniture Manufacturers' Federated Associations and the National Federation of Furniture Trade Unions.

The Natural Rubber Producers' Research Association

The research programme of the Natural Rubber Producers' Research Association, under the direction of Dr. L. Mullins, continues its fundamental investigations of the science and technology of natural rubber and related materials. The problem of the chemical constitution of natural rubber vulcanizates is, after many decades of hypothesizing, showing signs of yielding its secrets under the combined attack of chemists, physicists and technologists. One of the methods developed for establishing the identity of sulphur-containing cross-links in vulcanizates has been the use of 'chemical probes', and a recent publication of the Association (Publication No. 473, by C. G. Moore and B. R. Trego) deals with the rearrangement of alkenyl groups that occurs during the desulphuration of model organic trisulphides by the probe triphenylphosphine. On the biochemical side, the biosynthesis of natural rubber continues to be investigated, and experimental evidence has recently been obtained (Publication No. 479, by B. L. Archer, B. G. Audley, E. G. Cockbain and G. P. McSweeney) that mevalonic acid is converted into isopentenyl pyrophosphate in the aqueous phase of *Hevea brasiliensis* latex through the intermediate formation of mevalonate phosphates. Subsequent incorporation of isopentenyl pyrophosphate into the growing rubber molecule is believed to occur at the surface of the rubber particle and to involve the attachment of isopentenyl pyrophosphate at an allylic pyrophosphate group situated terminally in the rubber molecule. Three distinct aspects of the physics of natural rubber—namely, friction, reinforcement and foam elasticity—have been investigated and these typify the Association's concern with fundamental aspects arising out of the use of rubber. A theory of dynamic rubber friction (Publication No. 477,

by A. Schallamach) has been developed in terms of the number and average life-time of the bonds due to molecular adhesion being dependent on the velocity and temperature of the rubbing members. The enhanced strength properties of reinforced rubbers are shown to result from the effects of rubber hysteresis in reducing the stress at the tip of the growing crack (Publication No. 475, by E. H. Andrews), and the effect of filler particles is illustrated by electron microscope photographs. The deformation properties of foamed elastic materials are discussed (Publication No. 478, by A. N. Gent and A. G. Thomas) in terms of a model consisting of a three-dimensional network of thin threads. The theory is extended to open-cell foams by considering the viscous damping due to the flow of air through such a network.

Mineral Deficiencies in Rubber Cultivation

AGRICULTURAL science owes much to an understanding of signs displayed by the plants themselves and by the leaves in particular. In the sphere of rubber cultivation, as in so many others, painstaking pot culture and field experimentation have been rewarded by a knowledge of the mineral shortages linked with given leaf symptoms. A recently published monograph from Kynoch Press, entitled *Mineral Deficiencies in Hevea and Associated Cover Plants*, contains excellent reproductions, from colour photographs, of leaves affected by deficiencies of the essential major and minor nutrient elements (By Victor M. Shorrocks. Pp. viii+76 (53 plates). Kuala Lumpur: Rubber Research Institute of Malaya, 1964. 25 Malayan dollars). Research on the soil, that "frugal custodian of plant nutrients", to quote the author's own description, has been prosecuted by the Rubber Research Institute of Malaya since the late 1920's, and this publication, nourished on earlier work which resulted in the compilation of Bolle-Jones, shows a healthy response indeed. Alongside symptoms found in *Hevea brasiliensis* are set those in three commonly found leguminous ground cover plants, *Pueraria phaseoloides*, *Centrosema pubescens* and *Calopogonium mucunoides*, thus enabling the grower to turn these species to a new advantage as 'indicator' plants. Descriptions of deficiency symptoms and of their occurrence, not overlooking waterlogging symptoms, albeit strictly applicable to Malaysian conditions only, will be of value to workers in many other rubber-growing areas, as will the introductory section on plant nutrients to those studying different crops. Tables of published data are given on the results of leaf analysis, a technique which the author himself has used to no mean effect. To achieve successful corrective fertilizer application it will surely be essential to follow the advice given here. At the present time, known deficiencies in Malayan rubber trees include magnesium, potassium, calcium, nitrogen, phosphorus and manganese. We may look forward to progress, say, on such topics as yields and their relation to minor elements, and this timely publication marks an important stage in a field having world-wide economic implications.

Laminated Timber Structures

MODERN gluing techniques have rendered possible the use of laminated wood in timber structures of almost any size and shape. This form of construction is now apparently finding favour with architects concerned with certain types of building such as churches, schools, recreation halls, social centres, restaurants and, where practicable, industrial buildings. Laminated timber permits of combined structural strength and attractive design. Glued timber structures are free from corrosion hazards; thus maintenance costs are greatly reduced or even eliminated. A recent issue of *Technical Notes 258* (CIBA (A.R.L.), Ltd., Duxford, Cambridge, June 1964) is devoted entirely to "Timber Structures by Muirhead and Sons, Ltd.". Details and illustrations of several examples of laminated work incorporating 'Aerodux' resorcinol adhesive (made and supplied by CIBA) are given. These include a framework for a sports building at Newcastle upon Tyne, circular in shape, with an overall diameter of 206 ft.; a sports hall at Grangemouth with laminated timber portal frames spanning 35 ft.; the Church of the Holy Rood, Grangemouth, with laminated gothic arches with a span of 45 ft.; showrooms for the South of Scotland Electricity Board at Airdrie, in which very extensive use was made of laminated timber work incorporating 'Aerodux 185' adhesive; a cattle court in East Lothian having laminated portal frames spanning about 60 ft. The applications of laminated woodwork structures as described in this pamphlet are certainly impressive, but whether in the long run this type of timber construction ". . . is able to offer serious competition with steel and reinforced concrete", for example in the tower-block type of buildings now so conspicuous in London and elsewhere, likewise in modern school and factory designs, must, for many obvious reasons, remain a moot point.

Architecture of the Oviduct of the Domestic Hen

IN response to the article with the above title, which appeared on p. 699 of the August 15 issue of *Nature*, Prof. G. M. Wyburn has written to the Editor, pointing out that probably the most comprehensive histological investigation of the hen oviduct was carried out by Dr. K. Richardson in 1935. The work was undertaken in the Anatomy Department, University College, London, when Dr. Richardson was investigating the secretory phenomena in the oviduct of the fowl, including the process of shell formation, by means of the micro-incineration technique (*Phil. Trans.*, B, 225, 149; 1935). These investigations have recently been extended by electron microscope surveys in the Anatomy Department of the University of Glasgow. One of these, by Drs. R. M. C. Aitken and H. S. Johnston, was concerned with the fine structure of the infundibulum of the avian duct (*J. Anat. London*, 97; 1963). It showed that cells of the infundibular glands differ in certain respects from those of the succeeding albumen-secreting region and may be of greater significance in chalaza production than heretofore supposed and not simply additional albumen-secreting glands; although it is probable that these represent particular functional states of the same cell, this has not yet been confirmed. Ciliated non-secretory, ciliate secretory and non-ciliated secretory cells were distinguished in the surface epithelium, and the possibility of transformation from one type to another was suggested. The cilia present distinctive features, the apparent cross-striation of the rootlet in this species being due to its ensheathment by a fibre arranged as a helix. The structure is therefore reminiscent of the spermatozoon. Further investigations have been made by Drs. Johnston and Aitken and Prof. G. M. Wyburn into the fine structure of the avian uterus (*J. Anat. London*, 97, 3; 1963). This has revealed that the apical and basal cells show marked differences in their fine structure. Their significance in relation to the formation of the shell matrix and cuticle was also examined. The distinctive features of the junctional zone glands of the uterus were described, including the types of secretory granule. The uterine gland cells have secretory granules within vacuoles, and changes in the microvilli of these cells are possibly related to the passage of calcium ions during calcification of the organic matrix of the shell. Two types of dense cytoplasmic body have been demonstrated in the cytoplasm of the uterine gland cell. There is some evidence to support the view that the granular bodies, which may be lysosomes, are derived from mitochondria.

Spectrochemical Analysis of Soils, Plants and Related Materials

IT is about forty years since attention was first directed to the importance of minute amounts of certain elements in the healthy growth of plants and animals. Boron and then manganese and cobalt, followed by copper, zinc and

molybdenum, were proved to be essential 'minor' elements, but progress in this field of investigation was rather slow and uncertain until techniques were developed to deal adequately with the measurement of quantities of the order of a few parts per million and at the same time to overcome the difficulties associated with contamination. Our ideas on 'purity' have been completely altered by innovations in micro-analytical technique such that the classical chemical methods have been largely replaced by the use of equipment that enables the analyst to carry through large numbers of determinations in a short time. Flame emission and arc methods of spectrographic analysis rapidly achieved a precision and reliability under the lead of R. L. Mitchell, and in 1948 he wrote *Technical Communication* No. 44 of the Commonwealth Bureau of Soils, which was a review of the literature on spectrographic analysis relevant to soils, plants and related substances, with details of the methods devised at the Macaulay Institute. This was reprinted in 1956 and now, because of continuing demand, and major advances in methods, it has been brought up to date by the provision of an addendum and appropriate bibliography occupying 37 pages (*Technical Communication* No. 44*A*, *The Spectrochemical Analysis of Soils, Plants and Related Materials (with Addendum)*. Pp. ix+225. Farnham Royal, Bucks: Commonwealth Agricultural Bureaux, 1964. 40*s*.; 6 dollars). The original text is still relevant and is reprinted unaltered. The introduction of direct-reading instruments, with "the change in emphasis" from photographic to photoelectric assessment of the spectral line intensity, "has prompted" the change in title from spectrographic to spectrochemical analysis. Some idea of the nature of the modifications of the original methods and the development of new ones may be gained from the headings of the main sections of the addendum: the measurement of the photographic plate, direct current arc excitation, porous cup solution spark excitation, spark and interrupted arc excitation, flame photometry, atomic absorption, pretreatment of soils and plant materials, application to soil and plant investigations. The book also includes a brief description of special facilities for trace element work in the new Spectrochemistry Department of the Macaulay Institute.

Soil Survey of Great Britain

The aim of the Agricultural Research Council Soil Survey of Great Britain is to provide a record of the soils of the country, in the form of maps and memoirs, from field observations and laboratory analyses, designed to establish the derivation of the soil as well as its characteristics and distribution. The printed records are of the greatest value to those concerned with agriculture or with drainage, ecology, geography or land-use. The sixteenth report (Pp. vi+39. H.M.S.O., 1964. 5*s*.) describes the work done in 1963; two maps show those areas of the country where field work has been completed or is in progress. For example, soil maps were published in 1963 of the districts around Cambridge and around Church Stretton, while a further nine sheets are in the press; memoirs for the district around the Menai Straits, and the area around Aberdeen, Inverurie and Fraserburgh have been published, and another is in the press. Several other memoirs to accompany maps are in preparation. There are brief accounts of the general pattern of the soils being mapped in various parts of Britain, of the special surveys made for such bodies as the Nature Conservancy, the National Agricultural Advisory Service, the Forestry Commission, and for various drainage authorities, and of the advice and assistance given to a wide range of enquirers. There is also a list of the contributions to scientific journals by members of the Survey staff.

International Lactic Acid Prize

Stichting Ilra, International Research Association, 39 Overgaden neden Vandet, Copenhagen *K*, is offering two prizes, valued at 1,500 and 750 U.S. dollars, for the two best essays received on "The Position of Lactic Acid and its Derivatives in the Nutrition and the Metabolism of Ruminants". Entries will be assessed by an international committee of adjudicators selected by 'BIOCENTRALEN'—the Danish Society of Biotechnical Industry and Research. All papers entered for these prizes should be submitted in English, French or German to BIOCENTRALEN, Bülowsvej 13, Copenhagen *V*, not later than November 1, 1965.

Perkin Centenary Trust: Awards

Perkin Centenary scholarships for 1964 have been awarded to Mr. D. Harris, of Heaton Moor, Cheshire, tenable at the Royal College of Advanced Technology, Salford; to Mr. T. A. Moran, of Illingworth, Yorkshire, tenable at the Bradford Institute of Technology; and to Mr. J. A. Scott, of Burneside, Westmorland, tenable at the Royal College of Advanced Technology, Salford.

Financing Medical Research

The Office of Health Economics has issued the following correction concerning the medical research expenditures of two charities which were referred to in their report *The Finance of Medical Research* (*Nature*, 203, 999; 1964). The total grants made by the Nuffield Foundation for medicine in 1961–62 amounted to £669,000, of which only £245,000 were for buildings. The Leverhulme Trust do not finance buildings at all from their funds, but do make grants for the conduct of medical research. The estimated total amount granted or allocated for the conduct of research in Britain by all trusts supporting medical research therefore amounted to approximately £790,000 in 1961. The total from all private sources was about £2·5 million, in addition to £1·4 million for buildings. The estimated total of capital and current expenditures on medical research from all sources in Britain in 1961–62 remains as £27 million.

Announcements

The seventh British weed control conference, organized by the British Weed Control Council, will be held in Brighton during November 23–26. Further information can be obtained from Mr. W. F. P. Bishop, 140 Bensham Lane, Thornton Heath, Surrey.

A conference on "Magnetism and Magnetic Materials" will be held in Minneapolis, Minnesota, during November 16–19. Further information can be obtained from J. T. Elder, Minnesota Mining and Manufacturing Co., 400 McKnight Road, St. Paul, Minnesota 55119.

A symposium on "Mammalian Radiobiology at the Cellular Level", arranged by the British Institute of Radiology, will be held at the Institute on November 20. Further information can be obtained from the General Secretary, British Institute of Radiology, 32 Welbeck Street, London, W.1.

The effects on human beings of additives, colouring matter and foreign substances in foodstuffs will be the subject of an international conference to be held in Brno during November 19–21. Further information can be obtained from Dr. M. Totová, Katedra hygieny University J. E. Purkyně, třída Obr ánců miru 10, Brno.

A meeting of the Welsh Soils Discussion Group on "Chemical Means on Assessment of Soil Fertility" will be held at the Welsh Plant Breeding Station, Plas Gogerddan, on November 25. Further information can be obtained from Dr. A. H. Charles, Welsh Plant Breeding Station, Plas Gogerddan, near Aberystwyth.

OXIDASES AND RELATED OXIDATION-REDUCTION SYSTEMS

AN international symposium on oxidases and related oxidation-reduction systems was held at Amherst College, Amherst, Massachusetts, during July 15–19. The organizers of the symposium (T. E. King, H. S. Mason and M. Morrison) made use of the presence of many biochemists in the United States for the sixth International Congress of Biochemistry, which was held in New York at the end of the month, to bring together most of the leading workers in the field. The symposium was restricted to 49 participants, all of whom except two presented papers. The geographical distribution of the speakers was: United States, 28; Japan, 5; United Kingdom, 4; Sweden, 3; Australia, 2; Israel, 1; Switzerland, 1; Germany 1; The Netherlands, 1. The symposium was generously supported by grants from the National Institutes of Health and the Office of Naval Research. The organizers were assisted by an advisory committee consisting of B. Chance, P. George, M. D. Kamen and E. C. Slater.

The first session of the symposium, on fundamental chemistry, was opened by P. George, who took as his theme the "Fitness of Oxygen" (cf. Lawrence J. Henderson's *The Fitness of the Environment*, first edition, Macmillan, New York, 1913; Beacon Paperback, Boston, 1958) as a terminal oxidant. N. Sutin discussed the mechanisms of some electron transfer reactions in solutions. F. W. Cope proposed a new kinetic theory of electron and ion transport, based on electron conduction within semiconductive particulate and membranous solid structures. His claim that the application of solid-state physics to enzyme kinetics is more fruitful than the conventional theories based on the 'law of mass action' was rather strongly attacked both by Sutin and by Chance. In general, it appeared that the biochemist is not ready to abandon the conventional approach for one that does not seem to offer any advantages, and which, according to B. Rosenberg's paper on some problems in the electrical conductivity of proteins, appears at present to have very limited experimental support.

The second session on peroxidase–oxidases, model oxidases, and related systems was opened by a theoretical investigation of the structure of oxygen–metallic ion complexes by L. L. Ingraham. His molecular-orbital calculations supporting a $Fe^{3+}.O_2^-$ structure for oxyhæmoglobin were vigorously discussed, especially by Sutin and L. Orgel. Organic chemistry was now brought into the picture by R. O. C. Norman speaking on the mechanisms of aromatic hydroxylation and ring-opening reactions, and by W. S. Caughey on structure and medium effects on the reactions of iron(II) porphyrins with oxygen and carbon monoxide. M. E. Winfield presented a mechanism for the autoxidation of myoglobin and proposed the first of many mechanisms brought forward during the symposium on cytochrome oxidase. I. Yamazaka suggested a mechanism of the reaction in which peroxidase acts as an oxidase. W. E. Knox described his experiments on the activation of tryptophan pyrrolase by reduction and by hæmatin.

The third session was devoted to oxidases containing non-hæm iron or copper (with the exception of cytochrome *c* oxidase). D. I. Crandall discussed molecular oxygenation by iron-activated oxygenases. O. Hayaishi confined his attention to comparative investigations on pyrocatechase and metopyrocatechase, reserving the more general aspects of oxygenases for a brilliant exposition at the opening session of the International Congress of Biochemistry later in the month. Investigations on copper oxidases were opened by Bo G. Malmström, who discussed the two forms of copper which he has shown to be present in these oxidases by determination of the electron-spin resonance spectrum. E. Frieden reported on the catalytic activity of ceruloplasmin and its inhibition, and C. R. Dawson concluded the session, but not the discussion on copper, by his paper on the reaction between copper and the apoenzyme of ascorbate oxidase.

The chemists had the first word in the fourth session on flavin-containing oxidases. P. Hemmerich discussed the chemistry of flavin–metal interaction, dealing in particular with metal–ligand charge transfer, and A. Ehrenberg, the nature of flavin-free radicals. The biochemists continued with discussions by T. P. Singer on the comparative biochemistry of succinate dehydrogenase, by Y. Ogura on the action of mechanism of several flavin enzymes, by V. Massey on D-amino-acid oxidase, by R. C. Bray on xanthine oxidase, and by P. Handler on several metalloflavoproteins. The controversy which has raged for several years on whether the iron atom in iron-containing flavoproteins undergoes oxidoreduction in the course of the enzymatic reaction was not settled, even after an extra evening session. H. Beinert produced new and convincing evidence that iron is concerned in the $g = 1{\cdot}94$ signal in the electron-spin resonance spectrum which appears on reduction of these flavoproteins. This does not, however, prove that the iron atom is reduced, since the signal probably involves another atom, possibly a sulphur. V. Massey made a plea that his data, which show that the rate of reduction of the iron atom, as measured chemically, is several orders of magnitude slower than the overall reaction catalysed by the enzyme, should not be ignored.

M. D. Kamen opened the fifth session with a discussion on the status of the *Rhodospirillum* hæmoprotein and other atypical hæmoproteins as bacterial oxidases. I. C. Gunsalus considered the generation of active oxygen for mixed function oxidation, dealing in particular with investigations arising out of his discovery that this reaction is involved in the lactonization of camphor. Microsom[al] oxidases were discussed in four papers: by Hj. Staud[inger] on the mechanism of microsomal hydroxylation [with] Estabrook on spectral and kinetic investig[ations] microsomal pigments involved in this reacti[on] on the CO-binding hæmoprotein and NA[DPH] flavoprotein in liver microsomes and their [role] somal electron transfer, and H. S. Mason [on] and oxidase function of liver microsome[s].

A 'free' afternoon was devoted to a [discussion] controversial question of the separat[ion] chrome *a* and a_3. Most workers in t[his field] to accept the postulate of Keilin and [Hartree] made in 1939, that cytochrome *c* [is] differently bound hæm *a* molecules [which] with oxygen and respiratory inh[ibitor] does not. However, the so-calle[d] still supported by Onunuki and [Smith] assume that a single protein co[mplex] prosthetic groups (Siamese tw[ins]) a concept which can be formul[ated] is at present no compelling [evidence] separate proteins—cytochrom[e] although this cannot yet be ex[cluded].

Ten papers on cytochrome c[oxidase] the next two sessions, by K. [Okunuki] Takemori) on the reaction wit[h] chrome *c*, Q. H. Gibson on the re[action]

H. Beinert on the function and disposition of the copper, W. W. Wainio on some anomalous reactions of the oxidase, M. Morrison on its components and their reactions, E. C. Slater on its composition and oxidation-reduction potential, T. E. King on a cytochrome *c*–cytochrome oxidase complex, L. Smith on the reaction of the oxidase with cytochrome *c*, P. Nicholls on its mechanism and E. E. Jacobs on its reaction with high-potential electron donors. Comments from R. Lemberg and K. Okunuki, who were unable to be present, were submitted by E. C. Slater and S. Takemori. There is now general agreement that copper forms part of the enzyme, although its exact role remains to be determined. Q. H. Gibson agrees with B. F. van Gelder and E. C. Slater that equal amounts of the a and a_3 components are present in active cytochrome *c* oxidase. Gibson's discovery that cytochrome *c* oxidase preparations contain non-reducible cytochrome a_3 explains the higher ratios of $a : a_3$ reported by M. Morrison. P. Handler brought forward the coupling of sulphite oxidation with the cytochrome *c* oxidase reaction as evidence in favour of an iron–O_2^- radical as an intermediate in the enzyme reaction.

The last two sessions were devoted to oxidase systems and oxidative phosphorylation in intact mitochondria. A. W. Linnane reported on the induction by oxygen of the synthesis of mitochondria in anaerobically grown yeast cells. E. R. Redfearn discussed the interaction of electron carriers in the mitochondrial $NADH_2$ and succinate oxidase systems. Oxidative phosphorylation was dealt with in four papers. L. Ernster described a new method for investigating phosphorylation in the cytochrome *b*–*c* region, D. E. Griffiths examined possible reaction mechanisms at the pyridine nucleotide level and P. D. Boyer suggested carboxyl activation as a possible common reaction in substrate-level and oxidative phosphorylation, and in muscle contraction. Boyer stated that there is now no clear evidence for a role for phosphohistidine in oxidative phosphorylation, and that all data can be rationalized in terms of the succinyl-Co*A* synthetase being responsible for the incorporation of inorganic phosphorus-32 into mitochondrial protein-bound phosphohistidine. C. L. Wadkins reported interesting work on the oligomycin-inhibited, dinitrophenol-stimulated interaction between the ADP–ATP exchange enzyme and cytochrome *c*. B. Chance reported data on the reaction-velocity constants for electron transfer and transport reactions. He proposed that three types of reactions are volved in oxidative phosphorylation: (1) a rapid elec- transfer process, limited by the speed with which an departs from an iron atom and by the speed of its ugh the hæm-linked groups to the periphery of (2) an electron-transfer process which involves a thermal reaction in which the adjacent electron-bearing hæmatin becomes properly orientated; (3) a process involving the formation of high-energy intermediates of the cytochromes.

The last two papers dealt with the mitochondrial morphology as revealed by the electron microscope, with particular reference to the structure with projecting sub-units discovered by Fernandez-Moran. Those features of this structure which have been regarded as essential have gradually undergone a change, summarized colloquially by the sequences 'knobs', 'knobs on sticks' and 'the tripartite knobs-sticks-base plate'. There was general agreement that the morphological structure is not an artefact, but no agreement as to its physical significance. D. E. Green defended his suggestion that the structure represented the 'elementary particle' of electron transport. E. Racker, in a paper with D. F. Parsons, B. Chance and others, discussed the correlations between electron-transport activity, ATPase and the morphology of the submitochondrial particles, which led him and his colleagues to conclude that the projecting sub-units do not contribute an essential component to the electron-transport chain. A possible correlation between the sub-units and ATPase activity (coupling factor I) was suggested. Those attending the symposium were not disappointed in their expectations of a vigorous discussion, which was brought to a close by Racker's revelation that the 'knobs–sticks–base plate' structure shown by electron microscopy had been painted, in appropriate colours, by a Finnish artist in the 'thirties.

The papers presented at the symposium are to be published in due course. Since all but two of them were circulated to participants several weeks before the symposium, only 10 minutes were allowed for each presentation, hence maximum time was made available for discussion. The time-table for the symposium was planned on the hopeful assumption that the participants would have read the preprints (weighing 3·5 kg) before the meeting. Although in this respect most of the participants disappointed the organizers, the discussions were mostly lively, and there can have been few points in the field of oxidases which were left undiscussed.

If I may be permitted to record what struck me most about this symposium—and the identical thought was voiced by one of the most senior and most distinguished of the participants—it was the number, breadth and depth of the contributions made to the discussions by B. Chance, covering everything from theoretical chemistry, through physical chemistry, mechanism of enzyme action, oxidative phosphorylation to the morphology of mitochondria. It was a masterly performance, which contributed much to the high standard of the discussions. E. C. Slater

NAL AND SOCIAL FAILURE: SOME FURTHER OBSERVATIONS

By JOHN BRADLEY

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ine[1] gave an account of a new which had been used to provide ent of a concept of a family ildren of school age.

ven to primary school children xes and to a group of 1,600 boys and girls of 11–15 years, t the mother is regarded as the ily in the earlier primary years y displaced by the father, the ing complete for the majority enth year.

Groups of children committed to approved schools and a group of children who had failed to make satisfactory academic and social progress in a grammar school gave a significantly different pattern of test results.

It was shown that social and educational failure, which can be defined as an obvious deviation by the child from the social and educational standards acceptable to those in whose care he finds himself outside the home, have a similar ætiology and that both can be understood only through a study of the family authority relationships and a consideration of the child's response to these relationships. It was further shown that these relationships

are as relevant to the failure of girls as to that of boys; no significant sex differences in the responses to the projection test appeared either at the primary or secondary school level, while for the approved-school girls absence of the father or his serious defection was reported in a high proportion of cases and appeared to be reflected in the test results.

The emergence of the father as first in the family hierarchy during the years 7–11+ seems to be related to the development of a conforming, socially acceptable attitude of the child. It is interesting to note that in the groups of delinquent boys the mean age of the first recorded offence was 10·2 years.

The original primary school sample was small, and the test has now been repeated in three mixed primary schools with results which are consistent with the earlier findings.

Table 1

	F.	M.	Self	Other	
Year I	24	68	12	15	119
Year II	38	57	13	12	120
Year III	38	51	30	20	139
Year IV	80	41	12	18	151
				Total	529

It was argued in the earlier report that social deviations, of which frank delinquency is only one arbitrary category, stem from relationships within the nuclear family. If this argument is valid, it seemed likely that test results of children who showed deviations from 'normal' behaviour in the secondary modern school group would show significant variations from the 'normal' family patterns in the test.

The headmaster of the secondary modern boys' school was therefore asked to give details of all children in the school who were regarded as unsatisfactory in terms of academic effort or out of the ordinary in behaviour or attitude. This deviant group included those known as petty pilferers, repeated breakers of school rules, the extremely nervous and diffident, and boys who were irresponsible and impulsive. Those whose behaviour could reasonably be attributed to organic or functional abnormalities, for example, asthmatics and cases of epilepsy and head injury, were excluded.

Full longitudinal records were available for 659 boys, and Table 2 shows the relationship which was found for this group between deviant behaviour and the member of the family placed at the top of the circle.

Table 2

	F.	M.	Br.	Sis.	Self/other	
'Normal'	350	103	22	13	22	510
Deviant	43	67	21	10	8	149
						659

The relationship is significant.

The school serves a large housing estate and a town centre area, and the large number of cases of deviant behaviour indicated in the Table is likely to be a reliable estimate, particularly as the schools keep very detailed records for each pupil.

The patterns of family dominance hierarchy for deviants in this secondary modern school are similar to those obtained from a group of 317 delinquents made up of 268 boys in approved schools and 49 boys remanded for clinical reports by the courts.

Table 3

F.	M.	Br.	Sis.	Self/other
78	129	61	30	19

The same tendency for the mother to retain the salient position in the hierarchy is apparent and resembles the patterns obtained from the first-year primary school group.

It is evident that the non-deviant and deviant groups respond differently to the projection test. If the reasons for these differences of response are to be explored it seems necessary to arrive at an objective assessment, in so far as objectivity is possible, of the relationships between parents and child and of the parts played by both parents in the family group.

Information concerning the family relationships of non-deviant children is scanty and anecdotal as families, generally, are inaccessible to systematic observation and assessment. There are no norms for attitudes in the family and no means of assessing objectively the quality of the relationships between the members of the family group. The families of delinquent children, however, come under the scrutiny of children's officers and probation officers, and if the child is committed to an approved school all available reports are brought together by the classifying school and passed on to the approved school to which the child is allocated, with any additional information gathered during the classifying period.

It is clear that the norms of family life adopted by the social workers successively involved with the child's problem will be in no way standardized. Each worker will bring to the investigation his own experience of family life, his own response to training and his own private view of all that he feels family life should entail.

His contacts with the family will be stylized and formal and, however close his relationship to the family may appear to him to be, he remains an official; consequently no more than part of the truth can be recorded since only part of the truth is ever revealed.

Furthermore, the reports of the first social worker to be involved with a family may influence significantly the reports of subsequent workers. The 'good' family tends to remain 'good' throughout the records, while the 'bad' family tends to exhibit a stubborn resistance to the social workers' efforts to modify its attitudes.

Nevertheless, since for the delinquent group the bulk of information is to be found only in such reports, it is on this information, with the reservations mentioned above, that the next part of the investigation is based.

The records of a group of 173 boys, of 11–15 years, committed to approved schools, were examined and the reported parental attitudes were recorded separately for both parents on five-point scales.

The scale for fathers was:

Table 4

1	2	3	4	5
Overstrict Rigid Harsh Domineering	Inconsistently strict	Consistently reasonable but firm: interested in child	Plays little part in controlling but sometimes weakly involved	Disinterested: abdicating: leaves all control to mother or others

The scale for mothers was:

Table 5

1	2	3	4	5
Overprotecting against blame and correction: resists father's intervention: overindulgent	Inconsistent: tends towards overprotecting: overindulgent attitude	Consistent: reasonable and participates warmly	Inconsistent: at times sentimentally overindulgent but tends to reject	Disinterested: has little or no affection: rejecting

The following results were obtained:

Fathers' reported attitude to the family:

Table 6. HARSH—RIGID←→DISINTERESTED—ABDICATING

1	2	3	4	5	
13	18	7	47	88	Total 173

Mothers' reported attitude to the family:

Table 7. OVERPROTECTING←→REJECTING

1	2	3	4	5	Insufficient information 5
31	21	21	42	53	Total 168

Of the fathers 7 (4 per cent) were, from the reports, normal in their attitudes; of the mothers 21 (12·5 per cent) were regarded as normal. No control or training was given by either parent in 67 cases (38·7 per cent). Where the mother was reported to be 'normal', the attitudes of the fathers were distributed as follows:

Table 8. Harsh—Rigid←→Disinterested—Abdicating

1	2	3	4	5	
1	–	3	2	15	Total 21

Of the three families with both parents described as 'normal', that is, falling into category 3, the parents of one family were preoccupied for the first 9 years of the subject's life with an idiot spastic sibling; and one mother was described by the classifying school as having given no moral training. The third mother suffers from psychotic episodes. One father is psychotic.

Of the fathers 36 (20·8 per cent) were unemployed; 45 (26·0 per cent) were delinquent (convicted); 9 (5·0 per cent) were delinquent and unemployed. Of the 168 mothers for whom full information was available 9 (5·3 per cent) were delinquent. Of the 173 families 74 (42 per cent) had more than one delinquent child. For this group of 173 boys the protection test gave the following family dominance pattern:

At the top of the circle:

Table 9

F.	M.	Br.	Sis.	Self/other	
42	72	39	13	7	173

The pattern in cases with fathers delinquent was:

Table 10

F.	M.	Br.	Sis.	Self/other	
12	22	6	4	1	45

and the pattern where siblings were also delinquent but father non-delinquent:

Table 11

F.	M.	Br.	Sis.	Self/other	
17	29	23	4	1	74

The records of 53 girls of 11–15 years committed to approved schools, while far less detailed and comprehensive than those for the boys, were examined in the same manner.

Of this group 25 came from broken or incomplete families, fathers being absent in 15 cases, mothers in 5 cases; the information available was inadequate in 5 cases.

Of the 28 girls from intact families who were studied, reports on parental attitudes yielded the following information:

Table 12. Fathers' Reported Attitude to the Family

1	2	3	4	5
5	7	–	3	13

Table 13. Mothers' Reported Attitude to the Family

1	2	3	4	5	Insufficient information 5
1	3	1	8	10	Total 28

Of the fathers, 5 were unemployed; 5 were delinquent; 1 was delinquent and unemployed. Of the mothers, 5 were delinquent.

The attitudes adopted by parents of delinquents of both sexes seem from this small sample of girls to be very similar. However, mothers rarely exhibit to their daughters the overprotective attitude they often show towards their sons.

No evidence was forthcoming to suggest that early separation from the mother was a significant factor in the delinquency of either sex or that the quality of the discipline and control of the mother alone as suggested by Glueck[2] was of first importance.

Mead[3], as long ago as 1950, affirmed that the cohesion of society depends on the learned nurturing behaviour of the human male. It seems clear from this present research that both parents are important in the socialization of the child but that the role of the father is more vital than has hitherto been admitted. The father who chooses to abdicate or to dominate rather than to nurture or who, because of the defects in his own rearing, is incapable of learning to nurture adds to the difficulties of his children's socialization and appears from the investigation so far carried out to be responsible in a large measure for his children's social deviation.

[1] Bradley, J., *Nature*, **200**, 455 (1963).
[2] Glueck, Sheldon and Eleanor, *Family Environment and Delinquency* (Routledge, Kegan Paul, 1962).
[3] Mead, Margaret, *Male and Female* (Gollancz, 1950).

THE TROPICAL PRODUCTS INSTITUTE

In his report for 1963*, the director of the Tropical Products Institute, Mr. E. S. Hiscocks, states that investigations of aflatoxin and its presence in groundnuts and other materials remained the major project throughout the year, although by the end of the year the results of this work allowed all but a small nucleus of staff to return to other duties. The Institute is, however, still providing advice to most parts of the world on the problem and is maintaining close contact with other laboratories working in this field. In September, a member of the staff was seconded to Nigeria for six months to assist with work on testing samples for aflatoxin and to train local staff in this technique. In October, the Institute acted as host to an international meeting organized by the United Nations International Children's Emergency Fund on the subject of aflatoxin.

Two new projects were started during the year. The first, which arose from a growing concern about the widespread use of toxic insecticides and the concomitant problems of insect resistance and toxic residues, deals with a search for insect attractants. It is hoped that the use of such attractants, which may operate through sexual, food or ovipositional stimuli, may provide alternative methods of controlling certain tropical insects and may also overcome the problem of insect resistance to insecticides.

* Department of Scientific and Industrial Research. Report of the Tropical Products Institute 1963: The Report of the Tropical Products Institute Committee with the Report of the Director of the Tropical Products Institute. Pp. vi+33. (London: H.M.S.O., 1964.) 3s. net.

The second is an investigation of methods for local processing of tropical products with the view of improving the equipment or method, or of designing machinery where this does not exist. The first investigation, which was recently started, is concerned with the difficult problem of cashew nut decortication.

During the year, 838 enquiries were received from 78 countries and international bodies. This represents a slight decrease on the previous year which can probably be explained by the setting up of similar laboratories in various developing countries. Although such laboratories will eventually be able to take over much of the work that the Institute has carried out in the past, it means that the enquiries now received tend to come from such laboratories and concern aspects of work for which they do not have facilities. Thus, the enquiries received, although diminishing somewhat in number, tend to increase in complexity.

Among the many important investigations referred to in the report is one concerned with dried fish. The methods of drying used in the tropics are usually very primitive: the fish is laid out in the sun or placed over an open fire, and it is probable that these methods not only impair the quality of the fish but also reduce the biological value of the protein. A detailed investigation of dried fish from tropical countries has been carried out and a series of experiments was aimed at investigating the drying of fish under conditions approximating those which prevail in

both dry and humid tropical climates. The experiments were undertaken in a small Torry kiln in which tropical conditions were simulated.

Even in dry tropical climates, fish drying can normally be carried out for only 8 h a day because at sundown the temperature falls and the humidity increases. An attempt was made to determine whether hanging, stacking or pressing fish overnight gave the shortest drying time. The results of these experiments indicated that there might be some advantage in pressing during the later stages of drying, thus enabling the moisture to equilibrate. Further work with more refined apparatus will be required before definite conclusions can be drawn.

The 'available lysine' contents of three specimens of *Tilapia nilotica* from Uganda were determined by Carpenter's method in order to ascertain if any damage had been caused by the different methods of drying. The three specimens had been either fresh-frozen, or sun-dried, or fresh-frozen and later dried in a Torry kiln. It was found that the fresh-frozen fish contained most available lysine (10·7 g/16 g nitrogen) as would be expected, the sun-dried fish contained least (6·1 g/16 g nitrogen) and the kiln-dried fish gave an intermediate value (6·9 g/16 g nitrogen). Those concerned with this aspect of the Institute's work were surprised to find that the loss of available lysine in sun-drying was as great as that occurring in the manufacture of fish meal where much higher temperatures are used. However, as only three fish were used in the experiments the results were not, at the time of preparing the report, regarded as conclusive. They indicated, however, that further work on these lines would be worthwhile. Factors, other than protein deterioration, which are important in the nutritive value of fish, have not been examined as part of this investigation.

An officer was seconded to the Government of Aden during October 1962–March 1963 to investigate the problems involved in preparing dried fish for export. A survey of the curing methods in present-day use in Aden suggested that minor improvements in technique would enable the processors to make much better products. A series of fish-curing experiments was carried out and the crew of a Fisheries Department vessel was trained to demonstrate the methods evolved to local fishermen. These demonstrations included dry-salting in closed vessels instead of salting in open kench piles, proper washing of the fish using brine, and drying on raised racks in the shade instead of throwing the fish on the sand in the open sun. The success of these methods was illustrated by the fact that products made by the demonstrators fetched higher prices than the traditional products in local markets. Two fish-curing stations were designed for experimental use and built so that the experiments and demonstrations could be continued after the officers' departure.

The problem of insect infestation in Aden of dried fish during preparation and in storage was also examined. Early in the assignment it became obvious that this was a serious problem. A preliminary report on insect infestation was, therefore, prepared and forwarded to the Department of Technical Co-operation, since it was obvious that insect infestation should be investigated by an entomologist specializing in stored products.

The report recommends that demonstration of the improved curing methods should continue, that samples of the products made should be sent to overseas markets for evaluation and that experiments with species of fish at present under-exploited should continue. It is also recommended that the possibility should be investigated of erecting commercial fish-curing stations for communal use.

ROYAL INSTITUTE OF TECHNOLOGY, STOCKHOLM

PROF. H. ERDTMAN, of the Kungliga Tekniska Högskolan, Stockholm, has provided an interesting account of the Royal Institute of Technology, Stockholm, which was founded in 1827 as a 'humble' school of technology (*May and Baker Bulletin*, 6, No. 2; May 1964). The staff of the Institute then consisted of one director, two professors, two other teachers and eight other employees, including one steward and one porter. Instruction was in the fundamental subjects of "general natural knowledge, i.e., physics and chemistry, with particular reference to those of the general laws of Nature that find their most common applications in industry", and it was required to be "popular and practical rather than strictly scientific". In 1876 the Institute was transformed into an institute of technology, but in 1907 it still had only two professors of chemistry, one in 'technical chemistry' and one in 'general chemistry'. The present Kungliga Tekniska Högskolan, has nine departments and about eighty professors. There are about 750 other employees, of whom some 500 are teaching and research assistants, and in all about 4,000 students.

The Department of Chemistry now has twelve professors and three other senior lecturers, each in charge of his own branch of study: analytical chemistry, inorganic chemistry, organic chemistry, nuclear chemistry, physical chemistry, wood chemistry, chemical technology, polymer technology, paper technology, cellulose technology, food chemistry (biochemistry), fermentation chemistry, applied electro-chemistry and corrosion, heat technology, and chemical engineering equipment.

The degrees awarded by the Institute include *civilingenjor*, *teknologie licentiat*, and *teknologie doktor*. The time normally required for the first degree is four years, although in practice 4·5–5 years are often needed. The licentiate takes a further two or three years, and the doctorate yet another two or three or even longer. The licentiate corresponds roughly to an American, British or German Ph.D., and the doctorate approximates to a British D.Sc.

Students enter the Institute of Technology at the age of eighteen to twenty, and, owing to compulsory military service, male students are about twenty-five when they pass their *civilingenjorsexamen*. Approximately seventy freshmen are admitted annually to the Department of Chemistry, of whom about 10 per cent are women. Admission is competitive and far more students apply for admission than there are places. During the first two years, basic subjects are studied, for example, mathematics, physics, inorganic and organic chemistry, machine element design, and mechanics. The first courses in chemistry are elementary 'general courses', and all first- and second-year courses must be completed before starting the third-year course. During this and the fourth year, applied subjects are studied, and more advanced chemistry courses are available, but these are compulsory only for students who have chosen the relevant field of specialization. During the fourth year, specialization is much more intensive. Finally, for the *civilingenjorsexamen*, the student must carry out a small research project, requiring about three months work; about four months of practical experience in industry is also required.

Every student who has obtained a *civilingenjor's* degree may continue his studies for the licentiate and then go on to seek the degree of *teknologie doktor*. Foreign students who can show that they have taken a degree comparable to that of *civilingenjor* may be admitted to study for the licentiate and then for the doctor's degree by special

permission from the Governing Board of Sweden's three institutes of technology. The studies for the licentiate degree are much freer than those for that of *civilingenjor*. There are practically no lectures or any special courses, and only seminars to be attended. The main emphasis here is on scientific or technological research. For the doctor's degree a fairly long and thorough scientific training is required, and it is not uncommon for a *doktorand* (candidate for a doctorate) to have some ten to fifteen published papers.

Work for the higher degrees is generally sponsored by the research councils. As a rule, it is the professor who applies to these councils for fellowships for his students, and thus remains more or less responsible for the progress of their research work. In the applied subjects collaboration is often established with the appropriate industry. When the *doktorand* feels that he is ready for his *doktorsdisputation* he writes a review article covering his publications. This article constitutes his formal doctor's dissertation and is followed by a disputation.

THE ONTARIO RESEARCH FOUNDATION

THE annual report for 1963 of the Ontario Foundation* includes the reports of the director, the director of research and departmental directors, and a list of scientific and technical papers published during the year, as well as details of scientific and technical assistance given to outside organizations. Reference is made to the increasing number of requests received for the development of new instrumentation and measurement techniques for application in industry, and also to the growing acceptance of the Institute's project type of contract research. An intensive investigation of the chemistry of sporulation has been undertaken and an improved method of ballistically disintegrating spores has shown that the release of protein and dipicolinic acid by ruptured spores is followed by the release of an esterase.

Research was continued on high-purity iron and low alloy steel to determine the fundamental causes of fatigue by means of ultrasonic wave attenuation. In biochemistry much information was obtained regarding the part played by enzymes in the glucose metabolism of yeast. Further work was continued on the processing of fats and oils, emphasis being placed on the hydrogenation of fish oils and on the selectivity of hydrogenation of fats and oils in relation to operating variables. Fundamental information on the properties of chrysotile was accumulated with particular interest in the longer grades of fibres. In the Division of Organic Chemistry work continued to be concentrated on wood chemistry, including the utilization of wood, pulping, paper-making and paper additives. However, factors in the mechanism of absorption of wet-strength resins on paper were also investigated, and an investigation of the 4-methoxy*cyclo*hexene oxides and their ring opening reactions with nucleophilic reagents was completed. A detailed examination of many black spruce trees gave new information on the complex of interactions involving the character of the foliage, rate of growth and morphology of the wood.

In parasitology it was discovered that a lung worm of deer was responsible for paralytic disease in moose, and evidence was also gained that the same worm may cause paralytic disease in sheep. An intensive survey of small mammals was initiated to assess their role as intermediate hosts for the lung worms of carnivorous animals. Work on the attraction of different flies to different birds indicated the importance of smell, sight and touch stimuli. In physical chemistry, as part of a programme to develop non-nuclear uses of uranium, the possible application of uranium compounds as catalysts was investigated, for example, in the dehydrogenation of 1-butene to 1 : 3-butadiene. A process was developed for producing a flexible sheet for foamed-in-place shelters under field conditions, which can be operated down to −40° C, and the use of the sheet in shelter construction was demonstrated.

The work of the Physics Department was concerned chiefly with measurements on materials and development of instruments using new principles. The Department of Physiography completed a map of the surface features and glacial deposits for the area between the Kawartha Lakes and Algonquin Park, while a survey of 2,500 acres of the Humberstone peat bog was made to determine the amount of fibrous *Sphagnum* peat remaining.

* Ontario Research Foundation. Annual Report 1963. Pp. 70. (Toronto: Ontario Research Foundation, 1964.)

TIDAL COMPONENTS IN HURRICANE DEVELOPMENT

By DONALD A. BRADLEY

Research Division, College of Engineering, New York University

A TOTAL of 269 individual hurricanes evolved out of North Atlantic tropical depressions during the 60 years 1899–1958, as catalogued in a standard data source[1]. The criterion for ranking a tropical storm as a hurricane is its attainment of sustained winds of force 12 on the Beaufort scale, at which intensity stage the pressure drops rapidly and distinct organization of the system into inward-spiralling bands takes place. The dates of transition from tropical storm to hurricane status of the 269 historical cyclones in question have been investigated as frequency distributions within frames of reference defined by lunar cycles.

The convention among investigators of lunar relationships to weather[2–4] is to express the Moon's position in any cycle as hundredths of the cycle elapsed, measuring from the fiducial of the cycle. Each hundredth part thereby denotes a class-interval 3° 36′ in width. Table 1 presents the four lunar variables of key concern in this enquiry.

The lower limit of the first interval (decimal 0·00), it should be clarified, lies 1° 48′ in advance of the given fiducial point so that the interval straddles the fiducial

Table 1. Decimal Reckoning of Lunar Positions

Lunar cycle	Mean length (d)	Fiducial	Mean daily increment (r)	Moving total time equivalents 10-unit (d)	13-unit (d)
Synodic	29·5306	New Moon	0·03386	2·953	3·839
Tropical	27·3216	Vernal point	0·03660	2·732	3·552
Nodical	27·2122	Ascending node	0·03675	2·721	3·538
Anomalistic	27·5546	Mean perigee	0·03629	2·755	3·582

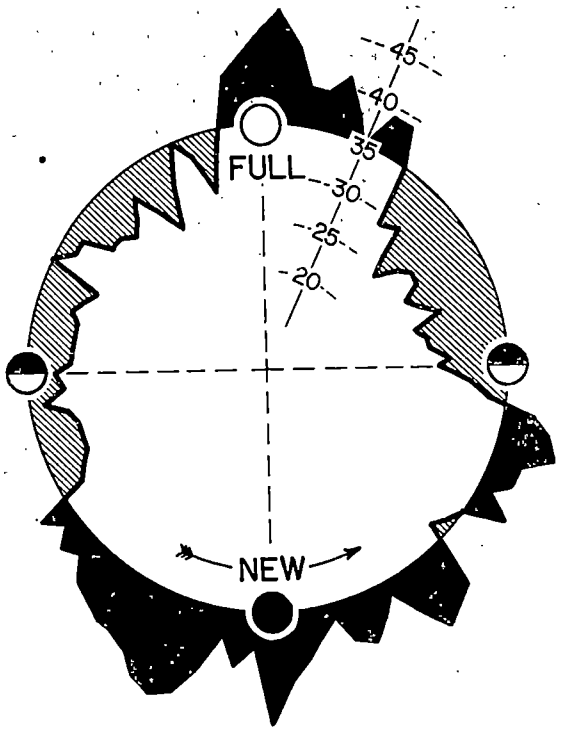


Fig. 1. Distribution along a luni-solar time-scale, shown in polar projection, of the 269 dates during 1899–1958 when North Atlantic tropical storms attained hurricane intensity. Thirteen-unit running sums of frequencies tabulated in hundredths of the 29·53-day synodic month, lunar positions taken as of 1200 G.C.T. on recorded maturation date

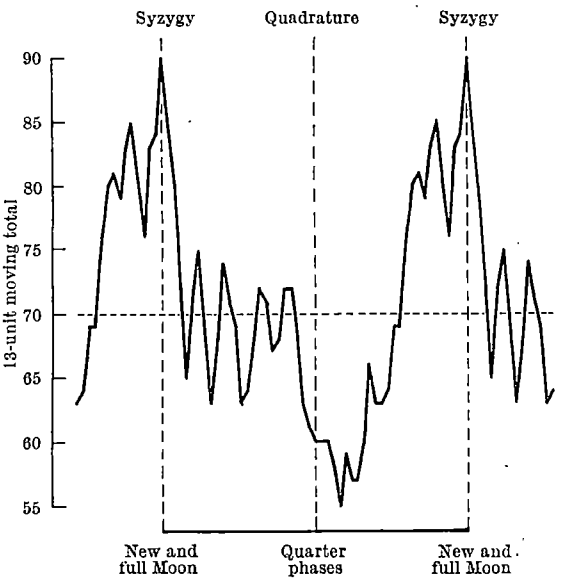


Fig. 2. The same distribution shown in Fig. 1 treated in terms of a 14·765-day cycle by superposing complementary halves of the 29·53-day synodic cycle divided at the axis of luni-solar quadrature

itself rather than commences at it. In the work recorded here, instead of using mean values, the true apparent positions of the Moon are used in two-place decimal form. As Table 1 shows, a 13-unit centred moving total, which was adopted for the distribution in synodic decimals to be discussed, roughly equates to a 3·8-day moving total, each figure thus signifying the occurrence within a segment of the cycle 46° 48′ wide. Therefore, the indicated frequency applies to activity within a range of ± 23° 24′ of an ordinate point.

Where necessary, as with handling of the lunar-distance frequencies, the curves are appropriately adjusted for differing probabilities arising from ellipticity and periodic oscillations of the Moon's orbit. For all practical purposes, it may be noted, synodic-cycle distributions of events spanning more than half a century need not be corrected for small inequalities since the divergence from linearity becomes progressively more negligible with the passage of time.

Drawing on the simplest precepts of tidal theory, if lunisolar gravitational forces impinge in any significant way on hurricane development, a statistical bias, symmetrically diphasic within the lunar month, can reasonably be expected. The two crests of such a model need not dovetail with any preconceived axis of alignment because of unknown or uncertain developmental time-lags which may be involved in generation of a hurricane. The anticipatory model requires only some statistically discernible reflexion of luni-solar variation in time and intensity.

When the 269 dates of hurricane maturation are plotted as singular events along the time-scale of the 29·53-day synodic month, a preference for near-syzygy segments of the Moon-phase cycle becomes evident. As can be seen in Fig. 1, the sunward half of the luni-solar circuit had above-average representation, the point of greatest departure from mean expectancy coinciding exactly with the event of Sun–Moon conjunction. The excess frequencies sprawl along a considerable span of the entire cycle, from shortly after last quarter phase to shortly before first quarter.

By contrast, during the other half of the cycle, excess incidence occurred only near the date of full Moon itself. The highest frequency in this hemisphere was reached within one-third of a day of the event of Sun–Moon opposition. Evaluated by several significance criteria, the distribution as a whole, with its rough symmetry around the axis of syzygy, is statistically aberrant. The primary requirement of the empirical model is therefore met.

Superposing the halves of the synodic cycle, which in effect is to treat the data in terms of the tidally fundamental 14·765-day period, yields the frequency curve shown in Fig. 2. The complete history of North Atlantic hurricane phenomena during 1899–1958 reveals that 90 of the 269 cyclones matured while the Moon was within

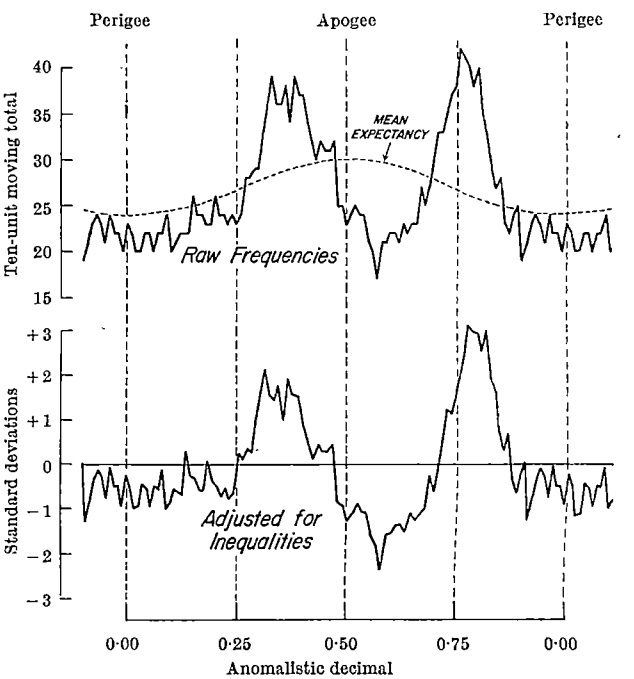


Fig. 3. Distribution of the 269 dates in terms of the Moon's longitude minus the longitude of mean perigee (top curve) and corrected for varying probabilities and orbital inequalities (bottom)

Table 2. Lunar North–South Frequencies

Group	Classification	Declination (equator)	Latitude (ecliptic)
I	Extreme north	55	56
II	Middling north	54	58
III	Near plane	57	47
IV	Middling south	48	56
V	Extreme south	55	52
	χ^2	0·870	1·428
	$n=4$	$P>0·90$	$P>0·80$

23° 24′ of the alignments known as astronomical new Moon and full Moon. In terms of mean lunar motion with respect to the Sun, this represents extreme limits of ±46 h, less than 2 days, from the hour of geocentric syzygy. The departure from mean expectancy amounts to $+2·79\sigma$ ($P < 0·01$), and, in view of its geometric agreement with expectations from accepted tidal theory, clearly argues in favour of the hypothesis of tidal components in hurricane formation suggested some time ago by L. Rodés[5] and more recently by C. H. Smiley[6,7].

Less gratifying from the point of view of theoretical expectations are the distributions of the 269 events in terms of angular departures of the Moon from the fundamental planes of either the equator or the ecliptic. Frequency tabulations of the Moon's positions in five groups for which probabilities of representation are equal in time show no significant departures from statistical likelihood (Table 2). On the basis of tidal theory and the showing made by the synodic-month distribution, a bundling of frequencies in lunar declination might reasonably be expected; but no such deviations are apparent in the north–south groupings as defined. Further investigation, however, in which the geographical co-ordinates of the individual storms are grouped with reference to the prevailing sub-solar and sub-lunar parallels of latitude, may produce quite different results.

Any model stemming from tidal theory must include, of course, the factor of varying lunar distance. The distribution of the 269 key dates along the time-scale of the anomalistic month, it turns out, is anomalous both statistically and theoretically. Fig. 3 shows both the observed and adjusted curves with their striking fold-over symmetry around points two days past the events of perigee and apogee. The trough shortly after apogee is no surprise in itself, this being the expected vicinity of minimal tidal force. What is surprising, however, is the absence of a complementary crest around perigee when tidal forces are maximal. Instead, the peaks of highest frequency were reached at points around 80° before and after the low point which lies at anomalistic decimal 0·57.

The near-orthogonality of this pattern suggested the experimental need for yet another adjustment which was incorporated into the bottom curve of Fig. 3. This adjustment was a taking into account of equal areas of *cis*-lunar space swept out by the orbiting Moon in equal intervals of time. When the proper displacements for ellipticity conditions were applied to the distribution, the two peaks were found to lie orthogonally with respect to the post-apogee low point. If the bottom curve in Fig. 3 were redrawn so that the fiducial lay at 1·9 days past the time of perigee, the peaks would fall rather neatly near 90° and 270° of 'anomalistic longitude', with the trough at 180°.

The situation thus reveals that hurricane maturation during the period 1899–1958 occurred most frequently on days when the rate of change in the intensity of the Moon's disturbing effect, and not the magnitude of the effect itself, was at a maximum. The sign of the rate of change does not appear to be important inasmuch as both the decelerating and accelerating quadrants appear to have been similarly represented in the observed frequencies.

These results suggest that the lack of significantly above-average counts around the time of perigee may be the chief reason why the possibility of tidal components in hurricane activity has been generally ignored until recently. Conceivably, previous investigators, who made probative spot-check counts of the incidence within, say, five days centred on perigee dates, may have prematurely concluded that tidal forces are of no great moment in hurricane production.

The history of hurricanes spawned over North Atlantic waters during the sixty years investigated reveals the existence of appreciably strong tidal components in the timing of their formation, as shown by significant anomalies of incidence within the luni-solar and lunar-distance cycles. The question of lunar factors involved in the generation and subsequent course of hurricanes is obviously of much importance in both theoretical and practical meteorology.

This research work was supported by the U.S. National Science Foundation, Atmospheric Sciences Program, grant *GP*–484.

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FIBRE OPTICS SPECTROPHOTOMETER FOR *IN VIVO* OXIMETRY

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REFLEXION and transmission spectrophotometry of specimens *in vitro* are well-established techniques. However, their use is difficult or impossible when the specimen is at a remote location or is in a dynamic state. For such applications, fibre optics probes in the form of flexible narrow tubes[1] or rigid hypodermic needles[2] can play an important part. Such an instrument capable of sampling remote and dynamic media consists of a probe composed of two fibre optics bundles: one to transmit light from an appropriate light source to the distal end, where it is in close proximity to the specimen, and the other to transmit the return signal from the specimen to a photodetector. An optical schematic of this instrument is shown in Fig. 1. The sensitivity and spectral response of this type of spectrophotometer would be a function of the source emission, the filter transmission (or monochromator), the fibre transmission and the photodetector response. Obviously, use can also be made of a single unaligned fibre bundle which conducts light from the source to the specimen as well as transmits the return signal to the photodetector. However, such a configuration suffers from noise because of scattering and 'cross talk' between the fibres. For this reason the use of a double-probe system, which consists of separate or intermingled fibres for the two functions, is preferred.

Various applications of such an instrument are apparent. Continuous monitoring of a liquid in process control would be possible, for example, in the chemical and food

industries. The spectral response of the system is primarily determined by the fibres, and with the availability of high-quality fibres capable of transmission in the regions from 3500 Å to 6μ and 1–10μ (ref. 3), sophisticated investigations of fluorescence and associated phenomena on remotely located specimens are possible. Furthermore, with the appropriate choice of wave-lengths, investigation can be made of dye concentration as well as flow velocities of dynamic fluid media. The application which has been of the greatest interest to us has been the use of this instrument in cardiac and vascular oximetry, which will be discussed in some detail in this article, and the role of the optical properties of blood under dynamic conditions in these measurements.

One of the principal measurements required by physicians is the oxygen saturation of the blood. A present method for this determination is to catheterize the heart by inserting a flexible hollow tube into a cardiac chamber and removing a sample of blood. The blood is chemically and physically prepared and then analysed by a Van Slyke or spectrophotometric process. This method requires a separate sample for each reading (a requirement which is medically undesirable in many clinical applications) and results in a serious time-delay before the oxygen saturation value is obtained. Since the time required to withdraw blood for an *in vitro* analysis is typically in the range of many seconds, small or rapid changes in blood conditions cannot be measured by this technique.

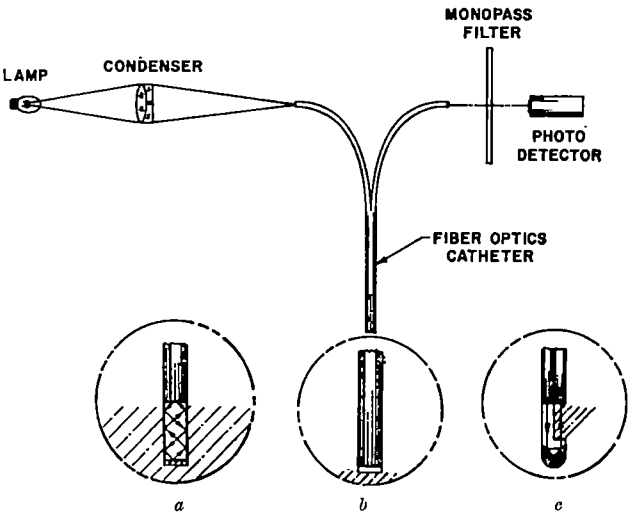


Fig. 1. Optical arrangement of *in vivo* oximeter using fibre optics, and optical designs of three different types of distal heads for: (*a*) multiple total reflexion; (*b*) reflexion; (*c*) transmission oximetry

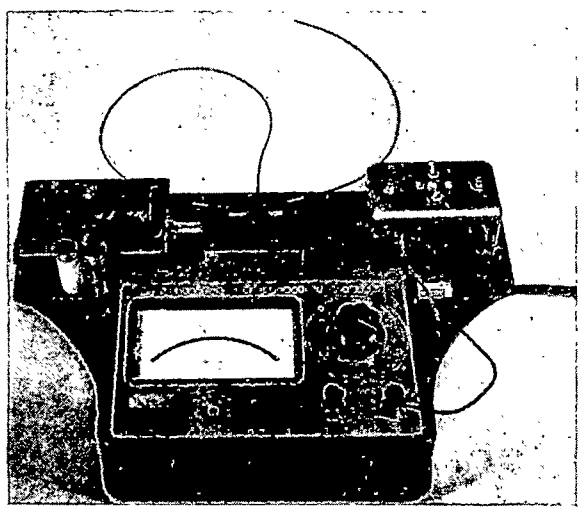


Fig. 2. Photograph of fibre optics spectrophotometer for *in vivo* oximetry. The flexible catheter consisting of fibre optics is shown at the top of the photograph

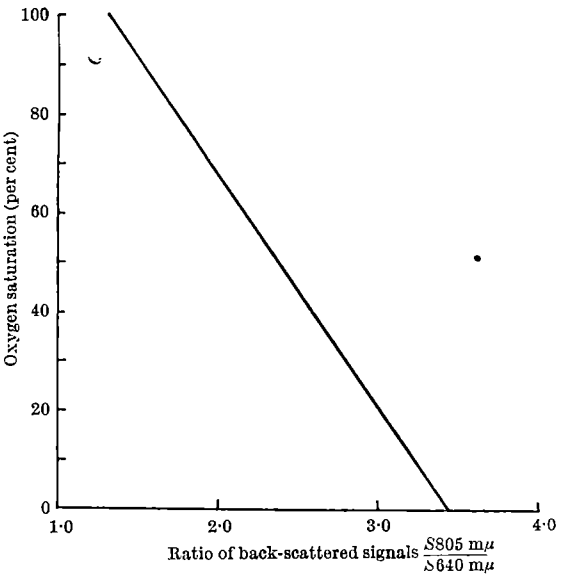


Fig. 3. Calibration of the *in vivo* fibre optics measurements against oxygen saturation determined by measurements made on conventional spectrophotometer

In vivo *oximeter*. The system illustrated diagrammatically in Fig. 1 was specifically designed to determine oxygen saturation *in vivo*. Three possible configurations of the distal head are shown. In the centre of the figure is a reflexion measuring head, which detects, at 640 mμ and 805 mμ wave-lengths, the signal generated by reflexion (back-scattering) of light from the blood cells. The head at the left is based on the multiple total internal reflexion principle similar to the photo-refractometer system[4]. The head at the right measures the signal transmitted through (or forward-scattered by) the blood within the gap. *In vivo* oximeters, of types illustrated in Fig. 1, which can be used both in 'transmission oximetry' and 'reflexion oximetry', have been constructed and tested.

Fig. 2 is a photograph of the *in vivo* oximeter. The fibre optics catheter shown at the top conducts the light from the source located on the left. A condenser lens is used to focus the light on to one of the fibre bundles and an appropriate filter (640 mμ or 805 mμ) is placed in the optical train. The return signal is transported by the fibres on to a photodetector located on the right. The signal is read out on the microammeter in the foreground, or on a strip chart recorder.

Tests of the oximeter on *in vitro* samples were satisfactory in both sensitivity and repeatability when the results were calibrated against spectrophotometric analyses of the same blood. Consequently, *in vivo* tests were initiated using live animals. The fibre optics catheter was inserted into the heart of a dog through the jugular vein. A second catheter, a standard hollow type, was passed through the femoral vein and into the same cardiac chamber. Under fluoroscopic control the two catheters were placed tip to tip. An endotracheal tube, inserted through the dog's mouth, was connected to gas cylinders of various percentages of oxygen, and in this manner the oxygen saturation of the blood was varied. The fibre optics oximeter was calibrated by matching its reading with samples that were taken simultaneously and analysed by standard laboratory techniques (Fig. 3). Similar work has been reported by Polanyi and Cournand *et al.*[5,6].

These investigations produced results so sensitive that both the respiration rate and heart beat were clearly

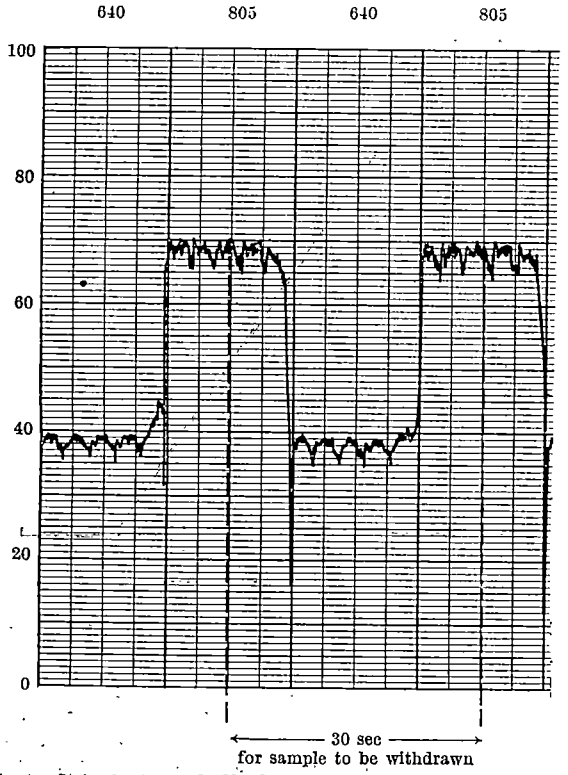


Fig. 4. Strip-chart record of back-scattering from the blood within the cardiac chambers of a dog. *In vitro* oxygen saturation, 79·4 per cent; heart rate, 186/min; respiration, 20/min

recorded on the output trace of the oximeter (Fig. 4). However, the oximeter readings versus the oxygen saturation, determined by a standard procedure, were not the same for each animal catheterization. Since the earlier *in vitro* results were satisfactory, we looked further into the optical properties of blood in a living animal to try to clarify this anomaly.

Optical properties of blood. Whole blood is a diffusing medium which does not obey Beer's Law, since the light is scattered from a random array of anisotropic non-spherical particles. Blood consists of cells and intercellular liquid, or plasma. The cells are classified as red cells, white cells and platelets. The white cells are of various sizes, but most of them in human blood are about 9μ in diameter. There are about 7,000/mm³. The platelets are short rods which are smaller than the white cells. Plasma comprises 55 per cent of the blood volume, and besides being the carrier of the cells contains many other substances, such as blood proteins, nutrients, salts and waste products. It is itself a clear, amber fluid which is 90 per cent water. The red cells, which number nearly 5 million/mm³, are disks which are 7–8μ in diameter and generally biconcave. They are a soft, flexible and elastic framework, and comprise about 44 per cent of the volume of blood. Hæmoglobin, which is deposited within the red cell, readily combines with oxygen to form an unstable compound called oxyhæmoglobin. In an oxygen-deficient environment, the oxyhæmoglobin loses its oxygen and becomes reduced hæmoglobin.

Optical oximetry is based on the colour of the blood (at 640 mμ and 805 mμ), which is dependent on the oxygen saturation. Oxygen saturation is defined as that portion of the total hæmoglobin which is oxygenated. Arterial blood, the most completely oxygenated in the body, is bright red, whereas venous blood, which has the lowest oxyhæmoglobin content, is darker. In a spectrophotometric analysis from 640 mμ to 700 mμ the largest signal difference will be indicated between oxyhæmoglobin and reduced hæmoglobin. When analysed at 805 mμ, the isobestic point, all hæmoglobin appears the same. The oxygen saturation, therefore, is proportional to the ratio of the signals received at these two wave-lengths.

In addition to oxygen saturation, many other parameters determine the optical properties of blood. These include the flow velocity, whether the flow is laminar or turbulent, the temperature, the hæmatocrit or the total number of red cells per unit volume of blood, and the concentration of any dye present. A detailed investigation into the scattering properties of blood as a function of these variables was, therefore, necessary.

For the investigation of angular scattering of blood the goniophotometer shown in Fig. 5(*a*) was designed in which an optical cell, made of two plane parallel glass plates of variable spacing, is connected to a flowing blood supply. The pump does not contact the blood itself, but partially compresses the tubing, causing pulsatile flow similar to that in a living being. The light is mechanically chopped and focused to a small spot on the cell. A photomultiplier tube, either with an *S*-20 or an *S*-1 photocathode, rotates about the centre of the cell and records the light-scattering properties of the blood as a function of angle, as various parameters of the blood are varied.

Fig. 5(*b*) is a photograph of the laboratory set-up. In the centre is the optical cell, which is connected by tubing to the blood supply in the bottle on the magnetic stirrer. The cell was carefully designed to make a smooth transition from the circular tubing to the rectangular cell so as to avoid any sharp edges that might cause turbulent flow. The pump is located at the far right. The long tube just to the right of the cell is the light source and chopper. The phototube, *x*–*y* recorder, power supplies and controls are on the left.

The equipment illustrated in Figs. 5(*a*) and (*b*) has been used for measurement of angular scattering of blood with

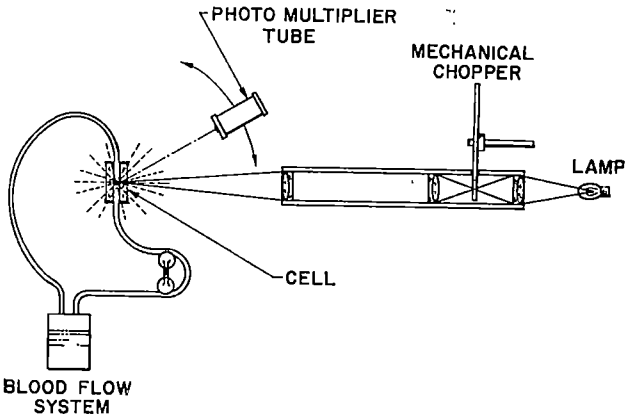


Fig. 5(*a*). Schematic diagram of the goniophotometer for measurement of forward and back-scattering of blood under static and dynamic conditions

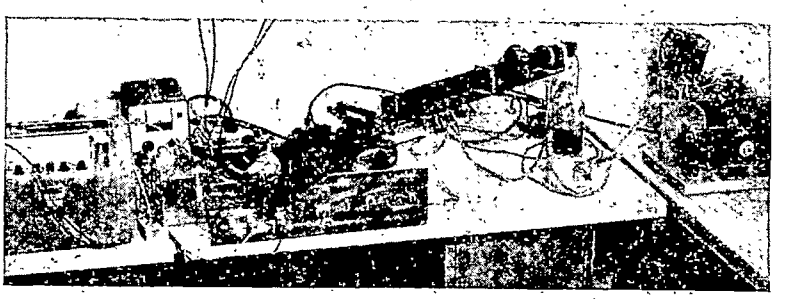


Fig. 5(*b*). Photograph of goniophotometer

different composition under static and dynamic conditions. Some representative test results obtained with the goniophotometer are shown in Figs. 6 and 7. Fig. 6(*a*) shows the scattered light-levels for 100 per cent oxygenated blood, while flowing at 0·21 l./min as a function of angle. Two wave-lengths near the region of maximum signal difference between oxyhæmoglobin and reduced hæmoglobin are shown, as are two wave-lengths near the isobestic point. It should be noted that the curves are not symmetrical. This is attributable to the asymmetrical cell orientation during laminar flow conditions. All results were collected with the photo-tube rotating in the direction of blood flow, and the highest readings are obtained in the two quadrants where the motions are in the same direction. The curves have been normalized to take account of the spectral response of the phototube and the transmission of the filters. The angular resolution of the system is approximately 1 deg. It is noted in the figure that the values fall to zero at 90 deg. This is a result of the plane parallel construction of the cell which gives rise to high internal reflexion coefficients, at the cell window, of the light scattered at highly oblique angles. For a blood thickness of 2 mm, the signal for back-scattering is almost twice as high as that for forward scattering.

Fig. 6(*b*) shows the effect of temperature on the angular scattering property of 100 per cent oxygenated blood while flowing at 0·21 l./min. This could be the result of a cellular change, since it is known that temperature variations can destroy and dissolve the red blood cells and release hæmoglobin into the plasma.

Fig. 7 indicates the effect of variation of the flow velocity on the scattering properties of blood with 100 per cent oxygen saturation. Forward scattering shows an increase in signal due to increased flow. When the flow is stopped there is a short period of decreased signal,

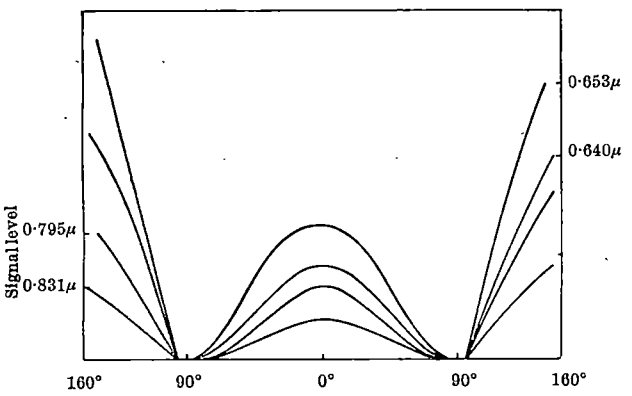


Fig. 6(*a*). Forward and back-scattering of blood with 100 per cent oxygen concentration, and flowing at 0·21/min, at 0·653, 0·640, 0·795 and 0·831μ

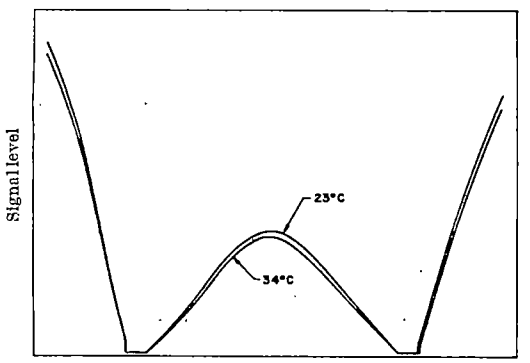


Fig. 6(*b*). Forward and back-scattering of blood at 100 per cent oxygen concentration, and flowing at 0·21 l./min, at 23° and 34° C

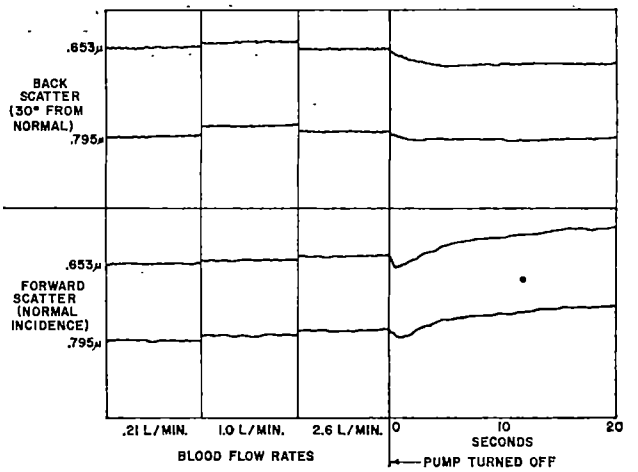


Fig. 7. Back- and forward-scattering of blood with 100 per cent oxygen concentration as a function of blood flow rate at 0·653 and 0·795μ

due to random orientation of the cells, and then there is a gradual increase in signal as the cells settle out. Back-scattering, on the other hand, shows an anomaly at the highest flow rate (well above what is normally found in the living body). It may have been that the laminar flow at the two lower flow rates changed to turbulent flow at 2·6 l./min, thereby causing a reduction in the back-scattered signal while forward scattering was increased. Additionally, there is a decrease in signal when the flow is stopped.

The results of these investigations into the optical properties of blood will be applied to the improvement of *in vivo* cardiac oximetry. They are of special significance to the photometric efficiency of the oximeter system inasmuch as the acceptance angle of the fibre optics is determined by the fibre numerical aperture. Since the ratio of the two red signals to the infra-red does not change measurably as a function of angle, the numerical aperture of the fibres does not affect the sensitivity of the *in vivo* oximeter. It is well known that fibres tend to integrate the incident light over the angle determined by their numerical aperture, and, since this does not affect the ratio of the signal at the two wave-lengths, as high a numerical aperture as possible should be used to increase the signal level.

Conclusions. *In vivo* spectrophotometry using fibre optics can be a most useful research and clinical tool in many areas of investigation, inasmuch as it permits the sampling of specimens in remote locations and in a dynamic state. The spectral properties of the specimen are immediately available, and there is no spatial or temporal integration to mask out small or rapid changes. Our experiments have shown, however, that an intensive investigation must be made when the specimen is not optically homogeneous.

It is clear that broad spectral transmission fibre optics that cover the near ultra-violet, visible and infra-red regions up to 6μ, when used with appropriate sources, filters and detectors, make possible the accurate measurement of spectral properties of remote specimens. The application of *in vivo* oximetry has been well demonstrated. An interesting example is the *in vivo* measurement of dye concentration in the heart. Furthermore, use can be made of this technique for fluorescence investigations of tissues under the skin using a fibre optics hypodermic probe.

Further detailed investigations of the angular scattering and other optical properties of the blood with different oxygen concentration, dyes and flow velocities are in progress along with investigations on the effect of laminar or turbulent flow conditions on scattering of polarized light.

We thank Dr. Herbert Hultgren and Harry Miller of the Stanford University School of Medicine and Kenneth Frame of Optics Technology, Inc., for their assistance.

This work was supported in part by Public Health Service research grant *HE* 07684 from the U.S. National Institutes of Health.

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FORMATION AND DECOMPOSITION OF ALKALINE 'PERNITRITE'

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THE reaction which leads to the formation of 'alkali–metal pernitrite' is usually carried out in the laboratory[1–3] by mixing a cold, fairly concentrated solution of alkaline nitrite with acidified hydrogen peroxide; this in turn is followed by fast alkalinization of the oxidizing mixture. The procedure, if carried out with sufficient promptness, leads to relatively fair yields and stabilization of the yellow 'pernitrite' solution, the rates of formation and decomposition of which are both strongly enhanced by the concentration of the hydrogen ion. It has so far been customary to assign the formula of H—O—O—N=O to 'pernitrous acid', although it was suggested[4] that the compound may have the structure of an acid nitrosyl-peroxide, $NO^+HO_2^-$. Neither the 'acid' nor its 'alkaline salts' have so far been isolated, and comparatively little work dealing with them has been carried out[3] despite the fact that it has been pointed out repeatedly[3,5] that solutions of the substance possess very strong oxidizing powers, in that 'pernitrite' decomposes yielding free OH radicals.

Considerable effort has recently been devoted to the examination of reactions induced in solid crystalline nitrates of alkali metals by ion bombardment[6], γ-radiation[7,8] and ultra-violet light[9], and the results obtained generally point to a decomposition of the substances examined, with ultimate formation, through free-radical steps, of nitrite ions and oxygen. At the same time recent work on both the radiolysis of alkali–metal nitrate solutions[10–12] and nitric acid[13] appears to have yielded information along a similar line, the overall reactions going through intermediate steps which may differ according to experimental conditions. It appears, therefore, quite surprising that very little work has so far been carried out on ultra-violet photolysis of nitrate solutions, although a recent contribution by Geissler[14] indicates that such alkaline solutions can be altered by ultra-violet radiation, yielding nitrites.

We feel that reaction of ultra-violet photolysis of alkali–metal nitrates may present considerable fundamental interest for a chemist, and may also offer broad interdisciplinary implications. It is in fact well known that atmospheric aerosol is largely formed of sodium chloride which, in turn, contains substantial proportions of nitrate[15–18], and that the amount of the substance in the air appears to vary with latitude[19–21]. The photolytic reaction was therefore investigated in this laboratory, and it was found that, although no apparent change in solutions of acid and neutral nitrates could offhand be obtained by ultra-violet irradiation, substantial amounts of both nitrite and 'pernitrite' appeared in concentrated alkaline solutions of sodium/potassium nitrates on irradiation, at room temperature, with the light of a wave-length shorter than 2800 Å, obtained from a high-pressure, 450-W, Hanovia mercury immersion lamp. Since it thus appears that a substance capable of slow decomposition with free OH radical yield may now be produced and temporarily 'stored' in some regions of the atmosphere during the day and according to the chemical parameters which may prevail at that time in that region, an investigation of the rates of the photolytic process has been begun in this laboratory. We shall limit ourselves here to presenting some qualitative results dealing with a few experiments on photolysis of concentrated solutions of sodium nitrate (of which terrestrial aerosol partly consists) as well as some quantitative data on the rates of decomposition of 'sodium pernitrite', under controlled conditions.

Experiments on photolysis of sodium nitrate solutions were carried out in a 5-l. 'Pyrex' flask. The flask was made to house a standard 450-W Hanovia high-pressure mercury ultra-violet lamp, which was supported in a water-cooled, quartz immersion well, built by Hanovia. The light could be filtered using filter sleeves which were inserted into the well and around the lamp. The sleeves were made of 'Vycor', 'Corex' and 'Pyrex', and therefore transparent to wave-lengths longer than 2200, 2800, 3500 Å respectively, according to specifications of the Hanovia Co. The well was immersed centrally in the solution and connected to the flask by means of a standard ground joint. The flask usually contained 3 l. of solution which could be vigorously stirred magnetically, thus enabling an intimate contact with a gas (which could be bubbled through the liquid) to be achieved during irradiation. The solution usually contained initially 500·0 $g \times l.^{-1}$ of reagent purity–sodium nitrate, and its *p*H was adjusted before each run to 12·0 with analytical reagent sodium hydroxide. A yellow coloration, due to 'pernitrite'[22], was noticed after about 1 h of irradiation with unfiltered light; the deep yellow solution was found to contain about 0·15 and 0·8 $g \times l.^{-1}$ of 'pernitrous' and nitrous acid respectively, after 24 h. The final yield appeared to be generally independent of the gas dissolved and dispersed in the liquid; the gases bubbled through the ultra-violet reactor during different tests were: nitrogen, oxygen, N_2O, all of chemical purity grade, and laboratory air. An experiment which was carried out in an evacuated flask also yielded the same result. There appeared to be no substantial changes of *p*H, as monitored by a Beckman 9600 instrument, during irradiation; no 'pernitrite' was found in solutions irradiated through 'Corex' and 'Pyrex' sleeves, after 24 h.

As observed by Gleu and Roell[1], dilute alkaline solutions of 'pernitrite' will promptly reduce permanganate to manganate only. Since it occurred to us that an estimation of 'pernitrite' by the arsenite method may yield somewhat inaccurate results[1,23], and since preliminary tests using this method led to rather scattered data, the possibility of analysing for 'pernitrite' by alkaline permanganate titration was investigated. It was found that potentiometric titration of 'pernitrite', which was prepared either by ultra-violet irradiation of nitrate solutions, or by synthesis from nitrite and hydrogen peroxide solutions, gave reproducible and satisfactory results, and all analyses were therefore carried out potentiometrically using smooth platinum versus calomel electrodes, and monitoring

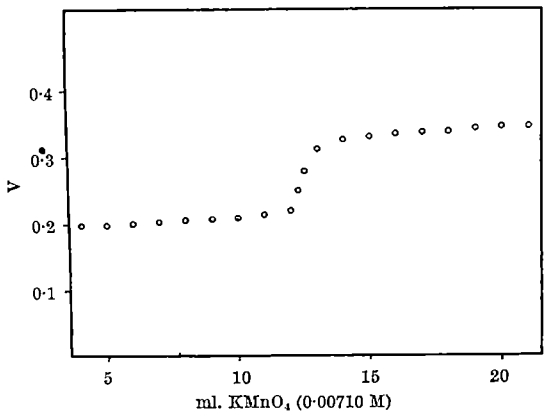


Fig. 1. Example of potentiometric titration, at room temperature, of 200 c.c. of 'pernitrite' solution prepared from $NaNO_2$ and H_2O_2, after decomposition of hydrogen peroxide by manganese dioxide

potentials by means of a Tinsley 3184-*D* instrument. It was assumed that 1 g-formula weight of 'pernitrite' corresponds, in the circumstances quoted, to 2-g-formula weights of permanganate; this was found experimentally to give results in agreement with analytical results previously quoted[1]. Fig. 1 gives an example of a representative titration.

Nitrites, in the range of concentrations used, did not appear to interfere with the procedure, and were determined on acidified solutions by the method of Lunge, as quoted by Treadwell[24]; all other salts which were used for the preparation of solutions were of analytical reagent grade and were desiccated at 140° C for 48 h, and afterwards weighed amounts thereof were dissolved in known volumes of solution.

In general agreement with the procedure described in ref. 3, aliquots of approximately 125 c.c. of aqueous solutions containing 5·0 g of sodium nitrite of analytical reagent purity were added with approximately 125 c.c. of distilled water containing 7·5 c.c. of 35 per cent hydrogen peroxide and 2·0 c.c. of 96 per cent sulphuric acid. The resulting mixture was promptly poured into 250 c.c. of aqueous solution containing 12·5 g sodium hydroxide. In further agreement with ref. 3, it was established that a delay of a few seconds in 'quenching' the acid 'pernitrite' with alkali would have brought about the decomposition of the substance. Since earlier work[23] pointed out that the decomposition of 'pernitrite' might be enhanced by hydrogen peroxide, and because hydrogen peroxide could not be found by the titanium sulphate test in the photolysed solutions, and since, probably, this peroxide is not contained in appreciable amounts in atmospheric aerosols, the excess of hydrogen peroxide was later destroyed by repulping for 5 min with about 2 g of finely subdivided manganese dioxide. This was followed by filtration through a layer of chromatography-grade silica gel, and the solution was refiltered again after standing for 2 h. The clear solutions were thereafter treated with known amounts of some of such salts as may be present in atmospheric aerosols, brought up to 1·000 litre of volume with distilled water, adjusted to *p*H 12 and left in a thermostat at 25·0° C, for periods of time ranging up to one week. Samples were taken at suitable time intervals and analysed for 'pernitrite' and nitrite by the methods outlined here.

If, in agreement with previous work[22], the process of 'pernitrite' decomposition is assumed to proceed unimolecularly, then, with other parameters constant, the following equation stands:

$$\frac{dp}{dt} = -K\,p \qquad (1)$$

Therefore:

$$\log_e p = -Kt + \log_e p_0 \qquad (2)$$

where p and p_0 stand for concentrations of 'pernitrite' at time t and t_0 respectively, and K is a constant.

Since it has already been established quantitatively[3] that there is proportionality between the rates of 'pernitrite' decomposition and the hydrogen-ion concentration, and since, on the other hand, it is known[23] that this rate strongly increases with temperature, all our experiments were carried out keeping those parameters constant. Fig. 2 gives an example of rates of 'pernitrite' decomposition with time and for different concentrations of sodium nitrate in solution, while Fig. 3 shows the relationship between constant K and the nitrate content. Concentrations of nitrite were here of the order of 1·5 g × l.$^{-1}$ and did not vary appreciably during the experiments. It was found, however, that decomposition of 'pernitrite' was 'accelerated', in our experimental conditions, by nitrite; this is substantiated by Fig. 4, which gives values of K as function of nitrite in solution and at nitrate concentrations of about 3 g × l.$^{-1}$.

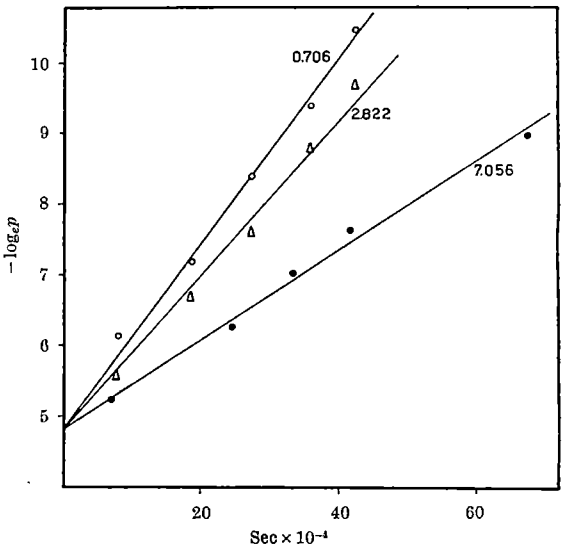


Fig. 2. Plots of natural logarithms of molar concentrations of 'pernitrite,' against time. The oxidant was prepared from nitrite and hydrogen peroxide, and the excess hydrogen peroxide decomposed by MnO_2. Molar concentrations of sodium nitrate are given near plots which were linearly regressed as a family of lines with common intercept (ref. 25)

The experiments which are illustrated in Figs. 2–4 were almost all carried out using 'pernitrite' solutions prepared from sodium nitrite and hydrogen peroxide. Preliminary results dealing with photolysed nitrate solutions gave rates of decomposition which were considerably higher than those quoted here. It was, however, noted that treatment of photolysed solutions of sodium nitrate with MnO_2, followed by filtration through silica gel, decreased the velocity of decomposition of the yellow product. The first-order rates obtained at 25° C by alkaline–permanganate potentiometry agreed very well with those obtained on peroxide-prepared 'pernitrite', as is substantiated by Fig. 3.

It can therefore be assumed that, as the photolysed solutions responded to all qualitative tests quoted in the literature in the same manner as solutions of 'pernitrite', and since they appear to decompose with identical rates at the experimental conditions described, the compound formed during ultra-violet photolysis of alkaline nitrate solutions of alkali metals effectively corresponds to the 'pernitrous' species, such as described in previous work which deals with the subject.

We have, furthermore, found that addition of chloride or sulphate ions to nitrate solutions prepared for photolysis tends to diminish the yield of 'pernitrite' formed by ultra-violet irradiation of alkaline nitrates dissolved. On the other hand, addition of small amounts of sodium chloride or sulphate to the solutions of 'perni-

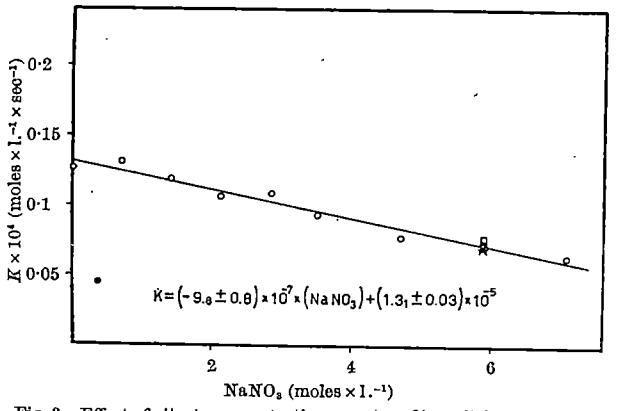


Fig. 3. Effect of nitrate concentration on rates of 'pernitrite' decomposition. All solutions were treated with MnO_2. ○, from hydrogen peroxide and sodium nitrite; □, △, ★, by photolysis of sodium nitrate with nitrogen, oxygen and air respectively

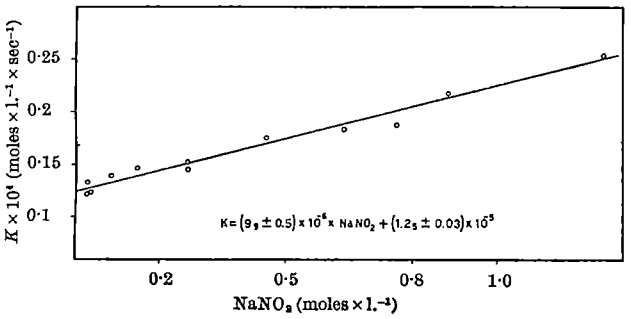


Fig. 4. Effect of nitrite concentration on rates of 'pernitrite' decomposition. All solutions were prepared from sodium nitrite and hydrogen peroxide afterwards treated with MnO_2

trite' prepared from hydrogen peroxide and sodium nitrite appears in both cases to increase slightly the rates of decomposition. Since, however, despite such additions, 'pernitrite' can still be found in the photolysed solutions of alkaline nitrate after more than 24 h of standing, it can safely be assumed that photolysed, basic, alkaline nitrate-bearing terrestrial aerosol may maintain its oxidizing activity for prolonged periods of time.

Refs. 3 and 5 strongly emphasize the role of 'pernitrite' in some organic reactions. Apart, therefore, from corrosion problems of both organic and inorganic nature, and problems which can be due to the processes of oxidation through OH-radical attack which is favoured in turn by 'pernitrite'-bearing aerosol, it is thought that the substance may have a bearing on some present-day problems, such as air-pollution and smog-formation, and that, more broadly, attention should be directed to its possible role in biology.

We thank Profs. V. Caglioti and G. Sartori for their advice. This work was supported in part by Cassa per il Mezzogiorno.

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USE OF PROTEOLYTIC ENZYMES IN STRUCTURAL INVESTIGATIONS OF WOOL KERATIN

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In order to understand the structure of keratin fibres at a molecular level we are attempting to dissolve molecules or aggregates of molecules from wool keratin by mild treatments. We hope that by carefully controlled extraction it will be possible to bring the molecules or aggregates into solution in the same configuration as they possess in the original fibre. Examination of the protein in solution by biochemical and biophysical methods might then suggest how the molecules are arranged in native wool keratin.

Proteolytic enzymes have been used to investigate the structure of proteins at two levels. Each enzyme hydrolyses protein chains at certain specific amino-acid residues, but for reaction to occur the enzyme molecule requires the part of the substrate protein containing the specific amino-acid residue to be able to assume a specific configuration in the enzyme-substrate complex[1]. With substrate molecules in a random coil this can occur, but if the molecules are in a definite native configuration, peptide bonds at the specific amino-acid residues can only be attacked if they are in parts of the molecule with some freedom of movement. For example, the triple helix of tropocollagen is resistant to pepsin and chymotrypsin, but small peptide chains attached to the molecule are digested[2]. Thus proteins in a random coil in solution are hydrolysed at all the specific amino-acid residues by trypsin, and sequence studies are then possible on the small peptides which are released[3]. When proteolytic enzymes react with proteins in their native configuration fewer peptide bonds break immediately and, instead of small peptides, large fragments of the original molecule result, which are still in a definite configuration. Limited digestion of myosin by trypsin gives rise to two large fragments[4] (S = 2·5 and 5·3). The same approach has been used in structural studies of γ-globulin[5] and fibrinogen[6].

Keratin fibres are digested only slowly by proteolytic enzymes[7], and it seems likely that stability in the presence of enzymes is necessary for keratin protein to be able to resist decomposition by micro-organisms in the absence of a blood supply as a source of antibodies. However, keratinolytic enzymes have been reported from clothes moth larvæ[8], where a high pH is necessary for attack, from the bacterium *Streptomyces fradiae*[9] which can dissolve about 30 per cent of wool at pH 9, and from other micro-

organisms[10]; in this last case losses in weight were not measured.

In this article we describe experiments designed to discover the reasons for the resistance of wool keratin to digestion by the enzymes trypsin, chymotrypsin and pepsin. In another communication we shall describe how the resistance to enzyme attack may be overcome without irreversibly denaturing the wool protein, and how large protein fragments may be brought into solution.

64's New South Wales Merino wool was used throughout this work. It was purified by soxhlet extraction for 24 h in ether, rinsed twice in ethanol at room temperature, rinsed for several days in tap water and distilled water, and allowed to dry at room temperature and humidity. It was then conditioned and stored for use at 22° C and 65 per cent relative humidity. During each run two accurately weighed samples were dried over phosphorus pentoxide *in vacuo* to determine the moisture content of the conditioned wool.

Approximately 50-mg samples of conditioned wool were accurately weighed and treated with 10 ml. of a 0·05 per cent trypsin solution in 0·1 M phosphate buffer adjusted to *p*H 7·8. The tubes containing the wool and trypsin solution were immersed in a thermostat at 35° C. Every 24 h the supernatant was poured off and a fresh aliquot of enzyme solution in buffer added. The enzyme solution was stored at 4° C. At intervals the contents of a tube were rinsed several times in distilled water, dried over phosphorus pentoxide *in vacuo* and weighed. Curve I of Fig. 1 shows the loss in weight of the wool in this treatment. In this run cortical and cuticle cells released from the fibres were lost while renewing the enzyme solution every day, and during the final rinsing. To discover whether the weight loss was due to protein dissolved from the fibre or to the loss of intact cells by the fibre, an identical experiment was carried out, except that in this run loss of cells was prevented by centrifuging the wool and enzyme solutions at 1,250*g* for 5 min before pouring off the solution and adding another aliquot, and between the final rinses. The results are shown in curve II. Comparison of curves I and II shows that the main weight loss in trypsin is due to freeing of cells from the fibres, not to protein dissolving. In all the following experiments, centrifugation was used to prevent loss of cells during and after the enzyme treatments.

During 19 days only small amounts of protein were released from wool by trypsin treatment under optimum conditions. Two explanations of this result are possible: Either peptide bonds in wool keratin cannot be hydrolysed by trypsin or, alternatively, certain bonds may be hydrolysed, but hydrogen bonds or disulphide bonds prevent release of any protein into solution.

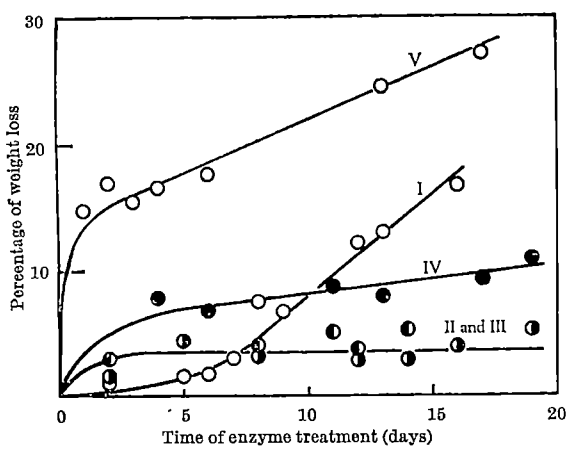


Fig. 1. Curve I: loss in weight of Merino wool treated with daily aliquots of trypsin at *p*H 7·8 and 35° C: this includes loss of cells detached by the enzyme from the fibres; curve II (◑), wool treated with trypsin as before. In this and the following runs the cells released in the treatments were collected by centrifugation and included in the final weighings; Curve III (◐), wool treated with chymotrypsin at *p*H 7·8 and 35° C followed by 8 M urea at 35° C for 12 h; curve IV, wool treated with trypsin at *p*H 7·8 and 35° C followed by 0·1 M thioglycollic acid buffered at *p*H 8·0 for 24 h at 22° C; curve V, powdered wool (see text) treated with trypsin at *p*H 8 and 35° C

Therefore, similarly weighed wool samples were treated in centrifuge tubes with chymotrypsin (wool–enzyme ratio 10 : 1) in 0·1 M phosphate buffer at *p*H 7·8 and 35° C, changing the enzyme solution daily, as before. Samples were treated for different times and then rinsed in distilled water and transferred to 8-M urea solution at 35° C for 12 h. After rinsing they were dried and weighed. Again, the weight loss in these experiments was small (curve III). Similar experiments with trypsin and pepsin at their optimum *p*H's also showed only small weight losses.

Thus a hydrogen-bond-breaking reagent did not release any protein from enzyme-treated wool. It was still possible that the enzyme might bring about some hydrolysis of peptide bonds but that protein fragments might be prevented from dissolving by disulphide cross links. Weighed wool samples were treated with daily aliquots of trypsin (wool–enzyme ratio 25 : 1) in *p*H 7·8 buffer at 35° C for up to 19 days, rinsed several times in distilled water, and then transferred to a reducing solution, 0·1 M thioglycollic acid, 0·1 M disodium hydrogen phosphate, adjusted to *p*H 8·0, for 24 h at 22° C. The enzyme treatment did not increase the amount of wool soluble in the reducing solution under these conditions (curve IV). Treatment with chymotrypsin before reduction at *p*H 8, and trypsin treatment followed by reduction at *p*H 10, gave similar results.

It appeared, therefore, that none of the wool protein was susceptible to attack by digestive tract enzymes. The small weight losses are indeed a measure of the small extent of enzyme reaction with keratin proteins. As a final check, base uptake to maintain the *p*H at 8·0 during 10 h reaction of wool with trypsin (50 : 1) at 20° C under carbon dioxide-free nitrogen in carbon dioxide-free 0·1 N sodium chloride, was compared with base uptake in a control experiment under identical conditions with no wool present. The curves are identical, showing that the only reaction which takes place is the autodigestion of trypsin.

What, then, is the cause of the resistance of the proteins of wool to attack by proteolytic enzymes? Perhaps one function of the fibre epicuticle and cell membranes[11] in Nature is to prevent enzymes from micro-organisms entering the fibre. Rather than use non-specific chemical methods to break the fibre and cell membranes, we have used mechanical methods. The same purified Merino wool was first ground under liquid nitrogen, and the light microscope shows that this produces very short lengths of fibre. After this treatment the ground wool was dried and then ball-milled in a 250-ml. glass jar five-eighths full of $\frac{3}{16}$-in. glass beads in the presence of benzene which had been distilled from sodium metal. After powdering in this way for one week at room temperature, the microscope showed pieces of fibre which were smaller than cortical cell dimensions, and, therefore, both fibre and cell membranes must have been broken. Dry 50-mg samples of the powdered wool were weighed into centrifuge tubes and allowed to react for times up to 17 days with daily aliquots of trypsin (powder–enzyme, 50 : 1) in *p*H 8 buffer at 35° C. There is an increase in the amount dissolved compared with intact wool (curve V), part of which may be due to some denaturation of the wool protein in the mechanical treatment. But the experiment shows that breaking cell and fibre membranes does not allow extensive enzyme attack. The membranes may contribute to the stability of wool keratin in solutions of proteolytic enzymes, but they are not the only cause.

Enzymes can hydrolyse peptide bonds in native proteins if part of the native structure has certain freedom of movement. Perhaps the extensive disulphide cross-linking and hydrogen bonding do not allow any part of the keratin protein molecules to come into the configuration the proteolytic enzyme requires to cause hydrolysis. Our final experiment was to extend, by 40 per cent, weighed 0·1 to

0·2-g bundles of Wensleydale fibres (purified as the Merino wool) mounted in metal frames. The extended fibres were treated with chymotrypsin (wool–enzyme ratio 20 : 1) in phosphate buffer *p*H 7·7 at 35° C for 2 days. The fibres were removed from the frame, rinsed in distilled water, dried and weighed, and the weight loss was less than 4 per cent. Altering the molecular structure of the fibres by stretching had not increased the rate of hydrolysis by chymotrypsin. Thus the configuration of the molecules in the native fibre is not the only source of enzyme resistance.

The molecules of wool keratin are, therefore, shown to be proteins which are almost completely resistant to hydrolysis catalysed by proteolytic enzymes. Deuterium exchange by native wool shows that about 25 per cent of the protein is in the α-helix structure[12], and this fraction would be expected to be resistant to enzymatic hydrolysis. Part of the remaining protein may be in a definite, repeating structure which for various reasons does not give rise to a wide-angle X-ray diffraction pattern, and again the definite structure would prevent hydrolysis. Some of the protein may also be in a random structure but so firmly held by disulphide cross-links that the molecules do not have enough freedom of movement to allow enzyme catalysed hydrolysis. Wool keratin is not highly swollen in water because of the cross-linked structure, so that enzyme penetration of the fibre may be slow.

We are at present examining chemical treatments which allow increased swelling in the fibre but do not irreversibly destroy the α-helix structure. From these swollen fibres we have evidence that large protein fragments are released by enzyme attack.

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A PROCEDURE FOR THE STANDARDIZATION OF TISSUE CULTURE MEDIA

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VARIATIONS in the purity of reagents used for Media preparations are one of the greatest sources of error encountered in the cultivation of animal cells *in vitro*. The situation is further confused by the publication of media formulæ without a precise recipe[1,2]. Both circumstances greatly influence the reproducibility of results within and between laboratories.

This problem is particularly important to those engaged in the distribution of large numbers of cell cultures having unique properties. In the course of distributing over 500 starter cultures of the human diploid cell strains *WI*-26 and *WI*-38, it soon became apparent that although the recipients used the suggested Eagle's Basal Medium[1,3] many had difficulty in propagating them. This was often traceable to variations in reagent purity and discrepancies in compounding a published formula for which no consistent recipe is available. The group of laboratories associated with the Cell Culture Collection Committee[4], which are charged with the responsibility of characterizing cultures of heteroploid cell lines distributed through the American Type Culture Collection[5], have also had similar experiences.

We have recently completed investigations with a number of widely used media formulæ which indicate that standardized media of constant composition can be prepared. The major factor contributing to its resolution was the finding by Swim and Parker[6] that, contrary to common practice, it is not necessary to dissolve, in specific groups or 'blocks', the reagents composing many media formulæ. For example, by this procedure all the amino-acids (requiring a very low *p*H), all the vitamins, and all the inorganic salts are dissolved in separate blocks. Often the constituents of each block must be added in an ordered sequence before the blocks are combined. These 'principles' of media preparation have been accepted apparently without challenge and were probably carried over to the preparation of synthetic media from the earlier and similar practices used in the compounding of balanced salt solutions. Thus, it had been tacitly assumed that the simpler procedure of pulverizing preweighed components with a pestle and mortar or ball mill and adding the mixture to water would not be successful.

The earlier report of Swim and Parker[6] indicated that although this approach was feasible under certain conditions, such compounds as L-cystine, glutamine, calcium chloride and some vitamins should be added separately. It is our purpose to report on the production of a number of the more common media by this method without any omissions and to point out the concomitant advantages.

The powdered media used in these investigations were supplied by General Biochemicals, Inc., Laboratory Park, Chagrin Falls, Ohio. Although these investigations were exhaustively performed with Eagle's Basal Medium, favourable results were obtained in our Laboratories and by others[7] with Minimal Essential Medium, Eagle[2], 199 (ref. 8) and N.C.T.C. 109 (ref. 9). The purity of the amino-acids used in these formulæ was equivalent to that recommended by the Committee on Biological Chemistry of the U.S. National Academy of Sciences[10]. The vitamins used were *United States Pharmacopoeia* grade and inorganic salts were analytical grade. Earle's, Hanks's and Gey's balanced salt solutions can also be prepared by this process. All media were prepared by combining the weighed ingredients and desiccating the aggregate over sodium hydroxide for 18 h under vacuum. The mixture was then ball-milled until a free-flowing fine powder was obtained, re-desiccated to remove moisture acquired during the milling procedure, and then packaged for use. The ball-mills are constructed of porcelain and contain 'Burundum' balls, 1·3 cm in diameter.

Eagle's Basal Medium, thus prepared, contains all the ingredients of the original formula with the one exception of sodium bicarbonate. The reason for excluding sodium

bicarbonate from the mixture was to allow wider variations in individual requirements for this substance which are dependent on: (1) the *p*H of the water used for reconstitution of the powder; (2) variations in gas-phase and liquid-phase ratios in culture vessels of differing design; (3) variations in acid-production and *p*H tolerance by different cell populations; (4) the interrelationship of these parameters with cell inoculation density. Thus the incorporation of a specific amount of sodium bicarbonate into a medium restricts its flexibility, while the absence of it permits adjustments to be made to suit individual requirements.

Powdered Eagle's Basal Medium, in aliquots sufficient to prepare 10 l., was reconstituted for use by adding 92·9 g slowly to 1 l. of de-ionized or distilled water at room temperature, agitating with a magnetic stirrer for 20 min. The resulting solution was sterilized by 'Millipore' filtration and, as a 10 *X* stock, was aseptically added to 9 l. of water sterilized in the autoclave. Alternatively, the powder can be added directly to 10 l. of water and sterilized by filtration. For cultivation of human diploid cells, the medium is supplemented with sufficient sodium bicarbonate to obtain a *p*H of 7·3, 50 μg of aureomycin per ml., and 10 per cent calf serum. The use of aureomycin instead of streptomycin and penicillin has completely prevented mycoplasma contamination in more than 5,000 tissue cultures examined during the past four years[11,12].

The assessment of efficacy of powdered Eagle's Basal Medium was made in comparison with other commercially available 10 *X* and 100 *X* liquid stock concentrates of this formula and also with liquid media prepared in our laboratories. The cell lines used in this work included HeLa, *L*, *WISH* and *H.Ep.* No. 2. The most exhaustive investigations, however, were done with the human diploid cell strains *WI*-26 and *WI*-38, which are generally considered to be more fastidious in their media requirements and also more likely to be sensitive to the presence of toxic factors. The plating efficiency, subsequent to passage for at least six generations, of *WI*-38 in powdered Eagle's Basal Medium was 18 per cent in comparison with 20 per cent obtained in control media. Cell counts made at each passage of cultures of *WI*-38 that had been growing on powdered Eagle's Basal Medium for as many as forty generations did not vary from control values. Results obtained with other diploid cell strains, primary cultures and heteroploid cell lines indicated that, of those parameters measured (for example, plating efficiency, growth rate, cell density per unit period of time, morphology, virus sensitivity, preservation and reconstitution from storage in liquid nitrogen), cell behaviour was similar in both powdered and control Eagle's Basal Medium.

The results of these experiments indicate that powdered Eagle's Basal Medium and other tissue culture media formulæ are identical in their growth-promoting properties to conventionally prepared media.

The most important of numerous advantages of dry powdered media is that large amounts can be prepared from a single source of reagents and their composition standardized. The product can then be used by large numbers of laboratories for long periods of time. So far, we have produced 100 kg of Eagle's Basal Medium for use by a number of laboratories engaged in investigations with the human diploid cell strain *WI*-38. Thus, 10,000 l. of media are available from a single standard source and the preparation of even larger quantities of powdered media is feasible. The following are also of importance:

(1) The hygroscopic powder, when kept at 5° C, dry and in the dark, has been found to be stable for at least a year. Stability investigations over longer periods are in progress.

(2) The media can be packaged to contain any volume of powder sufficient to prepare aliquots of 100 ml., 1, 5 or 10 l.

(3) Although long-term storage of the powder should be accomplished as recommended here, powdered medium is stable at room temperature for at least several weeks. Thus, it is possible to transport it in approximately 1/1,000 of the space and weight required to ship even stock liquid concentrates, thereby effecting a considerable reduction in shipping costs.

(4) Packaging of the powdered media is made in air-tight plastic bags, which all but obviates losses due to breakage or spoilage in transit.

(5) The cost of powdered Eagle's Basal Medium and other media is approximately 1/20 that of presently available commercial stock concentrates, and approximately 1/40 that of single strength commercial media.

(6) A significant cost reduction can also be realized by those laboratories which compound media from the chemical reagents themselves, not to mention the saving in time for weighing and preparation, which are liable to error.

Although not limited to Eagle's Basal Medium reconstituted from powdered media, a further refinement in the technique for sterility control of this media formulation has been attained. Contrary to the usual practice of storing prepared media at 0°–10° C, Eagle's Basal Medium is stable for at least a month at room temperature. Subsequent to reconstitution and filtration of powdered Eagle's Basal Medium, the *p*H is raised with the usual amount of sodium bicarbonate and the entire volume of media is held at room temperature for 3 4 weeks. If after this time it is found to be sterile by macroscopic observation, L-glutamine and the usual concentrations of aureomycin and serum are added before use. The question of the necessity for adding additional L-glutamine at this time is now under investigation. Media managed in this way have been found to differ not at all from Eagle's Basal Medium conventionally prepared and held at 5° C. This procedure ensures a sterility test on the entire media batch and obviates sampling errors inherent in the conventional methods.

The increasing interest in the utilization of a well-characterized human diploid cell strain for human virus vaccine production[13] results in a further practical advantage of a standardized medium available for use by numerous laboratories for long periods of time. The use of these passaged cell populations for the production of virus vaccines has resulted in the establishment of minimum criteria for vaccine production[14,15]. These criteria can now be made even more uniform by including in them the use of a standard medium.

This work was supported at the Wistar Institute by U.S. Public Health Service contract *PH* 43-62-157 and career development award 5-*K*3-*CA*-5938.

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MECHANISM OF INTESTINAL ABSORPTION: THE CONCEPT OF A SPECTRUM OF INTRACELLULAR PLASMA

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A GREAT deal of research has been carried out in the past forty years or so on the intestinal absorption of glucose, amino-acids and short-chain fatty acids, that is, nutrient substances which are now known to be actively transported against a gradient. In particular, the absorption of glucose has been intensively examined by numerous workers, yet the exact mechanism has persistently eluded all attempts at elucidation. Of the many hypotheses advanced for the mechanism of glucose absorption, I shall mention the only two which are seriously discussed in recent reviews[1–3]. The first involves chemical alteration of the compound during transport to maintain a steep gradient for free sugar. This hypothesis was originally suggested by Höber[4], and developed into the phosphorylation theory by Wilbrandt and Laszt (1933).

According to one variant of this theory, the glucose is converted to two triose phosphates and recondensed into glucose on the serosal side. Besides many objections to this theory, it was finally discredited when it was shown that both 1-deoxyglucose and 6-deoxyglucose, neither of which could be phosphorylated, were transported uphill just like D-glucose. The second hypothesis is that of the multiple hydrogen-bond theory put forward by Rosenberg[5]. According to this, reaction with a carrier involved hydrogen-bond formation between OH groups of the sugar and suitable receptors, so that each OH group contributed to the over-all affinity, but the carrier remained unspecified. Thus, little headway has in fact been made in characterizing the carrier transport mechanism, beyond a conjecture that it was probably protein in nature[6]. Further, investigations of intestinal absorption in general have so far yielded negative results, and no positive theory has been put forward successfully. Finally, none of the theories proposed has explained the anti-leakage mechanism by which glucose is prevented from diffusing back into the intestinal lumen.

The discovery of the potent action of cetrimide on intestinal absorption has provided a powerful new tool for the investigation of the complex mechanism involved. The striking reversal of glucose flow in the rabbit, guinea pig, rat and mouse (ref. 7, and unpublished observations) demonstrated the paralysis of the anti-leakage mechanism for the first time. Taylor[8] concluded from his investigations that the action of cetrimide was not specific, but resulted probably from interference with cellular aerobic metabolism or damage to the intestinal mucosa. This cannot be a correct explanation, however, for in the rabbit the absorption of methionine and butyrate continued undiminished at concentrations of cetrimide which caused the glucose to leak back into the lumen. The effect of cetrimide was reversible both *in vivo* and *in vitro* perfusion experiments, as the drug was gradually washed out of the mucosal cells (Nissim, unpublished observations). These findings, and others previously reported, on the mode of action of this drug made it clear that cetrimide combined with the enzyme or carrier concerned with the active transport of glucose, after passing through the first, phloridzin-sensitive, receptor[7]. It was thus envisaged early that labelled cetrimide might be used to isolate this second receptor, be it enzyme or carrier, but it was soon realized that cetrimide, being a cationic surfactant, combined easily with a large number of proteins. This fact indicated that the proposed method for identification might prove a rather difficult one technically.

Concurrently with the accumulation of data on the mode of action of cetrimide, evidence was gathered that many other substances which combined with protein in a non-specific or semi-specific manner possessed the property of inhibiting glucose absorption to a greater or lesser extent. Thus, earlier studies demonstrated that the anionic sodium lauryl sulphate, which has a similar affinity for binding protein generally, stimulated and reduced glucose absorption in appropriate doses just as cetrimide did[7]. Vital dyes, such as phenol red, and mercuric ions[9], are known to reduce glucose absorption, and both are protein-binding agents. Likewise, digitalis derivatives, which bind certain proteins with avidity, inhibit active sugar transport in frog intestine at concentrations of 10^{-7} (ref. 10). The multiplicity and variance of these protein-binding compounds strongly suggested some non-specific or partially specific mechanism.

The next important clue concerning the nature of the second glucose receptor resulted from attempts to understand the stimulation of glucose absorption with small doses of cetrimide and of sodium lauryl sulphate. Surface activity was excluded as a likely cause, for these stimulating concentrations produced marked inhibition when combined with small doses of phloridzin[7]. This fact therefore pointed to a mobile intracellular protein carrier as the second receptor effecting active glucose transport. Wilbrandt and Rosenberg[2], in an investigation of competitive and inhibitor mechanisms in carrier transport, described five possible models, only one of which is capable of producing stimulation with small doses of the inhibitor substance. The full expression for the velocity of transport (v) is[11]:

$$v = \frac{v_{max}\,(I' + 1 - S'_1S'_2)\dfrac{(S'_1 - S'_2)}{(S'_1+1)\,(S'_2+1)\,(S'_1+I'+1)\,(S'_2+I'+1)}}{} \qquad (1)$$

where S'_1 and S'_2 represent substrate concentration at the two aspects of the cell membrane, or two poles of the cell itself, divided by the respective K_m or dissociation constant for substance and carrier. I' represents the corresponding equilibrium value for the inhibitor drug, the concentration of which is assumed to be the same at the two termini of interaction. Stimulation of transport is obtained when $(I' + 1 - S'_1S'_2)$ is negative and inhibition when it is positive. As previously indicated[12], this second receptor would be a protein. Furthermore, it has to be mobile in order to be exposed to two different media in which the dissociation constant (K_m) would be different. Since such protein movement is unlikely to take place within the cell membrane, the second receptor or carrier would be intracellular. With the emergence of this definite hypothesis for the transport mechanism, it was decided to put it to the test. This was done by investigating the effects of chloroquine, thevetin and phloridzin at a wide range of concentrations. Chloroquine, an antimalarial drug, was chosen on account of its affinity for protein, for it gives 98 per cent binding in plasma[13]; furthermore, it has not apparently been tested previously for its effect on glucose absorption. Thevetin, a digitalis derivative, was shown by Csáky *et al.*[10] to be a potent inhibitor of intestinal glucose absorption in *Rana pipiens*, but has not

Table 1. EFFECT OF DIFFERENT DRUGS ON PERCENTAGE INTESTINAL ABSORPTION (MEAN ± *S.E.*) OF GLUCOSE FROM PERFUSION FLUID
No. of experiments in brackets

Drug concentration (w/v)	Chloroquine phosphate (*C3H* mice)	Thevetin (Albino *AG*-2 mice)	Phloridzin	
			(*C3H* mice)	(Albino rats)
0·0	34·6 ± 0·9 (34)	42·1 ± 1·7 (21)	34·6 ± 1·7 (34)	43·0 ± 1·9 (10)
10^{-9}				41·8 ± 1·2 (4)
5×10^{-9}			35·0 ± 3·4 (8)	39·2 ± 2·6 (4)
10^{-8}			43·6 ± 1·9 (12)	44·4 ± 2·9 (8)
5×10^{-8}			41·2 ± 1·6 (12)	38·9 ± 2·9 (4)
10^{-7}			37·1 ± 1·4 (4)	40·2 ± 3·2 (4)
5×10^{-7}			36·5 ± 0·7 (4)	32·5 ± 2·9 (4)
10^{-6}	32·9 ± 4·7 (4)		34·4 ± 0·9 (4)	29·7 ± 2·5 (4)
5×10^{-6}		41·8 ± 3·3 (4)	30·6 ± 3·0 (4)	
10^{-5}	38·5 ± 1·5 (6)	49·7 ± 1·4 (21)	29·8 ± 1·7 (4)	18·0 ± 1·6 (4)
$2{\cdot}5 \times 10^{-5}$	38·4 ± 6·2 (4)			
5×10^{-5}	42·1 ± 1·8 (17)			
10^{-4}	39·6 ± 1·7 (4)	35·6 ± 3·1 (2)	10·0 ± 0·5 (4)	12·2 ± 1·0 (4)
10^{-3}	33·8 ± 1·6 (4)	31·3 ± 0·9 (4)	−0·2 ± 1·4 (4)	3·7 ± 0·5 (4)
5×10^{-3}		28·6 ± 2·7 (4)		
10^{-2}	27·0 ± 0·7 (4)			
2×10^{-2}	21·9 ± 0·6 (4)			
P value for '*t*' test between control and group showing greatest absorption	$< 10^{-5}$	$< 10^{-3}$	$< 10^{-5}$	$< 0{\cdot}7 > 0{\cdot}6$

been examined in other species. Since phloridzin produced glucose reversal in the mouse but not in the rat, it appeared to block receptors 1 and 2 in the former, but only receptor 1 in the latter. Only combination with receptor 2 was likely to produce enhancement of glucose absorption, and the compound was therefore investigated at a full range of concentrations in both species.

Chloroquine phosphate was studied in male *C3H* mice, thevetin in male albino (*AG*-2) mice and phloridzin in male *C3H* mice and albino rats. Combinations of drugs were studied in *C3H* mice. In mice, 25 ml. of 0·2 per cent (w/v) of D-glucose, D,L-methionine and sodium butyrate in 0·9 per cent sodium chloride, containing the desired concentration of the drug or combination of drugs, was circulated for half an hour through the lumen of the small intestine as previously described[7]. In rats, 50 ml. of the same control solution with the required phloridzin concentration was circulated for 0·5 h. Only the glucose was estimated in these experiments, by a modification of the method of Haslewood and Strookman[14]. 10^{-3} Evans blue was examined in four *C3H* mice under parallel conditions.

Evans blue. Inhibition by 10^{-3} Evans blue was of the same order as that by 10^{-3} sodium lauryl sulphate. The mean percentage absorption of glucose in the four experiments was 25·0 ± 1·1 as compared with the control value of 34·6 ± 0·9 in 34 experiments ($P \simeq 10^{-3}$).

Chloroquine and thevetin. The experiments are summarized in Table 1. In Fig. 1 the new results are compared with those previously obtained with cetrimide and sodium lauryl sulphate[7]. There was evident stimulation of glucose absorption with both drugs, which showed maxima at 5×10^{-5} chloroquine phosphate ($P < 10^{-5}$) and 10^{-5} thevetin ($P < 10^{-3}$). The inhibitory effect of both drugs was moderate.

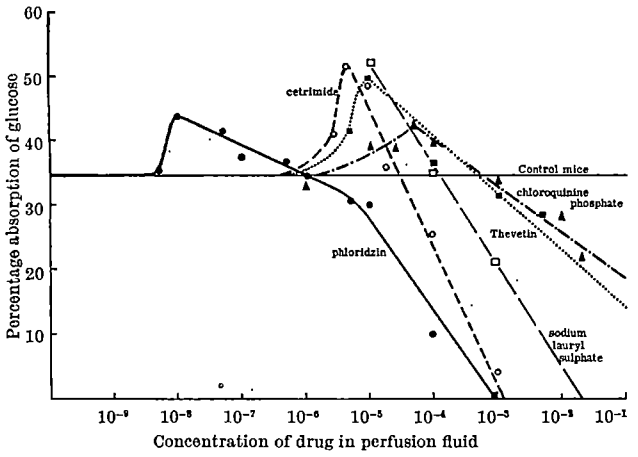


Fig. 1

Phloridzin. The results differed in the two species (Table 1, Figs. 1 and 2). In mice there was definite stimulation at concentrations of 10^{-8} ($P < 10^{-5}$) and of 5×10^{-8}. In rats there was only the random small variation about the control value with concentrations between 10^{-7} and 10^{-9}, so that no significant stimulant effect was obtained. Furthermore, the dose-response line was evidently composite in mice, with a sharp bend at about 10^{-6}, but straight in rats, right up to the point where it intersected the control line. There was marked inhibitory effect in both species with large concentrations.

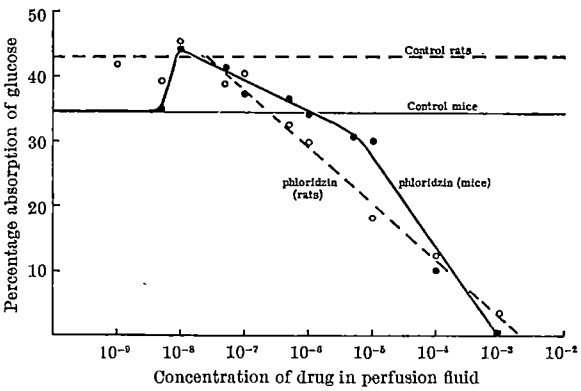


Fig. 2

Drug combinations. There was clear antagonism between phloridzin (10^{-4}) and chloroquine phosphate, increasing with the concentration of the latter drug ($P < 0{\cdot}01$). Thevetin also appeared to reduce the effect of phloridzin, but with the present number of experiments, P is just smaller than 0·1. The effect of 10^{-3} cetrimide was completely abolished with concentrations of 2×10^{-2} chloroquine phosphate ($P < 10^{-6}$). Curiously enough, the effect of chloroquine phosphate also seemed to be decreased by cetrimide ($P < 10^{-4}$), for the percentage absorption obtained with the mixture is significantly higher than with either drug alone.

The expected stimulation of glucose absorption has been substantiated, at very small P values, for both chloroquine and thevetin. Since neither compound is surface-active, the stimulation cannot be attributed to this physical effect. In the case of cetrimide and sodium lauryl sulphate, this was the first obvious mechanism which had to be excluded by other findings as mentioned before.

The striking stimulation of glucose absorption obtained with low concentrations of phloridzin was at first surprising, but when the significance of glucose reversal in the mouse was realized, namely, that phloridzin acted on the second receptor as well in this species, the result

became one of added interest. This composite action of phloridzin on the two receptors was evidently responsible for the sharp bend in the dose-response line in the mouse. The realization of this led to detailed testing of phloridzin in the rat, but neither stimulation nor reversal was ever obtained, and the dose-response line remained straight. Analysis of variance for the line between the doses 5×10^{-8} and 10^{-3} showed highly significant linear regression and non-significant deviation from regression.

The stimulation of glucose absorption in the mouse by these five totally different compounds, all of which are inhibitors of absorption at higher concentrations, leaves little doubt as to the mechanism in question. It is also clear that the drug-receptor combination cannot be very specific in nature. This view is strengthened by the antagonism between chloroquine and phloridzin and between chloroquine and cetrimide (Table 2), as it is also supported by the potentiation between phloridzin and cetrimide with regard to inhibition of α-D-glucosamine absorption when the concentration of the latter is low and their antagonism when the concentration is high[12]. The binding between protein and detergents, for example, is known to be a "totally unspecific reaction"[16]. This binding is through polar as well as non-polar groups and is dependent on *p*H. A protein of ovalbumin may bind up to 100 molecules of dodecylamine[15]. Evidently, cytoplasmic proteins can bind inorganic ions, various nutrient substances and numerous drugs. The results here indicate that these mobile proteins constitute the second receptor in the mechanism of absorption.

Table 2. Effect of Different Drug Combinations on Percentage Intestinal Absorption (Mean ± *S.E.*) of Glucose from Perfusion Fluid

Values represent mean of four experiments (except where indicated in brackets) in *C3H* mice

Drug concentration (w/v)	Percentage absorption	*P*
Phloridzin, 10^{-4}	10·0 ± 0·5	
Phloridzin, 10^{-4} + chloroquine, 10^{-4}	11·6 ± 1·6	
Phloridzin, 10^{-4} + chloroquine, 10^{-3}	12·6 ± 1·7	
Phloridzin, 10^{-4} + chloroquine, 2×10^{-3}	21·1 ± 2·6	< 0·01, compared with phloridzin, 10^{-4}
Phloridzin, 10^{-4} + thevetin, 10^{-3}	15·5 ± 2·7	< 0·1 > 0·05, compared with phloridzin, 10^{-4}
Cetrimide, 10^{-4}	25·2 ± 2·0 (14)	
Cetrimide, 10^{-3}	3·7 ± 1·5	
Cetrimide, 10^{-4} + chloroquine, 2×10^{-2}	22·2 ± 3·1	
Cetrimide, 10^{-3} + chloroquine, 2×10^{-2}	28·2 ± 0·4	< 10^{-6}, compared with cetrimide, 10^{-3} < 10^{-4}, compared with chloroquine, 2×10^{-2}

The foregoing results suggest therefore that, instead of a single and specific protein carrier for each nutrient, we have a spectrum of intracellular proteins—the intracellular plasma—with different affinities for nutrients as well as for drugs. The concept is supported by the fact that there is in intestinal absorption partial competition between different nutrients, for example, between glucose and amino-acids, and glucose and sodium butyrate[7,17]. This hypothesis of a spectrum of intracellular plasma fits in particularly well with the marked species differences and different activities of drugs previously reported[7]. The emerging model of the mucosal cell as an absorptive unit may be represented diagrammatically as in Fig. 3. The first receptor is on the inner aspect of the cell membrane. The association constant of the mobile second receptor must be greater at the mucosal pole of the cell, while the dissociation constant must be greater at the serosal pole. This greater affinity on the mucosal side between the intracellular proteins and actively transported substances must depend on different metabolic activities at the two opposite ends of the cell, namely, at *D* and *E*. These metabolic activities maintain an intracellular gradient in *p*H, redox potential, electrical potential or a combination of all three, and it is the maintenance of this gradient in the live cell which requires the expenditure of energy. When these energy-

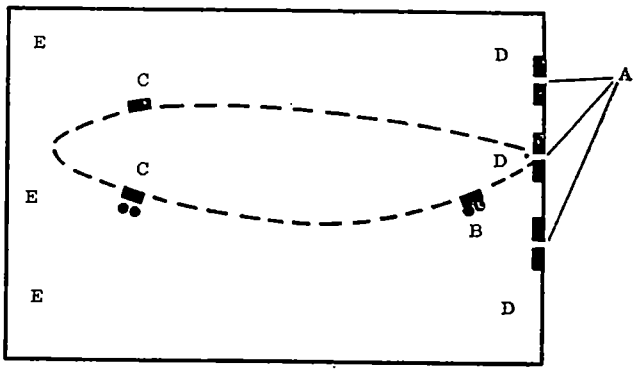


Fig. 3. Model of intestinal mucosal cell as an absorptive unit for nutrients, illustrating in particular the absorption of glucose. *A*, pore for sugar entry with first protein receptor on inner aspect of cell membrane—sensitive to phloridzin; *B*, second protein receptor, responsible for active transport, with molecules of sugar attached, at mucosal pole of cell—sensitive to cetrimide; *C*, second protein receptor, dissociated from molecules of sugar; *E* and *D*, sites of metabolic activities, at opposite poles of cell, responsible for *p*H or electrochemical gradient and giving different values for dissociation constant between nutrient and carrier protein

requiring metabolic processes are poisoned, for example, by 2,4-dinitrophenol, by iodoacetate or by other enzyme poisons, absorption is inhibited, for the second receptor cannot function properly with the same K_m value at the opposite poles of the cell. The theory explains all the odd assortments of facts which have accumulated so far, and which are too diverse to be enumerated here. It is pointed out, however, that the theory does not exclude relatively highly specific mechanisms for the absorption of some nutrients, for it is known that the absorption of cystine, for example, requires a special 'carrier', which is deficient in the congenital condition of cystinuria[18].

The concept advanced here of the mechanism of intestinal absorption may have wide applications in other fields of physiology and pharmacology, and may well result in the re-orientation of the whole of research on absorptive phenomena. It may also help in the detection of specific mechanisms, where they exist, more easily.

Finally, since cetrimide, at a concentration of 10^{-3}, completely paralyses the second receptor in the rabbit[7], so that glucose leaks into the intestinal lumen until its level equals that of the blood sugar, we may use data from these experiments to calculate the permeability constant (k) for glucose in this species. Using the well-known formula[1]:

$$k = \frac{V}{At} \cdot \ln \frac{C_{out} - C_{in}}{C_{out} - C'_{in}} \qquad (2)$$

where A represents surface area, V, volume of fluid, C_{out}, concentration of glucose outside the lumen, that is, in the blood, and C_{in} and C'_{in} the concentrations in the lumen before and after the interval of time t, we can determine k by substituting experimental values on the right-hand side of equation (2). The rise in glucose concentration per hour, in reversal experiments, shows an average of 40 mg or 0·01 per cent (C'_{in}) per sec—from zero (C_{in}). Taking C_{out} as equal to 200 mg per cent, $V = 250$ ml. and A as 6,000 cm²—after allowing for the 30-fold increase in surface area due to the convolutions in the brush-border or microvilli[3], we find, for $t = 1$ sec:

$$k = \frac{200}{6{,}000} \cdot \ln \frac{200 - 0}{200 - 199{\cdot}98} = 2{\cdot}295 \times 10^{-6} \text{ cm/sec} \qquad (3)$$

The energy required for preventing back diffusion is given by the formula[19]:

$$\text{Energy} = W = n_1 RT \cdot \ln(c_1/c_2) \qquad (4)$$

where n_1 represents the moles of substance transported in unit time, R, the gas constant, T, the absolute temperature, and c_1 and c_2 the final concentrations on the two sides of the membrane. Substituting in equation (4) from the same experimental data, and taking the total

weight of the rabbit small intestine as equal to 50 g, we obtain :

$$W = 43{\cdot}1 \text{ ergs/g/sec} \quad (5)$$

Parallel calculations for mice yield the same value for k, but the value of W is 77·7 ergs/g/sec, that is, nearly double the corresponding value in the rabbit. The explanation for the difference lies probably in the higher metabolic rate of smaller animals.

Reversal experiments with cetrimide in the rabbit and with cetrimide and phloridzin in the mouse have thus enabled us to determine the value of the permeability constant (k) for glucose *in vivo* for the first time. It is some 35 times smaller than the permeability constant for urea in red cells[1]. The calculated energy requirement for preventing glucose leakage in the mouse represents only about 1 per cent of the total energy expenditure by the intestinal mucosa, estimated at about 9,298 ergs/g/sec (ref. 20).

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PATHOGENESIS OF THE POST-NEONATAL THYMECTOMY WASTING SYNDROME

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DURING the past seventy years, several investigators have reported a cachectic or wasting disease following thymectomy in a variety of animal species including frogs[1], guinea-pigs[2], dogs[3] and goats[4]. It is difficult to determine whether this 'thymic cachexia' was the result of environmental factors, sepsis or actual lack of thymus tissue. Other investigations suggested that thymectomy in young animals did not lead to any alterations in growth or maturation[5]. Thymectomy, at or after 3 weeks of age, was performed in many strains of mice in work on experimental leukæmia. A review of these investigations has revealed no differences in growth, breeding behaviour, life-span, or susceptibility to common laboratory infections in thymectomized and control mice[6]. Some strains of mice thymectomized at birth grew normally for a time but then developed a syndrome characterized by marked wasting, lethargy, ruffled fur, hunched posture, sometimes diarrhœa and eventually death[7].

This condition closely resembles the syndromes seen in graft-versus-host reactions such as runt disease in mice injected at birth with allogeneic lymphoid cells[8], homologous disease in F_1 hybrid mice injected with parental lymphoid cells[9] and secondary disease in allogeneic[10], and even in some syngeneic[11], radiation chimæras. The earlier in life thymectomy was performed the higher the incidence of the wasting syndrome[12]. Thymectomy after 1 week of age was no longer associated with any significant incidence of this syndrome. About 50 per cent of wasted mice have been reported to show necrotic foci in the liver: granulomatous patches with aggregates of polymorphonuclear leucocytes, macrophages and giant cells[13,14]. These lesions have been associated with the presence of a hepatotrophic virus[14,15]. Other features of wasted mice are lack of subcutaneous fat, thinness of skin and intestinal walls, marked deficiency of small lymphocytes in blood and tissues[7,12,13], anæmia and proliferation of reticulo-endothelial elements in the liver, lymph nodes and spleen[16]. A small percentage of rats thymectomized at birth developed a similar wasting syndrome[17]. According to some investigators[18], thymectomy of male but not of female hamsters, as late as 2–4 weeks of age, was associated with the development of an essentially similar wasting syndrome.

The cause of the post-thymectomy wasting disease is obscure. Various theories can be proposed: infection, auto-immunity, loss of trephocytic lymphocytes, or loss of some thymus-specific growth-promoting factor. The fact that mice thymectomized at birth show immunological deficiencies[12], and are more susceptible to some effects of viral agents than sham-operated controls[14,19,20], suggests that their resistance to certain pathogens may be diminished. The fact that mortality in neonatally thymectomized mice was significantly reduced when they were "kept under nearly pathogen-free conditions"[21] and did not occur in "specific pathogen-free" mice[22] is in support of the infection hypothesis. Against this hypothesis can be cited the evidence that wasted mice were producing normal levels of γ-globulins and antibodies against certain standard antigens[23].

The similarities between graft-versus-host disease and the post-neonatal thymectomy wasting syndrome have been stressed[12]. These are: hyperplasia of the reticulo-endothelial system (proliferation of histiocytes, pyroninophilic cells, Kupffer cells), increased phagocytic activity, extensive extramedullary hæmopoiesis, diminished immunological competence, terminal lymphoid aplasia and trophic disturbances[16,24]. In graft-versus-host disease, pathological processes are initiated by immunologically competent cells with reactivities against xenotopes. After neonatal thymectomy, by analogy with graft-versus-host reactions, it might be postulated that immune, and

* This work was carried out while one of us (J. F. A. P. M.) was a recipient of an Eleanor Roosevelt International Fellowship at the National Cancer Institute.

therefore auto-immune, reactions are operating. In the absence of the thymus during the period of development of the lymphoid system, clones of cells with reactivities against idiotopes would develop unchecked, proliferate, and inflict damage on body constituents in the same manner that grafted lymphoid cells cause damage in the graft-versus-host reaction. The immunological incompetence of thymectomized animals would then be due to the 'immunological preoccupation' of the lymphoid cells with an auto-immune process. Against this hypothesis is the lack of any direct evidence in favour of the operation of auto-immune reactions, the failure to transfer the wasting syndrome to normal new-born hosts with lymphoid cells from neonatally thymectomized mice of the same strain[25], and the prevention of the syndrome by lymphoid cells from non-thymectomized donors of the same strain[26].

It was originally suggested by Carrell[27] that lymphocytes may be a mobile source of nutrients which promote growth and proliferation of other cells. Other investigators have supposed that the 'trephones' which lymphocytes might supply were nucleic acid fragments which were incorporated undegraded into the nucleic acids of proliferating cells[28]. The fact that failure to thrive is seen in conditions associated with deficiency of lymphocytes has led Loutit to postulate that the trophic disturbances seen in such conditions may be dependent on the loss of cells with trephocytic functions[29]. However, no direct evidence has been obtained to show that failure to thrive is dependent on a deficiency of any particular substance provided specifically by the lymphocyte.

The existence of specific thymus-growth promoting factors has been postulated by several investigators[30], but direct evidence for these is lacking.

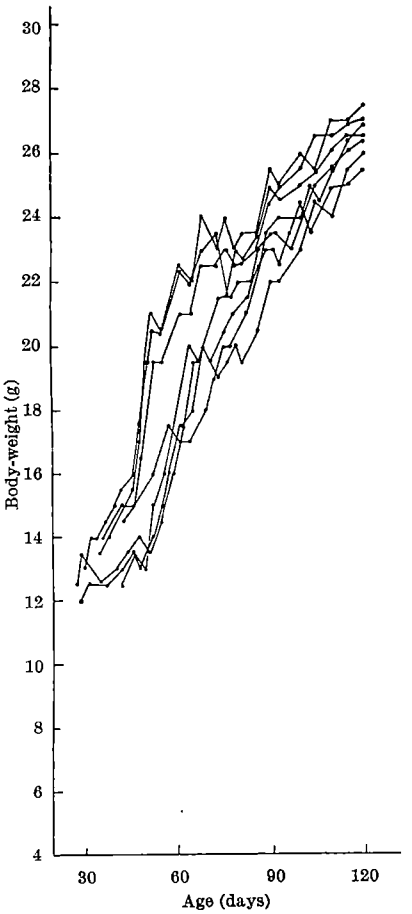


Fig. 1. Weight curves of conventional *C57BL* male mice sham or partially thymectomized at birth

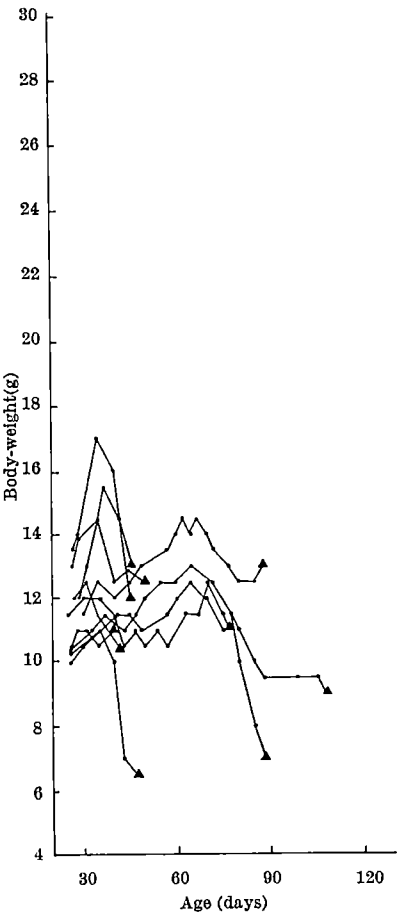


Fig. 2. Weight curves of conventional *C57BL* male mice completely thymectomized at birth. ▲, death from wasting disease

In order to determine whether infection plays a part in the pathogenesis of the post-neonatal thymectomy wasting syndrome, investigations were made on one highly inbred strain of mice under three environmental conditions: (1) conventional mice raised under ordinary laboratory conditions; (2) low pathogen mice originally obtained from germ-free mice and raised outside the germ-free environment, but isolated from all other mouse stocks; and (3) germ-free mice. All the mice were derived originally from the highly inbred *C57BL/Ka* strain maintained at the National Institutes of Health. Another highly inbred strain available in the germ-free environment was derived from the *BALB/c/An* line maintained in the same Institute.

In one experiment, *C57BL* mice of all three groups were thymectomized at birth by a method similar to that used for adult mice[31]. Body-weights were taken weekly and skin grafts from *BALB/c* mice were performed in some of the mice at 4–8 weeks of age. Controls were sham-operated at birth. Mice were killed and autopsied at appropriate periods and sections of thymus area, spleen, lymph nodes, liver, kidneys and ileum were fixed in Tellyesniczky's fluid and stained with hæmatoxylin and eosin.

There was a striking difference between the growth curves of conventional germ-free and low pathogen *C57BL* mice when thymectomized within 24 h of birth. More than 90 per cent of the conventional mice wasted between 5 and 10 weeks, whereas the germ-free and low pathogen mice showed no significant weight loss and no difference in size from untreated and partially thymectomized litter-mates. Individual weight curves of some of the mice in the various groups are shown in Figs. 1–7. The growth rates of an additional 49 completely thymectom-

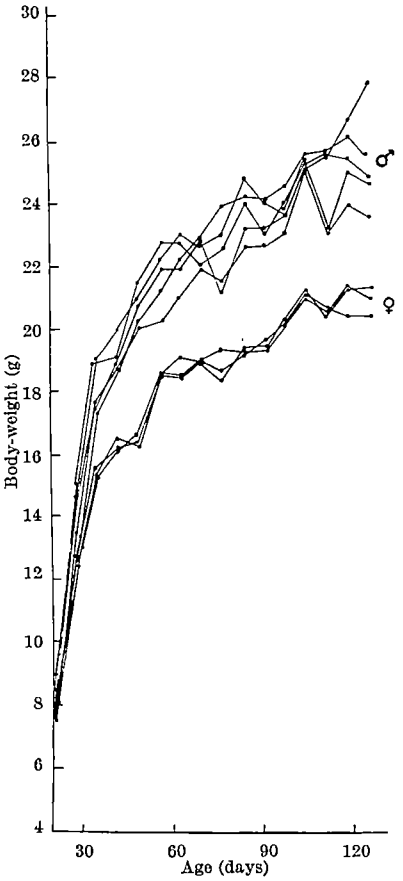


Fig. 3. Weight curves of untreated germ-free *C57BL* male and female mice

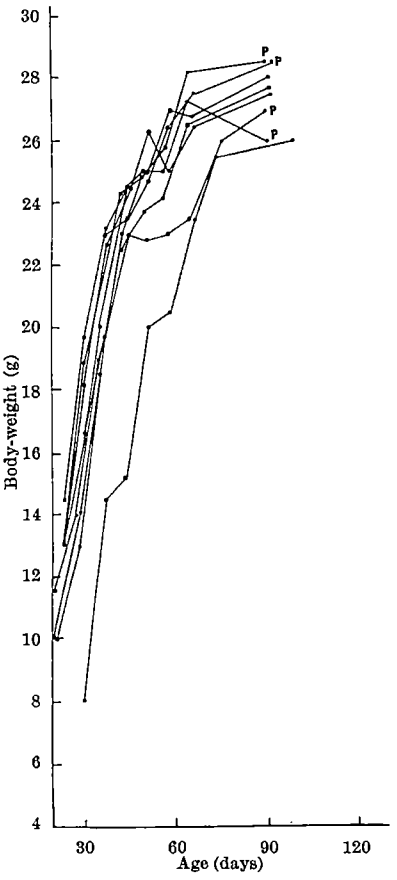


Fig. 4. Weight curves of germ-free *C57BL* male mice thymectomized at birth. *P*, partial thymectomy

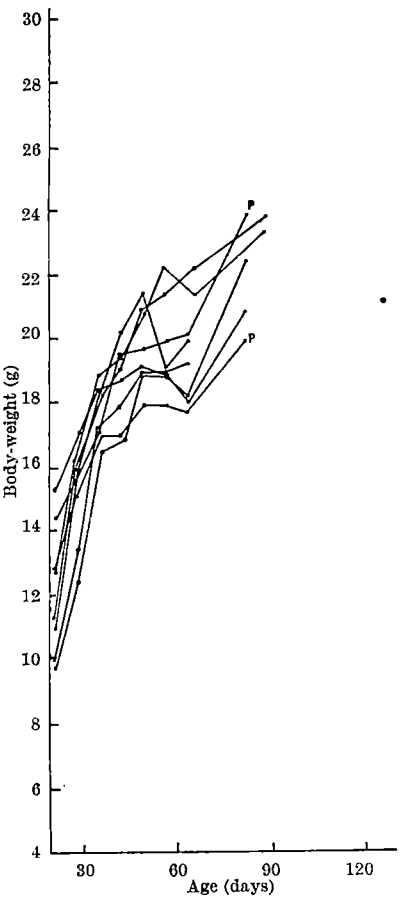


Fig. 5. Weight curves of germ-free *C57BL* female mice thymectomized at birth. *P*, partial thymectomy

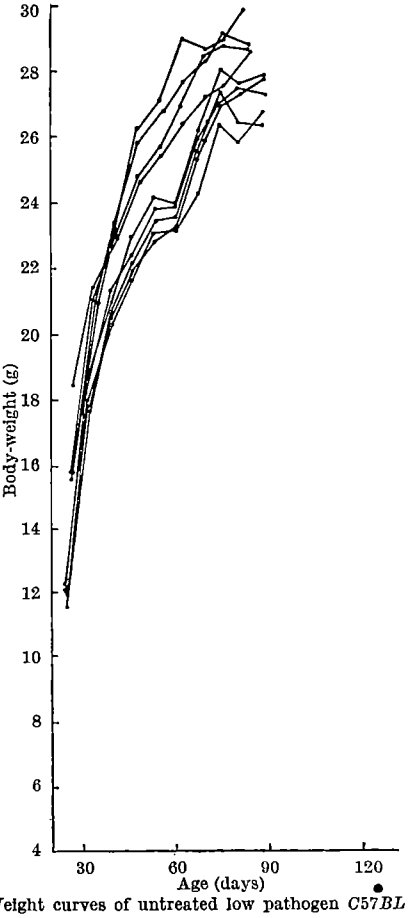


Fig. 6. Weight curves of untreated low pathogen *C57BL* male mice

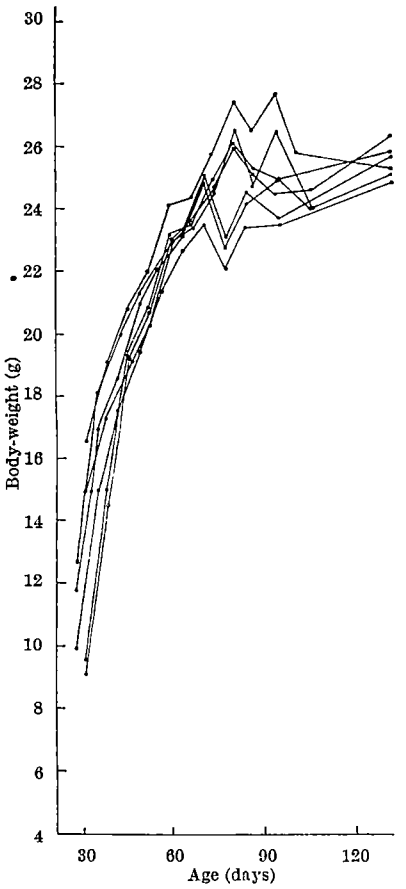


Fig. 7. Weight curves of low pathogen *C57BL* male mice thymectomized at birth

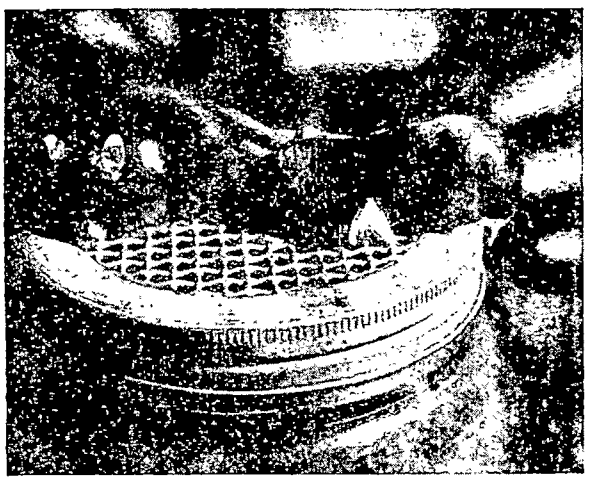


Fig. 8. Germ-free *C57BL* mice, 3 months of age, thymectomized at birth and bearing *BALB/c* skin grafts

ized germ-free and low pathogen mice are not shown in the figures: none of these 49 mice showed evidence of wasting at any time. Examination of the tissues of completely thymoctomized germ-free mice showed only a depletion of lymphocytes which was comparable with that seen in untreated germ-free mice. For skin grafting the only germ-free mouse strain available as donor was *BALB/c*, which differs from *C57BL* at the strong histocompatibility locus, *H-2*. None of the sham-operated mice showed prolongation of survival time of the graft, while 5 out of 20 of the thymectomized mice accepted the skin, which grew luxuriant tufts of hair (Table 1 and Fig. 8).

In another experiment an intraperitoneal injection of approximately 5×10^6 *BALB/c* and *C57BL* spleen cells were given to *C57BL* and *BALB/c* mice, respectively, on the day of birth and the growth of these mice was compared with that of non-injected litter-mate controls. One group was allowed to survive for longevity investigations and another group was killed at 12 days for estimation of organ weights and histology. In the case of *C57BL* recipients the body-weights of the injected conventional and germ-free mice were less than those of non-injected litter-mate controls (Fig. 9 and Table 2), and the mortality was very low in both groups. In contrast, the mortality of injected germ-free and conventional *BALB/c* mice was high: all the animals became severely runted by 11 days (Fig. 10) and 82 per cent of the germ-free and 94 per cent of the conventional mice had died by 18 days (Fig. 11).

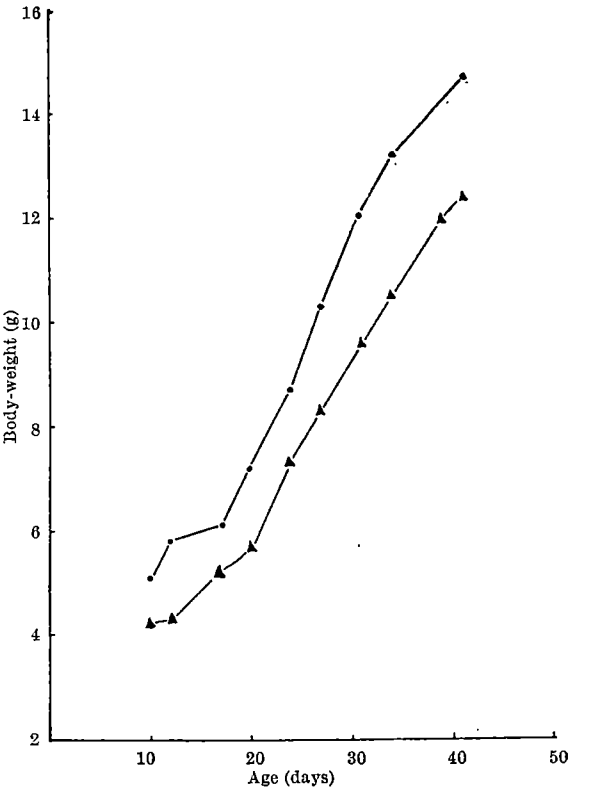


Fig. 9. Average weight curves of conventional *C57BL* mice. ●, uninjected litter-mate controls (8 mice); ▲, injected at birth with *BALB/c* spleen cells (16 mice)

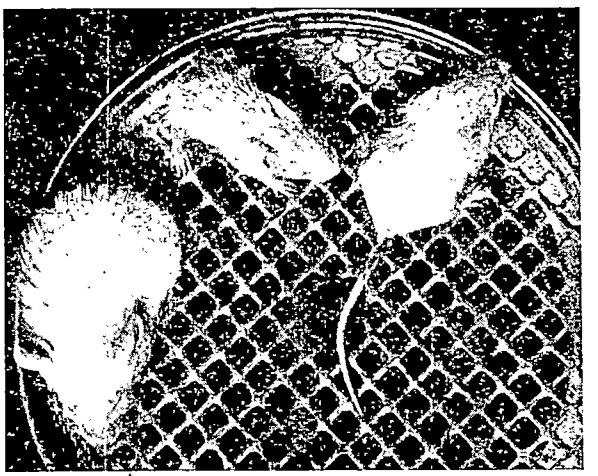


Fig. 10. Three-week-old germ-free *BALB/c* mice. The two runted mice were inoculated at birth with spleen cells from adult germ-free *C57BL* mice

Table 1. SURVIVAL OF *BALB/c* (GERM-FREE) SKIN GRAFTS ON GERM-FREE *C57BL* MICE THYMECTOMIZED AT BIRTH

Treatment at birth	No. of mice	No. of mice showing skin graft survival for: < 20 days	> 20 days
Sham thymectomy	10	10	
Total thymectomy	20	15	5*

*All grafts accepted for longer than 20 days grew luxuriant tufts of hair, and 4 of 5 were still healthy at the end of 50 days.

These results tend to rule out the absence of thymus-specific growth-promoting factors, the lack of trephocytes or the operation of auto-immune clones as primary factors in the pathogenesis of the post-neonatal thymectomy wasting syndrome. They do not exclude the possibility that some of these factors may operate as accessory factors. For example, in conventional thymectomized mice, an auto-immune process could still operate, but it may be secondary to cell destruction initiated by such agents as bacteria or their products, or viruses. The release of tissue breakdown products as a result of cell damage has been shown to produce an auto-antibody response[33]. The primary factor in the pathogenesis of the post-neonatal thymectomy wasting syndrome thus appears to be an environmental factor, presumably an infectious agent (or its product) to which only neonatally thymectomized mice, because of their diminished immunological capacity, are susceptible.

Table 2. BODY-WEIGHTS AND RELATIVE SPLEEN, LIVER AND THYMUS WEIGHTS OF 12-DAY-OLD CONVENTIONAL AND GERM-FREE MICE INOCULATED AT BIRTH WITH ALLOGENEIC LYMPHOID CELLS

Treatment	Strain*	Body-weights (g) ± *S.E.*	Spleen/body ± *S.E.*	Liver/body ± *S.E.*	Thymus/body ± *S.E.*
Nil	Conventional *C57BL*	5·7 ± 0·50	0·0058 ± 0·0003	0·0444 ± 0·0023	0·0067 ± 0·0002
I.P.I. adult *BALB/c* spleen cells at birth	Conventional *C57BL*	3·7 ± 0·33	0·0093 ± 0·0012	0·0518 ± 0·0036	0·0032 ± 0·0004
Nil	Germ-free *C57BL*	7·9 ± 0·22	0·0072 ± 0·0003	0·0371 ± 0·0008	0·0062 ± 0·0001
I.P.I. adult germ-free *BALB/c* spleen cells at birth	Germ-free *C57BL*	4·6 ± 0·30	0·0147 ± 0·0007	0·0711 ± 0·0057	0·0025 ± 0·0005
Nil	Conventional *BALB/c*	9·5 ± 0·52	0·0074 ± 0·0005	0·0417 ± 0·0014	0·0059 ± 0·0002
I.P.I. adult *C57BL* spleen cells at birth	Conventional *BALB/c*	4·7 ± 0·31	0·0193 ± 0·0002	0·0761 ± 0·0015	0·0018 ± 0·0001
Nil	Germ-free *BALB/c*	8·2 ± 0·07	0·0088 ± 0·0003	0·0480 ± 0·0076	0·0047 ± 0·0005
I.P.I. adult germ-free *C57BL* spleen cells at birth	Germ-free *BALB/c*	2·9 ± 0·50	0·0107 ± 0·0005	0·0431 ± 0·0014	0·0015 ± 0·0002

* Average of 5 mice per group.

The results of the first experiment clearly show that wasting did not occur after neonatal thymectomy in germ-free or low pathogen *C57BL* mice in contrast to conventional mice of the same stock. The fact that some germ-free *C57BL* mice were incapable of rejecting skin from *H-2* incompatible *BALB/c* donor mice indicates that immunological incompetence is a direct result of neonatal thymectomy and not secondary to either an infectious process or to whatever produces the wasting syndrome. It is known that the lymphoid tissues of animals kept in the germ-free state are not well populated with lymphocytes[32] so that thymectomy under these conditions may be expected to have little or no effect on such a population. This was indeed the case, as practically no difference was seen between the tissues of unchallenged thymectomized germ-free mice and their sham-operated litter-mate controls. Liver lesions, necrotic foci in lymphoid tissues, and splenomegaly were never observed in any of the killed germ-free *C57BL* thymectomized mice. On the other hand, evidence of graft-versus-host reactions developed in both conventional and germ-free *C57BL* and *BALB/c* mice inoculated at birth with adult *BALB/c* or *C57BL* spleen cells, respectively. This indicates that anti-host immune reactions can operate and produce runt disease in the germ-free environment. The low mortality of injected *C57BL* mice suggests that these mice may have developed, as early as the time of birth, some capacity for reacting against cells with foreign histocompatibility antigens. If this were true, it would explain why 75 per cent of neonatally thymectomized *C57BL* mice could reject *BALB/c* skin grafts.

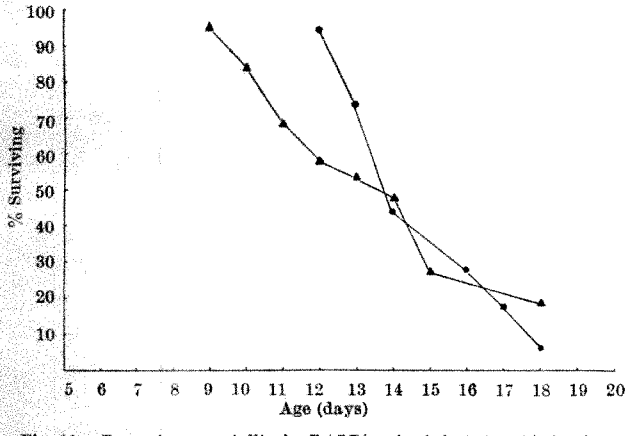


Fig. 11. Percentage mortality in *BALB/c* mice injected at birth with *C57BL* spleen cells. ●, conventional mice (18 mice); ▲, germ-free mice (19 mice)

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FAT-SOLUBLE VITAMINS AND BIOLOGICAL MEMBRANES

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PAUL KARRER, who elucidated the chemical structure of vitamin A more than thirty years ago, remarked in 1960 (ref. 1): "We are fairly well informed about the physiological functions of the water-soluble vitamins because they are parts of enzymes, the reactions of which are pretty well known. On the other hand, we have very little information about the biological role of the fat-soluble vitamins. This is true also for vitamin A". Evidence has accumulated in this Laboratory during the past few years that vitamin A, when added in excess to biological systems, acts on the membranes of cells and their organelles. Recently, it has been found that vitamin A alcohol is also able to penetrate and expand a monolayer of lecithin-cholesterol at an air–water interface[2]. Considerable molecular specificity was observed in the experiments with monolayers; the anhydro and hydrogenated forms of the vitamin, which are biologically inactive, were much less surface active than vitamin A alcohol. It was suggested, therefore, that ability to penetrate lipid films might be the basis for the molecular specificity observed in many of the biological actions of vitamin A.

Penetration of lipoprotein membranes by excess of vitamin A alcohol frequently leads to a disruption of their structure. Since the molecular specificity for lysis of rabbit erythrocytes by compounds in the vitamin A series is similar to that for the prevention of vitamin A deficiency, the working hypothesis was put forward that the site of action of vitamin A is at the lipoprotein membranes of cells and their organelles[3]. It was also suggested that the role of the vitamin within membranes might be to control permeability and that membranes therefore become excessively unstable in the presence of excess of vitamin A.

Degradation of the matrix of embryonic cartilage provides an example of extensive secondary changes resulting from alterations in the properties of membranes caused by vitamin A[4–6]. Thus, release of hydrolytic enzymes from lysosomes by the action of the vitamin is responsible for many of the changes observed in this tissue[7–9]. Alterations in metabolism[5] which occur in the presence of excess of vitamin A may be associated with the swelling action of the vitamin on mitochondria[10]. The electron microscope has revealed damage to the plasma membrane, and also to the membranes of mitochondria, the endoplasmic reticulum and the nucleus of rat dermal fibroblasts treated with vitamin A alcohol in buffered saline[11,12]. Changes in metabolism and alterations in the membrane-bound structures of these cells have also been observed during cultivation *in vitro* with excess of the vitamin[13], while embryonic chick skin cultured in the presence of excess of vitamin A shows alterations of mitochondrial structure in epidermal cells[14].

In this article, it is reported that a number of isoprenoid compounds, including certain derivatives of vitamins E and K, stabilize lipoprotein membranes against rapid disruption by the presence of an excess of vitamin A alcohol. This finding, and other observations on the membrane-active properties of vitamin A, are discussed in relation to some suggested biochemical roles for vitamin A and other fat-soluble vitamins within the lipid moieties of biological membranes.

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Inhibition of Vitamin A-induced Hæmolysis

Treatment of rabbit erythrocytes *in vitro* (1 in 40 suspension, v/v) with vitamin A alcohol[3] (10–20 μg/ml.) causes large indentations in the surface of the cells; vacuoles are then formed from the indentations by a process that resembles micropinocytosis[15]. Afterwards, the cells become spherical and lose hæmoglobin as breaks appear in the membranes. After 15 min at 37° C, most of the cells are lysed (Fig. 1*a*). In a preliminary communication, it was reported that the instability of red cell membranes produced by excess of vitamin A alcohol is largely prevented by DL-α-tocopheryl acetate[16]; mitochondrial swelling caused by vitamin A alcohol is similarly inhibited. When erythrocytes are incubated with both vitamin A alcohol (15 μg/ml.) and DL-α-tocopheryl acetate (50 μg/ml.), the sequence of changes in shape is similar to that seen with vitamin A alone (Fig. 1*b*). After 15 min, however, the cells are still intact though they contain vacuoles as a result of the initial expansions of the cell surface (Fig. 1*c*). Very few ghosts are present under these conditions. Cells treated with α-tocopheryl acetate alone (50 μg/ml.) resemble those treated with solvent (1 per cent ethanol) except that the cells tend to adhere to one another (Fig. 1*d*). It may be concluded that vitamin A alcohol penetrates the erythrocyte membrane both in the presence and absence of α-tocopheryl acetate, but that α-tocopheryl acetate prevents the subsequent rapid rupturing of the cell membrane that occurs with excess vitamin A alcohol alone. This stabilizing action of vitamin E may be related to its action in reducing the instability of a monolayer of lecithin and cholesterol containing a high proportion of vitamin A alcohol[17]. α-Tocopheryl acetate does not prevent penetration by vitamin A alcohol of the lipid monolayer, but its presence inhibits the subsequent slow fall in surface pressure[2].

The disruptive action of vitamin A alcohol on erythrocytes, lysosomes and mitochondria is highly temperature-dependent[3,7,10]. The inability of the vitamin to disrupt membranes in the cold may be due partly to a slower penetration of lipoprotein membranes by vitamin A alcohol at low temperatures. In experiments with lipid monolayers, marked isopiezic expansion was produced by vitamin A alcohol at and above 22° C, while at 13° C penetration and expansion of the lipid film were poor[2]. Electron micrographs of erythrocytes treated with vitamin A alcohol at 8° C for 15 min, however, reveal vacuoles within the cells, indicating that penetration of the cell membrane has occurred in the cold without causing hæmolysis (Fig. 1*e*). This expansion of the cell membrane and vacuole formation observed at 8° C closely resembles the action of vitamin A alcohol at 37° C when α-tocopheryl acetate is present, as may be seen from a comparison of Figs. 1*e* and 1*c*. It is interesting, therefore, that monolayers of lecithin-cholesterol penetrated by

vitamin A alcohol at the relatively low temperature of 13° C resembled those penetrated in the presence of α-tocopheryl acetate at room temperature and were stable for at least 15 min. Inhibition of lysis by vitamin E may thus be related to the inhibition associated with keeping the cells cold.

Although α-tocopheryl acetate inhibits loss of hæmoglobin caused by vitamin A alcohol during a 15-min period at 37° C, a significant loss of potassium still occurs. This leakage of potassium would be expected to cause complete lysis eventually, owing to the entry of water into the erythrocytes; longer incubation of the cells with α-tocopheryl acetate and vitamin A alcohol in fact produces hæmolysis. After 2 h, all the potassium has been released and loss of hæmoglobin is extensive (Fig. 2). Such relatively slow lysis may result from very small leaks in the membrane rather than from the comparatively large breaks seen both in the membranes of erythrocytes[15] (Fig. 1*a*) and in fibroblasts[11,12], treated with vitamin A alcohol alone. A possible mechanism for the production of these small holes in the membrane is discussed later.

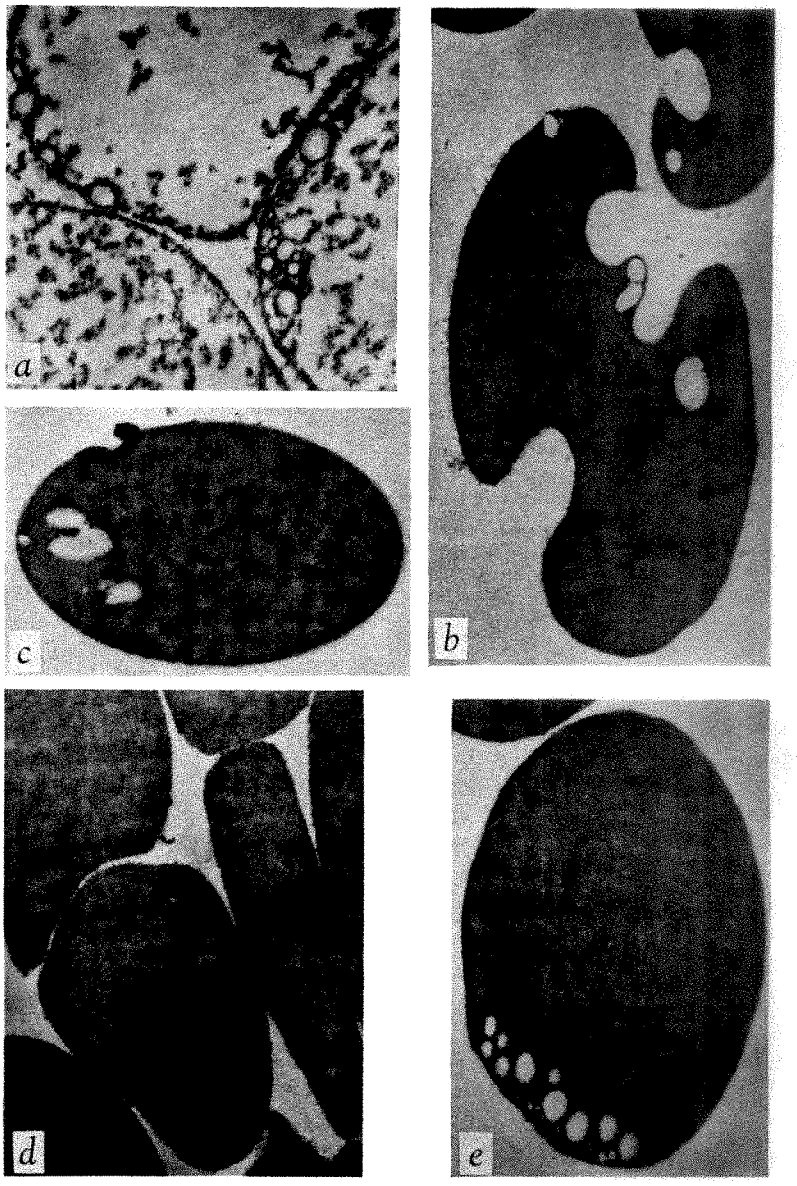


Fig. 1. Electron micrographs of thin sections of rabbit erythrocytes. Experimental procedures were as previously described[3,15]. (*a*) Ghosts formed on incubating cells with vitamin A alcohol (15 μg/ml.) for 15 min at 37° C. (*b*) At 25° C, indentations are produced in the cells 30 sec after addition to vitamin A alcohol (15 μg/ml.) and DL-α-tocopheryl acetate (50 μg/ml.). (*c*) An intact cell containing vacuoles after 15 min at 37° C with vitamin A alcohol (15 μg/ml.) and DL-α-tocopheryl acetate (50 μg/ml.). (*d*) Cells treated with DL-α-tocopheryl acetate (50 μg/ml.) for 15 min at 37° C. (*e*) An intact cell containing vacuoles after 15 min at 8° C with vitamin A alcohol (15 μg/ml.). All micrographs are × *c.* 14,500

Molecular Specificity for Inhibition of Lysis

It has been pointed out by Vasington *et al.* that although different ester derivatives of the tocopherols, including the phosphate, exhibit biological activity, this may be due to the liberation of free tocopherol by hydrolysis[18]. In order to investigate the relationships between molecular structure and activity, a number of different compounds were tested for their ability to inhibit lysis due to vitamin A alcohol.

DL-α-*Tocopherol.* The free alcohol form of vitamin E inhibited hæmolysis due to vitamin A alcohol (Table 1). It seems unlikely, however, that this is the active form when the acetate ester is used as inhibitor. Our standard suspension of rabbit erythrocytes was virtually unaffected by incubation at 37° C for 15 min with 500 μg/ml. of the ester, while extensive hæmolysis was produced by 200 μg/ml. of free α-tocopherol under the same conditions. Little hydrolysis of the acetate ester presumably occurs in this system.

D-α-*Tocopheryl acid succinate.* This compound, which is weakly hæmolytic, was active in inhibiting rapid lysis caused by vitamin A alcohol, although it was somewhat less effective than the acetate ester (Table 1).

Disodium DL-α-*tocopheryl phosphate.* The phosphate ester of α-tocopherol was strongly hæmolytic; extensive release of hæmoglobin occurred with 20 μg/ml., and lysis was virtually complete with 50 μg/ml. after 15 min incubation at 37° C. It is probably relevant that Rabinovitz and Boyer[19] have concluded that inhibition of the rat heart succinoxidase system by α-tocopheryl phosphate results from the ester behaving as an anion with a large non-polar group and that this inhibition is not necessarily related to the action of the phosphate as a vitamin.

Hydroquinone. It seemed possible that inhibition of lysis is associated with the antioxidant properties of vitamin E, and since hydroquinone, like the tocopherols, is soluble in ethanol this compound might be expected to inhibit rapid lysis due to vitamin A. No inhibition of lysis was observed, however, when quantities of up to 500 μg/ml. of hydroquinone were added simultaneously with vitamin A alcohol (15 μg/ml.). Hydroquinone was not lytic by itself under these conditions. The failure of hydroquinone to inhibit lysis due to vitamin A alcohol indicates that inhibition by the tocopherols may not be the result of neutralization of free radicals and the breaking of autoxidative chain reactions. This conclusion is supported by the fact that the acetate ester of α-tocopherol is particularly effective in inhibiting lysis, since the ester does not contain a hydroxyl group that can yield hydrogen and thereby neutralize free radicals.

Vitamins K_1, K_3 *and* K_5. Several authors have suggested that the reactivation by α-tocopherol, α-tocopheryl acetate and vitamin K_1 of succinic-cytochrome *c* reductase preparations that have been extracted with iso-octane is

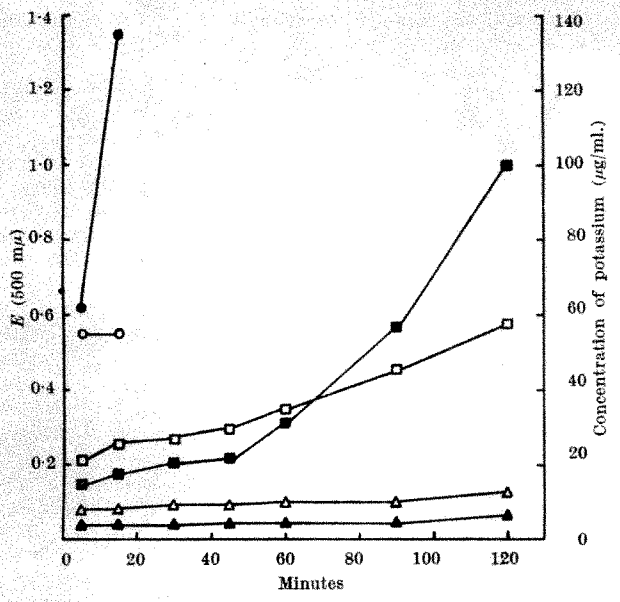


Fig. 2. Release of hæmoglobin and potassium at 37° C from the standard suspension[3] (1 : 40 v/v) of rabbit erythrocytes in sodium chloride solution (0·9 per cent). Closed symbols indicate release of hæmoglobin; ●, with vitamin A alcohol (15 μg/ml.); ■, with vitamin A alcohol (15 μg/ml.) and DL-α-tocopheryl acetate (50 μg/ml.); ▲, with ethanol (1 per cent). Open symbols indicate release of potassium; ○, with vitamin A alcohol (15 μg/ml.); □, with vitamin A alcohol (15 μg/ml.) and DL-α-tocopheryl acetate (50 μg/ml.), △, with ethanol (1 per cent)

a physico-chemical feature of the isoprenoid side-chains of these compounds rather than of any oxidation-reduction properties[20–23]. In view of this, and of the failure of hydroquinone to prevent lysis of erythrocytes by vitamin A alcohol, three compounds in the vitamin K series were tested. Vitamin K_1 inhibited lysis effectively, while menadione (vitamin K_3), which has the same quinone structure as vitamin K_1 but no isoprenoid side-chain, was inactive (Table 1) (cf. Crawford and Morrison[21]). 2-Methyl-4-amino-1-naphthol (vitamin K_5) was also inactive. Under the experimental conditions used, vitamins K_3 and K_5 caused only slight hæmolysis of erythrocytes when the compounds were tested by themselves. The inhibitory action of vitamin K_1 in vitamin A-induced lysis is interesting in the light of reports on interactions between vitamins A and K[24,25]. In particular, Walker *et al.*[26], in investigations on blood clotting, concluded that toxic excess of vitamin A induces a secondary deficiency of vitamin K. They also observed that massive doses of vitamin A caused an increase in plasma/cell ratio in the treated animals, which could sometimes be controlled by vitamin K.

Table 1. Percentage Inhibition of Vitamin A-induced Lysis

Compound	Concentration of inhibitor (μg/ml.)				
	10	25	50	100	200
DL-α-Tocopherol	65	78	78	—	—
DL-α-Tocopheryl acetate	52	84	87	—	—
D-α-Tocopheryl acid succinate	—	6	27	54	65
Disodium DL-α-tocopheryl phosphate	0	0	0	—	—
Hydroquinone	—	0	0	0	0
Vitamin K_1	38	44	72	86	—
Vitamin K_3	0	0	0	0	—
Vitamin K_5	0	0	0	0	—
Ubiquinone-30	77	93	86	88	87
Squalene	76	79	72	62	—
N,N^1-Diphenyl-*p*-phenylene diamine (DPPD)	0	0	0	—	—
Geraniol	11	5	0	0	0
Citral	7	7	4	0	0
Calciferol	5	3	8	0	0
L-Thyroxine, sodium salt	0	0	0	0	—

Rabbit erythrocytes were used as previously described[3]. Vitamin A alcohol was at 15 μg/ml., and the inhibitors were dissolved in ethanol (final concentration 1 per cent) with vitamin A. The phosphate ester of vitamin E in saline solution, and thyroxine in sodium carbonate solution (0·1 per cent w/v), were each added to the cells immediately before treatment with vitamin A. All tests were done at 37° C for 15 min[3].

Ubiquinone-30. This compound was very effective as an inhibitor of rapid lysis caused by vitamin A alcohol (Table 1), despite the fact that the antioxidant activity of ubiquinone-30 is much less than that of α-tocopherol[27,28].

Squalene and phytol. These two long-chain compounds, which have no activities as vitamins, are capable of reactivating preparations of succinic-cytochrome *c* reductase that have been extracted with iso-octane[20]. It is interesting, therefore, that both squalene (Table 1) and phytol inhibit lysis of erythrocytes by vitamin A alcohol. Phytol is itself hæmolytic at concentrations of 50–100 μg/ml., but at lower concentrations, which do not cause extensive lysis, it gave up to 60 per cent inhibition of lysis caused by vitamin A alcohol.

DPPD. The antioxidant, N,N^1-diphenyl-*p*-phenylene diamine (DPPD), which has some protective action against dialuric acid-induced hæmolysis[29], was tested for inhibition of lysis caused by vitamin A alcohol. No inhibition was observed (Table 1). The final concentration of ethanol was 2 per cent in this instance.

Other compounds. Geraniol, citral and calciferol were virtually inactive as inhibitors when tested in concentrations up to 250 μg/ml. Thyroxine, which is fully protective against both hæmolysis and lipid peroxidation caused by dialuric acid[30], was unable to inhibit lysis by vitamin A alcohol.

Structure and function. It is concluded from these experiments on the relationship of molecular structure to inhibition of lysis that inhibitory activity may be associated with the properties of the long isoprenoid chains of the active compounds.

Oxidation and Hæmolysis

Vitamin A is highly unsaturated and is easily oxidized[31,32]. Furthermore, interactions between vitamins A and E have been recognized for more than twenty years[33,34], and it is widely thought that vitamin E inhibits oxidative destruction of vitamin A. We have observed that, in the absence of vitamin E, the hæmolytic action of vitamin A alcohol is accelerated by the oxidizing agent, chloramine *T*, at a concentration of 0·015 per cent w/v (ref. 16); this concentration of oxidizing agent did not cause release of hæmoglobin in the absence of vitamin A alcohol. Rabbit erythrocytes were incubated with vitamin A alcohol (15 μg/ml.) for 2 min at 37° C, which is sufficient to enable the vitamin to penetrate the cell membrane, but not to cause release of hæmoglobin[15]. Chloramine *T* in saline solution was then added, and further incubation of the cells demonstrated the accelerating action of the oxidizing agent on vitamin A-induced lysis.

Rose and György[35,36] found that dialuric acid hæmolyses erythrocytes from vitamin E-deficient rats. There is a close relationship between hæmolysis induced by dialuric acid and lipid peroxidation, and agents that prevent hæmolysis also stop peroxide formation[30,37]. In the light of our experiments, it is interesting that both vitamin K_1 and phytol have some protective activity *in vitro* against hæmolysis by dialuric acid[29]. We have found that dialuric acid (0·02 per cent w/v) much accelerates the rate of lysis by vitamin A alcohol during a 15-min period at 37° C. Experiments with chloramine *T* and dialuric acid thus indicate that oxidation of the double bonds of vitamin A may be associated with the rapid hæmolysis that occurs at 37° C after penetration of lipoprotein membranes by the vitamin. Both these oxidizing agents, at the concentrations mentioned, can overcome the inhibition by α-tocopheryl acetate of lysis induced by vitamin A alcohol.

Since these experiments indicated that the disruptive action of vitamin A on membranes may involve oxidation of the vitamin, related experiments were done with oleic acid and the neutral detergent '*BRIJ* 35'. Vitamin E is known to inhibit the autoxidation *in vivo* of unsaturated fatty acids[38], and it was therefore interesting to find that the extensive lysis caused in 15 min at 37° C by oleic acid (30 μg/ml.) was progressively inhibited by increasing quantities of the acetate ester of α-tocopherol; 73 per cent inhibition of lysis was observed with a concentration of 200 μg/ml. of the vitamin. The dependence on temperature of lysis caused by oleic acid resembles that due to vitamin A alcohol, and lysis caused by oleic acid is also accelerated by chloramine *T*. In contrast to these observations, hæmolysis caused by '*BRIJ* 35' (100 μg/ml.) was unaffected by pretreatment of the cells with up to 1,000 μg/ml. of α-tocopheryl acetate. Chloramine *T* (0·015 per cent w/v) did not accelerate lysis by this concentration of detergent but, instead, inhibited it almost completely. Hæmolysis caused by the detergent has previously been shown to have different temperature characteristics from that caused by vitamin A alcohol[3].

Some Properties of Vitamin A within Membranes

It is conceivable that the functional presence of vitamin A within membranes may account for the broad syndrome of its biological actions. In this section, some properties of vitamin A in membranes are reported and their possible significance in the normal physiology of the vitamin is discussed.

The hypotonic release of hæmoglobin from a 1/200 (v/v) suspension of rabbit erythrocytes in dilute saline (0·6 per cent w/v), during incubation for 20 min at 23° C, is reduced by 60 per cent in the presence of 1 μg/ml. of vitamin A alcohol; loss of potassium is also diminished by the vitamin. Under these experimental conditions as little as 0·1 μg/ml. of vitamin A has a protective action, but the effect depends on the concentration of the cell suspension. Similar protection is given by vitamin A acid, vitamin A methyl ether and vitamin A aldehyde, while the palmitate ester is inactive. 3-Apo-β-carotenal, β-ionylidene acetaldehyde, β-ionone and citral have little or no effect on hypotonic hæmolysis. The stabilizing action of vitamin A alcohol, which is seen also in erythrocytes from rat, pig, chicken and human, can be prolonged either by the presence of α-tocopheryl acetate or by keeping the cells cold. The mechanism of this action of vitamin A alcohol is not clear, but one possibility is that stabilization towards hypotonic lysis is associated with expansion of the surface of the red cell by the vitamin[15], a process that may impart increased elasticity to the membrane.

Erythrocyte membranes that contain exogenous vitamin A alcohol, but are prevented from lysing rapidly by the presence of α-tocopheryl acetate or by a low temperature, have other demonstrably altered properties. The addition of serum albumin (0·5–1 per cent) to such cells causes virtually immediate and complete lysis which apparently results from combination of the added protein with molecules of vitamin A alcohol already present in the membrane; albumin that has been saturated with vitamin A alcohol is inactive[39]. Similarly, vitamin A alcohol has little effect on mitochondria at 3° C (ref. 10), but addition of albumin 2 min after adding the vitamin A alcohol causes immediate swelling. This lytic process is interesting in view of its similarity to lysis caused by the addition of calcium to erythrocytes previously treated with fluoride[40], and the possibility that it may provide a very simple test system for the study of immune hæmolysis[41]. At 25° C, lysis of red cells by the added albumin (1 per cent) occurs after only 1 min contact with the vitamin (10 μg/ml.), while at 1° C contact for about 8 min with the vitamin is needed before addition of the albumin produces a comparable degree of lysis.

This difference presumably reflects the slower penetration of vitamin A into the membrane at lower temperatures and appears to parallel the temperature dependence observed with penetration of lipid monolayers by vitamin A alcohol[2]. Calf thymus histone, bovine fibrinogen and human or bovine albumin, but not bovine γ-globulin, are effective in lysing vitamin A-sensitized cells. Any surface-active compound capable of penetrating membranes and subsequently binding strongly to protein may well cause lysis in this way, since '*BRIJ* 35' behaves like vitamin A alcohol when albumin, not previously saturated with the detergent, is added to red cells treated with '*BRIJ* 35' in the cold. The ability of a detergent to produce similar effects, however, does not detract from the need to consider these properties of vitamin A in relation to the vitamin's possible mechanisms of action, and it has been suggested that one of the physiological roles of vitamin A may be to stabilize membranes by acting as a cross-linking agent between lipid and protein since the vitamin is able to interact strongly with both molecular species[39].

It has recently been suggested that globular micelles of phospholipid, 40–50 Å in diameter, may be present in biological membranes under certain circumstances[42,43]. A theoretical micellar model for the lipids of biological membranes has also been discussed in which aqueous pores or holes, approximately 4 Å in radius, extend through the lipid layer of the membrane; such a micellar configuration might be in dynamic equilibrium with the bimolecular leaflet structure[44]. In the light of Haydon and Taylor's discussion[45], it has been suggested that a membrane containing a large quantity of vitamin A alcohol may have a high proportion of its structure in the more permeable, micellar form[44]. This suggestion would be consistent with the relatively slow leakage of potassium from erythrocytes incubated with vitamin A alcohol and α-tocopheryl acetate (Fig. 1). Release of hydrolytic enzymes from lysosomes by vitamin A might occur by a similar mechanism. α-Tocopheryl acetate has relatively little inhibitory action on the release of lysosomal enzymes by vitamin A[46], and enzyme release may not depend on oxidative damage to the membrane as appears to occur in vitamin A-treated erythrocytes and mitochondria, both of which contain hæmatin compounds that may catalyse oxidation. Even in the absence of oxidative changes, vitamin A might therefore exert a controlling influence on the permeability of cells and intracellular organelles under physiological conditions.

Discussion and Conclusions

Moore and Sharman[47] have recently shown that erythrocytes from rats fed with excess of vitamin A, like those from rats deficient in vitamin E, are easily hæmolysed by dialuric acid. In the present article, it is reported that vitamin A-induced lysis is accelerated *in vitro* by chloramine *T* and by dialuric acid; these observations suggest that lysis by vitamin A alcohol may at least in part be associated with oxidation of the vitamin. It has been reported that vitamin A behaves as a peroxidant of vegetable oils, and that antioxidants, including α-tocopherol, diminish peroxide formation[48].

The observations described here suggest that, under physiological conditions, damage to membranes by vitamin A may well be inhibited by the presence of suitable compounds such as certain other fat-soluble vitamins or the ubiquinones. α-Tocopheryl acetate does not prevent vitamin A alcohol from expanding the red cell membrane *in vitro*, and it appears that inhibition of lysis is associated with the prevention of rapid degradative changes which occur at 37° C after vitamin A

alcohol has penetrated the membrane. Since, however, the acetate ester of α-tocopherol is not an antioxidant in the usually accepted sense[49], and hydroquinone, menadione and vitamin K_5 do not inhibit lysis, the possibility that oxidation may not be involved in the degradative changes leading to hæmolysis cannot be categorically excluded at this stage.

Experiments on the molecular requirements for effective inhibition of rapid hæmolysis indicate that inhibition is a feature of compounds having a long isoprenoid chain. Thus, vitamin K_1, squalene and phytol are effective, but not menadione or vitamin K_5. It has been suggested that some kind of steric protection of the unsaturated chain of the vitamin A molecule by the chains of inhibitory compounds like vitamin K_1 may be concerned in this process[50], and it is conceivable that one of the functions of α-tocopheryl acetate, vitamin K_1, squalene and phytol in other systems[20,21], may also be the inhibition of the oxidation of unsaturated lipids. Cyanide, which under certain circumstances acts as an antioxidant, does not inhibit mitochondrial swelling caused by vitamin A alcohol[10], although swelling is prevented by α-tocopheryl acetate. This may provide a further example of apparent antioxidant activity of a compound possessing an isoprenoid chain since swelling of mitochondria induced by vitamin A has features in common with the swelling induced by ferrous ions, a phenomenon that has been shown to be associated with lipid peroxidation[51].

The red colour produced by the interaction of oxidized lipids, or their breakdown products, with thiobarbituric acid is commonly used as a measure of lipid peroxidation, although the exact mechanism of this reaction is not yet known[52,53]. In preliminary experiments, we have found that lipid peroxides, as measured by this procedure, may be concerned in lysis of erythrocytes by vitamin A alcohol, and that α-tocopheryl acetate decreases the formation of peroxides. The significance of these experiments is not clear, however, particularly since Green *et al.*[28] have concluded from their investigations on tissue homogenates that vitamin A must be considered as a factor in reducing lipid peroxidation as measured by the thiobarbituric acid test. Like them, we find it difficult to see why an unsaturated lipid such as vitamin A should protect against peroxidation. Nevertheless, it has been reported that vitamin A, but not vitamin K_1, inhibits mitochondrial swelling and lipid peroxidation caused by ferrous ions[51], and that both these vitamins inhibit swelling and peroxidation induced by ascorbate[54].

It seems possible that the physiological actions of fat-soluble vitamins may be concerned with the 'membrane-active' properties of these substances[12]. Vitamin D is important in maintaining the structure of mitochondria[55], in phospholipid metabolism[56], and in the release of calcium from mitochondria[57]; it is also capable of interacting with the membranes of Gram-positive bacteria as Kodicek[58] has shown. Willmer[59] has discussed many aspects of possible interactions of steroids, and of other molecules including vitamin A, with lipoprotein membranes. Vitamin E deficiency can lead to damage of the lipoprotein membranes of cells and their organelles, including lysosomes[60]. With regard to vitamin A, mitochondria from vitamin A-deficient animals are abnormally swollen[61], vitamin A drastically alters the morphology of certain viruses[62], and it has been suggested that the hypocholesterolæmic activity of vitamin A occurs before or during absorption of cholesterol through the intestinal wall[63]. These observations, the investigations on vitamins A, E and K reported here, and the related investigations discussed in this article, are thought to lend further support to the view that one biochemical site of action of vitamin A, and possibly of other fat soluble vitamins, is within membranes. Metabolic and structural changes occurring in vitamin deficiencies may therefore result from abnormalities in the structures and functions of the lipoprotein membranes of cells and their constituent organelles.

We thank Dame Honor B. Fell for her advice and Audrey M. Glauert for the electron micrographs. We also thank Miss J. Mizen and Mr. I. Armond for their assistance.

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MATHEMATICAL EXPRESSION OF SEASONAL CHANGES IN SOIL ORGANIC MATTER

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THERE are several concepts concerning changes in soil organic matter with time. On one hand there is the equilibrium concept which proposes that soils under a given land system eventually attain an equilibrium organic matter content. Under these conditions the amount of soil organic matter broken down is replaced by an equivalent amount of newly formed organic matter from plant and animal sources. Changes in organic matter content with time in this concept are considered as taking place from year to year.

On the other hand, considerable evidence has accumulated concerning seasonal changes in soil organic matter content[1]. These periodic changes occur during the year and even in situations which might be expected to be in equilibrium. They appear to be particularly pronounced under some forest conditions[2].

These two concepts of the dynamics of organic matter change are not necessarily mutually exclusive. It is conceivable that while over a year or growing season the net change in soil organic matter may be zero the march of decomposition and addition throughout the year may be quite different. Periodic or seasonal variation is thus theoretically possible even in situations where an annual equilibrium has been reached although the amplitude of such variation may vary in different environments.

Most deterministic equations proposed for expressing changes in soil organic matter have generally considered the equilibrium concept only. Seasonal variations have been ignored, and in most instances changes have been considered on a year-to-year basis only.

The differential equation most commonly used is:

$$\frac{dN}{dt} = -K_1N + K_2 \qquad (1)$$

where N represents soil organic nitrogen or carbon and K_1 and K_2 are decomposition and addition constants respectively. The solution to this equation is:

$$N_t = N_E - (N_E - N_0)\ \exp(-K_1t) \qquad (2)$$

where N_0, N_t and N_E represent the soil organic matter content initially, at time t and at equilibrium: t is generally expressed in years.

Though such an equation is comparatively simple to use it was considered[3] that a truer representation of such changes was to consider the decomposition and addition coefficients K_1 and K_2 as variable and time-dependent rather than constant. In this case equation (1) becomes :

$$\frac{dN}{dt} = -K_1(t)\,N + K_2(t) \qquad (3)$$

The general solution to this equation is :

$$N \exp\left(\int K_1(t)dt\right) = \int K_2(t) \exp\left(\int K_1(t)\,dt\right) dt + C \qquad (4)$$

When K_1 and K_2 are constant the solution to this equation is identical with (1).

In practice use of equation (4) presents problems. Soil organic matter changes are slow and while half-lives of less than 10 years have been calculated for some land systems in tropical regions[4] the changes appear slower in temperate regions and values in excess of 25 years are not uncommon[3]. (Half-life in this sense refers to the time taken for half the change from the original organic matter level to the new equilibrium level to occur.) Under these conditions of slow change it is difficult to estimate differences in the addition and decomposition coefficients except where detailed records are available over a long period.

However, equation (3) does provide a means of expressing seasonal or periodic change. Any property which varies periodically with time and whose magnitude or intensity can be measured or estimated can be represented by a trigonometric or Fourier series. Thus the change in the decomposition and addition coefficients throughout the year can be represented by Fourier series as follows :

$$K_1(t) = \tfrac{1}{2}\alpha_0 + \sum_{m=1}^{M} (\alpha_m \cos mt + \beta_m \sin mt) \qquad (5)$$

$$K_2(t) = \tfrac{1}{2}\gamma_0 + \sum_{m=1}^{M} (\gamma_m \cos mt + \delta_m \sin mt) \qquad (6)$$

For any given situation the relative series can be inserted in equation (3). This equation then becomes a first-order linear differential equation with periodic coefficients. That is :

$$\frac{dN}{dt} = -(\tfrac{1}{2}\alpha_0 + \alpha_1 \cos t + \beta_1 \sin t + \alpha_2 \cos 2t + \ldots.)\,N + (\tfrac{1}{2}\gamma_0 + \gamma_1 \cos t + \delta_1 \sin t + \gamma_2 \cos 2t + \ldots.) \qquad (7)$$

In this case t is expressed in radians and a year would be 2π radians, and although $K_1(t)$ and $K_2(t)$ may vary throughout the year their value from year to year is assumed to be constant.

Numerical solutions of equation (7) can be obtained by several methods. (I thank Dr. M. N. Oguztoreli, Department of Mathematics, University of Queensland, for assistance in the mathematical treatment.) If the initial condition is known an approximate solution can be obtained by Euler's method. This method lends itself to programming for a digital computer. Picard's method of successive integrations can also be used and a power series in the independent variable (t) obtained. However, unless the value M in the Fourier series is very small, integration beyond the second approximation presents difficulties due to the rapid increase in the number of trigonometric terms. This can be overcome to some extent by expressing the Fourier series as MacLaurin series and carrying out the repeated integrations in Picard's method with polynomials. This method is most accurate for small values of t. Where values over one year only are to be considered it may even be desirable to express $K_1(t)$ and $K_2(t)$ as polynomials (for example, by Lagrange's interpolation formula) rather than as Fourier series to reduce the computational difficulties in the successive integrations of equation (7) by Picard's method. Choice of method of numerical solution depends on whether a computer is available and also the period over which a solution is required.

In spite of the difficulties of numerical solution equation (7) is undoubtedly a more comprehensive representation of soil changes than equation (1). Equation (7) takes into account seasonal changes in soil organic matter during the year and also annual changes from year to year.

While such an equation should be of value in the field, it also provides a framework for testing soil organic matter changes experimentally under controlled environmental conditions, for example, in a 'phytotron'. The original equation (1) makes several assumptions concerning changes in soil organic matter with time. Where yearly changes only are considered, and half-lives of 25 years or more

occur, it is evident that the critical testing of this equation and its underlying assumptions, by experiment, is very difficult. The existence of periodic change in the decomposition and addition coefficients, however, does provide a means whereby the equation can be tested.

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ELECTROENCEPHALOGRAPH DURING 'FEIGNED' SLEEP IN THE OPOSSUM

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THE opossum state, or 'feigned' sleep, is a well-known phenomenon in American folk-lore. Behaviourally it is a defence mechanism by which an opossum discourages a would-be attacker by pretending to be dead. When attacked, the opossum assumes a characteristic posture, curling itself into a round ball. The head is bent ventrally and the body curled; the limbs are slightly flexed and the muscles relatively flaccid. The animal remains motionless and is apparently insensitive to sensory stimulation. The most effective releaser for this response appears to be a dog, which will attack the opossum, grab it by the neck and shake it. Since the response represents such a unique behavioural mechanism it was of interest to investigate the EEG correlates of the behavioural state.

Fifteen adult opossums (*Didelphis virginiana*) were prepared with chronic, silver ball electrodes. The electrodes were placed on the dura resting over the frontal, parietal, temporal and occipital cortical regions, bilaterally; a ground lead was inserted into the sagittal crest of the skull. The electrodes were cemented to the skull with 'Grip' dental cement and led to a small (5 mm × 12 mm), 9-connector external plug. The connector plug was held to the skull with stainless steel wires and 'Acrylic' dental cement. Because of the rapid rate at which the opossum clears 'Nembutal'[1], it was necessary to insert a venous cannula in order to maintain anæsthesia during surgery. EEG recordings were taken from unrestrained, waking animals with an 8-channel Offner dynograph. Normal sleeping and waking records are shown in Fig. 1.

The most successful artificial technique for inducing the opossum state was found to be a combination of shaking and barking. An artificial dog jaw, resembling a large (2½ ft. long) pair of pliers, was prepared to grab the opossum by the neck and shake it vigorously. Dog barking, which had been pre-recorded on magnetic tape, was played through a loudspeaker near the animal while it was being shaken. When an opossum was attacked, he would typically enter a rage state, hissing, growling and attempting to attack the artificial jaw; autonomic reactions (salivation and defæcation) were marked. As the attack continued, the opossum would suddenly enter the opossum state, autonomic and bodily activity ceasing. When induced by these artificial means, the opossum state would last from a few sec to 2–3 min. Unlike the rapid onset of the opossum state, recovery from the state was gradual; the animal would slowly assume a normal posture and respond to external stimuli. The same sequence could often be repeated two or three times on the same day and on successive days.

Fig. 2 shows EEG tracings taken before, during and after the opossum state. Because of the rather vigorous shaking required to induce the opossum state, it was not possible to record the transition into the state. It will be noted that there is a slight flattening and increase in high frequencies during the opossum state, as compared with the control period before shaking. These changes should not be considered unique to the opossum state, however, since they continue afterwards when the animal's

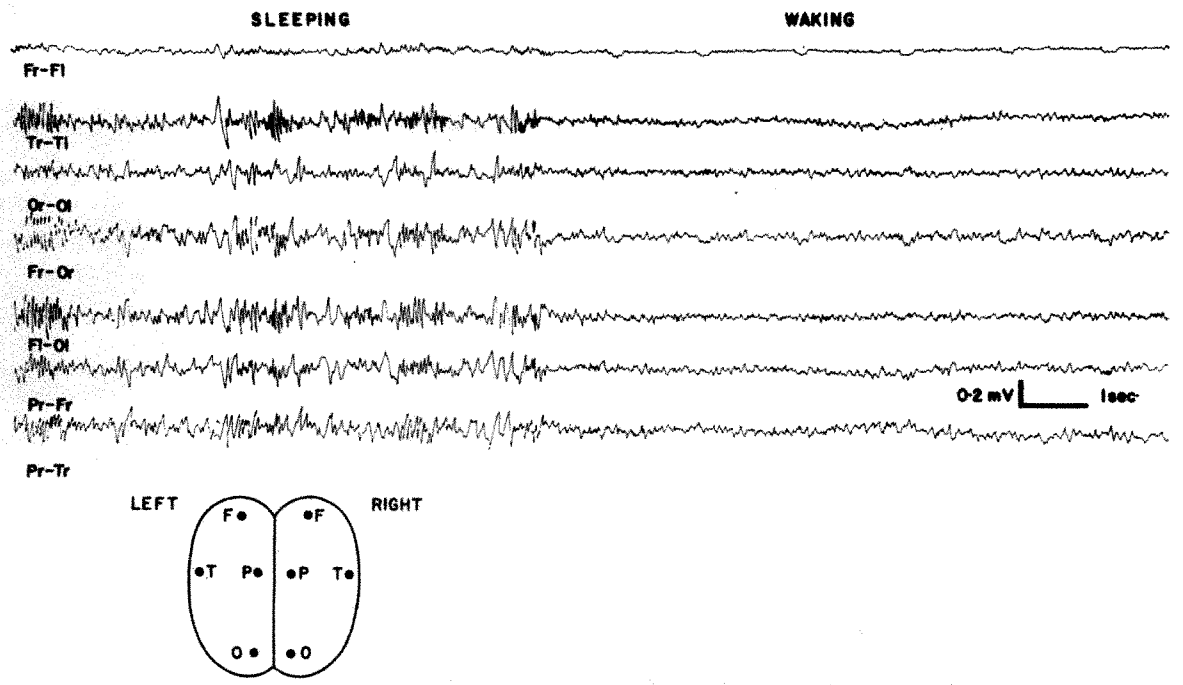


Fig. 1. Normal sleeping and waking EEG records in the opossum. Electrode placement lower left; time and voltage calibration lower right

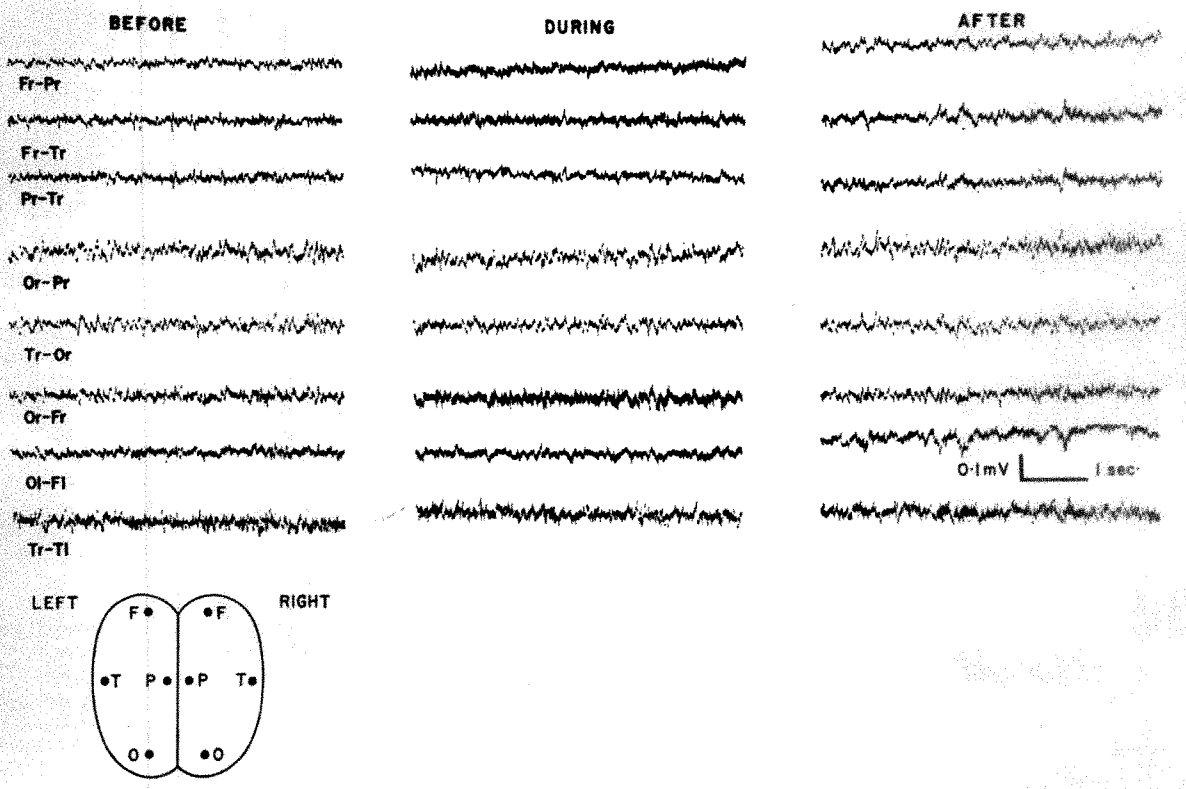


Fig. 2. EEG records before, during, and after an opossum state induced by artificial means. Electrode placement lower left; time and voltage calibration lower right

behaviour returns to normal, as seen in the third record. Such changes appear to reflect a more heightened arousal state induced by the attack situation. In animals which were highly aroused before entering the opossum state, changes before and during the state were not observed.

Because of the short time the animal would remain in the opossum state, and because of the failure to find EEG differences during the state, the question arose as to whether the state induced by our artificial methods was actually the opossum state familiar in folk-lore. In the course of the experiment it had been observed that although it was possible to induce the opossum state repetitively in the same animal, it became progressively more difficult to do so with each attempt. It seemed to hold generally that susceptibility to entering the opossum state decreased with increased contact with human experimenters. Thus, the following method was adopted to obtain more critical recordings during the opossum state.

Naïve opossums, newly brought into the laboratory, were isolated from human contact except for implantation of electrodes and daily feeding. After recovery from surgery and adaptation to the isolated living cages, they were subjected to the attack situation. In these critical experiments, the attacker was a real dog, supplied by a professional animal trainer. The dog would paw at the opossum, chase it into a corner, and then pick it up by the neck and shake it. The opossum was quickly shaken into the opossum state and would remain in the state up to 5–6 min. EEG recordings were taken during the opossum state and during recovery until the animal was behaving normally. Again no difference could be seen in the EEG between the opossum state and the subsequent control after recovery from the state. The EEG during the opossum state is that of a normal, waking, highly alert behavioural state. There is certainly no unique or unusual cortical activity during the state. It must be concluded that the folk-lore is correct; the animal is merely 'playing 'possum'[1].

This work was supported, in part, by grant *NB*–03453–03 from the National Institute of Neurological Disease and Blindness, U.S. Public Health Service.

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SIMULTANEOUS OCCURRENCE OF HÆMOGLOBINS C AND LEPORE IN AN AFRO-AMERICAN

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In 1958 Gerald and Diamond[1] described hæmoglobin Lepore, an abnormal human hæmoglobin which migrated electrophoretically in the position of hæmoglobin S and comprised 5–12 per cent of the hæmoglobin of affected individuals. Hæmoglobin $Lepore_{Boston}$ and similar abnormal hæmoglobins described afterwards in Greek (hæmoglobin Pylos)[2], Italian[3] and Papuan[4] families have been associated with the clinical findings of thalassæmia minor. Hæmoglobin $Lepore_{Hollandia}$[4] and hæmoglobin Pylos[2] have each been encountered in the homozygous state : hæmoglobin A was absent in the hæmolysates of both homozygotes, and hæmoglobin A_2 was absent as well in the hæmolysate of the homozygote for hæmoglobin Pylos.

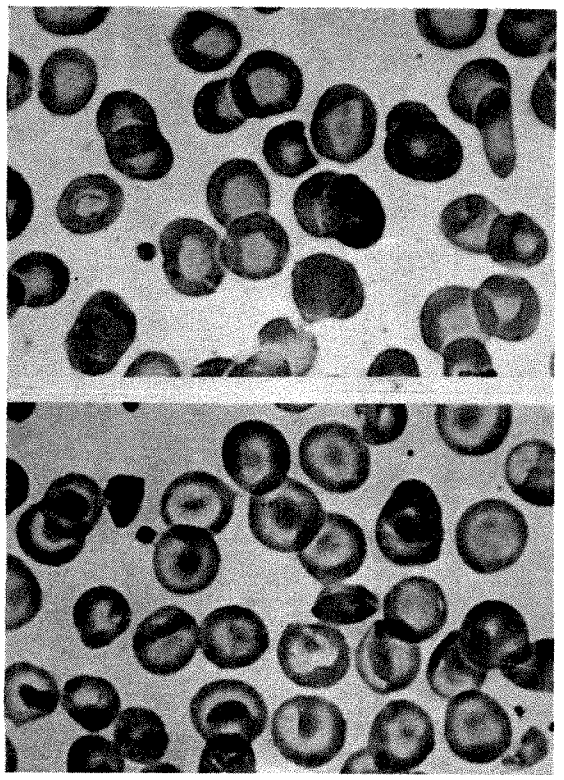


Fig. 1. *A*, Blood smear of II-2 (hæmoglobin Lepore$_{T.B.}$ trait). Hypochromia and stippled erythrocytes were noted. *B*, Blood smear of propositus III-5 (hæmoglobin Lepore$_{T.B.}$ and hæmoglobin C). Hypochromia, target cells and 'folded' red cells were observed

Baglioni[5] has provided an interesting hypothesis concerning the nature of hæmoglobin Lepore. He and Barnabas and Müller[6] found in hæmoglobin Lepore some tryptic peptides which appeared to be β-chain peptides, and others which resembled peptides of the δ-chains of hæmoglobin A_2. Recombination experiments[7] had indicated that the α-chain of hæmoglobin Lepore is identical with the α-chain of hæmoglobin A. Baglioni suggested that the remaining (non-α) chains, designated *x*, were composed in part of δ- and in part of β-chains as a result of unequal crossing over in the linked δ-β-locus.

This article is concerned with the presence of hæmoglobin Lepore in an American Negro family. One of the affected individuals had hæmoglobin Lepore in association with hæmoglobin C; in the other two individuals hæmoglobin Lepore occurred in association with hæmoglobin A. In accordance with recommendations concerning geographic designation[8], this hæmoglobin has been designated Lepore$_{\text{The Bronx}}$ (Lep.$_{T.B.}$).

Case report and family investigations. The propositus, a 17-year-old Negro high-school student, was first seen in January 1962, when she was referred for investigation of hæmoglobin C disease. This diagnosis had been made on the basis of a mild asymptomatic anæmia, the presence of target cells on blood smears, and a filter paper electrophoretic pattern which disclosed mostly hæmoglobin C. The patient was tall (6 ft.); she appeared healthy and the spleen was not palpable. Some laboratory data are listed in Table 1; hypochromic red cells and target cells were noted on blood smears (Fig. 1). Sickling preparations were negative and paper and agar electrophoresis disclosed hæmoglobin C and an increased amount of a minor hæmoglobin component, thought to be hæmoglobin F. When the hæmolysate was analysed by starch-gel electrophoresis[9] in borate buffer at *p*H 8·7, two hæmoglobin components both with mobilities intermediate between hæmoglobins C and A were observed (Fig. 2) in addition to the main C component. The components were quantified after electrophoretic separation on starch granules[10]: hæmoglobin C amounted to 80 per cent of the total hæmoglobin, hæmoglobin F (fastest component) to 6 per cent, and hæmoglobin Lepore to 14 per cent.

Family investigations (Fig. 3) were initiated, and the presence of a component resembling hæmoglobin Lepore was demonstrated in hæmolysates from the mother and from the maternal grandmother. The putative father had no abnormal hæmoglobin, and was of blood group AB. The mother's blood group was A, and the erythrocytes of the propositus were of blood group O. (The erythrocytes of the propositus were agglutinated by anti-H serum.) Therefore, on the basis of blood group investigations and of the hæmoglobin analyses, there appeared to be a lack of coincidence of the legal and the biological father. The latter was not identified; in all likelihood he had hæmoglobin C.

The hæmatological findings and hæmoglobin analysis of the mother, who was heterozygous for hæmoglobin Lepore$_{\text{The Bronx}}$, are included in Table 1. The maternal grandmother (Fig. 3, I-2) had received recent blood transfusions for anæmia secondary to metastatic carcinoma. The abnormal hæmoglobin was identified in her blood, but quantitative analyses were not carried out. The husband (Fig. 3, III-6) of the propositus had normal hæmoglobin, and their premature infant (Fig. 3, IV-5), born in April 1963, lived for only a few days. An umbilical cord sample obtained from the infant was found to contain mostly fœtal hæmoglobin together with a slow component which migrated in the position of hæmoglobin C and comprised 6 per cent of the total hæmoglobin.

The hæmoglobin of the propositus was analysed by starch-gel electrophoresis over a *p*H range of 8·0–9·0. While both minor components (F and Lepore) were readily identified, no component which had the mobility of hæmoglobin A could be identified in any of these gels.

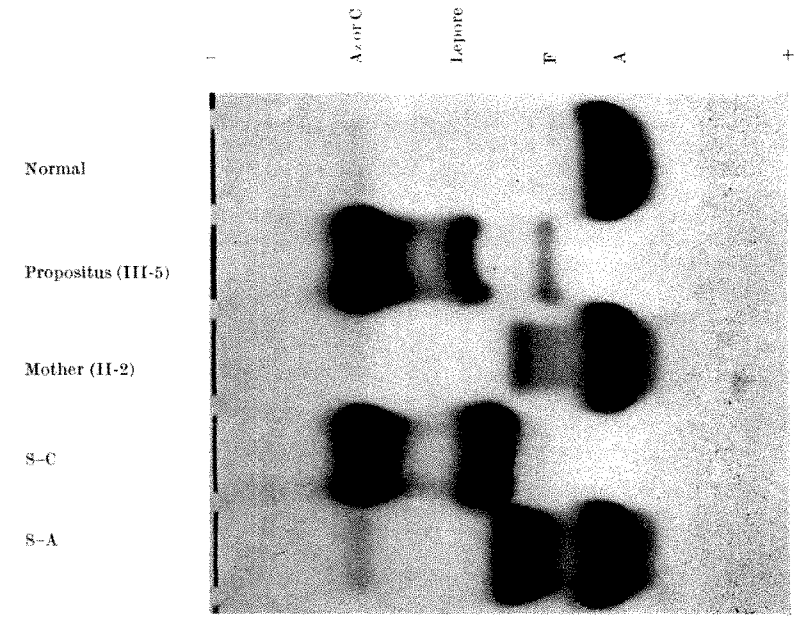


Fig. 2. Starch-gel electrophoresis (ref. 9) of hæmoglobins at *p*H 8·7. In hæmoglobin of propositus (III-5), 3 components (cathode to anode) C, Lepore and F, were observed. Note the greater anodal mobility of hæmoglobin Lepore in II-2, who had hæmoglobins A and Lepore. A similar alteration of mobility is noted in hæmoglobin S in a hæmolysate containing hæmoglobins A + S when compared with hæmolysate containing hæmoglobins C + S

Since the patient had hæmoglobin C, and her mother had not, the patient must have been heterozygous at the β-chain locus. The absence of hæmoglobin A in the patient's hæmolysate suggested that the other β-locus (that is, not β^C) in this patient did not determine the synthesis of normal β^A chains. If hæmoglobin $\text{Lepore}_{\text{The Bronx}}$ represented the product of a δ-β fusion on one chromosome, and a β^C gene was present on the opposite chromosome, no normal β-chains could be made, and hæmoglobin A would not be found.

Investigations of the structure of hæmoglobin $\text{Lepore}_{\text{The Bronx}}$. (1) *Recombination investigations.* Hybridization experiments were carried out at *p*H 4·7 by a modification of the method of Itano and Robinson[11]. Hæmoglobin Lep.$_{\text{T.B.}}$, isolated by two successive electrophoretic separations, was mixed with equal amounts of electrophoretically purified hæmoglobin S (a β-chain variant) or hæmoglobin $I_{\text{Burlington}}$ (an α-chain variant)[12]. Recombination, manifested by the identification of 4 hæmoglobin species after neutralization of the acidified mixture, occurred only in the mixture of hæmoglobin Lep.$_{\text{T.B.}}$ and hæmoglobin I. The abnormality in hæmoglobin Lep.$_{\text{T.B.}}$ must therefore reside in the non-α-chains:

$$\underset{\text{Hb I}}{\alpha_2^I\beta_2^A} + \underset{\text{Hb Lepore}}{\alpha_2^A x_2} \xrightarrow[\text{then neutralized}]{p\text{H } 4{\cdot}7} \underset{\text{Hb I}}{\alpha_2^I\beta_2^A} + \underset{\text{Hb A}}{\alpha_2^A\beta_2^*} + \underset{\text{doubly abnormal Lepore component}}{\alpha_2^I x_2} + \underset{\text{Hb Lepore component}}{\alpha_2^A x_2}$$

In experiments involving hæmoglobin Lep.$_{\text{T.B.}}$, and hæmoglobin I, hybridization was demonstrable only after the mixture of the hæmoglobins had remained at *p*H 4·7 for 72 h before neutralization. Demonstration of recombination of hæmoglobins containing α- and δ-chains has in general required prolonged acid treatment[13], although in hæmoglobins composed of α- and β-chains, recombination can be demonstrated after less than 4 h at *p*H 4·7.

(2) *Sedimentation investigations.* If hæmoglobin Lepore represented the combination of α^A polypeptide chains with a cross-over product of δ- and β-chains, examples of this abnormal hæmoglobin in which the cross-over product was significantly longer than the normal β- or δ-chain might be encountered. In that event, the molecular weight of the abnormal hæmoglobin might be larger than the molecular weight of normal human hæmoglobins A or A_2. Dr. Robin Briehl of the Department of Physiology determined the sedimentation constant of two purified samples of hæmoglobin Lep.$_{\text{T.B.}}$ in a Spinco model *E* analytical ultracentrifuge. In each determination, carried out at about 20° C and corrected to 20·0° C, the sedimentation constants (4·33 and 4·34 *S*) did not differ significantly from the values obtained for simultaneously purified hæmoglobin A (4·23 *S*) and hæmoglobin C (4·27 *S*).

(3) *'Fingerprinting' of hæmoglobin components.* Hæmoglobin components, C and $\text{Lepore}_{\text{The Bronx}}$, prepared from the hæmolysate of the propositus were 'fingerprinted' by the methods of Ingram[14] and Baglioni[15]. The hæmoglobin components were purified by chromatography on '*IRC*-50' resin columns with developer No. 2 of Allen *et al.*[16], and subsequent electrophoresis on starch granules. For isolation of hæmoglobin Lepore, two successive electrophoretic separations were carried out on the eluate from the '*IRC*' column. Electrophoresis of the tryptic digests of purified hæmoglobin components was carried out at *p*H 6·4 in pyridine–acetic acid–water[14], and ascending chromatography in the solvent mixtures utilized by Baglioni[5].

Fingerprints of hæmoglobin C isolated from the hæmolysates of the propositus appeared identical with fingerprints of hæmoglobin C published by Hunt and Ingram[17].

Three tryptic digests of samples of hæmoglobin Lep.$_{\text{T.B.}}$ were fingerprinted together with tryptic digests of hæmoglobin A and A_2. The analyses of the tryptic peptides are not completed; but observations on the fingerprints revealed 5 apparently significant findings: (1) Peptide A_2a, a δ-chain peptide (*Tp* δ XIII) (ref. 18) was absent in hæmoglobin Lep.$_{\text{T.B.}}$. (2) In the fingerprints in which good resolution of peptide 12 was obtained, it appeared to have an R_F comparable to that of peptide 12 of hæmoglobin A_2 rather than of hæmoglobin A. This observation was confirmed by ascending chromatography in *n*-butanol–acetic acid–water (3 : 1 : 1) of peptides 10–14 of hæmoglobins A, A_2, and Lep.$_{\text{T.B.}}$, eluted after one-dimensional ionophoresis[18]: the lowest tryptophan positive peptide of hæmoglobin Lep.$_{\text{T.B.}}$ and hæmoglobin A_2 were in corresponding positions. Peptide 12 thus resembled *Tp* δ II. (3) In hæmoglobin Lep.$_{\text{T.B.}}$ a peptide in the position of peptide 26 (an arginine-positive β-peptide of hæmoglobin A) was inconstantly present, but not

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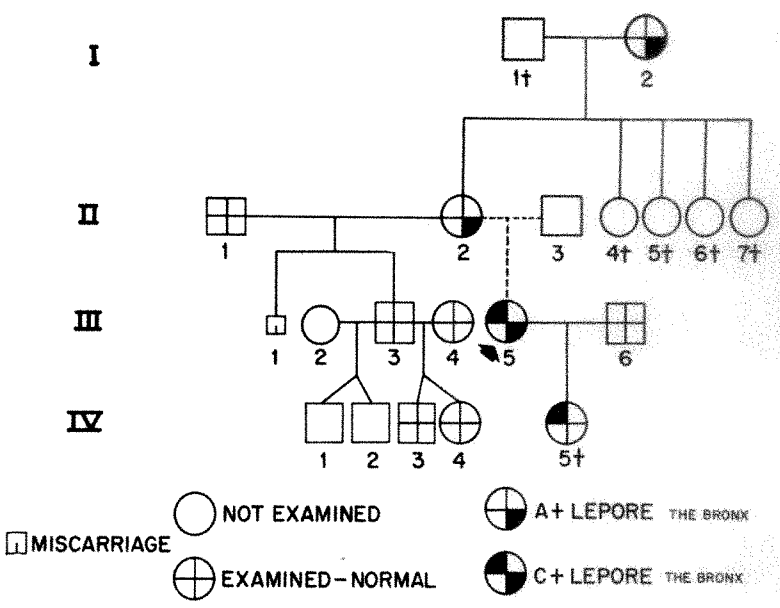


Fig. 3. No. IV-5 was a premature infant: examination of the cord blood showed small amounts of a component with the mobility of hæmoglobin C, together with hæmoglobins F and A. The amount of cord blood available was insufficient for fingerprinting

Table 1. Hæmatological Data and Hæmoglobin Analyses on 2 Subjects with Hæmoglobin $\text{Lepore}_{\text{The Bronx}}$

	Hb (g%)	Red blood cells ($\times 10^6$/mm³)	Reticulocytes (%)	Smear		Hæmoglobin analysis					
						Alkali resistance (F)%	Starch electrophoresis (ref. 10) % component eluted from starch				
				Targets	Hypochromia		A_2	C	Lepore	A	F
Propositus	10·7	5·3	2·2	+ + +	+ +	6	*	80	14	0	6
Mother	11·8	5·2	3·1	+	+ +	5	1	0	8	91	†

* Hæmoglobin A_2 not separated from hæmoglobin C. † Hæmoglobin F not separated from hæmoglobin A.

arginine-positive, in fingerprints prepared from the first tryptic digest. In collaboration with Dr. L. J. Reed, a second tryptic digest of hæmoglobin $Lep._{T.B.}$ was analysed and peptide 26 was consistently absent. It was therefore concluded that β *Tp* III (peptide 26) was absent in hæmoglobin $Lepore_{T.B.}$. The two remaining findings in the peptide maps of $Lepore_{T.B.}$ which were also present in $Lepore_{Boston}$ (ref. 5) were: (4) The R_F's of peptides 24 and 25 (β *Tp* V) were lower in hæmoglobin Lepore than in hæmoglobin A, and (5) the spot corresponding to peptide 5 (*Tp* β XIII or *Tp* δ XIV) was tyrosine-positive and sulphur-negative in hæmoglobin $Lepore_{T.B.}$. The last finding indicated that peptide 5 (*Tp* β XIII) resembled that of the β- rather than of the δ-chain. In summary, the fingerprints of hæmoglobin $Lepore_{T.B.}$ (prepared from hæmoglobin of an American Negro) contained no apparent differences from those of hæmoglobin $Lepore_{Boston}$ (prepared from hæmoglobin of a patient of Italian ancestry). In both $Lepore_{Boston}$ and $Lepore_{T.B.}$, tryptic peptides II, III and V resembled δ-chain peptides and tryptic peptides XII and XIII resembled the β-peptides.

Hæmoglobin $Lepore_{T.B.}$ resembled other examples of hæmoglobin Lepore in: (1) the amount of the component present; (2) the associated findings of thalassæmia; (3) the decrease in hæmoglobin A_2 in the presence of hæmoglobins Lepore and A.

(4) *Amino-acid analyses.* Amino-acid analysis of a 22-h hydrolysate of hæmoglobin $Lepore_{T.B.}$ was carried out on a Beckman amino-acid analyser. Isoleucine, found in hæmoglobin $Lepore_{Hollandia}$, was absent from hæmoglobin $Lepore_{T.B.}$. Unequal crossing-over might, as suggested by Nance[19], be a commonly occurring mechanism underlying the synthesis of protein variants. However, evidence for involvement of the γ-chain locus in hæmoglobin $Lep._{T.B.}$ was not obtained in our investigations.

Recently, the absence of hæmoglobin A was observed in two patients who were heterozygous for both hæmoglobin S and hæmoglobin Pylos[20]. The absence of hæmoglobin A in a subject heterozygous for both hæmoglobins C and Lepore is consistent with Baglioni's hypothesis that hæmoglobin Lepore results from crossing-over in the linked δ-β locus.

This work was supported by grant *A*–4502 from the Institute of Arthritis and Metabolic Diseases, U.S. Public Health Service, and by a grant (contract *U*–1039) from the Health Research Council of New York City. We thank Dr. R. Briehl for the ultracentrifuge investigations, and Dr. O. Blumenfeld for assistance with the amino-acid analyses.

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BEHAVIOUR IN EGGS AND TISSUE CULTURE OF THE INCLUSION BLENNORRHŒA (*LB*4) STRAIN OF THE TRACHOMA–INCLUSION CONJUNCTIVITIS VIRUS GROUP

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THE existence of a linear relationship between the amount of virus inoculated into the yolk sac and the average day of death of the embryo is now generally accepted for the psittacosis–LGV (PLGV) and the closely related trachoma–inclusion conjunctivitis (TRIC) virus groups. This relationship has been described in detail by Golub[1] for viruses of the PLGV group and by Jawetz and Hanna[2], Watkins[3] and by Reeve and Taverne[4] for viruses of the TRIC group. Another important observation was made by T'ang[5], who reported that the lethal action of the *TE*55 virus strain of trachoma for the embryo was increased on passage, and also that a steep rise in infectivity occurred between the tenth and fifteenth passage. Reeve and Taverne[4] have observed that some virus strains of the TRIC group changed their behaviour on passage in eggs, killing the embryos faster and acquiring also the capacity to form inclusions in HeLa cells. They explained these changes in growth characteristics by the emergence and subsequent selection of virus mutants.

The relationship between the reproductive capacity of TRIC viruses in tissue culture and their virulence for embryonated eggs poses many important questions; therefore it seemed desirable to conduct a comparative investigation of the behaviour in embryonated eggs and tissue culture of a virus strain of the TRIC group. The results of such an investigation are discussed in this article.

We used in this work the *LB*4 strain of inclusion blennorrhœa virus. This strain was described by Jones, Collier and Smith[6] and it was obtained in the third egg passage from the Medical Research Council Trachoma Unit, Lister Institute, London.

Many titrations of the *LB*4 strain at different egg passage levels have been carried out in eggs over a ten-day incubation period and in tissue culture; the results to be reported are representative of two years' investigation of this strain. At no time during experimental investigations of this strain were any other virus strains handled. Thus there was no possibility of contamination.

All egg passages were carried out using high concentrations of virus in the inoculum. This technique would seem to favour the chances of selecting out any mutations occurring in the virus population. *LB*4 did not produce inclusions in HeLa cells prior to the seventh egg passage, but beginning from the seventh, and on subsequent egg passages, the virus produced numerous inclusions in HeLa,

Table 1

Egg passage No.	Log_{10} ELD_{50}/ml. titres of 20% yolk sac suspensions of *LB*4 virus	Inclusion titre of 20% yolk sac suspensions of *LB*4 virus in HeLa cells, I.F.U./ml.
4	3·75	No inclusions
5	4·75	,,
6	5·25	,,
7	5·50	$5{\cdot}5 \times 10^5$
8	5·25	$5{\cdot}3 \times 10^5$
9	5·50	$2{\cdot}8 \times 10^5$
10	Not titrated	$1{\cdot}0 \times 10^6$
11	5·50	$3{\cdot}2 \times 10^5$
12	5·75	$4{\cdot}4 \times 10^5$

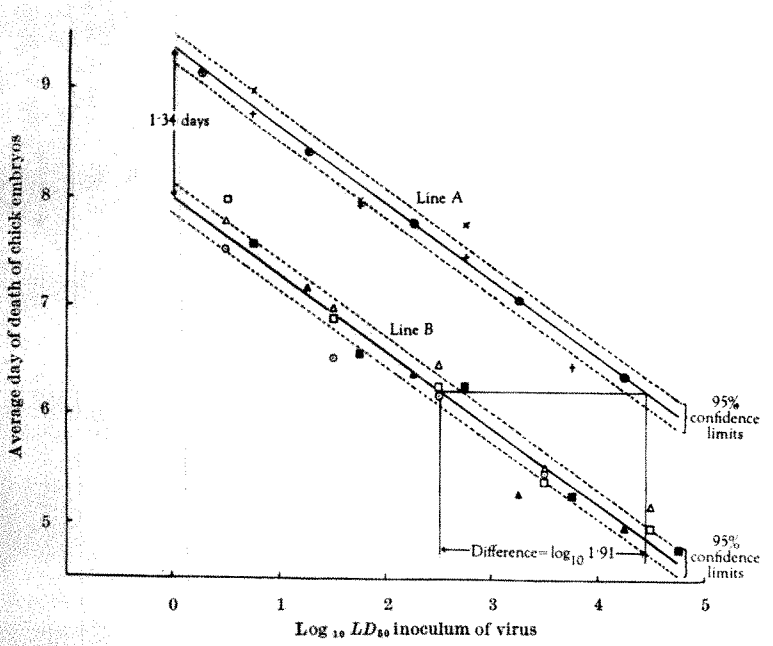


Fig. 1. Egg passage number: ×, 4; +, 5; ⊕, 6; △, 7; ▲, 8; ○, 9; □, 11; ■, 12

F.L. and *WI/38* cell systems; these were titrated by the method of Furness *et al.*[7]. Table 1 shows representative results obtained with 20 per cent yolk sac suspensions of *LB4* virus in HeLa cells. The ability to form inclusions in tissue culture was correlated with a steep increase in virulence of the virus for the chick embryo at the seventh egg passage as measured by the average day of death.

Fig. 1 shows that there is a linear relationship between the log concentration of virus in the inoculum and average day of death. A statistical analysis of these data revealed that this relationship was very similar at the fourth to sixth egg passages, and it could be represented by a straight line *A*. The linear relationship also held for the seventh to twelfth egg passages which could be represented by another straight line *B*. The slopes of *A* and *B* were not significantly different, and therefore they could be drawn as two parallel lines. The mean difference of the average day of death between the two lines *A* and *B* is 1·34 days with 95 per cent confidence limits of 1·21–1·47 days. This difference corresponds to an 81-times increase of virulence, that is, $\log_{10}$ 1·91 LD_{50}. Although the growth rate as estimated from the slopes of the *A* and *B* lines is similar, that is, 1·42 $\log_{10}$ LD_{50} per day, it is obvious that a steep increase of virus virulence for the chick embryos has occurred. However, there was no increase in the virus titres observed during the passages. Collier[8] noted that another inclusion blennorrhœa strain—*LB1*—lost its pathogenicity for the baboon conjunctiva after its eighth egg passage. We have not ascertained whether the changes described for *LB4* virus are associated with this characteristic.

In other experiments to be published elsewhere, we have observed that *LB4* virus pools before or after the seventh passage retained their immunogenic capacity, that is, when these virus pools were inoculated into baboons, the animals acquired immunity against a conjunctival challenge with homologous virus of the second egg passage.

The results of experiments just described show clearly that there was a relationship between the capacity of the *LB4* strain to form inclusions in tissue-culture cells and a steep increase in virulence for the chick embryo that has occurred at the seventh egg passage.

Since it is possible that several factors may have been responsible for this phenomenon, a reasonable interpretation of it would be as follows:

First, the third egg passage suspension of the *LB4* strain possibly contained a heterogeneous population of particles, some of high or low virulence and some uninfective for the egg embryo. In this context, the recorded average day of death for this strain may have depended on the ratio between particles of high and low virulence.

Secondly, the ability of this strain to form inclusions in tissue-culture cells may also depend on a ratio between particles of high and low virulence.

Finally, the highly virulent particles may have outgrown the ones of low virulence during the frequent consecutive passages in eggs, this resulting in a change of the ratio and consequently in an increase of the lethal action for the chick embryo and the capacity to form inclusions in tissue culture cells.

We thank Dr. L. H. Collier, Dr. W. A. Blyth and Dr. Janice Taverne of the Trachoma Unit, Lister Institute, London, for their advice and for kindly supplying us with the *LB4* strain. We also thank the Directors of Pfizer, Ltd., for permission to publish this communication.

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MITOCHONDRIAL AND CYTOPLASMIC ENZYMES FOR THE REDUCTION OF FUMARATE TO SUCCINATE IN YEAST

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In 1937 Fischer and Eysenbach[1] proposed the existence of an enzyme, which they named fumaric hydrogenase (fumarate reductase in present-day terminology), which catalyses the reduction of fumarate to succinate but cannot function as a succinate dehydrogenase. This hypothesis was based on the observation that partially purified samples of Warburg's "old yellow enzyme" catalysed the oxidation of reduced dyes of low potential, such as leucodiethylsafranin, in the presence of fumarate but failed to oxidize succinate with methylene blue as electron acceptor. It should be mentioned that this investigation antedated by many years recognition of the fact that mitochondrial succinate dehydrogenase does not react directly with methylene blue, but reacts rapidly with *N*-alkylphenazonium dyes, such as phenazine methosulphate[2,3]. In a later paper, Fischer *et al.*[4] stated that their preparation was inactivated by dialysis and that the activity was restored by the addition of FAD. Isolation of the enzyme

from a reproducible starting material, such as fresh yeast, was apparently not undertaken.

The concept of a unidirectional fumarate reductase in anaerobic yeast was widely accepted and remained unquestioned until about 1956. During this period some investigators, in fact, believed that yeast cells were devoid of succinate dehydrogenase activity, even when grown aerobically[5], until their apparent lack of succinate oxidation by intact yeast cells was traced to a permeability problem[6,7].

Succinate dehydrogenase was isolated in this laboratory from yeast mitochondria in 1956 (refs. 8 and 9), and its properties were found to resemble those of the enzyme from mammalian tissues in most respects, including the fact that the action of the enzyme is readily reversible. The ratio of rates of succinate oxidation to fumarate reduction remained essentially constant during purification and on treatment with inhibitors and denaturing agents, and no evidence was found for the existence of a separate fumarate reductase in the mitochondria[9,10]. Since the fumarate reductase activity ascribable to the mitochondrial enzyme was several orders of magnitude higher than the activities reported by Fischer *et al.*[1,4], it seemed possible that the German workers had been dealing with differentially inactivated succinate dehydrogenase samples. This reasoning was based on the fact that several steps in the isolation of the "old yellow enzyme" (the starting material for Fischer's preparation) would greatly inactivate mitochondrial succinate dehydrogenase, a notoriously unstable enzyme, and, further, that on ageing succinate dehydrogenase from yeast or animal tissues fumarate reductase activity declines much more slowly than succinate dehydrogenase activity. On the basis of these investigations it was concluded that "while the existence of a separate 'fumaric reductase' is not disproved, the 'reductase' activities of . . . yeast mitochondria may be readily accounted for by the reversible action of succinic dehydrogenase . . ." and it was suggested "that at present no unequivocal evidence points to the existence of a separate 'fumaric reductase' "[11]. The presumed reversible resolution into apoenzyme and FAD by dialysis[4] remained puzzling, however, since the flavin of yeast succinate dehydrogenase is covalently linked to the protein; but the possibility could not be excluded that added FAD acted as a non-enzymatic catalyst, rather than as a prosthetic group, in the experiments of Fischer *et al.*[4].

The problem was later reopened by Kováč[12], who confirmed the findings of Massey and Singer[10,11] but also noted that fumarate reduction in extracts and broken cell preparations of yeast is only partly inhibited by malonate, succinate, and *p*-chloromercuribenzoate, while succinate oxidation is completely inhibited by malonate and mercurials. Kováč concluded that this observation was suggestive but not unequivocal evidence for the existence of two fumarate-reducing enzymes in yeast, fumarate reductase and succinate dehydrogenase, and emphasized that other interpretations were equally conceivable. Differential response to inhibitors is, indeed, tenuous evidence for the existence of a separate fumarate reductase, since it has been shown that fumarate reduction by homogeneous preparations of succinate dehydrogenase from *Micrococcus lactilyticus* is far less sensitive to inhibition by malonate and *p*-chloromercuribenzoate than succinate oxidation[13,14]. Furthermore, treatment of respiratory chain preparations from aerobic yeast with *N*-ethylmaleimide, an –SH inhibitor, the action of which is irreversible, results in extensive loss of succinate dehydrogenase activity without detectable effect on the reduction of fumarate (Table 1).

Another indication of the possible existence of a unidirectional fumarate reductase came from the observations of Peck *et al.*[15] that extracts of the obligate anaerobe *M. lactilyticus* reduce fumarate very rapidly but oxidize succinate only extremely slowly, even in the presence of phenazine methosulphate and that, while succinate oxidation is readily inhibited by malonate and fumarate, the reduction of fumarate is virtually insensitive to inhibition by succinate and by low concentrations of malonate. Further, on preliminary fractionation of the *M. lactilyticus* extract succinate dehydrogenase appeared to be completely absent in certain fractions rich in fumarate reductase[17]. This last observation was traced by Warringa *et al.*[13] to the presence of a substance in crude preparations, which differentially inhibits the reduction of the enzyme by succinate but does not interfere with its reoxidation by fumarate. The other unusual properties of the *M. lactilyticus* enzyme were explained only after succinate dehydrogenase was isolated in pure form from this micro-organism[13]. It was found that a single enzyme is responsible for both the oxidation of succinate and the reduction of fumarate in this micro-organism and that its properties are remarkably different from those of succinate dehydrogenases from aerobic mitochondria. In contrast to mammalian succinate dehydrogenase, it catalyses fumarate reduction far faster than succinate oxidation and possesses a very low Michaelis constant for fumarate, while the K_m for succinate and K_I for malonate are rather large. Hence fumarate is a good inhibitor of succinate oxidation but not vice versa, and malonate competes much more effectively with succinate than with fumarate[13,14].

Table 1. Effect of *N*-Ethylmaleimide on Succinate Oxidation and Fumarate Reduction by Mitochondrial Succinate Dehydrogenase from Yeast

Sample	Succinate dehydrogenase activity		Fumarate reductase activity	
	μmoles succ. oxidized/min/ml. enzyme	Per cent inhibition	μmoles fum. reduced/min/ml. enzyme	Per cent inhibition
ETP	32·4		1·1	
ETP + NEM	6·4	80	1·3	0

The respiratory chain preparation denoted as ETP was prepared from Red Star brand baker's yeast by the method of Mahler *et al.* (ref. 15). Treatment with *N*-ethylmaleimide (NEM) was by incubation of the preparation with 0·5 volume of 10 mM NEM at *p*H 7·0 at 0° for 10 min, followed by removal of the unreacted NEM on 'Sephadex G–25'. Succinate dehydrogenase activity was measured by the phenazine–DCIP assay at V_{max} with respect to phenazine methosulphate at 30° with pre-activation, fumarate reductase activity by the $FMNH_2$ assay at 30° in the presence of 1·33 mM $FMNH_2$.

As has been emphasized in reviews on the comparative biochemistry of succinate dehydrogenases[18,19], these differences are encountered in diverse forms of life and may represent an adaptation to aerobic or anaerobic existence. Those organisms which live in an abundance of oxygen possess succinate dehydrogenases which catalyse the oxidative reaction much faster than the reductive one, and the dehydrogenase from such sources generally shows a high affinity for succinate and a low one for fumarate, while the reverse is true of organisms of a predominantly anaerobic habitat. Succinate dehydrogenase from facultative anaerobes, such as *Propionibacterium pentosaceum*[18] or *Proteus vulgaris*[20], usually shows properties in between those of the mitochondrial enzyme and of the dehydrogenase from strict anaerobes.

Very recently, Hirsch *et al.*[21] demonstrated that *E. coli* cells contain two succinate dehydrogenases, both of them reversible in their action, one of which resembles the mammalian enzyme in the properties mentioned and is found in aerobically grown cells, while the other shows properties typical of succinate dehydrogenases from anaerobes. Although neither enzyme has been purified, their simultaneous presence in the same cell has been established by the use of suitable mutants. This demonstration prompted the present authors to re-examine the possible presence of fumarate reductases in yeast. It will be shown in this paper that yeast cells contain, besides the well-known mitochondrial enzyme, at least two and possibly three additional enzymes concerned with the reduction of fumarate.

Mitochondrial succinate dehydrogenase in yeast. Since information on the properties of succinate dehydrogenase from yeast mitochondria was derived largely from studies on purified, soluble preparations, Rocca *et al.*[22] have recently investigated the properties of the enzyme in

more nearly intact particulate preparations (mitochondria and the sub-mitochondrial particle referred to as "*ETP*" in the literature[15]). The preparations were isolated either by blending with Ballotini beads or with the Nossal shaker, as described earlier[23]. Succinate dehydrogenase was assayed by an adaptation of the phenazine–DCIP method[24], with a fast-recording spectrophotometer, and succinoxidase polarographically. In all respects examined, the molecular and catalytic properties of the enzyme were found to be very close to those of bovine heart succinate dehydrogenase[6]. The turnover number of the dehydrogenase at 30° was 12,500 ± 1,000 moles succinate oxidized/min/mole of bound flavin, a value which is not very different from that reported for the heart enzyme[19] (17,000 ± 1,000 at 38°), considering the difference in temperature. The forward/reverse ratio (succinate + phenazine methosulphate/fumarate + $FMNH_2$) is about 34 ± 2 at 30°, again close to the value found in mammalian mitochondria. With the availability of improved assay techniques even the two differences which were earlier thought to exist between yeast and heart muscle succinate dehydrogenases (activation by the substrate and cyanide sensitivity) have now been shown not to exist[22]. The dehydrogenase is completely absent in 'petite' mutants and virtually absent in anaerobically grown normal cells[22,25]. Thus succinate dehydrogenase in yeast develops only under strongly aerobic conditions and is very similar to its counterpart in mammalian mitochondria.

Fumarate reductases in cytoplasmic preparations. Since yeast mitochondria have been shown to contain unambiguously only one enzyme for the reduction of fumarate to succinate, the respiratory chain-linked succinate dehydrogenase[9,10], it remained to examine whether the extra-mitochondrial phase contained additional proteins capable of catalysing this reaction. When yeast cells are broken by the method recently described by Mahler *et al.*[15] and the supernatant solution obtained in sedimenting the mitochondria is clarified by further centrifugation (20–40 min at 105,000*g* or 1 h at 59,000*g*), followed by titration with protamine sulphate until all detectable succinoxidase activity is sedimented, the resulting optically clear solution still oxidizes succinate in the presence of phenazine methosulphate and reduces fumarate with $FMNH_2$ as donor, but the ratio of the forward/reverse reactions is very much lower than in mitochondria. On passage of this preparation through a column of 'Sephadex *G*-200' the excluded part contains all the remaining succinate dehydrogenase activity: the enzyme responsible will be referred to as enzyme I. The included fraction does not appear to oxidize succinate but reduces fumarate vigorously. From this included fraction two fumarate-reducing enzymes have been separated by chromatography on hydroxylapatite (Fig. 1), which will be referred to as enzyme II and enzyme III.

For the isolation of these enzymes 2 kg of well-washed baker's yeast ('Red Star') were suspended in a final volume of 2 l. with 1 M sucrose–0·02 M *tris*–0·001 M ethylenediamine tetraacetate (EDTA), *p*H 8·0, and were blended with 2 l. of Ballotini beads at 0° with an overhead blender and circular glass blade, as described by Mahler *et al.*[15]. Blending was continued for 28 min, with interruptions each 7 min for cooling the suspension. The suspension was poured off and brought to 3 l. by washing with 250-ml. aliquots of a 4-fold dilution of the sucrose–*tris*–EDTA solution referred to above. Sufficient 1 M dipotassium hydrogen phosphate was added to adjust the suspension to 0·05 M phosphate concentration and the *p*H was brought to 7·5 with 1 N potassium hydroxide. After 30 min centrifugation at 2,000*g*, the turbid supernatant solution was further centrifuged for 1 h at 59,000*g* in the Spinco ultracentrifuge, and the residue was discarded. The supernatant solution was precipitated with 0·7 saturated ammonium sulphate at *p*H 7·0–7·5; the precipitate was redissolved in dilute phosphate buffer and titrated with protamine sulphate (1 per cent in 10 mM phosphate, *p*H 7·6) until all turbidity and succinoxidase became readily sedimentable. The clarified solution was then precipitated with solid ammonium sulphate at *p*H 7·0–7·5 and the fraction precipitating between 0·2 and 0·7 saturation was collected. Ammonium sulphate was removed on 'Sephadex *G*-25' and the preparation was passed through a column of 'Sephadex *G*-200', equilibrated with 0·03 M phosphate, *p*H 7·5. The excluded fraction, containing enzyme I, and the included part, containing enzymes II and III, were separately concentrated by precipitation with solid ammonium sulphate at 0·7 saturation and neutral *p*H. Enzymes II and III were separated from each other on hydroxylapatite, as shown in Fig. 1. Rechromatography of either of these enzymes on hydroxylapatite gave single, symmetrical peaks and no indication of contamination with the other enzyme. Considerable additional purification of enzymes II and III was achieved by fractionation with ammonium sulphate.

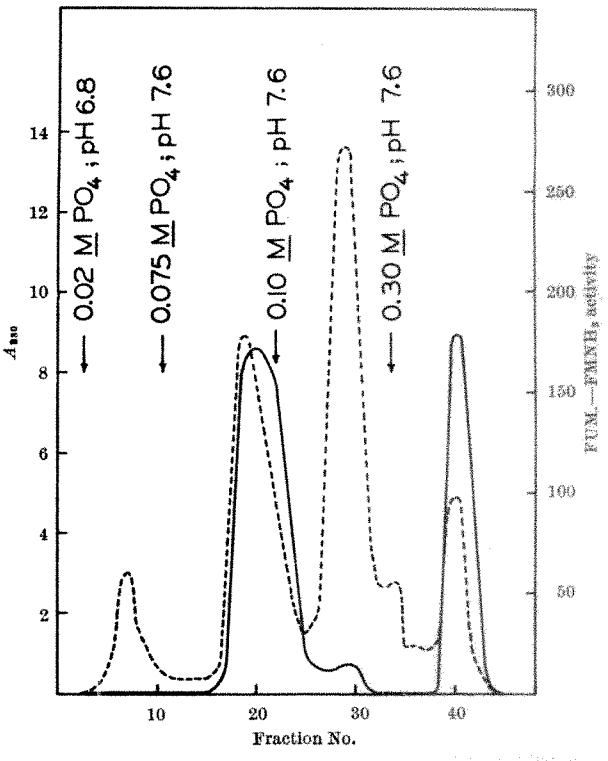


Fig. 1. Separation of the two low molecular weight fumarate reductases on hydroxylapatite. Solid line denotes activity in the fumarate–$FMNH_2$ assay, interrupted line absorbancy at 280 mμ. When rechromatographed on another hydroxylapatite column, both enzyme II (0·075 M phosphate eluate) and enzyme III (0·3 M phosphate eluate) gave single, symmetrical peaks

Known properties of enzyme I closely approximate those of succinate dehydrogenase from yeast mitochondria in soluble, purified preparations[9]. It is completely excluded on 'Sephadex *G*-200'; hence the molecular weight is of the order of 200,000 or higher. (The molecular weight of the mitochondrial enzyme is about 200,000.) Enzyme I is activated by the substrate and by competitive inhibitors; it is highly sensitive to malonate; it is present in aerobic but not in anaerobic cells; it oxidizes succinate with phenazine methosulphate as electron acceptor but does not react appreciably with cytochrome *c*, ferricyanide, or DCIP. The ratio of the forward/reverse reactions (succinate + phenazine methosulphate/fumarate + $FMNH_2$) is 7·6 at 30°, while the dehydrogenase solubilized from mitochondria shows a ratio of 9 in strictly fresh preparations, and this ratio declines gradually on storage[9,10].

Since enzyme I is present in very small amounts in the cytoplasmic preparation, at present it is uncertain whether it is derived from the mitochondria during the mechanical

Table 2. Comparison of Kinetic Constants of Fumarate-reducing Enzymes from Yeast

Constant	ETP from mitochondria	Cytoplasmic, Type II	Cytoplasmic, Type III
K_m fumarate	1·82 mM	1·43 mM	0·89 mM
K_I succinate	3·44 mM	6·1 mM	4·2 mM
K_I malonate	0·11 mM	0·79 mM	0·49 mM

Constants were determined by the manometric hydrogenase–$FMNH_2$ assay at 30°.

breakage of the cells and thus represents a small degree of solubilization of the mitochondrial enzyme, or whether it is a genuine cytoplasmic constituent, conceivably a precursor or breakdown product of the respiratory chain-linked enzyme.

In contrast, enzymes II and III bear very little resemblance to mitochondrial succinate dehydrogenase either in the soluble or the particle-bound form.

(1) They are completely included in 'Sephadex *G*-100' and '*G*-200' and partly included in '*G*-75'; hence their molecular weights may be about 50,000. (2) In contrast to the mitochondrial enzyme, they are present at undiminished levels in anaerobically grown normal yeast and in 'petite' mutants. (3) They are far more stable than the mitochondrial enzyme to a variety of treatments. (4) They do not appear to contain covalently bound flavin. (5) They do not oxidize succinate with phenazine methosulphate, ferricyanide, DCIP or cytochrome *c* as electron acceptors. Attempts to establish a bleaching of these flavoproteins by succinate under anaerobic conditions have thus far given negative results. Since succinate is a competitive inhibitor of both enzymes II and III (Table 2), clearly succinate combines with their active centres. Conceivably, the failure to detect a net oxidation of succinate may be the consequence of an unusually low redox potential of the flavin in these proteins. Lastly, they differ both from the mitochondrial enzyme and from each other in regard to kinetic constants (Table 2). Enzymes II and III have lower K_m values for fumarate and higher K_I values for succinate and malonate than the respiratory chain-linked enzyme, and thus also in this regard they resemble the fumarate-reducing enzymes of strict anaerobes[14].

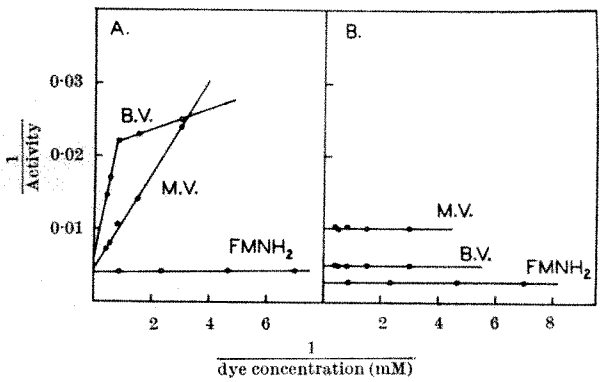


Fig. 2. Comparison of specificities of enzyme II (*A*) and enzyme III (*B*) for electron donors. *M.V.*, methylviologen; *B.V.*, benzylviologen. All assays were at 30°, *p*H 7·6 by manometric measurement of hydrogen uptake in the presence of excess hydrogenase from *Cl. pasteurianum* (ref. 26). The biphasic curve seen with benzylviologen in *A* is often noted with this dye and is due to impurities present even in recrystallized preparations of the dye (ref. 13)

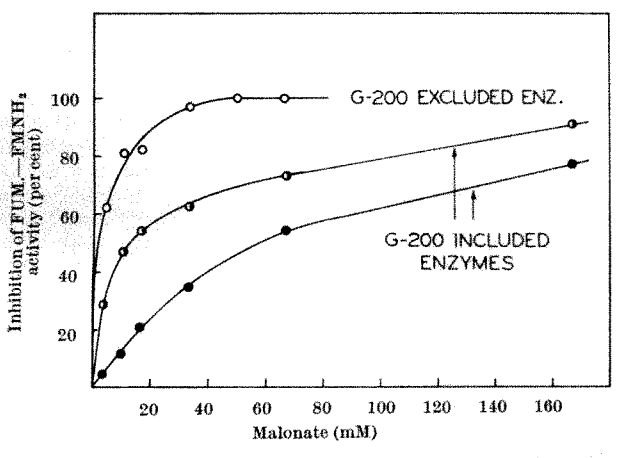


Fig. 3. Malonate sensitivity of fumarate-reducing enzymes from yeast cytoplasm. Activities noted on the abscissa refer to the fumarate–$FMNH_2$ assay at 30°, *p*H 7·6 in the presence of 2 mM fumarate. Open circles, enzyme I; solid circles, enzyme II; half-shaded circles, enzyme III

Known differences between enzymes II and III, besides those already mentioned, are as follows. They show somewhat different *p*H optima in the fumarate–$FMNH_2$ assay[26]; they precipitate at different concentrations of ammonium sulphate; enzyme II is more stable to storage and to exposure to acid *p*H than enzyme III; they show different reaction patterns with electron donors (Fig. 2) and different sensitivities to inhibition by malonate (Fig. 3). Further, while enzyme II is neither inactivated nor split into apoenzyme and flavin at *p*H 3·5 in the presence of ammonium sulphate, enzyme III is reversibly resolved into apoenzyme and flavin by this treatment.

It should be emphasized that, while the findings recorded here establish the presence of several fumarate-reducing enzymes in yeast, two of which appear to be incapable of oxidizing succinate, at least under the conditions tested, none of the enzymes discussed can be readily identified with Fischer's "fumaric hydrogenase" since they are neither inactivated to an appreciable extent nor resolved into apoenzyme and FAD by dialysis under the conditions used by Fischer *et al.*[4].

The biological function of these new enzymes is of considerable interest. Conceivably they are concerned with the production of succinate under conditions where the Krebs cycle is not functioning and thereby provide an important intermediate for synthetic reactions.

This work was supported by contract *Nonr* 1656 (00) between the Office of Naval Research, U.S. Navy, and this Institute, and by grants from the National Institutes of Health (*HE*-01995), the National Science Foundation, and the American Heart Association.

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LETTERS TO THE EDITOR

ASTRONOMY

A Case for Convection in the Moon

MODERN workers are agreed that the Moon has a small bulge, measured with reference to the mean radius of the limb, pointing in the direction of the Earth. The order of magnitude of this body-elongation is 0·1 per cent of the radius, but the actual value assigned to it depends quite critically on the selection of measured points used in the analysis.

A recent re-determination of the height of the bulge has been made by Baldwin[1], who separated points lying in the dark regions (excluding the deep, circular maria) from those of the bright regions (excluding the high mountains) and found values of about 1·2 km and 2·0 km, respectively. Conceding that the bulge is not significantly different in the low continents and in the irregular maria it seems that an explanation involving widespread uniformity of stress must be sought. For this reason I shall not detail the suggestion of Jeffreys[2], that the bulge was due to "irregular changes arising after solidification", or of Urey[3], that it was built up as a net deposit from differential impacts. Baldwin[1] has attributed the bulge to early tidal forces, while Runcorn[4] has proposed that currently operating convection cells keep the Moon's surface buoyed up as they rise in the centre of face.

The Moon's mechanical ellipticity, f, is found from observations to be at least 0·6 (cf. ref. 5). If the Moon had solidified as a synchronous satellite of the Earth the much smaller value $f = 0{\cdot}25$ would obtain[6]; hence it is difficult to believe that the bulge can be a fossil tide raised by the Earth. Baldwin[1] has postulated that the bulge was indeed raised when the Moon was a synchronous satellite bound close to the Earth, but that "the theoretical value of $f = 0{\cdot}25$ is more or less completely masked by the larger surface variations".

Yet on Baldwin's theory the original bulge formed more than 4×10^9 yr ago and, as the bulge is still present in the maria, their surfaces, too, must be very old. This seems to me to be a serious objection to the theory, for I find it difficult to believe that so few impact craters have formed in the maria in 4×10^9 yr. With different (I consider more realistic) assumptions about the rate of crater growth, based on crater-counts, I have found[7] that even the oldest maria are but one-seventh as old as the continents. It therefore seems to be at least equally reasonable to suppose that no mare is older than 7×10^8 yr. On this hypothesis the Moon would be little nearer to the Earth, when the maria were formed, than it is to-day; and the observed bulge in the maria could not have been raised by the appropriately weak value of the Earth's attraction.

Runcorn's convection theory[4] provides a solution to this problem: the general form of the bulge would be similar in the continents and in the maria if they were uplifted uniformly by rising convection currents. Regional isostatic adjustments could occur, as in the case of Baldwin's theory. There are, moreover, additional reasons for advocating a convection theory.

First, convection currents provide a means of carrying heat to the surface, and they would assist the mare melting and the Moon's differentiation that many authors advocate. Secondly, it is important to find an explanation for the remarkably uniform system of strike-slip faults[8] that traverse the Moon's face. A triaxial Moon adjusting to the equipotential shape is inadequate to explain this lunar grid system. Tidal forces are also unlikely to have been responsible unless, possibly, the Moon was warm at the time[9]. However, specially oriented, low-order convection cells would exert a drag on the crustal layers of the Moon, and the drag at any point would be fixed in direction for a very long period of time and, specifically, during the recent era of lunar history. In principle, this mechanism is capable of providing an explanation of the grid system.

Urey[10] has suggested that the lunar continents are between $3{\cdot}0 \times 10^9$ and $4{\cdot}5 \times 10^9$ yr old. I have estimated[7] that Mare Imbrium is only 1/19th of the age of the continents, so Mare Imbrium may have an age of 2×10^8 yr. Mare Frigoris appears to be of the same order of age[7]. The 'Frigorim Fault'[11] links Mare Imbrium and Mare Frigoris. It appears to be the longest strike-slip fault on the Moon, and has an offset of 200 km. It cannot be traced in the maria themselves, so it must be at least as old as they are; hence the maximum rate of movement along this major fault has been $\sim 0{\cdot}1$ cm yr^{-1}. This figure is not in conflict with Runcorn's estimate[12] of $\sim 0{\cdot}2$ cm yr^{-1} for the rate of creep of the lunar convection currents.

I thank Imperial Chemical Industries, Ltd., for a research fellowship.

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RADIO ASTRONOMY

Spectra of the Galactic Radio Emissions

RECENT observations of the spectra of the galactic emissions in the 1–10-Mc/s frequency range using satellite, rocket and ground-based receivers have produced apparently conflicting results[1–4].

The disagreement may be due partly to the difficulty of calculating the antenna efficiency in the ionospheric plasma for the orbital receivers, although the spectra obtained in the different ways have not been directly comparable since the low-resolution antennæ used above the ionosphere have looked at different areas of the sky from those surveyed from the ground. High-resolution studies[5], for example, have shown pronounced absorption within the galaxy, depending on galactic latitude and with an angular scale smaller than the beamwidth of the antennæ used for spectral observations. Below 3 Mc/s the absorption can be seen in the high-resolution profiles right out to the galactic pole[6].

It is suggested here that low-resolution spectra are meaningful only for directions near the poles where the brightness distribution is fairly uniform. The previously published ground observations did not meet this requirement since they were given for directions where the observed intensities were greatest and least during the right ascension scan. These directions were declination $-42°$, R.A. 2000 and R.A. 0400 h respectively. That is, galactic latitudes 30° and 45°, longitudes 325° and 214°. The subsequent high-resolution surveys showed consider-

able absorption in these directions compared with the polar direction.

Fig. 1 shows the intensities observed at Hobart at 1·65 Mc/s, 2·4 Mc/s, 4·8 Mc/s and 9·6 Mc/s for Dec. −42° and R.A. 0030 (galactic latitude −80°); that is, in the vicinity of the south galactic pole. The experimental details have been described previously. The antenna beamwidths were 32° × 40°. The attenuation in the ionosphere was calculated using observed *D*, *E* (ref. 7) and *F* (ref. 8) region density profiles and, at 1·65 Mc/s, amounted to 0·25 db for the ordinary mode and 480 db for the extraordinary mode. The effect of the ground on the antennæ was calculated after measuring the ground conductivity. The screening of the edges of the antenna beam by the ionosphere was calculated using observed values of f_0F_2. The attenuation allowed for by screening was 0·5 db at 1·65 Mc/s. Also shown in Fig. 1 are results obtained by Walshe *et al.*[3] and Hartz[4], although as mentioned earlier these are not strictly comparable.

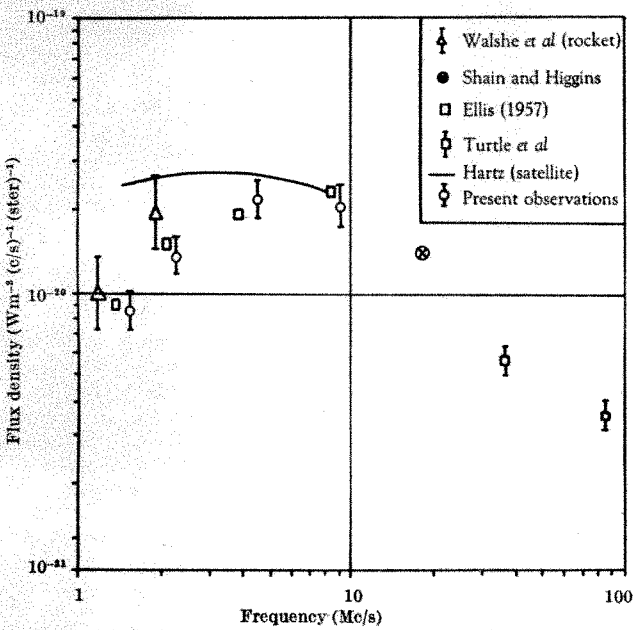


Fig. 1. Comparison of low-resolution cosmic radio noise intensities in the direction of south galactic pole (observed at Hobart) with satellite and rocket observations. Representative higher frequency points are also shown (refs. 10–12)

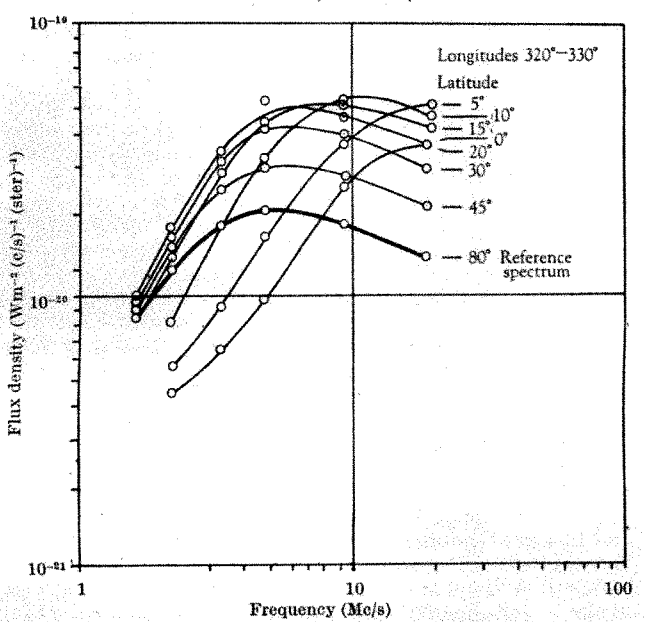


Fig. 2. High-resolution spectra at low galactic latitudes derived from Fig. 1 and brightness profiles of Ellis *et al.*[5]

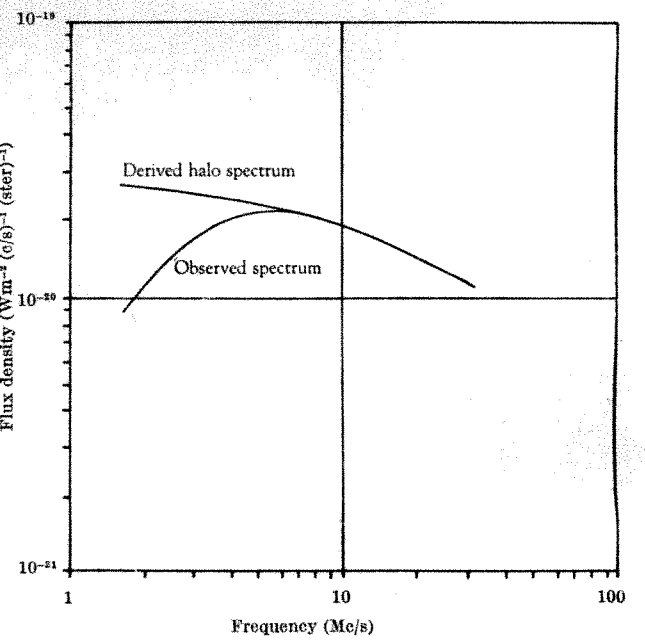


Fig. 3. Derived emission spectrum in the halo region

By using the spectrum in the direction of the pole as a reference we may construct the spectra in other directions using the high-resolution brightness profiles of Ellis and Hamilton[6]. These spectra are shown in Fig. 2. It is noteworthy that for low galactic latitude where the absorption is high the intensities at low frequencies have a spectral index of +2, that is, intensity $S \propto f^2$, as would be expected for a strongly absorbing interstellar medium[9].

Finally, it has been shown that the absorption coefficient in the galactic disk may be obtained from the change in shape of the high-resolution profiles only, and independently of absolute measurements of intensity. Ellis and Hamilton[6] found the absorption coefficient K to be $2{\cdot}5 \times 10^{12}$ cm^{-5} (°K)$^{-3}$ in the direction of the south galactic pole and the emission measure 1·92 cm^{-6} pc. The observed flux density S is proportional to $e^{-\int K dl}$ where $K = \dfrac{0{\cdot}4N^2}{T^{3/2}f^2}$.

We are able therefore to calculate the absorption in the disk at any frequency and hence, with the observed high-latitude spectrum as a starting point, calculate the emission spectrum for the radiating electrons outside the disk. This spectrum is shown in Fig. 3. It shows a flattening towards the low-frequency end with a spectral index of −0·15. This might be expected from ionization losses or some other process leading to depopulation of the lower-energy electrons[9].

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PHYSICS

Amorphous Magnetic Films

IRON has been vapour-deposited on to amorphous carbon substrates at room temperature and examined, while it was being deposited, by direct-recording electron diffraction[1]. Fig. 1 shows electron-intensity profiles along a radius vector of the Debye–Scherrer pattern. Successive traces recur every 50 sec at film thickness increments of about 20 Å. In Fig. 1*a* the profiles correspond to an amorphous structure; however, as the film grows sharp peaks appear, beginning in the final trace. These sharp peaks grow rapidly, initially at the expense of the diffuse ones; compare the first and fourth traces in Fig. 1*b*. The sharp peaks are those of normal b.c.c. iron, with measured lattice spacing 2·87 Å. If the deposition is continued beyond the stage represented by trace 4, Fig. 1*b*, there is little further change in the shape of the diffraction profiles but their intensities diminish as the film becomes more opaque.

Specimen growth may be stopped at any stage desired and the film transferred from the diffraction equipment to an electron microscope and there examined by diffraction, normal high-magnification and by Lorenz microscopy[2]. Fig. 2 shows a Lorenz micrograph of a film in the amorphous state. This was confirmed by diffraction photographs taken before and after the Lorenz microscopy. Fig. 2 shows a magnetic domain structure : from this and a knowledge of film thickness an estimate of the magnetization $M \simeq 0{\cdot}35$ Wb/m² may be made. Thicker films which have largely crystallized have a value of M of 1·4 Wb/m², which may be compared with the value 2·15 Wb/m² for bulk iron.

The amorphous-crystalline transformation appears reproducible: a total of 42 specimens have been grown on carbon and all show the phenomenon. The transformation also occurs when the substrate is a layer of SiO previously deposited on the carbon.

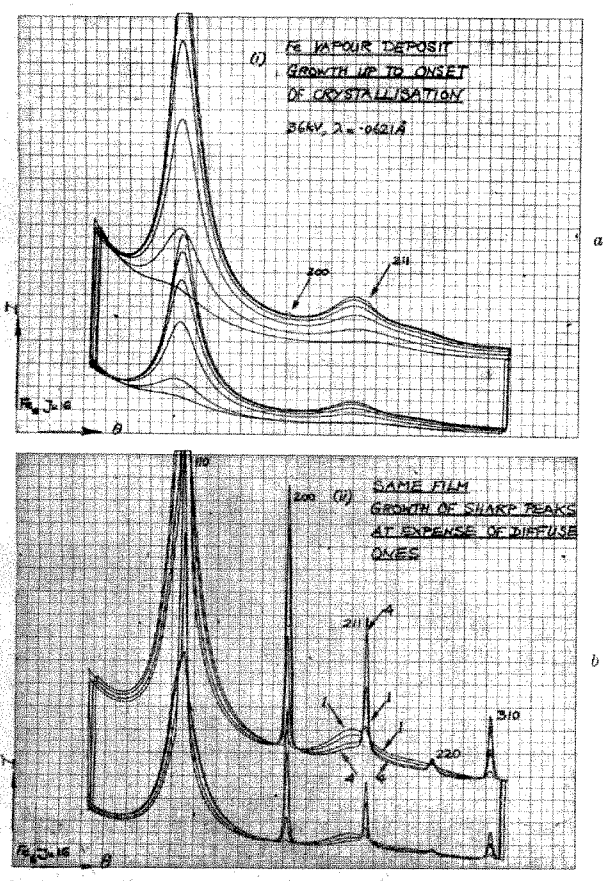


Fig. 1

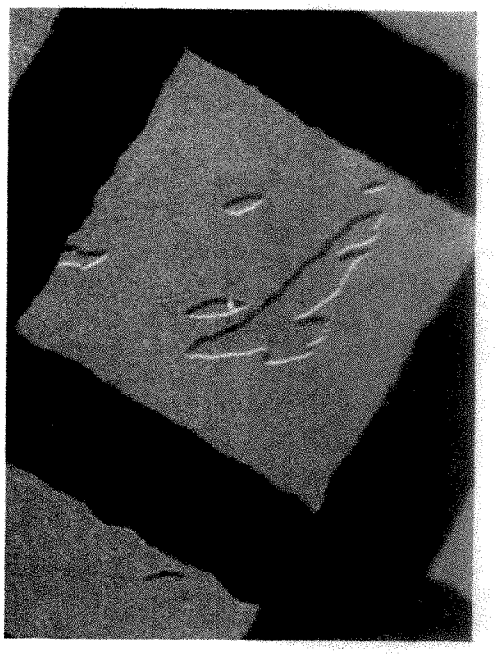


Fig. 2

Since the growths are made in a vacuum of 2×10^{-6} torr when the substrates will be covered with adsorbed gas molecules, there is some doubt whether the amorphous material is iron or some other compound. However, once recrystallization begins some of the amorphous matter itself transforms to crystalline iron. Moreover, if an amorphous film is heated, by increasing the illumination intensity in the electron microscope, it transforms to crystalline iron. These two facts suggest that the amorphous material consists chiefly of iron atoms.

We conclude that an amorphous magnetic state of matter has been found, for which the band theory model of Stoner[3] cannot apply; and that the amorphous material is probably iron.

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Quantity Equations and System Variation in Electricity

IN electricity there exists an unsettled dispute concerning the nature of system variation, that is, the transition from one system to another. According to one of the parties in the dispute, system variation is caused by a variation of units, whereas the other party maintains that system variation is caused by a variation of quantities[1,2]. In a recent article[3] I demonstrated that this variation consists in a change in the form of quantity equations without a change in quantities. As a consequence some units become incoherent and may be replaced by other units, which are now coherent with the changed equations. In the following a résumé of the problem in question is given.

The electromagnetic equations in the Giorgi system differ from the equations in the three classical systems (electrostatic, electromagnetic, symmetric) mainly in two points: (1) the 'sphere factor' 4π appears in the Giorgi system in equations other than in the classical systems ('rationalization'); (2) the 'permittivity of free space', ε_0, and the 'permeability of free space', μ_0, are introduced in the equations of the Giorgi system; these coefficients are unknown in the classical systems.

A typical example illustrating point (1) is the equation connecting magnetic field strength and current. This equation reads $\oint H\mathrm{d}s = I$ in the Giorgi system and $\oint H\mathrm{d}s = 4\pi I$ in the electromagnetic system. A typical example for point (2) is Coulomb's law, which reads $F = Q_1Q_2/(4\pi\varepsilon_0 r^2)$ in the Giorgi system and $F = Q_1Q_2/r^2$ in the electrostatic system.

There are two divergent explanations for these dissimilarities:

(I) The electromagnetic equations are 'measure equations'. In the different systems different units are used. For example, the Giorgi unit for H is 4π times that of the electromagnetic system, apart from the decimal conversion factors for the units of I and s; the electrostatic unit of Q is defined by the mechanical units of force and length; the Giorgi unit of Q may be considered as an independent electrical unit, hence the coefficient ε_0.

(II) The electromagnetic equations are relations between 'quantities', a quantity being symbolized by the product of a measure and a unit. In the various systems a certain number of physical laws are described by means of quantities which are defined in different ways. In our examples the relation between the rationalized H_r from the Giorgi system and the non-rationalized H_n from the electromagnetic system is:

$$H_n = 4\pi H_r$$

The quantity Q_G from the Giorgi system with four fundamental quantities and the quantity Q_e from the electrostatic system with three fundamental quantities are connected by:

$$Q_e\sqrt{4\pi\varepsilon_0} = Q_G$$

With regard to explanation (I), it is not very elegant to consider the symbols in the electromagnetic equations as measures. These equations formulate laws, that is, relations between physical quantities. These laws and quantities are independent of units. Measure equations apparently do not do full justice to this fact.

In explanation (II), to consider the symbols in the electromagnetic equations as quantities does emphasize the said independence. To consider system variation as a variation of quantities, however, is even less elegant than explanation (I), because in this case physical quantities seem to depend on the choice of the system. For example, there exists from the point of view of (I) only one quantity 'charge', whereas from the point of view of (II) one has to distinguish between the quantities Q_e and Q_G, thus violating the original intention of accentuating the independence of physical quantities from units and systems.

I have recently demonstrated[3] that the essential difference between the electromagnetic systems does not consist in a change of units or quantities, but in a change in the form of the equations and hence in a change in the coherence of units. The gist of the matter may be seen with the aid of our example regarding H. The underlying law is: $\oint H\mathrm{d}s \sim I$. This stands for:

$$\frac{\oint H\mathrm{d}s}{\oint H_1\mathrm{d}s} = \frac{I}{I_1}$$

where H, I and s are a combination with arbitrary values of I and s, and H_1, I_1 and s_1 are a combination with known values of field-strength, current and length. The right-hand side of this quantity equation may also be written: $kI/(kI_1)$. In the Giorgi system $k = 1$ has been chosen, in the electromagnetic system $k = 4\pi$. As a consequence, the 'coherent Giorgi unit' for H would be 4π times the 'coherent electromagnetic unit' for H, if one should use the same units for I and s in both equation systems. In fact, because 1 cm = 0·01 m and 1 amp = 0·1 e.m.u. of current, one has 1 amp/m = 1œ × 4π/1000.

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GEOLOGY

Implication of Certain Sedimentary Structures within the Cambrian Succession of the St. Tudwal's Peninsula, South-west Caernarvonshire

The source and depositional environment of the Cambrian sediments in the Lleyn Peninsula, North Wales, has been the subject of much dispute. Early work by Nicholas[1] described some of the sedimentary structures, but their importance in relation to the palæogeography of the Welsh geosyncline was first discussed by Kopstein[2] working in the Harlech Dome and later by Bassett and Walton[3] in the St. Tudwal's Peninsula. Each member of the succession can be correlated with similar horizons in the Harlech Dome[4].

Recent work (unpublished) in the Harlech Dome has shown the structural pattern to be more complex than has previously been supposed; consequently certain of the methods used by Kopstein are now known to be inapplicable. He concluded that, with the exception of the Dolwen Grits, palæocurrent evidence indicated derivation of the sediments from the south-south-west during the whole of the Cambrian. Bassett and Walton, however, concluded that the source of the Lower Cambrian Hell's Mouth Grits lay to the north. Knill[5] suggested that the Lower Cambrian Rhinog Grits of the Harlech Dome were derived from the north-east while the overlying Barmouth Grits appeared to have been largely derived from the south-west.

A limited number of readings taken from sedimentary structures within the Lower Cambrian Hell's Mouth Grits and Mulfran Beds are in agreement with those of Bassett and Walton, indicating a northerly source. The overlying Cilan Grits yield variable current directions, but within the Ceiriad Mudstones and Nant-Pig Mudstones there is evidence of a south-westerly source. There are, however, relatively few palæocurrent structures within this mid-Cambrian part of the succession.

Table 1. Relevant Part of the Succession of the Cambrian of St. Tudwal's Peninsula and the Harlech Dome

St. Tudwal's Peninsula	Harlech Dome
Lingula Flags	Vigra Flags
Nant-Pig Mudstones	Clogau Shales
Ceiriad Mudstones	Gamlan Shales
Cilan Grits	Barmouth Grits
Mulfran Beds	Manganese Shales
Hell's Mouth Grits	Rhinog Grits
	Llanbedr Slates
	Dolwen Grits

The Upper Cambrian Lingula Flags have a large number of exceedingly well-preserved structures which show that, during the deposition of the lower beds, currents from the south-westerly quadrant were active; a few hundred feet above the base, however, the current direction was from the south-south-west. On the east side of Porth Ceiriad there is a small exposure which probably represents a higher horizon in the Lingula Flags and here the current direction was from the south-south-east.

Thus, in general, currents appear to have travelled from the north in the Lower Cambrian and from the south in

the Upper Cambrian. The pattern of currents in the Cambrian is more complex and variable than has previously been suggested, consequently caution should be exercised in interpreting the palæogeographic importance of these and similar results, until more extensive regional investigations are completed.

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Iron-deficient Low-temperature Pyrrhotite

During the past few years considerable attention has been focused on the potential of pyrrhotite as a geothermometer. In 1962 Arnold[1] determined experimentally the solvus relationship between hexagonal pyrrhotite and pyrite, thereby demonstrating that the percentage of iron in pyrrhotite along this solvus was temperature dependent, but insensitive to pressure. The potential of this method was then tested on samples from the Highland-Surprise Mine[2] and the temperatures obtained from the pyrrhotite geothermometer were found to agree well with those obtained from associated sphalerite using the sphalerite geothermometer[3]. More recently, Buseck[4] and Kullerud *et al.*[5] have attempted to apply the method to monoclinic pyrrhotite–pyrite assemblages by inverting the monoclinic pyrrhotite to hexagonal symmetry by heating.

The occurrence of pyrrhotite in the northern Pennine orefield[6], although limited, is of interest because fluid inclusion studies have shown that depositional temperatures of fluorite and quartz, the two most abundant minerals in the central portions of the field, are below 250° C. To our knowledge the only reported instance of pyrrhotite formed under hydrothermal conditions at temperatures below 250° C is that described by Balitskii and Lyubofeyev[7]. Accordingly, these pyrrhotites were investigated in detail to ascertain whether they were in fact deposited at temperatures below 250° C and whether the pyrrhotite geothermometer could be applied to them.

The results obtained from pyrrhotite and associated minerals from localities in the Rookhope Borehole[8] and the Great Sulphur Vein[9] are summarized in Table 1.

At 1,083 ft. the borehole intersected a small replacement body of quartz and fluorite in the Smiddy Limestone. Here, the time relationships of the various minerals are obscure, but pyrrhotite-sphalerite and pyrrhotite–pyrite intergrowths occur on a very small scale. The veins intersected at depths of 1,355 ft. and 1,562 ft. in the Rookhope Borehole are in granite and have a maximum thickness of 6 in. The pyrrhotite appears to have formed at a very late stage for it invariably occurs in the centre of veins and in small vugs.

The Great Sulphur Vein is a large east–west vein which consists of quartz accompanied by small amounts of iron sulphides and traces of chalcopyrite. The available geological evidence (K. C. Dunham, personal communication) suggests that it represents the root of what must have been an extensive fluorite–quartz–galena vein at higher levels. This conclusion is supported by the reported occurrence of quartz and pyrrhotite in cores from drill holes in the lowest level of the Cammock Eals Mine of the Slitt Vein (K. C. Dunham, personal communication).

Arnold and Reichen[9] and Arnold[1] demonstrated that the d (102) spacing of hexagonal pyrrhotite is a function of its composition. The d-spacing of the pyrrhotite peak (102) was determined first on natural separated samples, and then on samples heated to 350° C in evacuated glass tubes following the method suggested by Buseck[4]. Silicon was used as an internal standard. The temperatures shown in Table 1 were then estimated using the graphs relating d-spacing to iron/sulphur ratio and then iron/sulphur ratio to temperature[1]. Full traces of the sample from 1,355 ft. in the Rookhope Borehole were run from 14°–90° 2θ to examine the changes resulting from the heating. The natural sample was monoclinic but on heating it inverted to hexagonal symmetry with cell parameters a = 3·45 Å and c = 5·67 Å. In Table 1 values for the twin peak (102) are given, but after heat treatment only a single peak was obtained. An X-ray spectrographic analysis of this material indicates that manganese, cobalt and lead are all present in amounts less than 1 per cent and nickel and copper in amounts less than 0·1 per cent. The appearance of an additional twin peak with d-spacings of 2·088 and 2·092 in the sample from 1,083 ft. suggests the presence of two pyrrhotites, one of which approaches stoichiometric FeS. This conclusion is supported by the occurrence of small amounts of pyrrhotite at 1,895–1,898 ft. with a d-spacing of 2·08 Å measured from a powder photograph.

The iron contents of banded sphalerite from 940 ft. and sphalerite intergrown with pyrrhotite from 1,083 ft. were estimated by calculating cell parameters from the d-spacings of the peaks (331) and (422). 'Analar' sodium chloride was used as an internal standard. These sphalerites proved to have identical cell parameters (a = 5·409 Å), indicative of temperatures less than 250° C. The absence of associated pyrrhotite in the sample from 940 ft., however, suggests that this temperature will be a minimum. Chemical analysis of this banded material by the method of Shapiro and Toulmin[10] gave 0·37 per cent iron for the light bands and 2·31 per cent iron for the darker variety. Both results are in broad agreement with the X-ray data.

The temperatures quoted in Table 1 for fluorite and quartz were obtained from the filling temperature of primary fluid inclusions. An estimated correction for pressure of + 20° C has been added to these filling temperatures to take into account the relationship between the filling temperature of the fluid inclusions and their true

Table 1. Data relevant to Pyrrhotite Crystallization

Sample	Assemblage	Pyrrhotite dÅ Natural	Pyrrhotite dÅ Heated	Pyrrhotite Temperature °C	Supporting temperature data
Rookhope Borehole. 1,083 ft.	Quartz, pyrite, pyrrhotite, sphalerite. Pyrite–pyrrhotite intergrowths.	(1) 2·0535 * (2) 2·088	2·0603	450	Sphalerite (ref. 18). a = 5·409 Å < 1% FeS. < 250° C.
	Pyrrhotite–sphalerite intergrowths. Mineralization in Smiddy Limestone	(2) 2·092	2·0855	Low ?	No suitable fluid inclusions
Rookhope Borehole. 1,355 ft.	Quartz, fluorite, pyrrhotite, calcite, pyrite. Sulphides in centre of vein, often lining vugs. Mineralization in Weardale granite	2·053 2·063	2·0581	485	Fluorite. 160°–174° C (8 readings) Quartz. < 220° C. Visual estimate
Rookhope Borehole. 1,562 ft.	Fluorite, quartz, calcite, pyrrhotite, pyrite. Sulphides in centre of vein, often lining vugs. Mineralization in Weardale granite	2·0565 2·063	2·0586	475	Fluorite. 140°–160° C (7 readings)
Great Sulphur Vein	Quartz, pyrrhotite, pyrite, chalcopyrite. Pyrite veining pyrrhotite. From sheared zone in sediments in Sir John's Level	2·0565	2·0575	530†	Quartz. 205°–230° C (5 readings)

* (1) From pyrrhotite–sphalerite intergrowth; (2) from pyrrhotite–pyrite intergrowth.
† A correction of 35° C was added to the determined temperature to allow for the presence of chalcopyrite (ref. 17).

temperature of formation. The precise relationship between filling temperatures and temperatures of formation of fluid inclusions can only be determined when the difference between the vapour pressure of the hydrothermal solutions and the total pressure is known[11]. As a result, whereas the filling temperatures are subject to an uncertainty of only ± 5° C, the temperature of formation of these inclusions is subject to an uncertainty of ± 25° C.

There is a marked discrepancy between the temperature results obtained from the pyrrhotite geothermometer and those obtained from fluid inclusions in minerals closely associated with the pyrrhotite. Further evidence for this discrepancy can be obtained from a consideration of the maximum possible pressures that could have existed during the emplacement of the Great Sulphur Vein. The maximum stratigraphic thickness that ever existed above the level from which the samples were taken was almost certainly less than 5,000 ft.[12,13]. Even if the rising solutions were subjected to the full lithostatic load of these overlying sediments the total pressure on them could not have exceeded 400 bars. At 530° C (cf. Table 1) the vapour pressure of aqueous solutions containing 5–20 wt. per cent sodium chloride (the most probable range of salinity for hydrothermal solutions, cf. Roedder[14]) is between 600 and 680 bars[15]. Thus, the pyrrhotite and associated quartz would have had to be transported by supercritical solutions. The results of calculations by Krauskopf[16] indicate that ore transport by supercritical fluids is unlikely.

The possibility that the pyrrhotite from the Rookhope Borehole represents an early high-temperature phase of ore deposition must be excluded since it invariably occurs in the centre of the veins and in some instances is intergrown with pyrite or sphalerite. In addition, the position of the pyrrhotite within the veins precludes the possibility of a large temperature gradient across the veins. The results of this investigation suggest that iron-deficient pyrrhotites can be deposited at temperatures below 250° C and that the pyrrhotite–pyrite solvus determined by Arnold cannot in all cases be applied to monoclinic pyrrhotites even after the pyrrhotite in question has been inverted to the hexagonal form.

We thank Prof. K. C. Dunham for his advice. This work forms part of the Rookhope Borehole Project financed by the Department of Scientific and Industrial Research, who also provided financial support for two of us (F. J. Sawkins and A. C. Dunham). The X-ray spectrographic data were supplied by Mrs. Maureen Kaye, and the iron determinations made by R. Lambert.

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MINERALOGY

Bonding of Hydrogen in Hydrogen-Montmorillonite

Research concerning bonding conditions for tritium and deuterium in micaceous minerals, as formerly mentioned by us[1], indicated the presence of apical OH in dioctahedral hydro micas. Burns and White[2] conclude from infra-red examination after hydrogen saturation and heating of $LiNO_3$-treated muscovite that hydronium ions H_3O^+ replace interlayer cations, giving rise to an infra-red absorption band at considerable bathochromic shift from the normal OH band of the mica (3,472 cm^{-1} respectively 3,600 cm^{-1}). In order to elucidate the bonding problem of the protons of hydrogen micas we have examined the very pure dioctahedral montmorillonite from Skyrvedalen, already described by one of us (I. Th. R.)[3]. As acid treatment tends to attack the silicate lattice we have obtained the desired hydrogen-substituted minerals by introduction of NH_4^+ in the exchangeable positions and subsequent dissociation of NH_3 by heat treatment.

The natural mineral corresponds to the following formula:

$$(Ca_{0\cdot24}Na_{0\cdot61})(Mg_{0\cdot36}Al_{1\cdot62}Fe^{III}_{0\cdot02})(Si_{3\cdot87}Al_{0\cdot13})O_{10}(OH)_2 \cdot 1\cdot08\ H_2O$$

0·49 exchangeable val.

This mineral has been treated for exchange with D_2O, NH_4Ac or ND_4Ac and afterwards heated to various temperatures in various atmospheres. Fig. 1 gives the infra-red absorption between 4,000 and 500 cm^{-1} of some characteristic samples. To facilitate comparison the diagrams have been redrawn to scale so that the Si—O band at 1,020 cm^{-1} is given the same intensity by all samples.

The infra-red absorption of the D_2O (and OD) in calcium montmorillonite is seen by comparing diagrams *A* and *E*. By heating to 420° C it was found that the NH_4^+ or ND_4^+ in interlayer position completely disintegrated: in air by oxidation to H^+, N_2 and water, in nitrogen by dissociation in D^+ and ND_3.

As seen from Fig. 1, *D* and *G* to *K*, H^+ from the hydrogen montmorillonite and D^+ of the deuterium montmorillonite do not give any other bands than those of OH and OD. By heating in moist air the OD band diminishes concordantly to the increase in the OH band. We therefore conclude that the dry hydrogen montmorillonite transforms in such a way that the interlayer H^+ respectively D^+ combines with an O of the montmorillonite lattice, giving rise to normal OH or OD bands. The presence of any H_3O^+ which may exist in interlayer positions in hydrogen montmorillonite in the moist state, is not revealed after heating either by infra-red or by nuclear magnetic resonance. Only very faint indications to the presence of bands that may correspond to the 3,472 cm^{-1} band described by White and Burns can be seen (Fig. 1, *J*, *K*). The bands at 3,520 respectively 2,590 cm^{-1} are, however, far too weak to equivalate the 0·49 protons respectively deuterons introduced by the exchange reaction.

The ammonium treatment and subsequent heating to 420° C reduced the base exchange capacity of the montmorillonite from 110 m.equiv. to 80 m.equiv. per 100 g, whereas the same heating of the calcium montmorillonite without previous ammonium exchange only reduced the exchange capacity at most from 110 to 105 m.equiv.

Introduction of ammonium and subsequent heat treatment reduced the *b*-axis of the mineral by 0·072 Å (a shift in the 060 line from 1·502 to 1·490). Such a contraction of cell dimension may be explained by the introduction of H's in vacant octahedrons, as predicted by us[4]. This will also necessarily reduce the negative charge and the base capacity. It seems to fit with the X-ray finding of

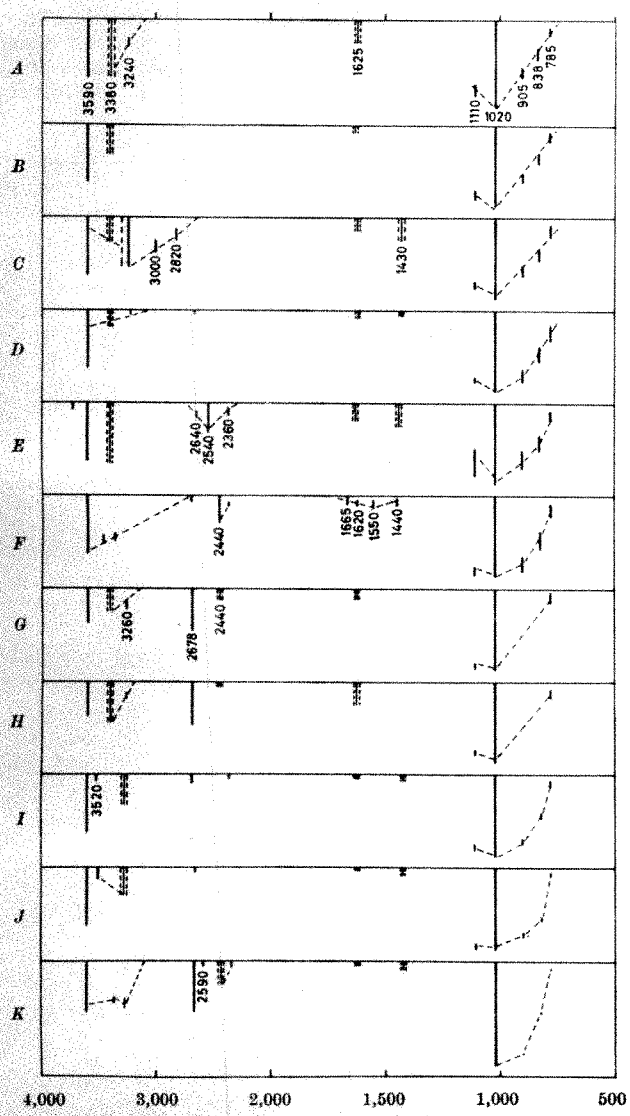


Fig. 1. *A*, Ca^{+} montmorillonite, untreated, air-dried; *B*, sample *A* heated for one day at 420° C in air; *C*, NH_4^{+} montmorillonite heated to 60° C in air ($pH_2O \approx 5$ mm Hg); *D*, NH_4^{+} montmorillonite heated to 420° C in air ($pH_2O \approx 5$ mm Hg); *E*, $Ca^{+}H_2O$ montmorillonite after 14 days in D_2O, saturated atmosphere, room temperature; *F*, $ND_4^{+} - D_2O$ montmorillonite dried above P_2O_5 for one week; *G*, ND_4^{+} montmorillonite heated to 420° C in dry air; *H*, sample *G* after one day in H_2O saturated atmosphere, room temperature ($pH_2O \approx 20$ mm Hg); *I*, sample *H* heated for one day at 420° C in air ($pH_2O \approx 5$ mm Hg); *J*, sample *H* heated for one week at 420° C in moist air ($pH_2O \approx 5$ mm Hg); *K*, ND_4^{+} montmorillonite heated to 420° C in dry oxygen-free N_2

White and Burns[5], who described a similar contraction by introduction of Li^{+} into the vacant octahedron of muscovite.

As a conclusion we arrive at the concept that the protons of hydrogen montmorillonite in anhydrous state form OH partly with the basal O's and partly migrate towards apical O's forming OH's in the vacant octahedrons. The basal OH's are able to dissociate, entering ion exchange reactions, whereas the extra apical (octahedral) OH's are not exchangeable. Thus we have had the following transformation:

$$Ca_{0.25}X_2Y_4O_{10}OH_2 \rightarrow (NH_4)_{0.5}X_2Y_4O_{10}(OH)_2 \rightarrow X_2Y_4O_{9.5}(OH)_{2.5} \rightarrow Ca_{0.18}X_2Y_4O_{9.86}(OH)_{2.14}$$

Dioctahedral hydrogen montmorillonites consequently seem to form structures corresponding to those previously reported for dioctahedral hydrous micas, namely, mixed crystals between $KAl_3Si_3O_{10}(OH)_2$ and $H_2OAl_3Si_3O_9(OH)_3$.

The absorption at 3,260 cm^{-1} in the heat-treated montmorillonites (Fig. 1, *I* and *J*) is the same as reported by Serratosa[6] for rehydrated montmorillonites. In our case it is shown that the band appears although no dissociation of water from the OH groups had yet taken place. The strong bathochromic shift of the OH band could be due to another modification of the lattice obtained by a rotation of the $A\begin{matrix}OH\\OH\end{matrix}Al$ planes, bringing some OH's in more favourable position for hydrogen bonding against O, or because of a disorder in the octahedron cations as demonstrated by Lindqvist[7] in case of muscovite:

AlAlAl□□Al□AlAl□ disorder
AlAl□AlAl□AlAl□ order

The disordered arrangements of the Al's will obviously polarize the O's, diminishing the stretching frequency of the HO band. Similar bathochromic shift is observed in Li-muscovites, and may be the reason for the 3,472 cm^{-1} band of White and Burns[5].

This work was sponsored by the Norwegian Research Council for Science and the Humanities.

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METALLURGY

Poisoning of the Surface of Platinum Metals by Intermetallic Compound Formation

Several years ago[1] it was reported that the platinum metals differed in their relative effectiveness in catalysing the corrosion of less noble metals in boiling acids. The purpose of this communication is to point out that the lowered relative effectiveness of particular platinum metals in the presence of ions of a specific, less noble metal may be explained by the formation of an intermetallic compound on the surface of the platinum metal when it is polarized to a potential close to the corrosion potential of the less noble metal. Quantitative experiments on the system, platinum metal–tin–boiling 2 M hydrochloric acid, are cited to support this point of view.

An extensive series of experiments[2] on the effect of metal ions on the corrosion of tin afforded conclusive evidence that the rate of dissolution of tin in boiling 2 M hydrochloric acid is completely under cathodic control. Thus measurements of the anodic half-reaction (tin dissolution) are in reality measurements of the rate-controlling cathodic half-reaction (hydrogen evolution).

The rate of dissolution of 99·999 per cent pure tin was determined under the following conditions when coupled to a platinum metal exposing the same surface area: (1) directly coupled, tin ions which formed freely bathed platinum metal; (2) coupled externally in separate chambers connected by a diffusion barrier, tin ions added to both platinum metal and tin chambers to a concentration of 0·01 M; (3) coupled externally in separate chambers connected by a diffusion barrier, no tin ions added. The results of these experiments are summarized in Table 1.

The major conclusions to be drawn from these experiments are the following: (1) In the absence of tin ions (column 4) all the platinum metals are very effective in catalysing the dissolution of tin. (2) Platinum and

Table 1

Pt metal coupled to tin	Directly coupled, Sn(II) ions freely bathed Pt metal	Coupled externally, separate chambers, Sn(II) conc. 0·01M	Coupled externally, separate chambers, no Sn(II) ions in Pt metal chamber
	3 (mg/cm²)	3 (mg/cm²)	3 (mg/cm²)
None			
Platinum	160	141	158
Iridium	154	66*	136
Rhodium	46	26	111
Palladium	32	17	162

* This value is not directly comparable with the other values because this set of experiments was carried out, as a result of breakage, in a different reaction vessel, with a higher internal resistance. Evidence obtained from many other experiments indicates that this value should be closer to the value for platinum.

iridium are not seriously poisoned by tin ions in their ability to catalyse the dissolution of tin. (3) Tin ions greatly decrease the ability of palladium to catalyse the dissolution of tin. Quantitative experiments were then carried out on the palladium–tin system.

A sample of palladium exposing a surface area of approximately 1·5 cm² was coupled externally to a tin sample exposing the same surface area in boiling 2 M hydrochloric acid containing 0·0001–0·01 M Sn(II). When the connexion between the palladium and the tin was broken and the potential of the palladium was followed as a function of time on a recording instrument, it was noted that a plateau in the potential decay curve occurred at a potential approximately 50 mV more noble than that of tin in the same medium. Smaller plateaux were also observed at potentials 300–350 mV more noble than that of tin in the same medium. X-ray powder analysis of scrapings from the surface revealed the presence of $PdSn_4$. X-ray diffraction analysis of the surface after the end of the plateau period revealed the absence of $PdSn_4$ and the emergence of a new pattern with strong lines at 2·21, 3·06, 2·87 and 2·29 Å. The compound yielding this pattern was not identified. A sample of palladium on which $PdSn_4$ was formed by coupling to tin was heated in mineral oil at 180° for 17 h. The intense lines in the resulting diffraction pattern were characteristic of PdSn, which is one of the products which might be expected from the thermal decomposition of $PdSn_4$. Corrosion current measurements and kinetic studies of the formation of $PdSn_4$ indicated that this compound begins to form on the palladium surface from the very beginning of the experiment.

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Interdiffusion in Zirconium–Aluminium and Zircaloy–Aluminium System

According to Kidson[1], the characteristics of layer growth in a multiphase binary system are influenced considerably by diffusion rate constants of individual phases. The rate constants for various phases differ either on account of differences in crystal structure or due to atomic processes by which diffusion occurs. Zirconium–aluminium system is a multiple phase binary system exhibiting low solid solubility at either ends of a phase diagram. The present note reports certain features of layer growth in zirconium–aluminium and zircaloy–aluminium systems which are presumably affected by the said rate constants.

High-purity aluminium (99·997 per cent) and zirconium (99·9 per cent) were used in the work recorded here. For preparing the diffusion couples, properly cleaned (after Storchheim[2]) Al plugs were tight pressed into holes 0·25 in. in diameter, in zirconium blocks. Prior to insertion, the surfaces of the holes were chemically polished using nitric acid ~ 45 c.c., hydrofluoric acid ~ 10 c.c., and water ~ 45 c.c. solution. In the case of zircaloy, thick-walled 0·25 in. inner diameter tubes were used. The diffusion couples were sealed in silica tubes in vacuum (better than 0·1μ) and later diffusion-annealed at 400°–600° C. The temperature of the furnace was controlled within an accuracy of ± 5° C.

The metallographic and X-ray examination of both zirconium–aluminium and zircaloy–aluminium couples revealed the presence of only two phases in the diffusion zones, which were identified as $ZrAl_3$ and Zr_3Al. The $ZrAl_3$ phase, nearer to aluminium, was found to be relatively more predominant and also more brittle. The presence of only two phases, $ZrAl_3$ and Zr_3Al, out of nine intermetallic compounds reported in phase diagram[3] suggests that the rate constants for the growth of other phases in the system are quite small. Our marker movement investigations showed that the atomic mobility of aluminium atoms is higher than that of zirconium atoms. $ZrAl_3$ lattice is f.c.c. tetragonal (a = 4·013 Å, c = 17·321 Å) with presumably larger interstitial holes. As such the predominance of $ZrAl_3$ in the diffusion layer can be attributed to the interstitial diffusion of aluminium atoms in the $ZrAl_3$ lattice. Zr_3Al has Cu_3Au type ordered lattice (a = 4·372 Å). Hence, assuming diffusion to occur by vacancy mechanism in Zr_3Al lattice, the relative distribution of

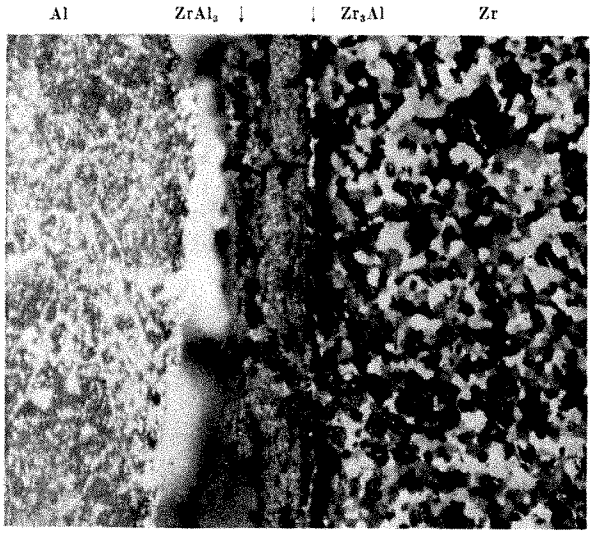


Fig. 1. Distribution of $ZrAl_3$ and Zr_3Al in zirconium–aluminium diffusion couple annealed for 162 h at 600° C (etched and polarized light) (× 120)

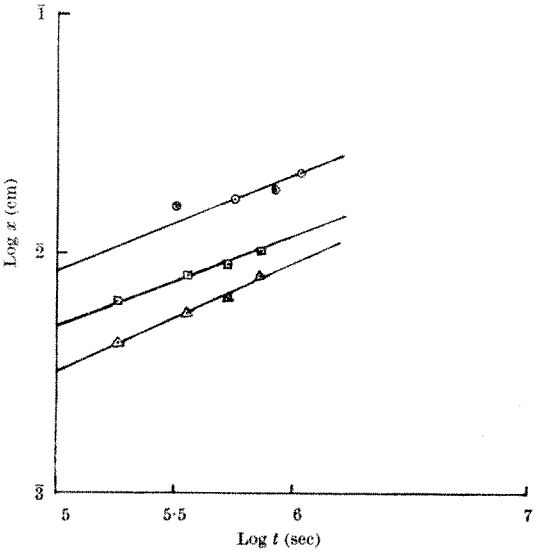


Fig. 2. Log x versus log t plot for zirconium–aluminium system. Diffusion anneal temperature: ○, 600° C; □, 550° C; △, 500° C

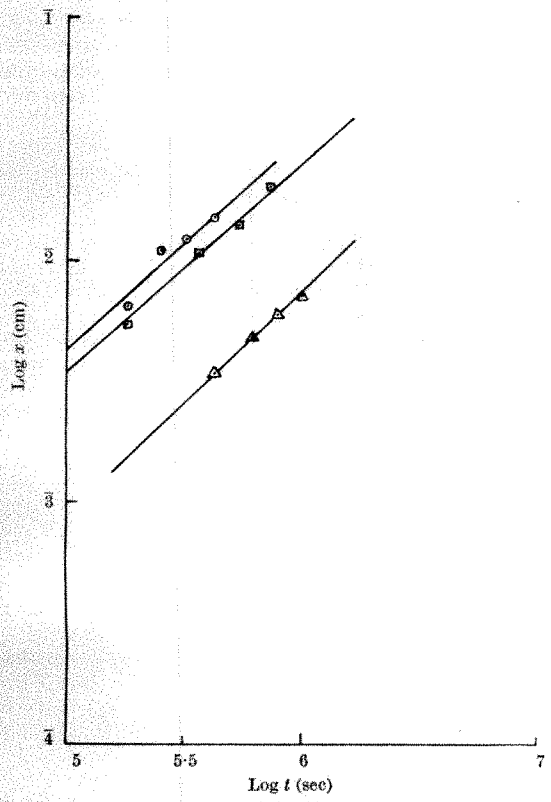


Fig. 3. Log x versus log t plot for zircaloy–aluminium system. Diffusion anneal temperatures: ○, 600° C; □, 500° C; △, 400° C

Zr_3Al and $ZrAl_3$ in diffusion zone can be easily understood (Fig. 1). The rate constants for layer growth could be a factor of miscibility gap or the range over which the phase occurs in the equilibrium phase diagram or the ease with which the mass transfer takes place. It appears that either one or all of these could be responsible for the absence of other phases in the diffusion zone.

Table 1. Showing the Values of n, K and Q for Diffusion of Aluminium in Zircaloy and Zirconium

Zircaloy-aluminium				Zr-Al			
Temp. °C	n	K (cm/sec/units)	Q (kcal)	Temp. °C	n	K (cm/sec/units)	Q (kcal)
600	1·13	$2·11 \times 10^{-8}$		600	2·45	$8·24 \times 10^{-11}$	
500	1·11	$1·7 \times 10^{-8}$	6·74	550	2·49	$4·78 \times 10^{-11}$	7·02
400	1·04	$6·14 \times 10^{-9}$		500	2·21	$3·18 \times 10^{-11}$	

An interesting feature of the interdiffusion of aluminium in zircaloy and zirconium relates to the layer growth. It was noticed that layer growth in zircaloy–aluminium diffusion was comparatively faster. For comparison the total width of the diffusion zone (X) was plotted logarithmically (Figs. 2 and 3) as a function of annealing time (t) by substituting the values in equation $x^n = Kt$, where n and k are reaction index and reaction constants respectively. Any value of $n \neq 2$ represents a deviation from Fick's law and could be characteristic of structural changes occurring in diffusion[4]. Table 1 shows the least square values of n and k for the two systems. (Activation energy for the layer growth (Q) has been obtained by writing the reaction constant $K = K_0 \exp(-Q/RT)$.)

The results seem to indicate faster interdiffusion in zircaloy–aluminium as compared with zirconium–aluminium system. A similar behaviour is reported for tracer diffusion of uranium in zircaloy and zirconium[5]. The Young's modulus for zircaloy decreases more rapidly with temperature[6]. The increased interdiffusion in case of zircaloy could, therefore, be explained due to 'weakening' of elastic bonds in zircaloy as compared with zirconium lattice.

We thank Dr. K. Tangri, head of the Metallurgy Division, and Dr. R. P. Agarwala of the Chemistry Division, for their advice. Shri R. V. Patil helped with the experimental work.

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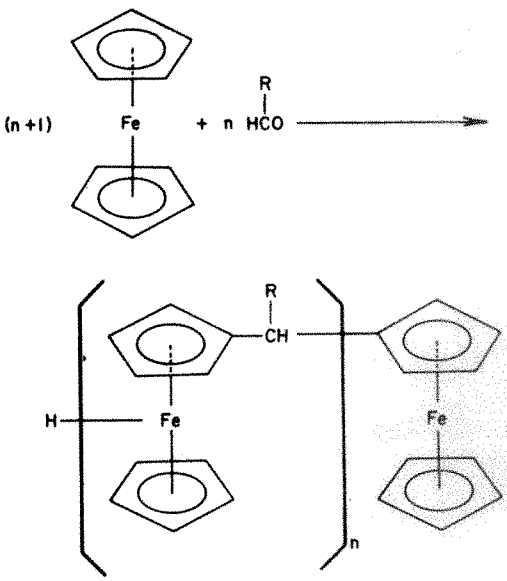
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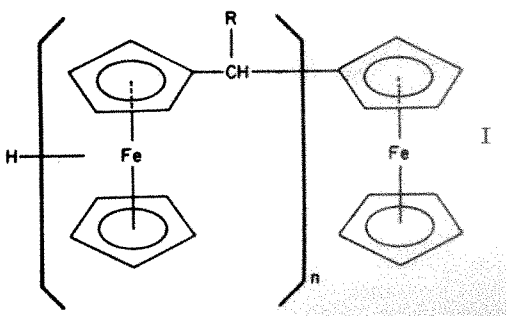
CHEMISTRY

Ferrocene-containing Polymers : Polycondensation of Ferrocene with Aldehydes

After earlier investigations[1] had shown the practicability of self-condensing ferrocenyl carbinols to give polymers containing ferrocenylene units interconnected by methylene or methine bridges, it was of interest to investigate the direct condensation of ferrocene with aldehydes. These components, if used in proper molar ratios, should interact according to equation 1:



with ferrocenyl carbinols and corresponding metallocarbonium ions expected to occur as intermediates, and the resulting polymers should possess structure I:



While, in previous work[2], with benzaldehyde as co-reactant and Lewis acids as catalysts, ferrocene was found to condense smoothly to give I (R = phenyl), aliphatic aldehydes such as formaldehyde and acetaldehyde failed to undergo such polycondensation under the conditions

applied. It has now been found that these aliphatic aldehydes can readily be polycondensed with ferrocene according to equation I if the reactions are conducted in a closed system. The mixture of ferrocene, aldehyde (paraformaldehyde, *s*-trioxane or, most advantageously, dimethoxymethane in the case of formaldehyde) and Lewis acid (preferably zinc chloride; concentrations 3–30 per cent by weight of ferrocene) was heated under nitrogen in a pressure vessel (sealed tube or autoclave) at temperatures sufficiently high to achieve homogeneity of the melt (130°–180°). Suitable work-up by extraction and reprecipitation gave soluble and partially fusible polymers with structures essentially conforming to I (R = H,CH_3) and yields up to 70 per cent. As a result of equilibrium conditions, the average molecular weights attained were generally below 2,500. In a similar manner, crotonaldehyde gave I (R = propenyl); however, yields of soluble polymer were less satisfactory (20–30 per cent) owing to competing cross-linking reactions involving the olefinic double bond.

In the course of this work, the polycondensation behaviour of such other aldehydic reactants as furfural and derivatives of benzaldehyde comprising various side-groups (*p*-hydroxy, *p*-carboxy, *p*-cyano, *o*-methoxy) was also investigated. Here, conditions of atmospheric pressure sufficed, and the resulting soluble polymers exhibited molecular weights up to 5,000 (unsubfractionated). Minor deviations in elemental composition from the expected composition of I were observed in most cases, depending, among other factors, on the steric and electronic effects of the substituent R (equation I) and its propensity toward competing side-reactions. In selected instances cross-linking reactions involving the functional side-groups were accomplished in the presence of diepoxy compounds, leading to insoluble and infusible cured resins.

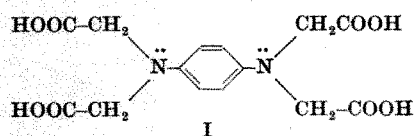
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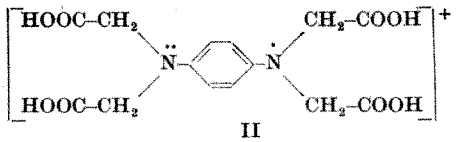
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Photochromism of *p*-Phenylenediaminetetraacetic Acid absorbed in Porous Glass

The compound *p*-phenylenediaminetetraacetic acid, *p*-PTDA (I), can be chemically oxidized to give a blue radical cation (II).



I



II

We have now found that this reaction can be carried out in a novel photochemical manner. When an aqueous solution of the diamine is absorbed by porous glass and irradiated with ultra-violet light, it gives what appears to be the same blue radical-ion species. On interruption of irradiation, the photo-induced coloration rapidly bleaches. Since photochemical reactions leading to reversible colour changes (photochromism) are of fundamental and practical importance, we have undertaken the following investigation of this system.

Aqueous or ethanolic solutions of approximately 0·005–0·06 M *p*-PDTA were absorbed into porous glass 2 mm thick. This glass slowly absorbed solution at atmospheric pressure; however, the process was facilitated by evacuating the glass prior to introducing a solution. Although the entire glass matrix could be saturated, only several tenths of a millimetre penetration were needed to give the results described. Under conditions of normal humidity this glass–diamine system persisted intact for up to 20 min, after which solvent escaping from the pores led to opacification of the sample. The samples could be stored under diamine solution for several days, after which noticeable chemical decomposition occurred.

The porous glass ('Vycor No. 7930', obtained from Corning Glass Works with flat surfaces) was previously cleaned by washing with water and drying at 450° C, using a slow rate of temperature elevation. At least 15 per cent of the volume of this glass is occupied by a continuous network of pores the average diameter of which is 40 Å.

The *p*-phenylenediaminetetraacetic acid[1] was freshly prepared before use as follows: In a vessel under nitrogen flow and heated to 50° C, 0·2 moles of phenylenediamine were slurried in 200 ml. of water. Over a period of 45 min, 1·0 mole of sodium chloroacetate was added with stirring to this mixture, and concentrated sodium hydroxide was intermittently added to compensate for a lowering of *p*H during the course of reaction. For 1 h after completion of chloroacetate addition, sodium hydroxide was added until the *p*H remained constant at 8. While still hot, the reaction solution was filtered under nitrogen and the desired product precipitated with 25 ml. of concentrated hydrochloric acid. After cooling, the precipitate was separated by filtration and washed with a small portion of cold dilute hydrochloric acid followed by washing with acetone. The yield based on phenylenediamine was 86 per cent. The product is a pinkish-white powder which decomposes from 150° to 160° C. Calculated for $N_2O_8C_{14}H_8$: C, 49 per cent; H, 4·71 per cent; N, 8·24 per cent: found, C, 49·5 per cent; H, 4·74 per cent; N, 8·23 per cent.

On irradiation of a 0·01 M aqueous solution of *p*-PDTA absorbed in porous glass with a 'G.E. *RS*-1 Sunlamp' at a distance of 12 in., a change from colourless to blue occurred in 30 sec. The induced change in optical density is shown in Fig. 1. When irradiation was interrupted, this absorption bleached with a half-life of approximately 8 min. An activation analysis showed that coloration was induced by radiation in the 3350–4200 Å region with an activation maximum between 3650 Å and 3950 Å. The coloration

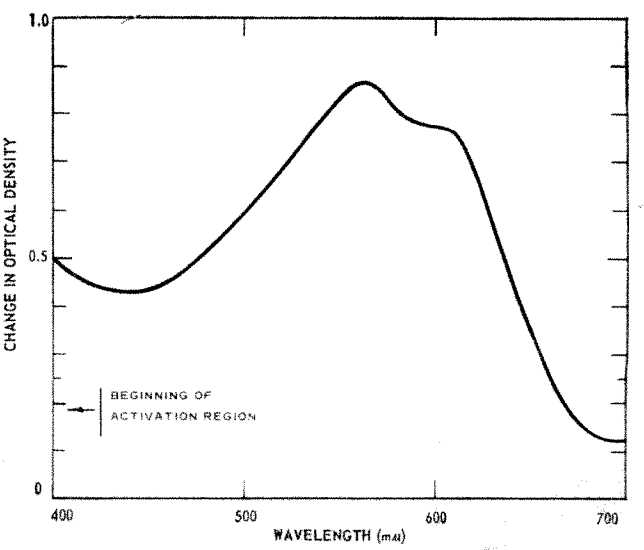


Fig. 1. Change in optical density of *p*-PDTA absorbed in porous glass on irradiation with ultra-violet

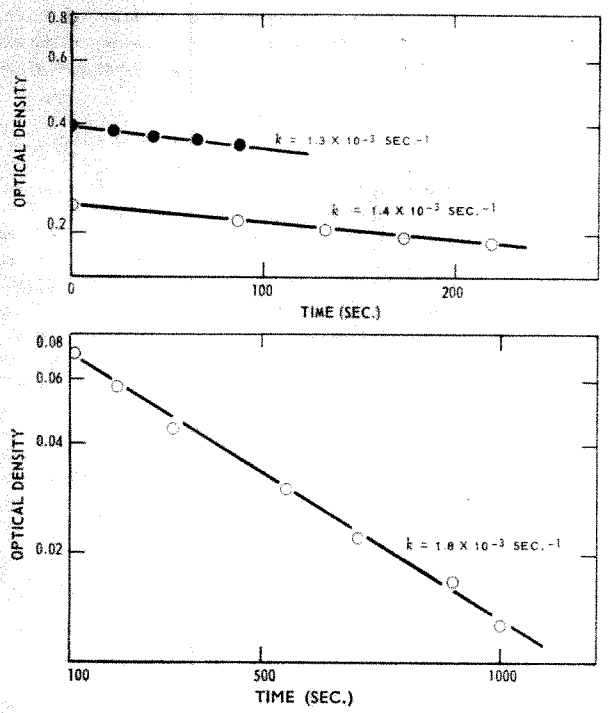


Fig. 2. First-order plots at $\lambda_{max} = 560$ mμ for bleaching of *p*-PDTA absorbed in porous glass. $k_{AVG} = 1{\cdot}5 \times 10^{-3}$ sec^{-1}. $t_{1/2} = 8$ min

and bleaching cycle could be repeated until evaporation of solvent occurred, but, if stored under diamine solution to prevent solvent loss, it could be repeated until chemical decomposition took place (after two days). These results were similar when ethanol was used as a solvent. Also, an aqueous or an ethanolic solution of *p*-PDTA irradiated in the absence of porous glass gave no observable absorption change. These solutions are very slowly attacked by air to give small amounts of the radical cation, especially at *p*H above 2, and care was necessary to avoid this in preparing solutions and fabricating glass samples. Solid *p*-PDTA, which is also susceptible to air oxidation, was freshly prepared before use and stored under nitrogen.

The radiation-induced absorption spectrum shown in Fig. 1 is identical with that of a solution of *p*-PDTA treated with hydrogen peroxide to give its radical cation, structure II, given here. The radical nature of this chemically prepared species was established from electron spin resonance data.

Although it was difficult to reproduce our kinetic results over a diamine concentration range, we did obtain first-order kinetics for the bleaching reaction in several sets of experiments where dilute solutions of *p*-PDTA were used. These results are shown in Fig. 2. Also, as the solutions absorbed in porous glass became more concentrated in diamine, the radiation-induced absorption became slightly lower.

Our results indicate that aqueous or ethanolic solutions of diamine absorbed in porous glass undergo a surface-catalysed photochromic change, that is, are activated by ultra-violet light of 3650–3950 Å and bleach in ambient light. The induced absorption spectrum corresponds to the radical cation of diamine independently prepared and characterized.

Although we conclude that the blue radical cation II is produced photochemically from *p*-PDTA absorbed in porous glass, our experimental results could fit two more specific interpretations for the overall reaction. (1) The diamine in close proximity to the glass matrix may react with ultra-violet light to dissociate an electron which is captured by a matrix centre, and on interruption of the activating light, optical bleaching could occur by electron–radical recombination; or (2) instead of a truly reversible reaction, we could postulate that photo-ionization is followed by radical–radical combination and optical bleaching. Of course, the latter mechanism implies a gradual consumption of active material, but we were not able to test this implication directly due to the fairly rapid chemical decomposition time of the system coupled with the exposure limit created by solvent loss from the glass pores. There are two conflicting pieces of evidence which support the suggested mechanisms: (*a*) bleaching appears to obey first-order kinetics in certain of our experiments; and (*b*) colour development for a given radiation dose becomes lower as the concentration of absorbed diamine increases, indicating the possibility of a self-quenching (radical–radical) mechanism which depletes the product of the forward photo-ionization reaction. We intend to continue this work by establishing a clearer picture of the bleaching reaction mechanism.

We thank Dr. Norma Searle for her activation analyses and Dr. William Hodgson for performing the electron spin resonance investigation.

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A Possible Mechanism for the Extra-terrestrial Synthesis of Straight-chain Hydrocarbon

Straight-chain hydrocarbon material[1,2] and long-chain fatty acids[3] have been found in meteorites belonging to the carbonaceous chondrite group. Two possible explanations have been advanced for the origin of this material. Nagy, Meinschein and Hennessy[1] have suggested that it is the result of extra-terrestrial life, since in Nature living processes are the only observed source of linear hydrocarbon molecules. An alternative hypothesis has been proposed by Wilson[4]: that there exists in free space a non-biological mechanism for the synthesis of hydrocarbon in a straight-chain configuration. The proposed mechanism (Fig. 1) involves crowding the reacting chains on to a surface so that only the ends are available for reaction, thus preventing branching.

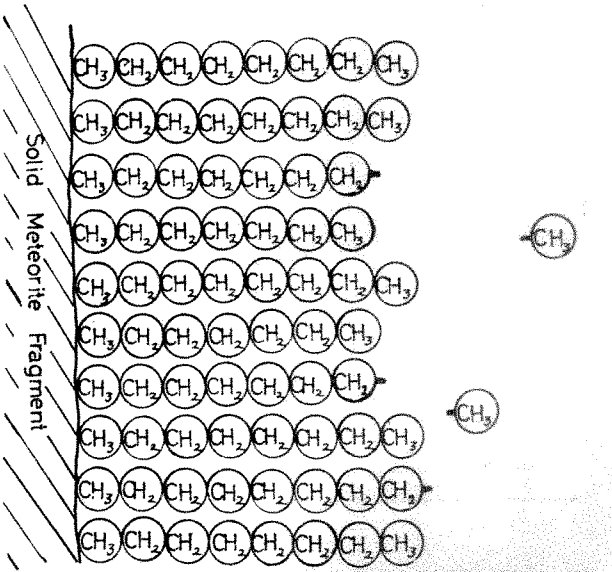


Fig. 1. Proposed mechanism for extra-terrestrial synthesis of hydrocarbon in a straight-chain configuration

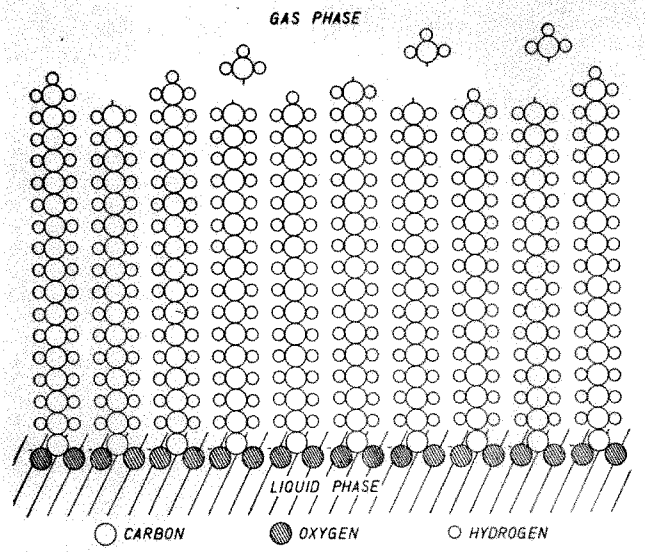


Fig. 2. Proposed mechanism for reaction of methyl radicals with a palmitic acid monolayer on water

The work reported in this communication is a demonstration in the laboratory of the synthesis of linear molecules using this mechanism. Methyl radicals generated by pyrolysis of di-*t*-butyl peroxide[5] were reacted with a monolayer of a straight-chain fatty acid on water, illustrated diagrammatically in Fig. 2.

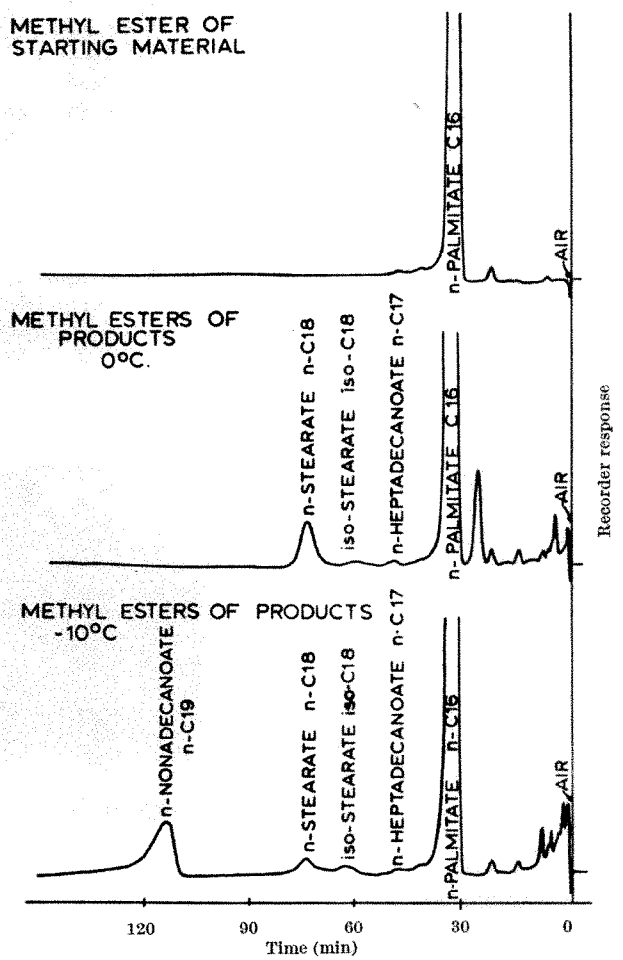


Fig. 3. Gas chromatogram of reaction products—fatty acid methyl esters. Column ¼ in. × 4 ft., packed with 'Apiezon *L*' grease on 80–120 mesh 'Celite' (10 per cent w/w); temperature, 200° C; carrier gas, argon; flow rate, 60 c.c./min; detector, argon ionization

The reaction products were acidified, extracted with petroleum ether, esterified using diazomethane, washed with sodium bicarbonate solution and analysed using a Pye argon gas chromatograph.

Typical results are shown in Fig. 3.

In this experiment it was shown that palmitic acid (n–C_{16}) can be built up stepwise to n-nonadecanoic acid (n–C_{19}) in a 3 per cent overall yield with the production of negligible quantities of branched-chain acids.

Thus it can be seen that it is possible to build up normal hydrocarbon material on a surface by the reaction of methyl radicals with that surface and thus make plausible the extra-terrestrial synthesis of the material on meteorite surfaces as postulated in Fig. 1. The consequences of this type of synthesis have been explored in an earlier communication[4].

We thank Dr. F. B. Shorland of the New Zealand Fats Research Laboratory, Department of Scientific and Industrial Research, for samples of pure fatty acids, and acknowledge financial assistance from the Victoria University of Wellington and the University Grants Committee.

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Photolysis of Benzene in *Cyclo*hexane: a Water-soluble Product giving a Positive Thiobarbituric Acid Test

Substances other than malonaldehyde give a characteristic pink coloration on heating with aqueous thiobarbituric acid (TBA). One such substance produced by photolysis of linolenic acid was tentatively assigned an unsaturated carbonyl component[1].

Photolysis of solutions of benzene in *cyclo*hexane produces a water-soluble material, some properties of which have been described[2,3]. Infra-red spectra of the dried material suggest an unsaturated carbonyl component[4]. Paper chromatography and ultra-violet absorption spectra exclude mucondialdehyde, and it has not been possible to correlate the properties of the material with any substances so far described as photolysis or radiolysis products of benzene[4]. It was possible in view of the unsaturated carbonyl assignment that the material might give a positive TBA test; this has been found.

The benzene was three-times recrystallized 'AnalaR'; *cyclo*hexane (Hopkins and Williams, chromatographic grade) was purified on a 75 cm × 1 cm column of silica gel, purity being assessed by the optical densities at 260, 220 and 200 mμ. A 0·2 per cent solution of benzene in *cyclo*hexane was prepared. Ten ml. were irradiated in an open Petri dish, 9·5 cm diam., with a mercury resonance lamp giving 2537 Å filtered by 2 mm 'Vycor': the intensity at the surface of the solution was 2×10^{15} quanta cm^{-2} sec^{-1}. The solution was stirred. After irradiation, the solution was evaporated in a current of cool air and the almost imperceptible film of residue extracted with 10 ml. of M/100 hydrochloric acid. The ultra-violet absorption spectrum was measured and the TBA test of Waravdekar and Saslaw[5] was applied to half the extract.

A broad absorption band, λ_{max} 278 mμ, increased in intensity with irradiation time, as did the TBA test colour at λ_{max} 531 mμ. There was considerable scatter

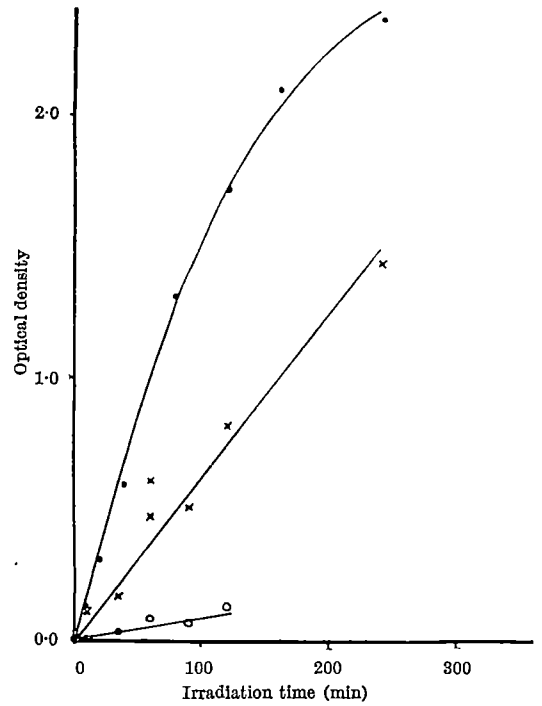


Fig. 1. Production of water-soluble substances absorbing at 278 and 330 mμ and increase in TBA test activity of irradiated solution. (Aliquots of the more concentrated solutions were taken for the TBA test.) ×, absorption at 278 mμ; ○, background absorption at 330 mμ; ●, TBA, test absorption at 531 mμ

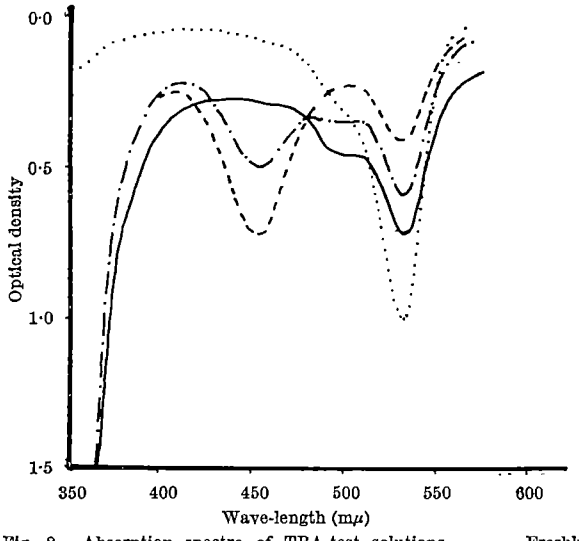


Fig. 2. Absorption spectra of TBA test solutions. ——, Freshly prepared heated solution; — · — ·, similar solution 48 h after preparation; - - - -, different solution, not heated, after 48 h at room temperature; · · · ·, TBA-test from autoxidized linseed oil, heated solution 48 h after preparation, for comparison. Spectra obtained on a Perkin–Elmer 137 ultra-violet spectrophotometer, 1 cm light-path, with water blanks

in the results which was attributed to partial volatility of the photolysis products. If the irradiated solution itself was used for the TBA test, a smooth curve was obtained of TBA test intensity against irradiation time (Fig. 1). (Although there is a correlation with the absorption of extracts at 278 mμ, the non-selective absorption at longer wave-lengths also increased slightly and the TBA-positive material may not be that giving the absorption maximum.)

The freshly-prepared TBA test solution from the photolysis products has λ_{max} 531 mμ but also shows a shoulder at 496 mμ; if the solution is allowed to stand for 24 h, a further maximum appears at 454–56 mμ. It has been shown that peroxides in hot acid solution produce a coloured derivative of TBA which is not a condensation product but has a similar absorption spectrum to the malonaldehyde–TBA product; it was suggested that the TBA test should be performed in neutral solution at room temperature for 15 h (ref. 6). The benzene photolysis product does not oxidize acid potassium iodide, but when the TBA test is thus applied no colour is produced in 15 h; at 48 h the intensity at 531 mμ is about half that of the freshly heated solution. In this aged solution the maximum at 454–56 mμ is much more intense than that at 531 mμ.

To summarize, the material produced by photolysis of a solution of benzene in *cyclo*hexane gives a positive TBA test; this, however, may not be due to a condensation with the unsaturated carbonyl component detected in the dry residue. Side reactions producing additional maxima in the TBA test also occur. This chemical relationship may increase the possibility that there is a connexion between the untested suggestion that benzene products may act as co-carcinogens in the carcinogenic aqueous extracts of photolysis of 3 : 4-benzopyrene in benzene solution[4,7,8] and the demonstration that the aqueous extract of ultra-violet irradiated linolenic acid induces hyperplastic changes in the liver[9].

I thank Dr. J. M. Nosworthy for gifts of three-times recrystallized benzene. This work was supported by the British Empire Cancer Campaign for Research.

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Rapid Reaction of Lime with Hydrous Alumina

Calcium aluminate hydrates have recently been reported to form as products of reaction between aluminium-bearing clay minerals and calcium hydroxide at or near room temperatures[1,2]. The investigation recorded here was initiated to determine whether hydrous alumina would react with calcium hydroxide under similar conditions.

Hydrous alumina ('pseudo-boehmite') was prepared by treating a 1 N solution of aluminium chloride with sodium hydroxide, the neutralization being carried out to *p*H 7. The resulting precipitate was washed repeatedly with water to remove the salt formed. The hydrous alumina yielded very weak, broad X-ray diffraction bands at spacings corresponding approximately to several of the strong peaks of boehmite ($Al_2O_3 \cdot H_2O$); however, the ignition loss after oven-drying corresponded almost exactly to that required for the composition $Al_2O_3 \cdot 2H_2O$. A high surface area is characteristic of this material, one such preparation yielding a surface area of 270 m^2/g by adsorption of water vapour. Differential thermal analysis of the dried material revealed broad overlapping endothermic responses with peak temperatures at about 220° and 400° C.

It was observed that mixtures of dried hydrous alumina and calcium hydroxide underwent a very rapid reaction when moistened. The X-ray diffraction peaks for calcium

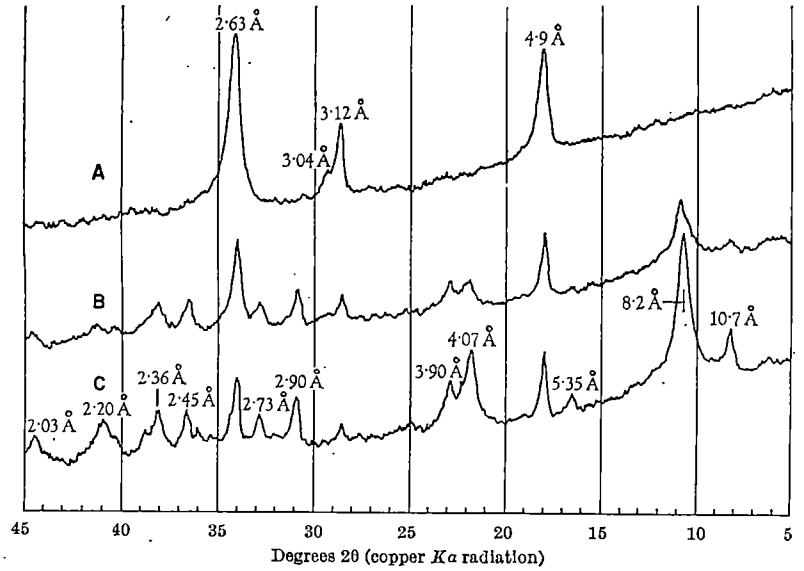


Fig. 1. X-ray diffractometer traces of calcium hydroxide–hydrous alumina preparations. *A*, Dry mixture; *B*, same mixture after wetting, X-ray scan begun 10 min after addition of water; *C*, same mixture 24 h after addition of water

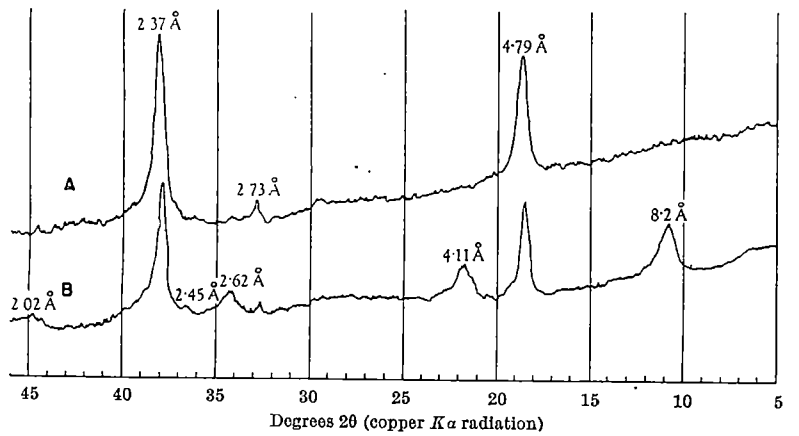


Fig. 2. X-ray diffractometer traces of magnesium hydroxide–hydrous alumina preparations. *A*, Dry mixture; *B*, same mixture 120 h after addition of water

hydroxide were immediately reduced or disappeared completely (depending on the proportion of calcium hydroxide in the mixture). Simultaneously, a new peak appeared at about 8 Å. Within a few minutes a complete X-ray diffraction pattern for the compound $4CaO{\cdot}Al_2O_3{\cdot}13H_2O$ appeared, and the intensities of these peaks continued to increase for some hours. It was observed that if the calcium hydroxide was present in excess, substantial decreases in the intensities of the calcium hydroxide peaks were confined to the first few minutes after moistening the dry mixture, suggesting that the reaction with the hydrous alumina is completed almost immediately. The additional increase in the intensity of X-ray diffraction pattern for the product beyond this short interval is thought to be attributable to progressively improving crystallinity. X-ray diffractometer tracings of a typical mixture before wetting, 10 min after wetting, and 24 h after wetting are given in Fig. 1.

The reaction does not require a large amount of water; in one instance a dry mixture set aside in a corked vial for several weeks was observed to have reacted appreciably, presumably through the agency of moisture adsorbed from the laboratory atmosphere while being mixed.

When a dry mixture is generously moistened (of the order of 50 per cent water by weight of solids), the product formed usually displays X-ray diffraction peaks at 10·7 Å and 5·35 Å in addition to those for $4CaO{\cdot}Al_2O_3{\cdot}13H_2O$. These peaks indicate the formation of some tetracalcium aluminate hydrate in the higher hydration state characterized by the composition of $4CaO{\cdot}Al_2O_3{\cdot}19H_2O$[3]. Occasionally in these experiments additional peaks were observed indicative of the formation of the cubic calcium aluminate hydrate $3CaO{\cdot}Al_2O_3{\cdot}6H_2O$. Seligmann and Greening[4] have directed attention to the ready interconvertibility of this compound and the hexagonal $4CaO{\cdot}Al_2O_3{\cdot}19H_2O$.

Two distinct polymorphic varieties of $4CaO{\cdot}Al_2O_3{\cdot}13H_2O$ have been described, the α-phase being marked by a (001) spacing of 8·2 Å, the β by a spacing of 7·9 Å[3]. In repeated trials in the present work both varieties have been observed to form. Alegre[5] concluded that these polymorphic varieties readily transform to each other depending on temperature and relative humidity; in contrast, Seligmann and Greening maintain that the α-form is in reality a solid solution incorporating a definite proportion of atmospherically derived carbonate, and that it does not spontaneously transform to the β-polymorph. The present products do incorporate some carbonate, the reagent grade calcium hydroxide used containing traces of carbonate observable in infra-red analysis. Furthermore, when the reaction is carried out in the ambient atmosphere additional carbonate is probably incorporated into the product. Nevertheless, the very rapid reaction between hydrous alumina and lime was found to proceed in an apparently identical fashion in a pure nitrogen atmosphere, suggesting that atmospheric carbonation is an accidental rather than an intrinsic feature of the reaction process.

Calcium hydroxide contaminated with traces of carbonate often shows remarkably strong infra-red adsorption maxima at about 1410 and 1470 cm^{-1}. Hunt[6] interpreted both of these maxima as being derived from the ν_3 vibration of the carbonate group, which usually appears at about 1430 cm^{-1}. He attributed the fact that two peaks are observed instead of one to the supposition that this normally degenerate vibration undergoes a loss of its degeneracy in the asymmetrical environment characteristic of the surface location of the adsorbed carbonate. In the work recorded here it was found that after reaction with hydrous alumina these two maxima were replaced by a single sharp maximum at about 1410 cm^{-1}. This might be interpreted as a restoration of the degeneracy of the vibration as a result of the incorporation of the carbonate within the lattice of the newly formed calcium aluminate hydrate.

Another noteworthy feature of the infra-red spectrum of the product is the appearance of a new and fairly strong adsorption band at about 3100 cm^{-1}. If this band is attributed to an OH stretching vibration, as seems reasonable, its low frequency suggests that some of the OH groups of the product are very strongly H-bonded.

Examination of the product by differential thermal analysis revealed the presence of three strong, sharp endothermic peaks at 110°, 235°, and 330° C. These were followed by a small exotherm, and finally the endothermic response marking the dehydroxylation of calcium hydroxide at 540° C. These peaks presumably are indicative of

the complex sequence of thermally-induced rearrangements in $4CaO{\cdot}Al_2O_3{\cdot}13H_2O$ which were reported by Buttler, Dent-Glasser and Taylor[7].

It has been determined that a reaction somewhat similar to that described above takes place between wetted mixtures of hydrous alumina and magnesium hydroxide. In this case the reaction is not rapid, the X-ray diffraction peaks for the new phase becoming evident only after several hours and slowly increasing in intensity over a period of several days. This product is apparently a double hydroxide of magnesium and aluminium. The X-ray pattern observed (Fig. 2) is very nearly identical with that given by Feitknecht and Gerber[8]. The present product yields a (001) spacing of 8·2 Å, which is slightly higher than the 7·9 Å spacing reported by these authors and the 7·95 Å and 7·63 Å spacings reported for different magnesium–aluminium double hydroxide preparations by Mortland and Gastuche[9].

I thank J. L. White, W. L. Dolch and E. B. Kinter for their advice.

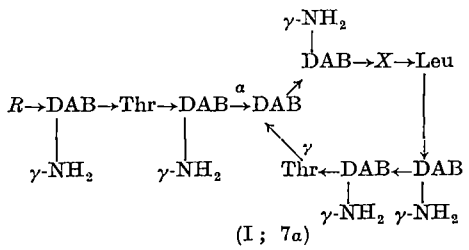
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Structures of Polymyxin *B*2 and Polymyxin *E*1

FROM the degradative investigations of Suzuki *et al.*[1], and finally by its synthesis[2], the structure of polymyxin *B*1 has now been established as (I): (R = (+)-6-methyloctanoyl, X = D-Phe), in which each of the six αγ-diaminobutyric acid units is of the L-configuration[1,3].



(I; 7α)

Suzuki *et al.*[4] have also shown that the structure of colistin *A* is identical with that of polymyxin *B*1, except that the D-Phe in the latter is replaced by D-Leu (that is, 1; R = (+)-6-methyloctanoyl, X = D-Leu).

From the partial acid hydrolysate of polymyxin *B*2 by fractionation on 'Dowex 50', using gradient elution with ammonium formate–ammonium acetate buffers, we have now isolated and identified the following peptides:

6-methylheptanoyl$\xrightarrow{\alpha}$L-DAB ; Thr$\xrightarrow{\gamma}$DAB ; Thr$\xrightarrow{\alpha}$DAB ;

D-Phe→L-Leu ; Leu$\xrightarrow{\alpha}$DAB ; DAB→Phe ;

Thr$\xrightarrow{\alpha}$DAB→DAB$\xleftarrow{\gamma}$Thr ; D-Phe→Leu$\xrightarrow{\alpha}$DAB ;

Thr$\xrightarrow{\gamma}$DAB$\xrightarrow{\alpha}$DAB ; DAB→Phe→Leu ; DAB$\xrightarrow{\alpha}$(DAB)$_n$;

Thr$\xrightarrow{\gamma}$DAB$\xrightarrow{\alpha}$DAB→Phe and Leu$\xrightarrow{\alpha}$DAB$\xrightarrow{\alpha}$DAB

With the exception of the first of these peptides, which is replaced by (+)-6-methyloctanoyl$\xrightarrow{\alpha}$DAB, they are identical with those obtained from a comparable hydrolysate of polymyxin *B*1.

We have also re-examined more thoroughly the partial acid hydrolysate of polymyxin *E*1 and by the procedure outlined above have isolated and characterized the following peptides:

D-Leu→L-Leu ; (+)-6-methyloctanoyl$\xrightarrow{\alpha}$L-DAB ; Thr$\xrightarrow{\gamma}$DAB ;

Thr$\xrightarrow{\alpha}$DAB ; L-Leu$\xrightarrow{\alpha}$DAB ; Leu→Leu$\xrightarrow{\alpha}$DAB ;

DAB$\xrightarrow{\alpha}$DAB→Leu→Leu ; DAB→Leu→Leu ;

Thr$\xrightarrow{\alpha}$DAB→DAB$\xleftarrow{\gamma}$Thr ; Thr$\xrightarrow{\gamma}$DAB$\xrightarrow{\alpha}$DAB→D-Leu ;

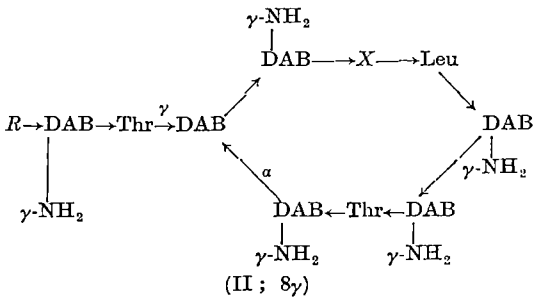
Thr$\xrightarrow{\gamma}$DAB$\xrightarrow{\alpha}$DAB ; Thr$\xrightarrow{\gamma}$DAB$\xrightarrow{\alpha}$DAB→Leu→Leu ;

DAB$\xrightarrow{\alpha}$(DAB)$_n$; DAB$\xrightarrow{\alpha}$DAB→Leu ; Thr→DAB→DAB
↑
DAB$\xleftarrow{\alpha}$Thr

Thr$\xrightarrow{\alpha}$DAB$\xrightarrow{\alpha}$DAB→Leu→Leu ; Leu$\xrightarrow{\alpha}$DAB$\xrightarrow{\alpha}$DAB

and Leu→Leu$\xrightarrow{\alpha}$DAB$\xrightarrow{\alpha}$DAB

The re-assembling of each of these two series of peptides in turn indicates that the structure of polymyxin *B*2 is restricted to either (I) or (II) (R = 6-methylheptanoyl and X = D-Phe) and that of polymyxin *E*1 to either (I) or (II) (R = (+)-6-methyloctanoyl and X = D-Leu).



(II; 8γ)

In the determinations of the unequivocal structures of both polymyxin *B*1 and colistin *A* by Suzuki *et al.*[1,4], the differentiation between formulæ (I) and (II) was not forthcoming from the products of partial acid hydrolyses but in each case only by the isolation of the peptide $R\xrightarrow{\alpha}$DAB→Thr$\xrightarrow{\alpha}$DAB by enzymatic hydrolysis. The close relationship between the physical properties of polymyxins *B*1 and *B*2 and their identities in the case of polymyxin *E* and colistin have already been described[5] so that fundamental differences between the structures of these pairs of antibiotics would not be expected, particularly taking into consideration, for example, the difference in optical rotation between the 7α and 8γ-type peptides synthesized by Vogler *et al.*[6]. It is intended to subject polymyxin *E*1 and polymyxin *B*2 to enzymatic degradation to confirm the correctness of structure (I).

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Peptizing Action of Some Polar Substances on the Benzene Solution of Cobalt Oleate

We have found that the viscosity of the solution of cobalt oleate in non-polar solvents such as benzene and carbon tetrachloride is unusually high even at low concentrations and, in addition, shows a tendency to increase more and more with time of ageing of the solution, while in polar solvents such as pyridine and chloroform no such reactions have been observed (Fig. 1). The high viscosity found for benzene or carbon tetrachloride solutions of cobalt oleate is to be ascribed to the formation of linear high polymeric structure of cobalt oleate in these solvents. We have also found that the addition of small amounts of certain polar substances to the benzene solution of cobalt oleate decreases its viscosity to almost the value for the solvent alone.

In Fig. 2 is shown the effect of additives (mole ratio to soap 2 : 1) on the viscosity of benzene solution of cobalt oleate. Among various additives the strength of the peptizing action was found in the order: 3,4-dimethylpyridine > pyridine > quinoline > 2,4-dimethylpyridine > 2,6-dimethylpyridine > aniline > chloroform. This order corresponds to that of the tendency for these substances to co-ordinate with a cobalt atom. Water and alcohols were non-effective as peptizers.

The high viscosity of aluminium disoap–hydrocarbon systems was investigated by various workers and the high polymeric structure of aluminium disoap was established[1]. The properties of cobalt oleate–hydrocarbon systems are similar in many respects to those of aluminium disoap–hydrocarbon systems. But the mechanism of the peptizing action of polar substances is apparently different. In the case of aluminium soap, Bauer *et al.*[2] have reported that ethyl alcohol, *m*-cresol and *m*-toluidine are good peptizers, but pyridine and *t*-butyl alcohol are not effective. It has been suggested by these authors that the substances which are capable of forming hydrogen bonds with the carbonyl group are good peptizers for aluminium soaps. However, on the contrary, for cobalt oleate the substances which have strong co-ordinating ability with cobalt atom (for example, pyridines, quinoline, etc.) are very effective peptizers and those which have strong hydrogen bonding ability (for example, water, ethanol, etc.) are not effective.

Cobalt oleate used in this investigation was prepared by metathesis from cobaltous chloride and oleic acid in water–ethanol solution. The composition was $Co_2(C_{18}H_{33}O_2)_3(OH)$. Probably a high polymeric structure will be formed through —OH—Co— linkages. The action of peptizers is assumed to be the destruction of this polymeric structure by co-ordinating with the cobalt atom. We have confirmed this by infra-red and ultra-violet spectroscopy.

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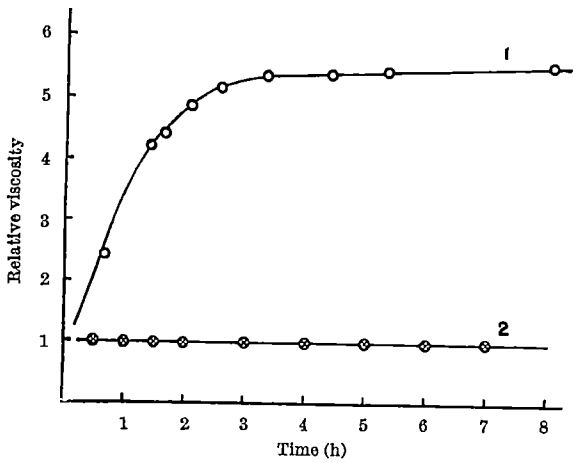


Fig. 1. The viscosity of cobalt oleate solution in organic solvents at 35° C. 1, Benzene ; 2, pyridine or chloroform

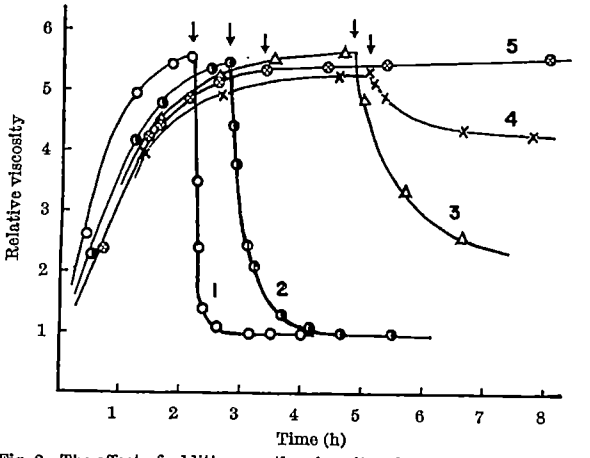


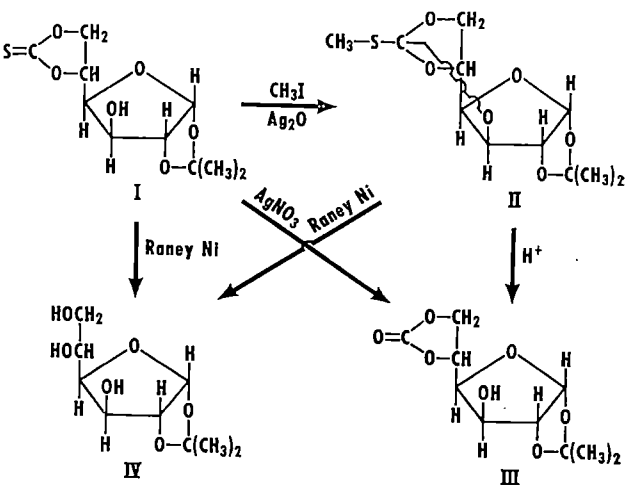
Fig. 2. The effect of additives on the viscosity of cobalt oleate solutions in benzene at 35° C (the arrow shows the point of addition). 1, 3,4-Dimethylpyridine; 2, pyridine; 3, quinoline; 4, 2,6-dimethylpyridine; 5, ethanol or water

BIOCHEMISTRY

A Unique Sugar Orthoester

The isolation and identification of a D-glucose derivative in which the hydroxyl groups at positions C-3, C-5 and C-6 and an *S*-methyl group are commonly linked to form an orthocarbonate-type structure is reported. Such a carbohydrate derivative has not been shown previously.

In previous work[1] we found that *bis*(1,2-*O*-isopropylidene-3-*O*-thiocarbonyl-α-D-glucofuranose) disulphide underwent a rearrangement–fragmentation reaction on standing in various organic solvents to give equimolar quantities of 1,2-*O*-isopropylidene-α-D-glucofuranose 5,6-thionocarbonate (I), 1,2-*O*-isopropylidene-α-D-glucofuranose (IV), carbon disulphide, and free sulphur. The structure of I was proved by its conversion to known compounds and by its independent synthesis. When pyridine solutions of I were treated with acetic anhydride or *p*-toluenesulphonyl chloride, the 3-*O*-acetyl and 3-*O*-*p*-tolylsulphonyl derivatives were formed. In an attempt to prepare the



3-*O*-methyl derivative for subsequent investigations, I was treated in dioxane solution with silver oxide and methyl iodide at room temperature. None of the expected product was formed, but rather 1,2-*O*-isopropylidene-α-D-glucofuranose-3,5,6-(*S*-methyl monothio-orthocarbonate) (II) was isolated as large transparent needles in 54 per cent yield, m.p. 178°–180° C, $[\alpha]_D^{20} - 3{\cdot}3°$ (*c*. 3, chloroform). The structure of II was formulated on the basis of the following experimental findings.

Elemental analysis of II gave: C, 47·8; H, 5·7; S, 11·6, indicating an empirical formula of $C_{11}H_{16}O_6S$, formula weight 276. Molecular weight determination by the Rast method in camphor gave a molecular weight of 280 as an average of three determinations. The infra-red spectrum of II showed no absorption band for a hydroxyl group in the 3,400–3,500 cm^{-1} region. The absence of a hydroxyl was confirmed when an anhydrous ethereal solution of II evolved no hydrogen on treatment with metallic sodium.

Analysis of the ultra-violet spectrum of II at 234 and 288 mμ showed that the $\rangle C{=}S$ group was no longer present. The spectrum of I had absorption maxima at 234 and 288 mμ with respective molar extinction coefficients of 15,000 and 44. When an acetone–water solution of I was treated with silver nitrate, silver sulphide was formed immediately and 1,2-*O*-isopropylidene-α-D-glucofuranose, 5,6-carbonate (III) was recovered from the filtrate in nearly theoretical yield. Similar treatment of II gave no silver sulphide confirming the absence of the $\rangle C{=}S$ group in this product. Desulphurization of I and II with Raney nickel gave IV in yields exceeding 90 per cent.

Compound II exhibited a behaviour characteristic of orthoester-type structures[2] when treated under alkaline and acidic conditions. Accordingly, when an aqueous–acetone solution of II was treated with 5 per cent barium hydroxide solution under reflux for 30 min, only unchanged starting material was recovered. Treatment of I under similar conditions produced a sulphur-free product. When II was treated with 0·01 N hydrochloric acid at room temperature, a strong odour of methyl mercaptan was immediately detected. Following neutralization with silver carbonate, evaporation of the filtrate under reduced pressure gave III, identified by its infra-red spectrum, m.p., and mixed m.p. with an authentic sample. The methyl mercaptan evolved on nitric acid hydrolysis of II in chloroform was determined quantitatively by a slight modification of the procedure of Malisoff and Anding[3]. Under such conditions, 1·0 mole of II gave 0·98 mole of methyl mercaptan. The presence of one alkyl sulphide group per mole of II was also shown by titration of II with a standard solution of potassium bromate in acetic acid[4].

Examination of a Dreiding stereomodel of II reveals a rigid, strained molecule basically comprised of two 1,3-dioxolane rings; one oxolane ring (the furanose ring); and one 1,3-dioxane ring. One of the 1,3-dioxolane rings forms a bridge structure with the 1,3-dioxane ring where the orthocarbonate carbon is the bridgehead atom bonded to three oxygens and one sulphur.

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A Polysaccharide resembling Alginic Acid from a *Pseudomonas* Micro-organism

A *Pseudomonas* micro-organism isolated from the sputum of a patient with cystic fibrosis produced unusually large mucoid colonies on agar culture media. This *Pseudomonas*, identified as No. 8906 by us, tended to revert to a less mucoid form and occasionally demonstrated phage plaques. The mucoid *Pseudomonas* was grown on Oxoid sensitivity test media. The bacterial growth was scraped from the surface of the media, and a yield of 664 mg of crude polysaccharide was obtained by initial precipitation with ethanol, followed by extraction with 0·01 N potassium hydroxide at *p*H 10 and repeated precipitation from aqueous solution with 68 per cent ethanol containing 1 per cent sodium acetate.

At a concentration of 0·5 per cent of the polysaccharide, a very viscous aqueous solution was obtained. Analyses showed only insignificant amounts of hexoses[1], hexosamines[2], phosphate and protein. A small amount, about 5 per cent, of nucleic acid was shown to be present by measuring absorption at 260 mμ. Analyses for uronic acid are shown in Table 1. The polysaccharide appeared to be a polyuronide and was insoluble in weak acid. The carbazole to orcinol ratio of 0·20 suggested the presence of mannuronic acid[3]. Because of the similarity to alginic acid, a commercial sample of this polyuronide (K. and K. Laboratories, New York) was also analysed.

Small samples of alginic acid and the crude polysaccharide were hydrolysed in 88 per cent formic acid for 5 h at 98° C, aliquots chromatographed on paper in a pyridine–ethyl acetate solvent[4], and the spots developed with anisidine reagent[5] (Fig. 1). The hydrolysate of the *Pseudomonas* polysaccharide showed spots corresponding to mannuronic and guluronic acid and one corresponding to mannuronolactone. The same results were obtained when the hydrolysates were chromatographed in a butanol–acetic acid solvent[6]. No additional spots could be detected when chromatograms were sprayed with aniline[7], silver nitrate[8] or ninhydrin reagents.

Table 1. Uronic Acid Analysis*

	Carbazol[11] (%)	Orcinol[12] (%)	Carbazole/Orcinol
Crude polysaccharide	8	41	0 20
Alginic acid	18	83	0 22
Purified polysaccharide	18	92	0 20

* Glucuronic acid is used as a standard.

The chemical analyses and the paper chromatography indicated no significant constituents other than uronic acids; but lower values were obtained for the uronic acid colour reactions for the unknown polysaccharide than for the commercial alginic acid. Therefore, the following procedures were carried out on the crude polysaccharide.

Equivalent weight by potentiometric titration was found to be 214, indicating a uronic acid content of 80 per cent. A small amount of the sodium salt of the *Pseudomonas* polysaccharide was dissolved in water and the solution acidified with 1·0 N hydrochloric acid. The gelatinous precipitate which formed was collected by centrifugation, washed with water, ethanol, ether and dried. The yield was 75 per cent, but no change in analyses could be detected.

Infra-red spectra of the polysaccharide sodium salt and of sodium alginate are shown in Fig. 2. No major differences between the polysaccharides in either intensity or position of the peaks were observed. About 25 mg of the crude polysaccharide were purified by precipitation with cetylpyridinium chloride[9]. The analysis of the material, obtained in a 65 per cent yield, is shown in Table 1. Despite the doubling of the orcinol value, no changes in either intensity or position of infra-red peaks could be detected. Several explanations for this and the foregoing results are possible: (1) A substance which had interfered with the colour reaction had been removed during purification. (2) The colour reaction was unreliable, due to

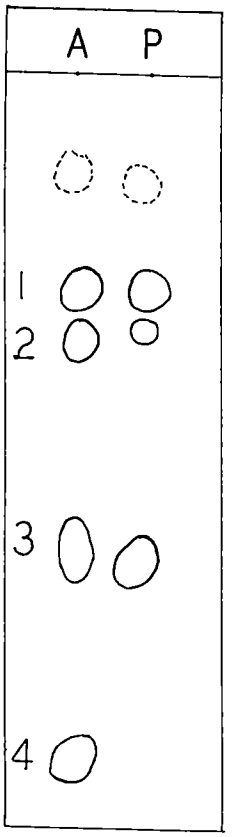


Fig. 1. Tracing of paper chromatogram of acid hydrolysates. All spots showed the red colour, typical for uronic acids, with the anisidine spray. *A*, alginic acid hydrolysate: 1, D-mannuronic acid, 2, L-guluronic acid, 3, D-mannurono lactone, 4, L-gulurono lactone; *P*, *Pseudomonas* polysaccharide hydrolysate

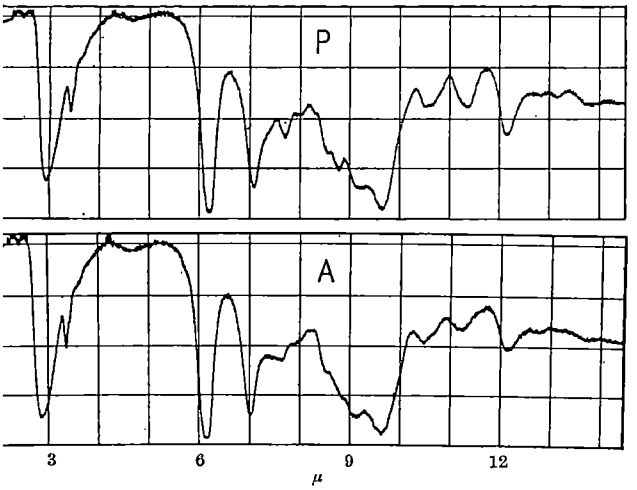


Fig. 2. Infra-red spectra of polysaccharides. *A*, sodium alginate; *P*, sodium salt of *Pseudomonas* polysaccharide. The spectra of the samples (1 mg) in potassium bromide pellets were run on a Perkin–Elmer model 21 spectrophotometer

either the high molecular weight of the polymer or glycosidic linkages different from alginate. (3) A uronic acid-containing polysaccharide not giving the colour reactions was removed by cetylpyridinium fractionation.

The results presented show the production, by a pathogenic *Pseudomonas* organism, of a polysaccharide very similar to alginic acid, a polyuronide usually obtained from brown seaweed. Both mannuronic and small amounts of guluronic acid appear to be present. In this connexion it is of interest that another strain of *Pseudomonas* has been found to degrade alginic acid[10].

The presence of the polysaccharide-producing organism in a patient with cystic fibrosis or mucoviscidosis is of unknown significance, but the thick mucoid secretions in the bronchi would not be improved by the presence of a bacterial polysaccharide which is insoluble in acid solutions and cannot be degraded by endogenous enzymes.

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Isolation of Methylcobalamin from Natural Source Material

Investigation of the form of vitamin B_{12} in human blood plasma by chromatographic-bioautographic analysis revealed as the major component a 'fourth factor' besides *DMB* coenzyme (coenzyme B_{12}) and traces of hydroxocobalamin and cyanocobalamin[1]. This factor was also found in liver, *E. coli* and *Streptomyces rimosus*. In order to identify this analogue we have tried to isolate it from a natural source material rich in coenzyme B_{12} and have found good evidence that this analogue is methylcobalamin. Methylcobalamin was prepared by partial synthesis a couple of years ago[2] and was shown by Guest *et al.*[3] to be active in the enzymatic synthesis of methionine by extracts of an *Escherichia coli* mutant. However, methylcobalamin has not previously been shown to occur naturally.

All operations were carried out in the dark or in dim light and all vessels were covered with black cloth or metal foil.

An alcoholic extract of cells from a commercial preparation, kindly supplied by Glaxo Research, Ltd., Greenford, Middlesex, containing about 500 mg of coenzyme B_{12}, was concentrated in an evaporator under reduced pressure to a final volume of 4 l. and until no ethanol remained. From this aqueous solution the vitamin B_{12} analogues were extracted and then displaced from phenol back into water by addition of acetone and ethyl ether (according to Barker *et al.*[4]). After removal of phenol, and concentration, the solution was adjusted to *p*H 5·0 with 0·1 M hydrochloric acid and was passed through a '*CM*-cellulose-Na^+' column (20 cm × 4 cm). The non-adsorbed B_{12} analogues were again purified via phenol and the aqueous solution was concentrated to 3 ml. 3 ml. of normal-butanol and 2·1 ml. of isopropanol were added and this mixture was passed through a column of cellulose 25 cm high × 4·5 cm diam., equilibrated with *n*-butanol, isopropanol and water (10:7:10). The chromatogram was developed with the same solvent mixture and two fractions were collected. Everything moving faster than *DMB* coenzyme (which was the main B_{12} analogue in the preparation) was collected in fraction 1 and the clearly visible zone of *DMB* coenzyme in fraction 2. An equal volume of ethyl ether was added to each fraction and the water-phases were collected and concentrated by evaporation. Paper chromatographic examination showed that fraction 1 contained one fast moving

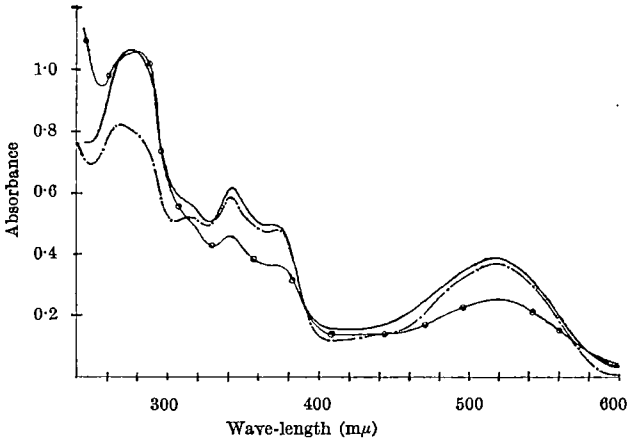


Fig. 1. Spectra of crystalline methylcobalamin (— · —); coenzyme analogue from micro-organism (—); coenzyme analogue from calf liver (—○—)

red compound and a little *DMB* coenzyme. Fraction 2 was mainly *DMB* coenzyme.

Fraction 1 was further purified by adjustment to *p*H 5·0 and passage through a 5 cm × 1·0 cm column of 'Dowex 50*W* × 4-Na$^+$'. The effluent was adjusted to *p*H 10 and was washed through a 'Permutit *FF*-OH$^-$' (strongly basic) column (5 cm × 1·0 cm). The effluent was purified via phenol and the aqueous solution concentrated to a few μl. It was then chromatographed on paper with *n*-butanol, isopropanol and water (10:7:10). The paper was dried and the coenzyme analogue (about 1 mg) eluted with water and again concentrated by evaporation to a small volume.

Calf liver was processed in a similar manner except for some additional purification steps and gave a very small yield of the same substance.

Paper chromatographic investigation in two different solvent systems (I, *n*-butanol, isopropanol and water, 20:7:10; II, *sec*-butanol, acetic acid and water, 100:3:50) gave R_F values for all the preparations including the 'fourth factor' from blood plasma corresponding to that of methylcobalamin. Electrophoresis at *p*H 6·5 showed that the coenzyme analogue had moved the same distance as methylcobalamin.

The spectra of preparations from both micro-organism and liver in water solution (Fig. 1) are in good agreement with the spectrum of crystalline methylcobalamin made by partial synthesis. Both preparations, and methylcobalamin, change colour from red to yellow on acidification of aqueous solutions. The intensity of absorption in the 260 mμ region, relative to that of longer wavelengths, is not great enough to accommodate a purine moiety, so that the 'fourth factor' cannot be derived from *DMB* coenzyme.

The natural occurrence of methylcobalamin is not surprising since Guest *et al.*[3] have already found it to be active in enzymatic synthesis of methionine. It seems unlikely, however, that this is its main function in the human blood stream. Our findings may, on the other hand, help to elucidate the much discussed functional relationship between folic acid and vitamin B_{12}.

I thank Dr. E. Lester Smith, Dr. Å. Nordén and Dr. Thorild Dahlgren for their advice.

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PHARMACOLOGY

Inhibition of Phenylquinone-induced Writhing by Narcotic Antagonists

Since nalorphine was demonstrated to have potent analgesic properties[1,2] and to be non-addicting in man[3], a considerable effort has been directed towards finding narcotic antagonists with similar activities, but without the undesirable psychotomimetic effects produced by nalorphine[4]. This effort has been complicated by the fact that nalorphine not only is inactive in most analgesic tests in animals, but also will antagonize the analgesic activity of most narcotic drugs[5]. Only the rat flinch-jump test of Evans[6] has been reported capable of detecting the analgesic activity of nalorphine. This finding, however, has yet to be reported by other workers.

Recently, a series of benzomorphans with narcotic antagonist activity[7] also has been shown to possess potent clinical analgesic efficacy[8,9]. These compounds, too, were found inactive in several animal analgesic tests[7]. One of them, cyclazocine, was shown to increase the aversive shock threshold of trained monkeys, but nalorphine was found to be inactive in the same procedure[10].

The work recorded here has demonstrated that the phenylquinone writhing test[11] is a sensitive indicator of the analgesic activity of narcotic antagonists. Using the method of Hendershot and Forsaith[12], groups of five male *CFW* mice (19–21 g) were injected intraperitoneally with phenylquinone (10 ml./kg of 0·025 per cent solution) 15 min after the subcutaneous administration of the test drugs. The mice were then placed in an observation beaker, and the number of 'writhes' (arching of the back and hind-limb extension) per animal was counted during a 10-min period starting 5 min after the phenylquinone treatment. At least two groups of five animals each were used for each dose, and the total number of writhes for each dose as compared with the total number of writhes for a saline-treated control group was used for calculating the percentage protection. The mean and standard deviation for 72 saline-control groups, representing 360 mice, was 98·8 ± 17·0. Using the method of Litchfield and Wilcoxon[13], the dose–response curves were plotted and the ED_{50}'s and relative potencies were determined. The drugs compared were: morphine sulphate, nalorphine hydrochloride, cyclazocine ('*WIN* 20740', 2-*cyclo*propyl-methyl-2′-hydroxy-5,9-dimethyl-6,7-benzomorphan) and pentazocine ('*WIN* 20228', 2′-hydroxy-5,9-dimethyl-2-(3,3-dimethylallyl)-6,7-benzomorphan).

Both nalorphine and the two benzomorphans were potent antagonists of phenylquinone-induced writhing in mice (Fig. 1 and Table 1), and seemed active in the same order of potency as seen in man[8,9], that is, cyclazocine > nalorphine > pentazocine. An estimate of the relative potency of these drugs with respect to morphine could not be made because of significant differences in the

Table 1. Comparison of the Phenylquinone Antagonism of Morphine and Narcotic Antagonists

Drug (subcutaneous route)	ED_{50}	95% confidence limits	Slope
Cyclazocine	0·10	(0·04–0·28)	10·0
Morphine	0·45	(0·27–0·77)	2·4*
Nalorphine	0·92	(0·33–2·6)	9·0
Pentazocine	2·00	(0·67–6·0)	8·8

*Statistically significant difference from all other slopes ($P = < 0·05$).

Table 2. Correlation between Phenylquinone Antagonism and Clinical Analgesic Potency

Drug	Mice ED_{50} (mg/kg)	Mice Relative potency	Human clinical dose (mg/kg)	Ref.
Cyclazocine	0·01	10·0	0·004	9
Morphine	0·10	1·0	0·11	2, 8, 9
Nalorphine	0·12	0·8	0·13	2
Pentazocine	0·20	0·5	0·14–0·28	8

All doses calculated in terms of mg/kg of free base ; human doses calculated on basis of 70 kg man.

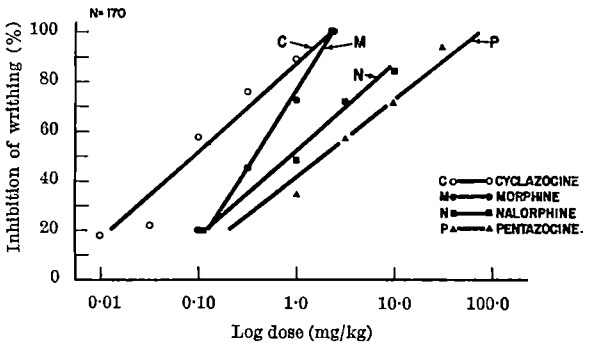


Fig. 1. Phenylquinone antagonism of morphine and narcotic antagonists

slope of the dose–response curves (Table 1). However, when the drugs are compared at a point (ED_{20}) where morphine and nalorphine are equi-effective, as has been shown in man, both the relative potency and the actual mg/kg doses seem highly correlated with established clinical results (Table 2).

The dissimilarity between the narcotic antagonists and morphine, both in the spectrum of activity in other analgesic tests and the slope of the dose–response curve in this procedure, suggests that the antagonists may, in part, exert their analgesic effect through a mechanism different from that of morphine. At present, we are investigating the effects of various combinations of morphine and nalorphine on the writhing response to phenylquinone in an attempt to elucidate these differences further.

We thank Dr. S. Archer, of Sterling-Winthrop Laboratories, for the supply of cyclazocine and pentazocine.

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Inhibition of Uridine Ribonucleotide Synthesis by 5-Halogenated-2′-deoxyuridine Analogues

Previous *in vitro* research[1] with the halogenated analogues of orotic acid demonstrated the following basic experimental facts: First, to inhibit the conversion of orotic acid to the uridine nucleotides it was necessary for a drug to be a 5-substituted analogue of orotic acid. For example, it was found that 5-fluoro-orotic acid would inhibit orotic acid conversion, but that 5-fluoro uracil would not. Second, the order of activity of the 5 halogenated orotic acid analogues was fluorine > chlorine > bromine, and the 5-iodo compounds showed little or no activity.

The systems used in the research reported here used a high-speed supernatant fraction from rat brain (20 per cent homogenate in 0·25 M sucrose centrifuged at 114,000*g* for 1 h) as the enzyme source. This preparation will convert either orotic acid or uracil to the uridine nucleotides, the end-product being predominantly UTP. The reaction mixtures contained phosphoglyceric acid, $6{\cdot}6 \times 10^{-3}$ M; $MgCl_2$, $5{\cdot}0 \times 10^{-3}$ M; KH_2PO_4, $3{\cdot}3 \times 10^{-3}$ M; ATP, $3{\cdot}3 \times 10^{-4}$ M; (6-^{14}C) orotic acid, $1{\cdot}0 \times 10^{-4}$ M; or (2-^{14}C) uracil, $1{\cdot}66 \times 10^{-5}$ M; with ribose-5-phosphate, 2×10^{-3} M; or ribose-1-phosphate, $1{\cdot}3 \times 10^{-3}$ M; with the orotic acid and uracil systems respectively. The reactions were carried out at 37° C for 90 min and chromatographed on 'Dowex-1-formate' columns as described in a previous communication[2]. The brain was used as a source of enzyme because both the orotic acid and uracil pathways are operative in this tissue.

Table 1 demonstrates the activity of a number of 5-halogenated-2′-deoxyuridine analogues against both the uracil and orotic acid systems. It is evident that practically all the compounds tested had significant activity against the uracil system in 60 : 1 drug/metabolite ratio. It is noteworthy that thymidine and deoxyuridine both had marked activity. When the drug concentration was lowered to a 6 : 1 drug/metabolite ratio only thymidine and the iodo-, chloro-, and bromo-derivatives retained their activity.

Table 1. Inhibitory Effect of Deoxyuridine Antimetabolites on Uridine Ribonucleotide Synthesis by Brain High-speed Supernant Fraction

Uracil system	Drug/metabolite ratio	% Inhibition of conversion to uridine nucleotides 60 : 1	6 : 1
Thymidine		64·6	18·6
2′-deoxyuridine		72·3	0·0
5-fluoro-2′-deoxyuridine		79·8	0·0
5-iodo-2′-deoxyuridine†		92·0	31·2
5-iodo-uridine		78·7	0·0
3,5-diacetyl-5-iodo-2′-deoxyuridine†		94·1	57·0
5-chloro-2′-deoxyuridine		*	32·6
5-bromo-2′-deoxyuridine		*	27·5

Orotic acid system	Drug/metabolite ratio	10 : 1
Thymidine		0·0
5-fluoro-2′-deoxyuridine		0·0
5-iodo-2′-deoxyuridine		0·0
3,5-diacetyl-5-iodo-2′-deoxyuridine		0·0
5-chloro-2′-deoxyuridine		33·0
5-bromo-2′-deoxyuridine		0·0
Deoxyuridine monophosphate		6·3
Thymidine monophosphate		33·3

* Indicates that the drug was not tested at this concentration.
† Obtained through the courtesy of International Chemical and Nuclear Corporation, City of Industry, California.
All drugs listed under orotic acid system were inactive at a 2 : 1 ratio.

The orotic acid system is much less sensitive to these agents; only thymidine monophosphate and 5-chloro-2′-deoxyuridine showed any significant activity. All drugs were inactive at a 2 : 1 ratio. It should be pointed out that phosphorylation of the deoxynucleoside analogues in the orotic acid system is minimal, and that the deoxynucleotides of these analogues would be better test agents.

The order of activity of these halogenated derivatives against the uracil system is reversed from that of the 5-halogenated orotic acid analogues mentioned earlier, in that iodine > bromine > or chlorine, with fluorine showing the least activity. It should also be noted that normal deoxyribose metabolites inhibited this system. The inhibition of ribonucleotide synthesis by deoxynucleotides has been shown previously[3]. There is a possibility that the 5-halogen substituted compounds used here may exert their effect through a similar mechanism.

It cannot be said if the action reported here can be correlated with the known inhibitory action of 5-iodo-2′-deoxyuridine against herpes infections of the cornea[4]. However, the actions demonstrated here could be expected to inhibit protein synthesis in tissues which are partially dependent on uracil utilization rather than orotic acid for uridine nucleotides. The high drug/metabolite ratios used in this work are not unrealistic in view of the high concentrations of 5-iodo-2′-deoxyuridine used in the local

treatment of herpes. Experiments utilizing corneal epithelium are in progress.

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HÆMATOLOGY

Dizygotic Twins with XX/XY Chimerism

CHIMERAS, dizygotic twins who have two populations of blood cells, arise when hæmatopoietic cells are exchanged or shared at some time in early embryonic life. The first case of chimerism in man was reported by Dunsford *et al.*[1].

The twins here reported are clinically normal male and female infants. They had a single dichorionic placenta with no obvious anastomosis of the surface blood vessels. Chimerism was discovered by blood grouping of the red cells of the twins by the Rh Laboratory of Winnipeg. Two red-cell populations were found in the same proportion in each twin, approximately 85 per cent being group O and the rest group A. The inherited ABO types were established by testing the saliva: the boy was group A and the girl group O. Details of the blood group investigations have been reported elsewhere[2].

Peripheral blood samples were obtained for chromosomal investigations. Karyotype analyses of 100 cells of each twin revealed normal chromosome complements except for the presence of numerous cells in the male twin with a female karyotype and some cells in the female twin with a male karyotype in the following proportions: 70 XX : 30 XY cells in the male and 78 XX : 22 XY cells in the female twin. The parents and sib have normal karyotypes. These proportions are similar to, and probably not significantly different from, those found for the red-cell populations.

The phenomenon of chimerism is best known in cattle, and was first described by Owen[3]. The sterility of the female chimera is believed to be caused by androgens circulating in the common placental vascular system. Cattle chimeric twins have been observed to have the same proportions of each of the two types of blood irrespective of their genotype. This suggests that each twin has drawn its primordial blood cells from a common pool rather than having received the foreign cells by partial exchange[4].

Among marmosets chimeras are even more common than among cattle since 87·5 per cent of births are reported to be twins, the majority of which are suspected to be dizygotic[5]. Extensive placental anastomoses between dizygotic twins are present but freemartins are unknown. The actual proportions of the two types of red blood cells in marmosets are not known. However, Benirschke *et al.*[6] have demonstrated by bone marrow culture that in one female and two males the cells were chimeric with respect to the sex chromosomes. In each case the major population of cells was its own.

There have been occasional reports of vascular anastomoses in human dichorionic placentæ[7]. However, in a series of 409 fused dichorionic placentæ (238 isosexual and 171 heterosexual twins) examined by injection of the blood vessels, we have found no instance of a vascular communication. This suggests that such intercommunication is a rare event. On the other hand, it is possible that a vascular communication could have existed some time in early embryonic life and disappeared during later development.

With respect to the blood group types, the previously reported human chimeras are similar to marmosets in that the major proportion of cells corresponds to the genotype of the individual. Chromosomal analyses in the only case studied gave frequencies of XX and XY cells consistent with the blood group proportions[8,9]. These twins are unmarried. Two other female chimeras have produced children[1,10]. The present pair of twins differs from the other human chimeras and from marmosets but resembles cattle in the proportion of blood types.

This work was supported by the National Foundation (U.S.A.) and U.S. Public Health Service grant *GM*-07950-02. Colcemid was kindly supplied by the Ciba Co. of Canada. We thank Dr. B. Chown and Miss Marion Lewis for referring these twins to us and Dr. K. Christie for his assistance.

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Alkaline Phosphatase Activity of the Leucocytes in Animals

THE study of alkaline phosphatase activity in neutrophilic leucocytes has become a useful tool in clinical laboratory work. Although the practical usefulness of the test has been demonstrated, the significance of differences in activity in different conditions is still obscure.

A convenient histochemical method has been described by Kaplow[1]. Since many factors influence the outcome of the test, it is important to have a positive control when examining the blood of unknowns. It has been found that pregnant women have increased alkaline phosphatase activity of the leucocytes[2] and therefore they can always be used as positive controls. However, pregnant women are not always available or the maternity wards may not be convenient to the laboratory.

The work recorded here had the following objectives : (*a*) to find laboratory animals with constant and physiological high alkaline phosphatase activity of the leucocytes, which can be used as positive control any time in the laboratory; (*b*) to contribute to the understanding of this enzyme metabolism in leucocytes.

Using the method of Kaplow[1], I examined 157 laboratory animals of different species, hamsters, rats, mice, guinea-pigs, rabbits and monkeys. In rabbits, guinea-pigs and monkeys, the blood can easily be obtained from the ear, while in hamsters, mice and rats it is easier to get it from the tail. The scoring was carried out in duplicate in the first 37 animals, but after it had been found that the duplicate findings were rather close to each other, this practice was discontinued.

The hamsters, rats, mice, guinea-pigs and rabbits were adult and healthy, and reared in the laboratory. Some were young unmated adults, others had one or more litters. None was pregnant. The results are shown in Table 1. In 53 normal healthy men and women we

Table 1. Alkaline Phosphatase Activity of the Leucocytes in Animals

Species	No. examined	Sex	Age (months)	Mean score	Range
Guinea-pigs	31	M 16 F 15	1–7	311·5	251–351
Hamsters	27	M 13 F 14	1½–7	335·8	231–392
Rabbits	12	M 6 F 6	2–9	171·0	88–262
Rats	10	M 6 F 4	2–12	188·9	111–254
Mice	22	M 12 F 10	1–8	0	0
Total	102	M 53 F 49	1½–12		

Table 2. Alkaline Phosphatase Activity of the Leucocytes in Monkeys

Species	No. examined	Malaria	Mean score	Range
M. mulatta	27 (M 15, F 12)	Pos. 18, neg. 9	0·4	0–11
M. nemestrina	12 (M 7, F 5)	Pos. 3, neg. 9	319·5	255–384
M. iris	10 (M 6, F 4)	Pos. 5, neg. 5	315·5	262–386
Hylobotes spp.	5 (M 1, F 4)	Pos. 2, neg. 3	346·4	283–378
Presbytis cristatus	M 1	Pos. 1	343·0	
Total	55 (M 30, F 25)	Pos. 29, neg. 26		

* M, male; F, female.

found a mean score of alkaline phosphatase activity of 10·3, with a range of 3–65. Compared with the findings in man, there is a definite increase of alkaline phosphatase activity in the leucocytes of guinea-pigs, hamsters, rabbits and rats. Especially in guinea-pigs and hamsters the alkaline phosphatase activity of the leucocytes is extremely high. Nearly all neutrophilic leucocytes look dark-brown or black. Since, in man, a high alkaline phosphatase activity of the leucocytes is often associated with leucocytosis, we performed the total white blood cell count in 6 guinea-pigs and 3 hamsters. It was not increased in comparison with the count found in man. It was interesting to see that the leucocytes in mice were devoid of alkaline phosphatase activity. The lack in alkaline phosphatase activity in the leucocytes of mice on one side and the high activity in those of guinea-pigs and hamsters on the other side is so striking and constant that their blood can effectively serve as negative and positive controls respectively, when stained at the same time as unknown smears. No definite difference was found between the scores in males and those in females. Since hamsters, guinea-pigs and rabbits are found in most laboratories, this will be of great help to those examining alkaline phosphatase activity in leucocytes.

The group of monkeys included different species. Some of them were healthy, others were infected with various forms of malaria. They ranged in age between eighteen months and three years except two baby *M. nemestrina* monkeys who were 5 months old. It is interesting to find that different species showed entirely different alkaline phosphatase activity of the leucocytes. The activity seems to be more dependent on the species than on the health condition of the animals. In the *Macaca iris*, *M. nemestrina* and *Hylobates* spp. the enzyme activity is very high in healthy as well as in malaria-infected animals. There was no significant difference between the mean scores of monkeys with and without malaria. The leucocytes of the *M. mulatta*, however, were devoid or nearly devoid of all alkaline phosphatase activity, also in those most heavily infected with malaria. In fact, in all *M. mulatta* monkeys examined the leucocytes were found to be devoid of any alkaline phosphatase activity, except in one which had a score of 11. No significant differences in activity were found between male and female animals.

This investigation demonstrates that in animals genetic factors play an important part in the metabolism involving the enzyme alkaline phosphatase in leucocytes. It is possible that genetic factors also play a part in human beings in certain conditions.

Similar interesting differences in metabolism involving glucose-6-phosphate dehydrogenase in red blood cells have been shown in closely related animals[3].

I thank Mr. Lau Kheng Khuan of the animal house, Institute for Medical Research, and Dr. M. Warren and Dr. Gordon Bennet of the Far East Research Unit, U.S. Public Health Service (Kuala Lumpur), for their co-operation.

This work was supported in part by the U.S. Public Health Service research grant *GM*–11329–03 from the Office of International Research, National Institutes of Health, for the University of California International Center for Medicine Research and Training.

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Changes in the Human A Antigen of Erythrocytes with the Individual's Age

The human A and B antigens on fœtal red cells are weaker than those on erythrocytes of adults and undergo marked quantitative changes during life. Reports describing these changes appeared in the literature as early as 1929. Morville[1] stated that in most instances the A and B antigens seemed to have reached maximal strength by the time the child was four months of age. However, according to Thomsen and Kettel[2], the strength of the antigens increased more slowly, and maximal values were not reached until 15–20 years of age, afterwards remaining constant. This view was challenged by Lodenkämper and Stienen[3], who provided data indicating that the A and B antigens reach maximal strength at one or two years of age and remain at this level to 30–40 years of age; thereafter the strength of the antigens decreases and approaches zero. Obviously, these reports disagree with respect to the age at which the A and B antigens reach adult strength, and they also disagree as to the strength of these antigens in old age.

A quantitative investigation of the sources of variation within the human A blood types has provided evidence bearing on this question of age and antigen strength. It is this evidence which is summarized in the present communication.

Type A blood samples were obtained from three different sources: (1) from all A_1 individuals tested during the period from November 1, 1962–December 31, 1963, in a population investigation; (2) from families selected for having an infant with ABO hæmolytic disease of the new-born; but young infants who had suffered severe neonatal hæmolytic disease are not included. These families were ascertained from the records of the University Hospital in Ann Arbor, Michigan; (3) cord blood samples of type A_1 infants born to A_1 mothers delivered in the Maternity Clinic of the University Hospital. All individuals from the different sources were Caucasians. Since the strength of the A antigen is depressed in the presence of B, AB individuals were not included.

The strength of the A antigen was determined by an immunohæmolytic system which was previously described in detail elsewhere[4]. Briefly, the technique consists of incubating a constant amount of a photometrically standardized cell suspension with a constant amount of hæmolytic rabbit anti-human-A antibody and complement for 90 min at 37° C. After centrifugation, the density of hæmoglobin in the supernatant fluid is determined photometrically and the percentage hæmolysis is taken as a

Table 1. THE STRENGTH OF THE A_1 ANTIGEN IN RELATION TO THE AGE OF INDIVIDUALS

Age (years)	No. tested	Average strength of A_1 antigen	S.D.
Cord cells	30	26·9	6·56
< 2	18	43·3	6·37
2 and 3	19	52·9	5·71
4 and 5	18	53·3	5·69
6–10	88	52·1	6·40
11–15	85	53·4	6·19
16–20	33	53·7	4·03
21–30	53	54·7	5·89
31–40	84	53·9	7·34
41–50	50	53·4	6·35
51–60	29	54·2	5·84
61–90	26	52·2	5·58

measure of antigen strength. Fresh blood samples are essential for reproducible results. Although the sub-type of A could generally be predicted from the hæmolytic test, it was always verified with *Dolichos biflorus* and *Ulex europaeus*.

The results are presented in Table 1. An increase in antigen strength with an increase in the individual's age up to 2 years of age is obvious. The difference in antigen strength between cord cells and children less than 2 years of age is highly significant ($P < 0.001$), as is the difference between children less than 2 years of age and those 2 and 3 years of age ($P < 0.001$). The differences in antigen strength among the age-groups above 2 years of age are relatively small and only the difference between age-groups 6–10 and 21–30 is significant ($P < 0.05$). The cells in the 61–90 age-group were similar in behaviour to those of other adults, both in the immuno-hæmolytic and agglutination tests. The cells of the oldest individual tested, who was 90 years of age, showed 47·5 per cent hæmolysis, a value which is well within the range of younger adults. The sources of variation among type A adults will be the subject of a forthcoming publication.

The values for children up to 4 years of age are plotted in Fig. 1. A freehand curve has been applied to these points to show the average trend in strength of the A_1 antigen with age. The term 'adults' is being used for individuals 6 years of age and older. As the figure illustrates, antigen strength increases very rapidly during the first few months of life up to about 5 months and then increases with slower rate until adult values are reached. The data indicate that there is considerable variation in the age at which the infant's red cell antigen reaches its adult strength, but 2–4 years or shortly thereafter may represent a general average.

For sub-type A_2, similar trends of change in the quantitative aspects of the A antigen were observed, the main difference being that A_2 was weaker than A_1 during all

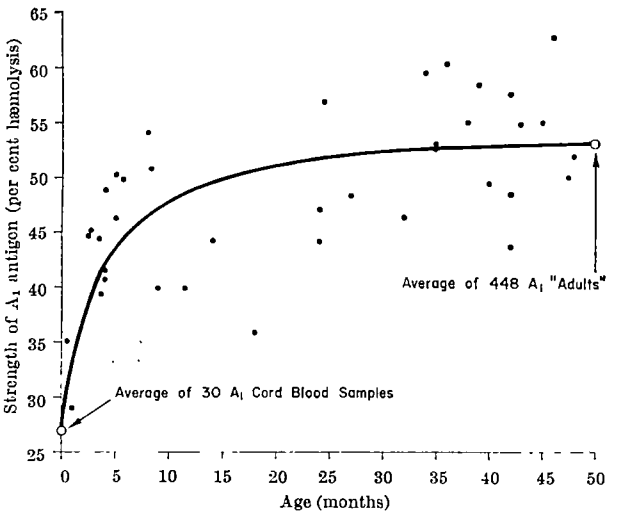


Fig. 1. Change in the strength of the A_1 antigen among infants up to 4 years of age. The dots represent individual values

phases of the individual's life. The B antigen undergoes similar changes, although it has not been investigated here. What will be said in the following discussion regarding A probably also applies to B.

Compared with the previously mentioned reports in the literature, the present findings indicate intermediate values with respect to the age at which the A antigen attains parental strength. That the present values are intermediate may result from the fact that none of the previous authors made any distinction between A_1 and A_2 sub-types, a fact which could have increased the variation in their investigations. Also, different antisera may be responsible for some of the differences in results. The present findings disagree with the conclusion of Lodenkämper and Stienen[3] that A antigen strength decreases to zero in old age. The reason for this discrepancy is not clear. It is known, however, that in rare cases of certain types of leukæmia the A antigen may no longer be present on the red cells of formerly recognized type A individuals[5,6], but it is not known whether or not this was a factor in the investigation of Lodenkämper and Stienen. The results recorded here agree with those of Thomsen and Kettel[2] in showing that the red cells maintain their sensitivity in old persons.

Erythrocytes of cord blood and of new-born infants possess antigenic sites which are not present on the red cells of adults[7,8]. This fœtal antigen is not a property of the Lewis system[7]. The presence of this antigen may be the cause of the relative insensitivity of the A and B antigens of fœtal erythrocytes. This may provide a protective mechanism *in utero* against maternal antibodies in ABO incompatible pregnancies. If fœtal cells were to have adult strength, few A_1 fœtuses would probably remain unharmed in pregnancies of type O mothers. The quantitative aspects of the A antigen as an etiological factor in ABO hæmolytic disease of the new-born have been discussed elsewhere[9].

The change in strength of the A and B antigens may result from a gradual maturation of the erythropoietic system. This maturation may be partly associated with the change in erythropoietic sites which occurs during fœtal life and early infancy. The mesenchyme and then liver and spleen are the main sites of erythropoiesis up to the fifth month of fœtal life, thereafter this function is gradually transferred to the bone marrow, so that at birth erythropoiesis is almost exclusively a function of the bone marrow[10].

Of the human red cell antigens only a few undergo marked changes during the individual's life, while most appear to remain relatively constant[6]. A similar pattern seems to prevail among the bovine red cell antigens. There the J antigen, which is serologically related to the human A antigen, is not present at birth and likewise undergoes changes during life[11]. Although the time of change of the A antigen in man and the bovine J antigen differs, a phylogenetic relationship cannot be excluded.

The evolutionary mechanisms underlying the marked change in the strength of the A antigen are as yet not clear. However, selective forces may have been operative in opposite directions in fœtal life and post-natally. One force may have been towards the selection of a weak fœtal A_1 antigen, thereby reducing the hazards of fœtal-maternal ABO incompatibility. The other force would seem to have been directed towards selection of a strong A antigen in post-natal life. Since a weak A antigen has been favoured in fœtal life, the A antigen would be expected to remain weak to post-natal life. The fact that it does not do so implies that some selective force has favoured a change to a stronger post-natal A antigen. Selective advantage in epidemics, as proposed by Vogel *et al.*[12], or heterotic advantages might be examples of such selective forces. The examination of monkeys and higher primates, such as the baboons, which possess the A and B antigens but not O (ref. 13), may provide some insight into the selective mechanisms concerned.

This work was supported in part by Atomic Energy Commission contract *AT*(11–1) while I held a U.S. National Institutes of Health post-doctoral fellowship (*GPD*–15, 166). I thank Dr. D. C. Shreffler and Dr. H. Gershowitz for making available their blood-typing records. I am also indebted to Dr. D. E. Anderson for his assistance with the manuscript and to Dr. T. N. Evans and his staff for collecting the cord blood samples. A portion of the samples used was made available by the Cardiovascular Research Center, University of Michigan, supported by programme project grant *H*–6378 from the National Heart Institute.

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PATHOLOGY

Antigenicity of Trypanosome Nuclei: Evidence that DNA is not coupled to Histone in these Protozoa

The sera of patients with certain diseases (for example, systemic lupus erythematosus) often contain autoantibodies which react with various constituents of cell nuclei[1,2]. We have detected antibodies reacting specifically with free DNA[2,3], DNA-histone[2,4], a saline soluble protein[2,5] and an unidentified constituent of the nucleolus[2,4] in such sera by the fluorescent antibody technique. Most sera from patients with connective tissue diseases contain a mixture of two or more of these antinuclear antibodies, but occasionally a serum may contain only one of them. Monospecific antinuclear sera have proved valuable cytochemical reagents for the tentative identification of the corresponding antigens since they show relatively little species specificity[6], but they have not been used previously to investigate protozoa.

Several strains of trypanosomes were examined. Blood forms were available in smears of blood from rats infected with *Trypanosoma gambiense*, *T. rhodesiense*, *T. rhodesiense* 200, *T. cruzi*, *T. congolense*, *T. congolense K*71, *T. equiperdum*, *T. evansi S.A.K.* and *T. lewisi*. Cultures of *T. rhodesiense*, *T. congolense*, *T. lewisi*, *Leishmania donovani* and *Strigomonas* sp. were suspended in 0·15 M sodium chloride and smeared on slides. Unfixed air-dried preparations were treated for 30 min with monospecific human antinuclear sera in a moist chamber at room temperature to allow the antibody to become attached to its antigen. The unattached human serum proteins were removed by washing in barbitone buffered 0·15 M sodium chloride (*p*H 7·2) for 10 min and thereafter the slides were stained with a fluorescein-conjugated rabbit antiserum to human γ-globulin for 30 min. The slides were then washed and, after mounting in glycerol containing 10 per cent (v/v) barbitone buffer (*p*H 7·2), examined with dark-ground illumination using blue violet/ultra-violet light on a horizontal optical system similar to the instrument designed by Young[7]. Excitor filtration was achieved with a 2·0-cm optical cell containing 10 per cent copper sulphate and a glass mounted Kodak Wratten 18*A* filter; a Kodak Wratten 2*B* gelatin filter was used for absorption of ultra-violet light scattered into the microscope tube. Photographs were taken in colour on high-speed 'Ektachrome' and also on monochromatic Agfa *KB*14 film.

The specificity of the first stage of the staining procedure was controlled by demonstration of absorption with the appropriate antigen and failure of absorption with the other antigens as described previously[4,5], except in the case of the antinucleolar antibody because the corresponding antigen has not been identified.

The non-specific staining properties of the fluorescein-conjugated rabbit anti-human-γ-globulin were removed by absorption with acetone-dried rat liver powder. The specificity of this reagent was then established by absorption experiments in which it was shown that its capacity to stain attached human γ-globulin could be removed by treatment with human γ-globulin (prepared by DEAE 'Sephadex' chromatography of normal human serum) but not by other serum fractions.

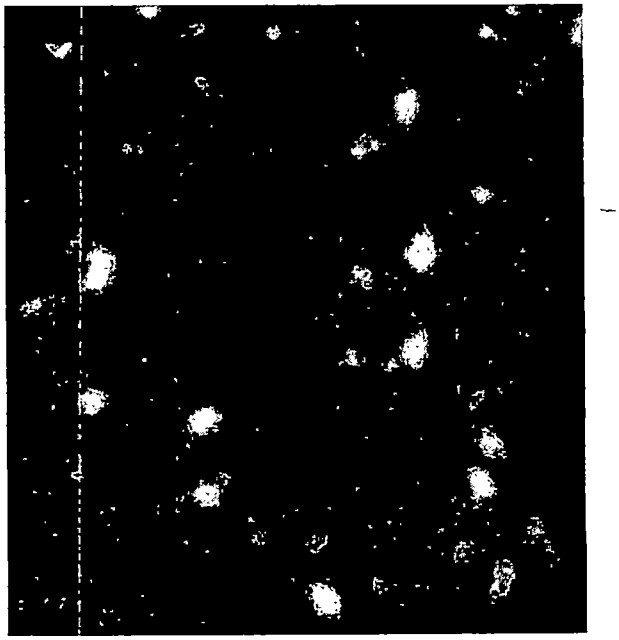


Fig. 1. *Trypanosoma cruzi* in blood film from infected rat: nuclei stained with antinuclear serum containing anti-DNA (× 1,650)

Similar results were obtained with all the trypanosome strains investigated. Trypanosome nuclei were stained by sera containing anti-DNA antibodies (Fig. 1) but they remained unstained after treatment with normal sera or sera containing antinuclear antibodies to other antigens (Fig. 2). The potency of the antinuclear antibodies in all sera used was demonstrated by their ability to stain the nuclei of the host leucocytes in films of the infected blood.

Further investigations of the staining of trypanosome nuclei by sera containing anti-DNA have shown that these sera lose this property after absorption with calf thymus DNA (Worthington Biochemical, Inc.) but remained unaffected after treatment with RNA (Worthington Biochemical, Inc.); in these experiments sera were absorbed for 45 min with 25 mg purified antigen/ml. serum at 37° C. Furthermore, the nuclear antigen with which these sera react was destroyed by pretreatment with DNase (Worthington Biochemicals, Inc.) (0·1 mg/ml.)

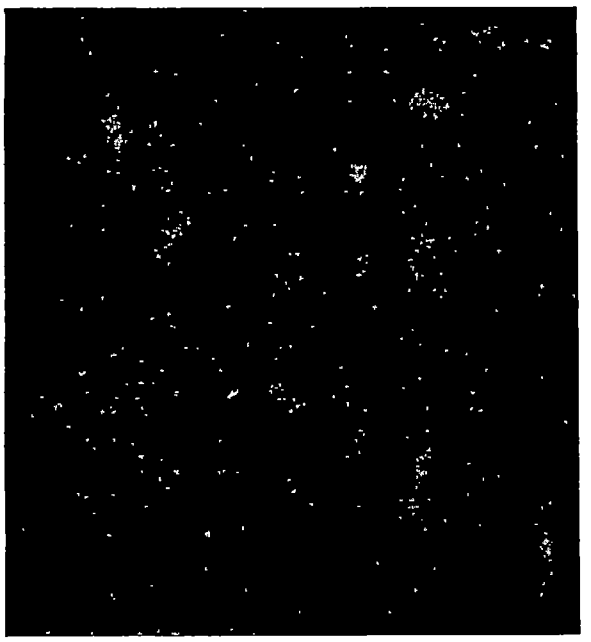


Fig. 2. *Trypanosoma cruzi* in blood film from infected rat: nuclei unstained after treatment with antinuclear serum containing anti-DNA-histone (× 1,650)

in 0·15 M sodium chloride containing 40 μg Mg^{++}/ml. for 1 h at 37° C whereas the antigen was unaffected by treatment with buffer control or with RNase (1·0 mg/ml.) in ion-exchanged water for 1 h at 37° C. These experiments confirm the specificity of staining by anti-DNA sera which has demonstrated DNA antigen in trypanosome nuclei.

The nuclei of all species of trypanosomes investigated were stained with sera containing anti-DNA antinuclear antibody. This staining was immunologically specific since it was prevented by absorption of these antinuclear sera with purified DNA or by predigestion of the antigen by DNase but not by control treatments. This demonstrated that antigenicity of the DNA in trypanosome nuclei was identical with that in higher forms[2]. Stollar *et al.*[8] had shown previously by a complement-fixation technique that similar antigenic determinants for anti-DNA antinuclear sera were present in DNA extracted from normal and neoplastic mammalian tissue, many varieties of bacteria, several strains of bacteriophage, *Euglena gracilis* and tobacco leaf, so that it was not surprising that the present investigation showed that trypanosome DNA conformed to the general biological pattern.

The failure of sera containing antinucleohistone, the potency of which was demonstrated by its reaction with host leucocyte nuclei, makes it highly probable that the DNA of trypanosome nuclei is not present as DNA–histone complexes of the type found in metazoan cell nuclei. Therefore, trypanosome DNA may either be free or it may be combined with a protein other than histone. The first alternative appears more attractive since there is considerable evidence that bacterial and viral DNA are not associated with basic protein[9–11]. The possibility that the DNA is combined with a non-histone protein cannot, however, be excluded since the only previous investigations on protozoal DNA have been concerned with analysis of its base composition[12–15]. There have been indications that the nuclei of trypanosomes circulating in the blood may be in some stage of division[16], but it is unlikely that this will have altered the state of combination of the DNA since it has been shown that in HeLa cells DNA is complexed as nucleohistone in interphase cells and remains so during all stages of mitosis[17].

The failure of the antibody to the saline soluble protein of mammalian nuclei to stain trypanosome nuclei is not surprising as this antigen has not been demonstrated previously in the nuclei from animals other than mammals[4]. Sera containing the antinucleolar antibody failed to stain trypanosome nuclei, demonstrating that the corresponding antigen was not localized in discrete nucleoli. This observation is of interest as structures with the appearance of nucleoli are seen in electron micrographs of trypanosomes, but the nature of these intranuclear inclusions is undecided since no intranuclear RNA staining with acridine orange is seen on fluorescence microscopy[16,18].

All human sera stained the kinetoplast area in all species of trypanosomes investigated, but markedly brighter staining in kinetoplastic forms was produced with sera containing anti-DNA antinuclear antibody than with normal sera, or other antinuclear sera which react with DNA-histone, the soluble protein antigen or the nucleolar antigen. In most species the stained area corresponded in size, shape and position to the kinetoplast, but a similar area of staining was seen in the strain *T. evansi S.A.K.*, in which neither kinetoplasts nor their DNA could be demonstrated with the light microscope at the base of the flagellum[19]. Recent electron microscope investigations have shown that the kinetoplast is a modified mitochondrion containing a band of DNA[20] and that similar structures, lacking only the DNA band, are seen in drug-induced and spontaneously occurring akinetoplastic trypanosomes[21–23]. It is, therefore, probable that in the non-specific staining of the kinetoplast region produced by human serum, the human γ-globulin is attached to the kinetoplast at some point other than in the DNA band, although it must be remembered that the resolution of the optical system used in this investigation is such that it is not possible to localize the staining with great accuracy to the kinetoplast. Alternatively it is possible that this staining has resulted from trapping of human serum in the invagination of the cell membrane at the base of the flagellum which Vickerman[24] has demonstrated close to the kinetoplast.

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RADIOBIOLOGY

Protection with 2-β-Aminoethylisothiourea, Glutathione and Serotonin of Mice against Whole-body Irradiation

2-β-Aminoethylisothiourea (AET)[1,2], glutathione[3,4] and serotonin[5], administered separately, have been shown to protect X-irradiated mice. Many attempts have been made to increase the protective action by combining two or more drugs, and we have shown previously that AET in combination with serotonin increases optimal protection offered by either compound alone[6,7]. Results are reported here on the survival of mice treated with glutathione, AET and serotonin prior to exposure to a supralethal dose of X-irradiation.

Groups of C^+ male mice 11–12 weeks old ($LD_{50/30}$ and $LD_{100/30}$, respectively 650 and 900 r.), weighing 25–30 g, received a whole-body dose of 1,350, 1,800 and 2,000 r. of X-rays. The conditions of irradiation were: 200 kV, 10 m.amp, 1 mm aluminium, dose rate in air 100 r./min, target to object distance 54 cm. The mice were exposed in groups of 11 in a partitioned 'Lucite' cage. Times of administration and doses of AET, glutathione and serotonin used in the different experiments are given in Table 1.

Table 1 shows that after a radiation dose of 1,350 r. all the non-treated mice as well as the mice treated with glutathione died within 8 days. 10 per cent of the mice treated with serotonin and 20 per cent of those treated with AET survived after 30 days. 40 per cent of the mice treated with AET + glutathione and 70 per cent of those treated with AET + serotonin were still alive after a 30-day period, whereas 90 per cent of the mice treated with AET, glutathione and serotonin survived the same period of time. The combined treatment with serotonin + glutathione was less effective than the protection offered by the combined treatment with AET + serotonin or AET + glutathione. The mortality curves obtained after a radiation dose of 1,800 r. for treated and non-treated mice is presented in Fig. 1, and a corresponding curve for a dose of 2,000 r. is shown in Fig. 2. It is evident from these curves to what extent combination of AET, glutathione and serotonin increases survival rate and survival time if compared with unprotected mice.

Burnett and Doherty have demonstrated that AET approximately doubles the $LD_{50/30}$ days survival of X-irradiated mice[1]. A similar dose reduction factor for AET protection was observed for damage to bone-marrow[8] and small intestine[2]. Bone-marrow and small intestine are also protected to some extent by serotonin[6,7]. Glutathione administered subcutaneously or orally protects

Table 1. Survival time of C^+ mice treated with AET, glutathione and serotonin given separately or in combination before a whole-body dose of 1,350, 1,800 and 2,000 r. of X-rays

Treatment	Amount of compound (mg)	Route of administration	Time of treatment (min before irradiation)	Dose of radiation (r.)	Medium times of survival (days)	Percentage of survival (30 days)
Control				1,350	5	0
				2,000	4	0
Serotonin*	1	IP+	5	1,350	8	10
Glutathione†	32	OR+	25	1,350	6	0
AET‡	10	OR	25	1,350	10	20
AET	8	IP	15	1,350		70
Serotonin	1		5	1,800		0
Glutathione	32	OR	25	1,350	7	20
Serotonin	1	IP	5			
AET	10	OR	25	1,350	15	40
Glutathione	32					
AET	8	IP	10	1,350		90
Glutathione	16	OR	25	1,800	10	37
Serotonin	1	IP	5	2,000	9	30
AET	8	IP	10	1,350		90
Glutathione	75	OR	25	1,800	9	37
Serotonin	1	IP	5	2,000	6	20
AET	10	OR	25	1,350	6	50
Glutathione	32	IP	5	1,800	8·5	13
Serotonin	1	IP	5	2,000	5	10

+IP = intraperitoneally. OR = Orally.
* Serotonin creatinine sulphate.
† Reduced glutathione.
‡ AET bromide.

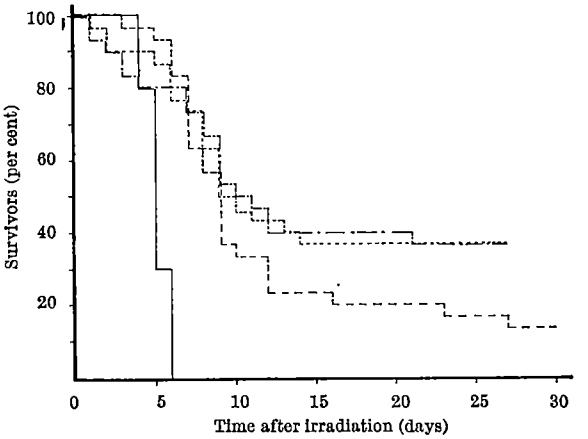


Fig. 1. The protective effect of different combinations of AET + glutathione + serotonin to whole-body X-irradiated C^+ mice with 1,800 r. (30 mice were used per group). —·—, Glut. 16 mg (orally) 25′ + AET 8 mg (intraperitoneally) 10′ + ser. 1 mg (intraperitoneally) 5′ + X-irradiation, 1,800 r.; - - - -, glut. 75 mg (orally) 25′ + AET 8 mg (intraperitoneally) 10′ + ser. 1 mg (intraperitoneally) 5′ + X-irradiation 1,800 r.; — — —, AET 10 mg (orally) 25′ + glut. 32 mg (intraperitoneally) 10′ + ser. 1 mg (intraperitoneally) 5′ + X-irradiation 1,800 r.; ——, X-irradiation 1,800 r.

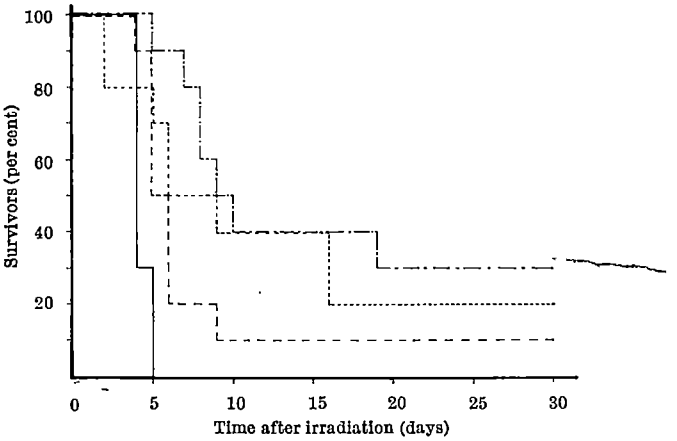


Fig. 2. The protective effect of different combinations of AET + glutathione + serotonin to whole-body X-irradiated C^+ mice with 2,000 r. (10 mice were used per group). —·—, Glut. 16 mg (orally) 25′ + AET 8 mg (intraperitoneally) 10′ + ser. 1 mg (intraperitoneally) 5′ + X-irradiation 2,000 r.; - - - -, glut. 75 mg (orally) 25′ + AET 8 mg (intraperitoneally) 10′ + ser. 1 mg (intraperitoneally) 5′ + X-irradiation 2,000 r.; - - -, AET 10 mg (orally) 25′ + glut. 32 mg (intraperitoneally) 10′ + ser. 1 mg (intraperitoneally) 5′ + X-irradiation 2,000 r.; ——, X-irradiation 2,000 r.

animals against an $LD_{100/30}$ days[3,4]. We have shown previously that combined treatment of AET and serotonin increases the survival of mice irradiated with 1,350 r.[6]. This increased survival time is dependent on the dose of AET and is essentially due to an increased protection to the bone-marrow[7]. Concentrations obtained in the marrow, as well as the dependence of the degree of protection of the combined treatment on the sulphydryl compound levels, suggest that AET and serotonin act through different pathways. Serotonin offers protection, presumably, through local anoxia[9], and AET partly either by a temporary formation of mixed disulphide bonds with sensitive sulphydryl groups of proteins[10,11], or by a radical-trapping action[12,13], or by an inhibiting action on synthesis of DNA[14].

Thus glutathione which alone is not a strong radioprotector becomes a potent supporting agent for the radioprotection due to AET. We do not think that the fundamental mechanisms of action of AET and glutathione are different, but rather that the low toxicity of the latter compound allows the administration of relatively large amounts of glutathione in combination with AET and thus increases the protection of AET. Glutathione could, for example, either increase the time during which AET is available in the cell, or react with certain structures of the cells which are relatively little radiosensitive, but which are capable of binding AET. In this manner more

AET would become available in the cell for action on radiosensitive structures which either do not react with glutathione or are only little protected by it.

Our results show that combined treatment with AET, glutathione and serotonin still offers some protection to mice subjected to whole-body X-irradiation at 2,000 r.

This work was supported in part by a grant from Euratom and the International Atomic Energy Agency.

Note added in proof. An experiment now completed indicates: (1) that the $LD_{100/30}$ days of mice treated with glutathione, AET and serotonin before X-irradiation is 2,200 r., as compared with 900 r. in the controls; (2) that glutathione added to AET or to AET plus serotonin decreases the toxicity of AET.

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Oxygen Effect with 14-MeV Neutrons

The modifying effect of oxygen on the biological response to 14-MeV neutrons has been discussed in relation to the use of these neutrons in radiotherapy[1–5]; but no direct experimental data were cited. We have recently obtained for another purpose some data for 14-MeV neutrons on yields of chromatid-type aberrations in meristematic cells of bean root tips in aerated and anoxic conditions. The dose modification factor for true aberrations was about 1·5 and for non-staining lesions ('gaps')[6] about 1·3. These values are very little greater than those found for chromosome damage in the same biological material with 3-MeV neutrons[7] or in *Tradescantia* microspores with fission neutrons[8].

It has been estimated[9] that half the yield of chromosome damage seen with 14-MeV neutrons is due to the α-particles produced by inelastic nuclear reactions[10]. The dependence upon oxygen of the effect of these α-particles should be appreciably less than for the recoil protons, so the comparatively low overall value of oxygen dose modification factor found by us is not unexpected. It seems to be a reasonable presumption that for other biological effects besides chromosome aberration induction the oxygen factor for 14 MeV neutrons should be small and that, as regards oxygen dependence of effect, 14 MeV neutrons should be almost as suitable for radiotherapy as fast neutrons of lower energy.

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BIOLOGY

Ultrastructure of the Sensory Hair-cells in the Labyrinth of the Ammocœte Larva of the Lamprey, *Lampetra fluviatilis*

In a previous publication[1] the ultrastructure of the sensory epithelia of the labyrinth of *Raja clavata* was described and brought into relation with what is known of the responses of single sensory cells to angular acceleration, tilting, and to vibrational stimuli. It was found that the compound hair-processes of the sensory cells show a topographic polarization which could in most cases be correlated with the directional functional properties of the receptor cells.

The hair-cells are secondary sensory cells, and as such they synapse with the end branches of the bipolar neurones which constitute the first neuronal stage of the afferent pathway. Synaptic membrane structures were described but there was very little demonstrable evidence for the presence of synaptic vesicles associated with them.

We have now extended our electron-microscopic studies to the labyrinth of the ammocœte larva of the

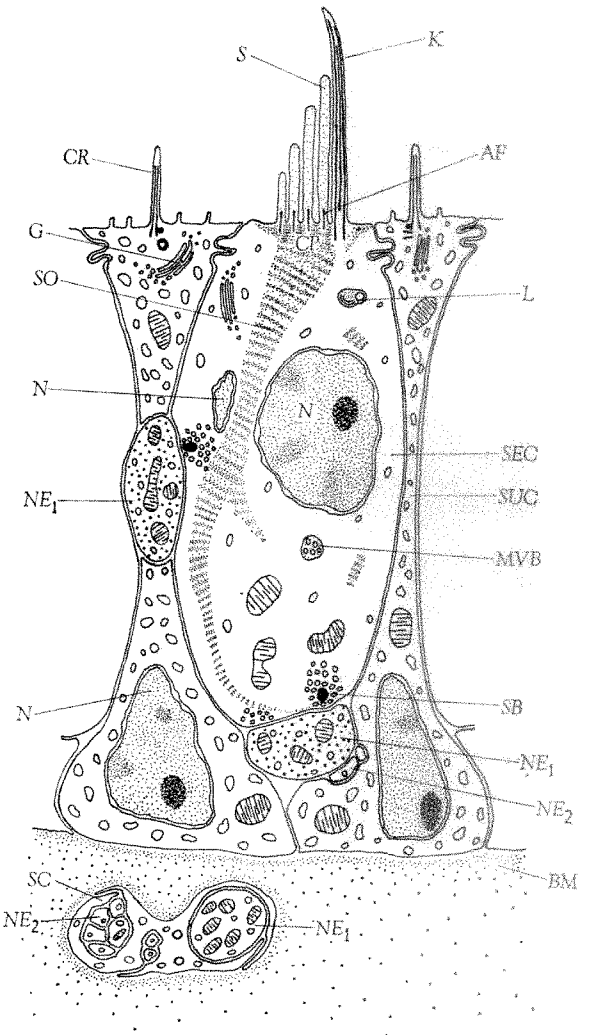


Fig. 1. Schematic drawing of a single sensory cell from a crista of the ammocœte labyrinth. Note the striated organelle which originates from the cuticular plate and runs to the base of the cell where it terminates in close proximity with synapse. Nerve fibres synapse with basal and nuclear regions of the cell. *AF* = axial fibre; *BM* = basement membrane; *CR* = ciliary rod; *CP* = cuticular plate; *G* = Golgi apparatus; *K* = kinocilium; *L* = lysosome; *MVB* = multivescular body; *N* = nucleus; *NE*$_1$ = large nerve endings; *NE*$_2$ = small nerve endings; *S* = stereocilia; *SB* = synaptic bar; *SC* = Schwann cell; *SEC* = sensory cell; *SO* = striated organelle; *SUC* = supporting cell

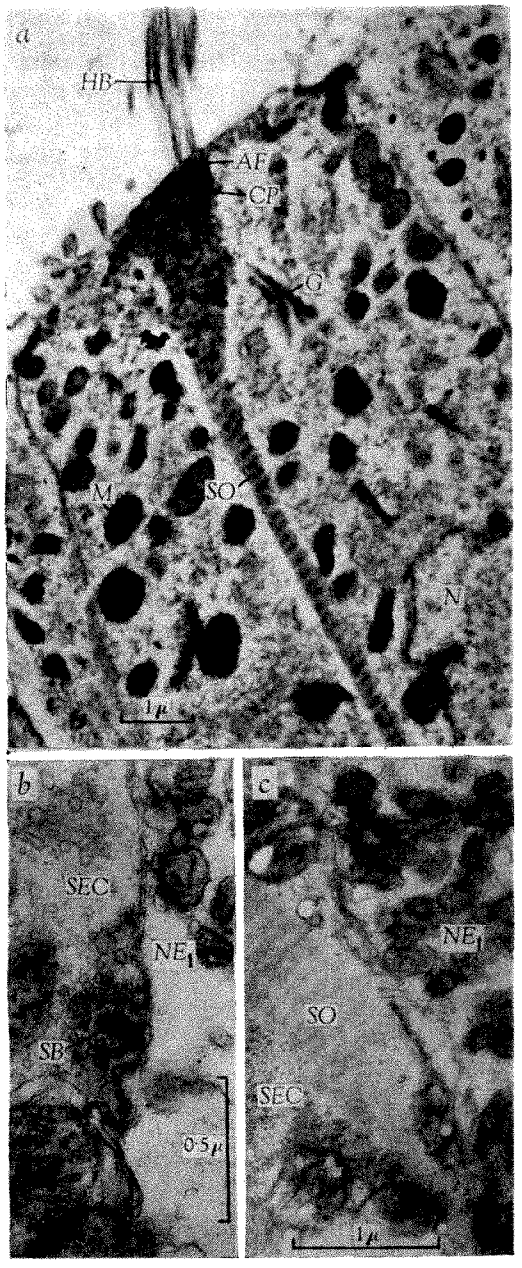


Fig. 2*a*. An electron micrograph of the apical region of a sensory cell showing the striated organelle continuing from the cuticular plate down past the level of the nucleus. *AF* = axial fibre; *CP* = cuticular plate; *G* = Golgi apparatus; *HB* = hair bundle; *M* = mitochondria; *N* = nucleus; *SO* = striated organelle. *b*, A micrograph showing the synaptic region at the base of a sensory cell. Note the electron dense synaptic bar surrounded by a cluster of vesicles. NE_1 = nerve ending; *SEC* = pre-synaptic cytoplasm of sensory cell; *SB* = synaptic bar. *c*, A micrograph of the synaptic region at the base of the sensory cell showing the striated organelle closely associated with the synaptic membrane. NE_1 = nerve ending; *SEC* = pre-synaptic cytoplasm of sensory cell; *SO* = striated organelle

lamprey. On the whole the ultrastructure of both the sensory cells and of the supporting cells conforms with the pattern described for the elasmobranch labyrinth. However, some new features were observed which appear to us worthy of early communication, before the report on the complete survey of the lamprey labyrinth is published elsewhere.

Fig. 1 shows a schematic representation of the sensory hair-cell. It appears to us of considerable interest that the cell contains typical synaptic bars surrounded by an accumulation of vesicles. The organelles appear to be identical to those described by Smith and Sjöstrand in the external hair-cells of the guinea-pig cochlea[2]. Figs. 1 and 2*b* show such synaptic bars (*SB*) in close association with nerve endings (NE_1). Similar bars have also been found in the sensory cells of electro-receptors in electric fishes[3]. These cells, too, belong to the acoustico-lateralis system.

A second feature of potential functional importance is an organelle, which runs from the cuticular plate at the top of the sensory cell, in which the roots of the stereo-cilia are anchored, down through the cytoplasm towards at least some of the synaptic structures at the base of the cell. The organelle is a cross-striated extension of the cuticular plate (Figs. 1 and 2*a*, *CP*). Its periodicity ranges from 120–190-μm with an average repeat of 130-μm. When seen in cross-section it appears to be composed of longitudinal fibrillæ. In passing down the cell the organelle divides either above or below the level of the nucleus. The nucleus is usually grooved along one side, forming a longitudinal invagination in which runs a branch of the striated organelle. Where the organelle approaches the base of the cell, it curves round and its striations thus continue to run perpendicular to the cell membrane, which it can approach very closely.

Essentially similar striated organelles have been described by Mountford in the light-sensitive cell of the rat retina[4]. They, too, are closely associated with the synaptic regions of the cell and also run in close proximity to the nucleus. No such organelle has, to our knowledge, as yet been described in the labyrinth of any other animal. Its functional significance is obscure, but it is tempting to suggest that its association both with the cuticular plate and with synaptic structures may mean that it is in some way concerned with the relaying of excitation caused by the mechanical deformation of the hair-bundle and the cuticular plate to the synapses and nerve endings at the base of the cell.

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Tissue Cultures on Solid Media

When tissue cultures of mouse tumours were routinely tested for mycoplasma by inoculation in Petri dishes containing Difco PPLO agar (7 parts), 25 per cent yeast extract (1 part) and horse serum (2 parts), a nodule of tumour cells 2 mm in diameter was seen on the agar surface of one dish examined after 14 days at 37° C. Several cell lines were then inoculated into this medium with substitution of fœtal calf serum for horse serum and the addition of 100 μg/ml. each of penicillin and streptomycin.

Differences in the appearance of various cell lines were noted when these cultures were examined after cultivation in a sealed aluminium desiccator for 2 weeks. Established cell lines routinely maintained in Eagle's medium, 10 per cent fœtal calf serum and antibiotics, including fungizone (2·5 μg/ml.), were each trypsinized to fresh culture medium and sedimented at 500 r.p.m. in centrifuge tubes; the supernatant fluid was discarded and cellular sediment was distributed with a Pasteur pipette to the surface of 1 ml. of agar medium contained in a Leighton tube, so that cell groups approximately 1 mm in diameter were produced.

After incubation for 4–5 days, the appearance of the cell aggregate was distinctive. $P388D_1$ mouse lymphoma had relatively dense, monolayer cell clusters with irregular

borders. A variant of this cell line altered by chronic infection with polyoma virus (PV) for 11 months had irregularly rounded cell nodules with regular, fairly sharp borders. A second variant of $P388D_1$ chronically infected with PV for 1 year was essentially non-viable on this medium; only a few scattered cells survived. A spontaneous mouse mammary carcinoma known to contain mammary tumour virus (MTV) had relatively round nodules of cells with indistinct cytomembranes and had a peripheral, irregular fringe of attached cells. A mammary tumour induced in *BALB-c/Crlg* mice with MTV had irregular, monolayer clusters of loosely associated cells. This cell line infected with PV for 2 months had dense nodules of cells and an extensive peripheral, monolayer outgrowth of polyhedral cells. Hamster embryo tissue culture, which grew as sheets of epithelioid cells in fluid media on glass, had on agar media dense, irregularly shaped nodules with relatively sharply defined nodular borders; this strain infected (1 year previously) with PV had the same appearance. Mouse embryo fibroblasts had irregular, relatively loose clusters of cells in a monolayer with irregular border. This strain infected (1 year previously) with PV had tight nodules of varying size with irregular borders and composed of cells of uniform appearance. A second strain of the mouse embryo cells infected with PV for the same length of time was non-viable on agar.

Agar layers were removed from the tubes with the aid of a spatula; contact impression smears of the cells were made on glass slides and stained. Varying numbers of viable cells were found for the cell lines. Similar cultures were made using 'Colab', 3 per cent ionagar (3 parts), Eagle's solution (5 parts), fœtal calf serum (2 parts). The appearance of several cell lines after incubation for the same time-interval differed somewhat from that seen on 'PPLO' agar. These observations suggest that colonial morphology of murine and hamster cells may be characteristic for a given strain cultured on a given solid medium.

This work was supported by grants from the U.S. National Institutes of Health and from the Attending Staff Association of the Los Angeles County Hospital.

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Participation of Cytochrome Oxidase in Lignification

INVESTIGATIONS during recent years on the more exact characterization of 'redoxases' involved in the lignification process have led, in the first instance, to the suggestion that this enzyme may be a laccase[1]. But since the distribution of laccase is principally restricted to lower plants, the consideration of laccase as the sole dehydrogenating enzyme in the formation of lignin cannot be accepted. Siegel[2,3] especially directed attention to another enzyme, namely, peroxidase. This enzyme can form lignin-like products from eugenol in the presence of hydrogen peroxide. Hence it is quite possible that also *in vivo* in the zone of lignification of a woody plant, peroxidase is an essential part of the mechanism of lignification. In fact, investigations of Wardrop and Bland[4] on the topological distribution of peroxidase by means of benzidine/hydrogen peroxide in the lignification zone have shown a positive reaction in the cambium cells and in the walls of the first dividing xylem cells, but as soon as a lignification did occur, peroxidase could no longer be detected.

This principle of reciprocal exclusion of lignin reaction and peroxidase activity seems to be universal, especially in structures of the wood[5] and the bark (bast fibres). Investigations here[6] have confirmed the results of the Australian workers. Furthermore, during this work we have found that the bulk of peroxidase is not localized within the cambium area or the differentiating xylem, but in the bark. Moreover, the peroxidase activity detected in the walls of young xylem cells disappears when lignification begins. The lignification of the middle lamella, however, is not the end, but the commencement of the lignification process; the formation of the secondary wall requires further quantities of lignin, which are produced likewise under the action of a redoxase. We were able to demonstrate that the enzyme necessary for this purpose is cytochrome oxidase (indophenol oxidase).

Cross-sections of shoots of 1-year-old *Pinus sylvestris*, *P. strobus*, *Abies alba*, *Acer platanoides*, *Populus nigra*, and *Aesculus hippocastanum* were investigated during the growth period in the second year (March–June). Since cytochrome oxidase is an extremely sensitive enzyme, any fixation and embedding procedures are out of the question; furthermore, one cannot obtain a certain thickness of sections without serious damage to the cell structure and therefore the activity of enzyme. On the other hand, a diffusion of the enzyme is scarcely to be expected, since it is closely bound to cell structures (mitochondria). Hence, we have worked with fresh slices, occasionally also with fresh frozen sections (cold-knife sections). For the histochemical detection of cytochrome oxidase we have utilized the *G*-Nadi reaction according to Moog[7] (0·01 M dimethyl-*p*-phenylene diamine + α-naphthol, *p*H 5·8; post-treatment with potassium acetate).

Both deciduous and coniferous woody species give approximately the same results. In each case, the walls of bark and cambium cells show no activity. The plasm of cambium cells is, if at all, only very weakly coloured. In this case, however, when the middle lamella of the differentiating xylem begins to lignify (detected by the phloroglucinol/hydrochloric acid reaction), a deep violet coloration appears within the cell wall after treatment with the Nadi-reagent. The reaction is so strictly located that with post-treatment of the same section with phloroglucinol/hydrochloric acid only those cell wall parts react which also contain the enzyme. Simultaneously with the lignification of the secondary wall the enzyme appears instantly; the coloured area increases with the thickening of the secondary wall. As soon as the wall

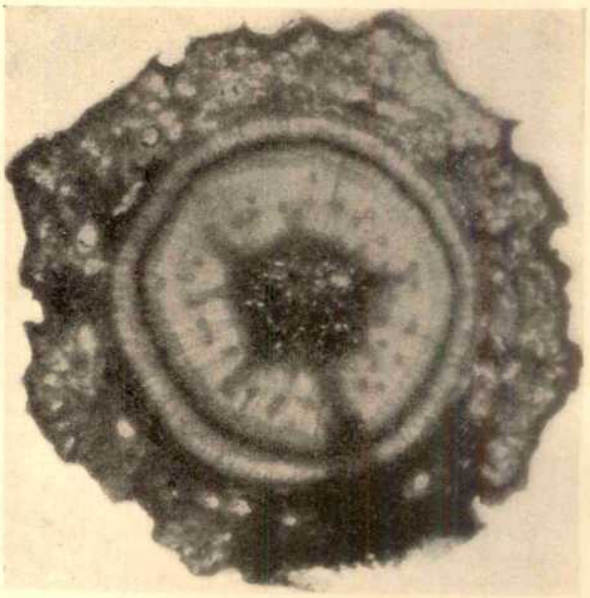


Fig. 1

has reached its full development, the activity disappears from the middle-wall layers, and a short time afterwards also the entire wall is free of enzyme.

According to conditions of growth and species of plant the number of cell series showing in their walls an enzyme activity differs. In each case, the walls of xylem cells, which are formed first and the lignification of which is complete, are already free of any enzyme activity. Fig. 1 shows a cross-section through *Pinus sylvestris*, in which the unbroken circle of indophenol blue in the differentiating xylem can be recognized. The same situation can be observed in the differentiating bast fibres of the bark, which lignify likewise, at least partially. During a certain development stage also here appears an important activity of cytochrome oxidase, which conforms with the lignification process. Tests on woody shoots many years old have shown the same results as such shoots one year old: cytochrome oxidase is found only in the differentiation region of the xylem; all older wood regions react negatively.

From the results of the investigation recorded here, we suggest that the principal redoxase involved in the lignification process of cell walls of higher plants is cytochrome oxidase. In order to establish the place on which the proton of the phenolic hydroxyl entered in the oxido-reduction chain of the succinic oxidase system, further investigations are necessary; presumably cytochrome c is primarily reduced.

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Piricauda Bubak from India

Bubak (1914) recognized *Stigmella* Lev. as a pycnidiaceous, phæophragmosporous fungus and so erected the genus *Piricauda* to accommodate *S. uleana* Sacc., a simple dematiaceous and phæodictyosporous member. Moore[1], working on 'sensu Saccardo', where many forms producing phæophragmo and dictyospores were immixed with simple dematiaceous, pycnidial, sporodochial and acervular forms, separated genera like *Cheiromyces* Berk and Curt., *Dictyosporium* Corda., *Sporidesmium* Link. ex Fr., *Steganosporium* Corda., *Berkleasmium* Zoebel., *Stemphylium* Wallr., and *Piricauda* Bubak, with monacrogenous conidia. Moore's[2] monograph on the genus *Piricauda*, in which 38 species were considered, further establishes and clarifies this.

The genus *Piricauda* is characterized by producing micronemic, dematiaceous colonies, consisting of continuous or separate, simple conidiophores arising from the creeping hyphæ, cutting off phæodictyospores monacrogenously. The conidiophores are sometimes reduced to a single cell or a peg-like structure or entirely absent. During an investigation of Hyphomycetes of Hyderabad a species of *Piricauda* was collected by us and described as new, *Piricauda obclavata*, since it differed from the known ones. This is not only the first record of this fungus from India but also an addition to the known species.

Piricauda obclavata sp. nov. This fungus produces black, pin-head-sized colonies on the substratum. Colonies are micronemic, cushion-shaped with dark brown, septate, sparingly-branched hyphæ. Hyphæ are characteristically

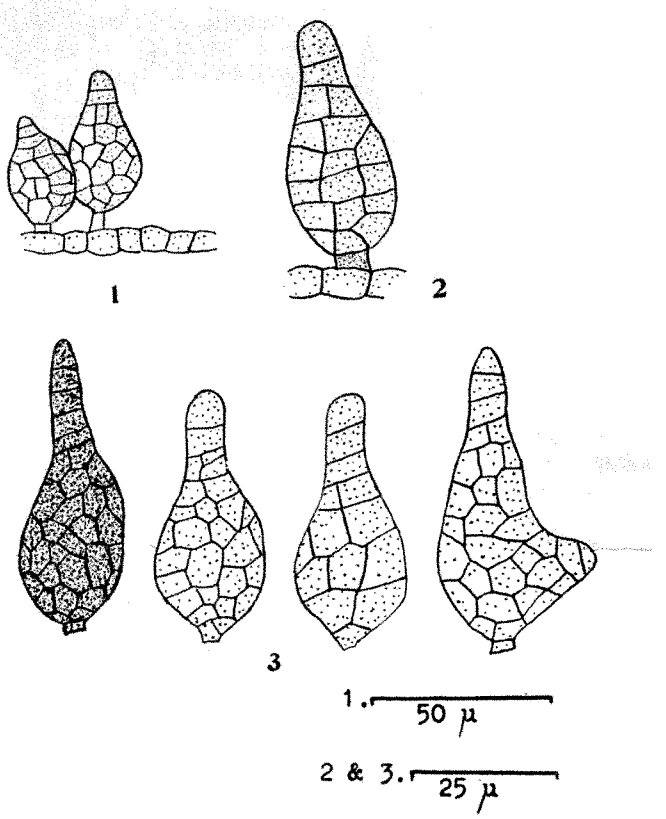


Fig. 1. 1, part of colony; 2, conidiophore and conidium; 3, conidia

attenuated at the septa, thick-walled, 3–7·2μ broad and the septa are 7·2–10·8μ apart. Conidiophores arise singly from the cells of the creeping hyphæ. The conidiophores arise continuous, yellowish-brown, very short, 3–7μ long, 1·5–3·6μ broad, producing conidia monacrogenously. Conidia are dictyosporous, black to blackish brown, opaque when mature, obclavate, 32–47μ long, 14–22μ broad at its widest, with a short or slightly elongate apical beak, 13–35 celled. Rarely are conidia observed with a lateral beak also.

We thank Prof. M. R. Suxena, Department of Botany, Osmania University, and Dr. S. D. Satwalekar, principal, Vivek Vardhini College, Hyderabad, for facilities and advice.

Full diagnosis of *Piricauda obclavata* can be obtained from the author.

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MICROBIOLOGY

Sub-microscopic Structure of Injured Surface of *Paramecium caudatum*

Paramecium caudatum is a very suitable object for observing the regeneration of the surface of Protozoa. The cell surface of *Paramecium caudatum* is composed of pellicula and cilia which are distributed evenly all over the cell surface.

The defect in pellicula of *Paramecium* was made in such a way that the front or back part (about 0·25 of the cell) was removed. The nuclear fragments were fixed with buffered 1 per cent osmium tetroxide (*p*H 7·2) for

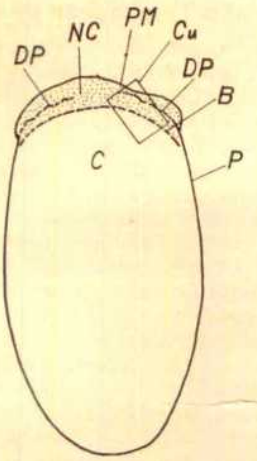


Fig. 1. Diagram of defect in surface of *Paramecium caudatum* 15–30 min after cutting. *P*, Pellicula; *DP*, partly damaged pellicula; *PM*, precipitation membrane; *C*, normal healthy cytoplasm; *NC*, necrotic cytoplasm; *B*, boundary between necrotic cytoplasm and normal cytoplasm; *Cu*, cutting presented in Fig. 2

10 min at different times (1, 2, 5, 7, 15, 30, 60 min, 2, 8, 24 h) after cutting. The fixed fragments were dehydrated and embedded in methacrylate mixture in such a way as to enable us to attain serial ultra-thin sections upright to the regenerating surface. Ultra-thin sections were cut on a Niklowitz ultramicrotome and examined with Tesla *BS* 242 electron microscope. The results were obtained by examining serial ultra-thin sections.

In cross ultra-thin sections (2–5 min after cutting) we can observe the partly damaged pellicula surrounding the wound of the injured end of the fragment. This partly damaged pellicula does not include cilia; but the kinetosomes are present. Towards the centre of the wound only a few remnants of pellicula remained. A small amount of necrotic cytoplasm which poured out when cutting the cell continues to be connected with the wound. The necrotic cytoplasm is coarsely and uniformly granulated and it does not contain any organelles (mitochondria, trichocysts, vacuoles, endoplasmic reticulum) which are present in normal cytoplasm. The surface of the necrotic cytoplasm is covered with a membrane; it is probably the surface precipitation membrane which seems to be connected with the membrana interna pelliculæ. The partly damaged pellicula extends, as a rule, under the remnants of extended necrotic cytoplasm. A small amount of necrotic cytoplasm occurs under the partly injured pellicula, also. After a short period following the cutting (5 min) the transition between the necrotic and the healthy cytoplasm is gradual without any boundary. In sections of fragments regenerating 15–30 min, a distinct boundary between necrotic and healthy cytoplasm is to be seen (Figs. 1 and 2). The boundary is formed by a layer of very fine granulated osmiophilic material, the thickness of which is 300–500 mμ. With the fragments regenerating for 24 h the unbounded pellicula is decreased in size. This decrease is probably brought about by the shifting of the remaining healthy pellicula. The membrane covering the remainder of the defect in pellicula is smooth without a complicated structure of normal surface. The value and further destiny of this boundary are so far unknown.

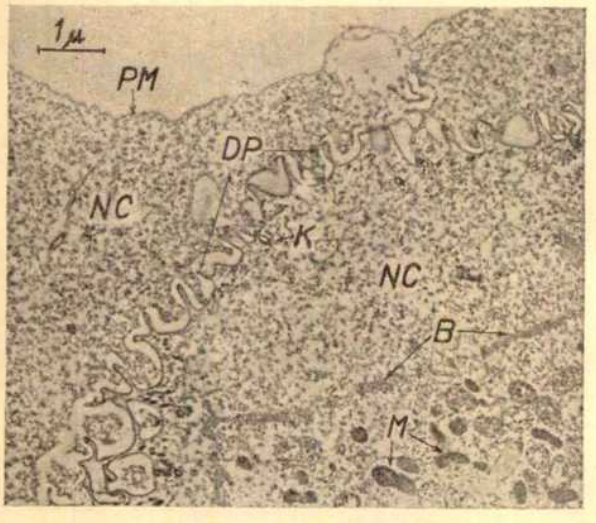


Fig. 2. Ultra-thin section through regenerating surface of fragment of *Paramecium caudatum* 15–30 min after cutting. *K*, Kinetosome; *M*, mitochondria (× *c.* 8,660)

There are also numerous changes in the organelles of the regenerating fragment. The mitochondria in the vicinity of the wound are swollen. The macronucleus of the regenerating fragment is more lobular in comparison with the macronucleus of the normal *Paramecium*. We cannot as yet determine whether all these observed changes are secondary results of changed physical and chemical conditions after cutting, or whether it is an active physiological reaction, which is directly connected with the processes of regeneration.

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A Culture Medium proposed for the Isolation of *Streptococcus faecalis, sensu strictu*

Recently, a medium has been proposed[1] for the specific detection of *Streptococcus faecalis, sensu strictu*, with the implication that this micro-organism resides almost exclusively in the intestinal tract of man. The functional ingredients of the medium depend on the ability of *S. faecalis, sensu strictu*, to ferment sorbitol, utilize tyrosine, and reduce 2,3,5-triphenyltetrazolium chloride (TTC) when incubated in agar at 45° C after a preliminary incubation at 37° C.

Unless one has rather an intimate knowledge of the source of the pollution, the implication that recovery of typical colonies indicates pollution only of human origin appears untenable in the light of recent investigations. The typical *S. faecalis* is not, as has been commonly assumed, a resident exclusively of the human intestinal tract. In all probability the concept came into being during the earlier years of bacteriology when the complexities of the genus *Streptococcus* were appreciated, and when workers concerned with *S. faecalis* were oriented strongly toward medical bacteriology. It and *S. liquefaciens*, which must be considered together with *S. faecalis*, are widespread among truly wild animals in an environment remote from the influence of man[2], and on plants in both truly wild[3] and agricultural environments[4]. Natural populations of several hundred thousand per gram of selected plant material have been noted, and ability of the bacteria to grow with the plant from inoculated seeds has been demonstrated[5]. The bacteria are not uncommon in the intestinal tracts of hogs[6], domesticated mink[7], calves[8], and sheep[9]. Kjellander[10] has also recovered these bacteria from farm animals.

One may question the characteristic described for the medium with ability to select only *S. faecalis, sensu strictu*. Except for the proteolytic enzyme, *S. liquefaciens*, whether isolated from human beings, plants, or animals, is indistinguishable from the non-proteolytic, non-hæmolytic *S. faecalis*. 255 strains isolated from all sources in our laboratory initiated growth vigorously in broth at 45° C

without prior incubation at 37° C, all reduced TTC to produce maroon-coloured colonies, and only 7 cultures failed to ferment manitol. Distinction between strains or species with respect to utilization of tyrosine was not pursued when it was noted that 10 of 47 cultures of the strongly reducing group of enterococci isolated from plants failed to utilize it, and the remaining 37 cultures utilized it in varying degree from slightly to extensively. On Mead's medium, strains of *S. liquefaciens* respond in a fashion identical with that of *S. faecalis, sensu strictu*.

Table 1. Growth and Utilization of Tyrosine on Mead's Agar by Selected Strains of *Enterococci*

Species, source and strain	Ratio of growth, Mead's medium/*MRS*	Clearing around colony	Clearing on streak plates
Human			
H 33 *a S. faecalis*	2·6	Good	Good
H 41 *a S. faecalis*	0·17	Detectable	Good
H 46 *a S. faecalis*	No colonies	—	Good
Animal			
R 76 *b S. liquefaciens*	0·04	Good	Good
R 100 *c S. liquefaciens*	0·16	Good	Good
R 107 *b S. liquefacients*	1·1	Poor unless colonies concentrated	Good
R 26 *a S. faecalis*	No colonies	—	Slight
Plant			
a 70 *S. faecalis*	1·0	Good	Good
137 *a S. liquefaciens*	0·9	None	*
140 *bl S. liquefaciens*	0·01	Poor	*

* No observation.

Other points at issue involve the quantitative aspects of recovery, and the inability of selected strains to produce typical colonies on Mead's medium. From our stocks, 10 cultures, 5 each of *S. faecalis* and *S. liquefaciens* including 3 obtained from human stools, 4 from wild animals, and 3 from plants, were selected at random. After overnight incubation in broth, the cultures were plated serially at 1×10^{-7} and upwards in parallel on the medium of De Man, Rogosa and Sharp (*MRS* medium)[11] and on Mead's medium.

Counts of colonies on *MRS* medium ranged from $1{\cdot}9 \times 10^{7}$ to $6{\cdot}8 \times 10^{8}$. Three cultures, one from each source, produced numbers of colonies equal to the number produced on *MRS* medium, as shown by the ratio Mead's medium/*MRS* in Table 1. Two cultures, one human and one animal in origin, failed to produce colonies at the lowest dilution plated, while the numbers of colonies of the remaining 5 cultures were 1–17 per cent of those on *MRS* medium. The colonies of all cultures which grew were maroon in colour; however, 4 cultures decomposed tyrosine well, as indicated by clear zones around the colonies, 1 culture with a high plate-count ratio produced no clearing, while 4 cultures decomposed tyrosine so slightly that in routine work the clearing would be undetectable. When streaked on Mead's medium, 7 of 8 cultures developed excellent clearing, especially where colonies were concentrated.

From these observations one must conclude that the Mead's medium is not selective for the detection of *S. faecalis, sensu strictu*; that colonies appearing on the plates of the medium could come from sources other than human, and that enterococci originating from the human may neither be detected nor produce typical colonies.

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Action of D-Tryptophan on *E. coli*

DL-Amino-acids as substitutes for L isomers are often used in culture media of the microbial organisms and mammalian cells. The common absence of growth inhibition by using DL-amino-acids in the culture media led to the notion that D isomers are nutritionally inert. While this remains generally true in the sense of the culture of wild-type strain of micro-organisms, it is now apparent in our report that D-amino-acid even in the low concentration of 10^{-5} M inhibits the growth of the L-isomer-requiring mutant. Although several D-amino-acids have been found to inhibit cell division and growth[1–3], the effect of D-tryptophan for cell growth has never been reported except that the high concentration of DL-tryptophan ($6{\cdot}8 \times 10^{-3}$ M) caused formation of extremely long cells of *Erwinia* after 16 h incubation and that higher levels of the D-amino-acid resulted in growth inhibition[1]. This paper presents evidence that D-tryptophan in low concentration (10^{-5} M) was found to inhibit the growth of an L-tryptophan-requiring mutant of the *E. coli*, and the inhibition of the active transport is one of the bases of the growth inhibitory action of D-tryptophan on *E. coli*.

The mutant of *E. coli K*-12 (Yale) used in this report is the tryptophan auxotroph, *B*-8, which forms normal *A* component and trace amount of extremely labile *B* component of tryptophan synthetase[4]. Bacteria were grown in 5–10 ml. of the minimal medium supplemented with 0·2 per cent of glucose and 5 μg of L-tryptophan per ml.[5]. D-Tryptophan (2·5–20 μg per ml.) added to the medium inhibited cell growth of *B*-8 strain (Table 1).

Table 1. Growth Inhibition of *B*-8 Cells by D-Tryptophan

Incubation (h)	Addition of D-tryptophan μg/ml. 0	2·5	5·0	10·0	20·0
	Klett unit*				
9	115	74	72	64	64
12	205	123	115	86	73
15	220	173	162	113	89

Cells were grown for 9–15 h in the minimal medium supplemented with 5 μg of L-tryptophan/ml. in the presence of D-tryptophan with shaking at 37°
* The turbidity was measured with a Klett–Summerson colorimeter with filter 54 and was expressed as Klett unit.

The time course of growth of *B*-8 which was markedly inhibited in the presence of D-tryptophan is shown in Fig. 1.

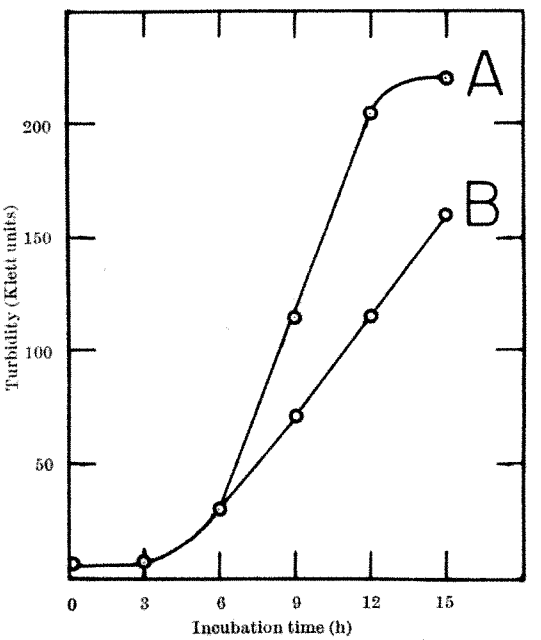


Fig. 1. Growth curve of *B*-8 cells in the presence or absence of D-tryptophan. The experimental details are the same as those described in Table 1. Curve *A* shows the growth of *B*-8 cells in the absence of D-tryptophan and curve *B* shows the one in the presence of 5 μg of D-tryptophan per ml. Both contain 5 μg of L-tryptophan/ml. and 0·2 per cent of glucose on the minimal medium

Table 2. EFFECT OF D-TRYPTOPHAN ON RADIOACTIVE AMINO-ACID UPTAKE

Radio-active amino-acid*	Control c.p.m.	D-Tryptophan (50 μg/ml.) c.p.m.
L-tryptophan-^{14}C	321	180
L-proline-^{14}C	1,094	1,080
L-valine-^{14}C	1,185	1,053

The reaction mixture contained per ml.: 50 μg of dry weight of cells; 40 μg of chloramphenicol; 17 μmoles $MgCl_2$; 10 μmoles sodium formate; 10 μg L-amino-acid-^{14}C (10^4 c.p.m./μg); 25 μmoles *tris*; final *p*H 8·0[7]. After incubation for 1 min at 35° each reaction mixture was filtered on 'Millipore' filter *HA*. The filter was washed with 10 ml. of 0·9 per cent sodium chloride, dried and counted[7].

*All amino-acids; L-tryptophan, benzene ring uniformly labelled, prepared enzymatically[6]; L-proline, uniformly labelled; L-valine uniformly labelled; were adjusted to 10,000 c.p.m./μg with addition of carrier ^{12}C-amino-acid.

Even at the highest level employed (100 μg/ml.) growth of the *B*-8 cells was not completely inhibited by D-tryptophan. Morphologically, no shortening or elongation of the cells was observed in the concentration range of D-tryptophan (2·5–100 μg/ml.). The fact that *B*-8, a tryptophan auxotroph, is unable to grow in a minimal medium containing D-tryptophan (5–100 μg/ml.) indicates that D-tryptophan cannot substitute L-tryptophan for the growth of the auxotroph. The possibility was examined that D-tryptophan may inhibit bacterial growth by interfering with the uptake of L-tryptophan. To determine the effect of D-tryptophan on the uptake of L-tryptophan by the cells, washed bacteria were incubated in the minimal medium containing ^{14}C-L-tryptophan at 35°. In the presence of D-tryptophan uptake of radioactivity into the cells was significantly reduced (Table 2). Uptake of L-proline-^{14}C or L-valine-^{14}C was little affected in the presence of D-tryptophan. In the intracellular site, activation of L-tryptophan (L-tryptophanyl–adenosine monophosphate) and synthesis of L-tryptophanyl–RNA, which participate on protein synthesis, were not influenced in the presence of D-tryptophan[6].

A tryptophan transport system in *E. coli* reported by Boesi and DeMoss[7] and our findings that D-tryptophan inhibited uptake of L-tryptophan into a tryptophan auxotroph of *E. coli* suggest that a mechanism of the growth inhibition of a tryptophan-auxotroph, B-8, by D-tryptophan is the disturbance of L-tryptophan transport into the cells.

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Transformation of *Leptospira*

THE well-known pioneer experiments of Griffith[1], in 1928, proved the phenomenon of transformation in the *Pneumococcus*; the genetic characteristic transmitted was the virulence, of which the elaboration of the capsule is the phenotypic expression. Avery, MacLeod and MacCarthy[2] elucidated the cause: the genetic material, freed in solution, is sensitive to deoxyribonuclease; it is thus DNA. This pattern of 'transfer' has since been observed in other bacteria.

From our investigation on recombinations through transformation in *Leptospira* we used a strain of *Leptospira icterohaemorrhagiae* isolated in 1958 from a rat (*Rattus norvegicus*) captured in a coal-mine; the strain was named 'Cheratte' after its place of origin in the region of Liège. Cultivated in our laboratory, it has, as usual, quickly lost its pathogenic power.

Starting in February 1963 from the twelfth sub-culture, we succeeded through serial passages on guinea-pigs in restoring its virulence: it kills the animal within 6–8 days, showing the usual ictero-hæmorrhagic picture. The mixture of 1 ml. of virulent culture disrupted by successive freezing and thawing and 1 ml. of an initially non-virulent living culture has twice, in May 1963 and July 1964, caused the death of the guinea-pig together with icterus, numerous hæmorrhages and the presence in the organs of leptospires which can be re-inoculated in series. All these experiments were conducted with the necessary control experiments.

Besides this homotransformation, we succeeded in heterotransformation. The transfer principle came from the 'Cheratte' strain made virulent; the receiving microorganism was the 'Kantorowicz' strain, belonging to the serotype of group *Icterohaemorrhagiae*, for a long time designated as *A* or incomplete. This strain, isolated in 1931 at Amsterdam from the blood of a patient, lost its pathogenic power many years ago.

During the same experiment which allowed us, in July 1964, to succeed for the second time in our homotransformation, 1 ml. of the live culture of 'Kantorowicz', mixed with the same quantity of the killed culture of virulent 'Cheratte', resulted in the death of the guinea-pig on the eleventh day, with hæmorrhagic icterus. The 'Kantorowicz' strain thus made virulent remained so during several passages.

This work was supported by a research grant of the Fonds National de la Recherche Scientifique, Belgium.

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CYTOLOGY

Distribution of Phthalocyanine Dye (Heliogen Blue *SBL*) in Ultrastructures of Vital Dyed Axons of the Crab *Carcinus maenas*

VITAL molecular disperse dyes are widely used in the investigation of permeability mechanisms and structural chemical changes in the cell[1]. The possibility of revealing such substances by means of the electron microscope was suggested by Isenberg[2] and later confirmed experimentally on preparations stained after the fixation[3,4]. Using an electron microscope Barrnett[5] revealed the azodye vitally formed in the cell as a result of a certain histochemical reaction.

The distribution of the acid substantive dye, heliogen blue *SBL* (HB) containing one copper atom, has been investigated with the aid of the electron microscope in vital-dyed axons of the crab, *Carcinus maenas*[6,7]. HB as observed on the film or in ultra-thin sections is characterized by a high electron density.

Axons were stained with 2 per cent HB solution diluted in artificial sea water without bivalent cations at *p*H 6·8 (phosphate buffer) for 2 h at 18°–20° C. Under such conditions diffusion equilibrium between the axon and the dye solution was reached in 1 h. The action potential of the axons in 2 per cent HB solution persisted for 3 days. Quantitative measurements of HB in crab's nerves prove that a half of the dye was dissolved or weakly bound, whereas the remaining portion was bound more strongly (Table 1).

For the electron-microscope investigation the stained and unstained axons were fixed in 2 per cent isotonic



Fig. 1. Part of cross-section of two adjacent axons of the crab. Unstained axons were fixed in OsO_4 (×35,000)



Fig. 2. Part of cross-section of axon stained in 2 per cent HB. Membranes of the sheath are characterized by increased electron density. The arrows indicate HB accumulations in axolemma (*ax*). Collagen fibres (*c*) show increased electron density (×30,000)

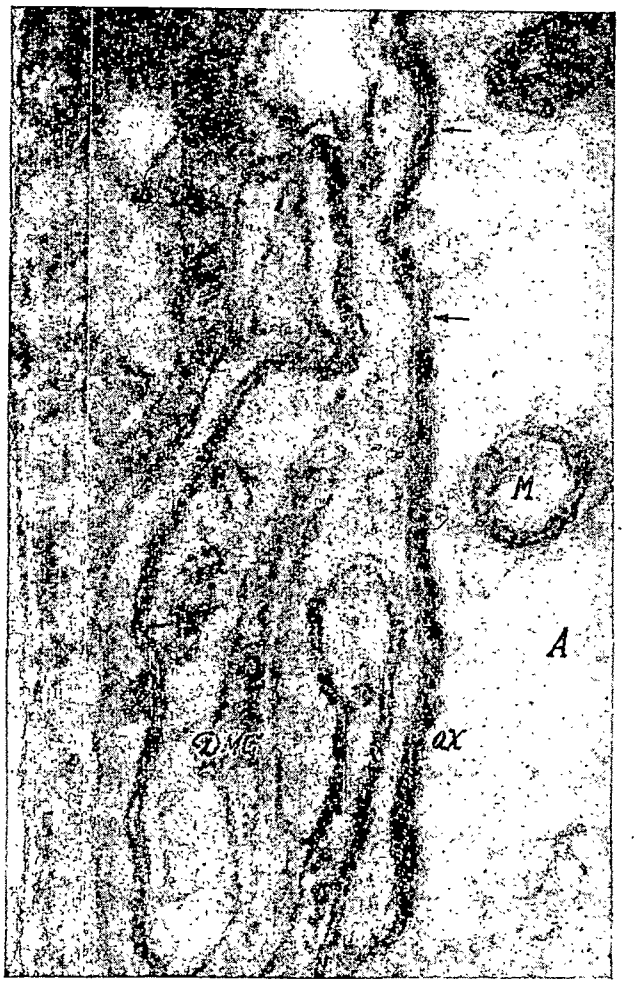


Fig. 3. Part of cross-section of axon stained in 2 per cent HB. As in Fig. 2 one can observe an increase in electron optical contrast of membrane structures of the axon sheath. The dye is localized in axolemma (*ax*) chiefly as discrete granules. Some mitochondrial membranes (*M*) in axoplasm (*A*) have increased electron density. Local dilation of mesaxon gap (*DMG*). The arrows indicate sites with triple-layered structure of axolemma. Some parts of the middle layer of axolemma exhibit increased electron density (×100,000)

solution of osmium tetroxide buffered with veronal buffer at *p*H 6·8, then dehydrated in ethanol and embedded in 'Araldite'. The analysis of ultra-thin sections of the stained axons has shown that HB is revealed in the membrane structures of fibre sheaths. The dye causes the latter to stand out in contrast as compared with the control object (Figs. 1 and 2). In some cases areas of increased electron density were found in axoplasm on mitochondrial membranes (Fig. 3). Moreover, HB is intensely bound in collagen fibres (Fig. 2).

The dye has a negatively-charged chromophore group and is not soluble in hydrophobic solvents. It would be only natural to suggest that the dye is bound by protein components of the membrane and not by phospholipides. This is in agreement with the facts of the acid dye binding by proteins[8]. In the region of axolemma wherein its triple-layered character is evident the dye is localized in external and probably protein layers (Fig. 3). This fact seems to be an indirect confirmation of our suggestion.

In some areas of axolemma there are accumulations of HB in the shape of granules which fill up the whole

Table 1. Amount of HB in the Nerves of the Crab's Leg when Stained with 2 per cent HB

Extracting fluid	HB concentration as percentage of net weight
Artificial sea water (*p*H 6·8)	3·2 ± 0·03
70 per cent C_2H_5OH (*p*H 12·8)	1·51 ± 0·1
2 M NaOH	1·63 ± 0·17

axolemma and vary in size from 50 Å to 400 Å (Fig. 3). These accumulations of the dye were observed only in some regions of axolemma. Such uneven distribution of the dye indicates structural heterogeneity of axolemma, unlike other cytoplasmic membranes. All the mesaxones in the investigated giant axones exhibit a winding character, their length varying from 4μ to 10μ. The width of gaps in mesaxons and axon-swann membranes is about 100–150 Å (Fig. 3). Sometimes in stained axons one sees mesaxon gaps widened to 500 Å or cavities with the maximal size about 0·7μ (Fig. 3). It is most probable that HB reaches axolemma through the mesaxon and axon-swann membrane gaps. This suggestion is based on the fact that the time of diffusion through a 150 Å gap (10μ long) is 10 times less than that required for attaining diffusion equilibrium between axons and the media. The absence of accumulations of electron dense dye in mesaxon gaps may be due to the washing out of the dye during fixation and dehydration of the object. Another possible way for the dye to reach axolemma is through plasmic membranes of the swann cell. However, no evidence was found to indicate the penetration of HB through these membranes.

Electron-dense granules in axolemma sometimes exceed the bounds of the latter and penetrate axoplasm (Fig. 3). It is possible that these regions of axolemma can be regarded as sites for HB influx in axoplasm.

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VIROLOGY

Resistance of Synthesis of Influenza Virus Ribonucleic Acid to the Action of Actinomycin *D*

It has been found that replication of some myxoviruses (Sendai and NDV) was stimulated by actinomycin *D* (refs. 1 and 2) but in influenza and fowl plague viruses multiplication was inhibited by this antibiotic[3–5]. Based on actinomycin-induced inhibition of influenza hæmagglutinin production, Barry *et al.*[3] concluded that influenza virus RNA synthesis, unlike NDV, requires participation of cell DNA.

To decide whether actinomycin *D* prevents influenza and fowl plague RNA synthesis rate of ^{14}C-cytidine incorporation into strips of chorioallantoic membranes (CAM) and chicken fibroblasts was determined and autoradiographic investigation was carried out.

Four replicate strips cut from 11-day-old chick embryos and infected with a saturating dose of *PR*8 and Weybridge strain of fowl plague virus were used for each concentration of actinomycin (2, 4 and 8 μg/ml.). Chicken fibroblasts grown for 3 days in Hanks's salt solution containing 0·5 per cent lactalbumin hydrolysate and 2 per cent calf serum were infected with 10 ID_{50} p.c. of fowl plague virus; 0·3 μg/ml. of actinomycin *D* was used. Labelling was carried out with 2 μc./ml. of ^{14}C-cytidine added every 2 h to strips containing Hanks's solution and every hour to chicken fibroblasts. Strips and trypsinized cells were washed twice with cold phosphate-buffered solution, twice with cold 5 per cent trichloroacetic acid and twice with 96 per cent alcohol and once with ether. The results of three identical experiments (*A*) and a typical experiment (*B*) expressed as percentage of controls are shown in Fig. 1.

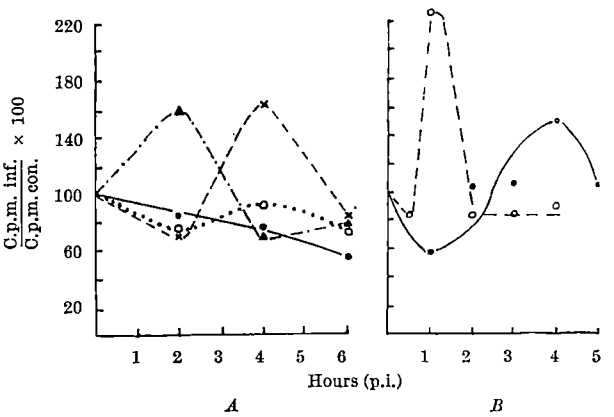


Fig. 1. Rate of ^{14}C-cytidine incorporation into *PR*8 virus infected CAM strips (*A*) and fowl plague infected chicken fibroblasts (*B*). ●, Cells without actinomycin *D*; ○, CAM strips treated with 2 μg/ml. of actinomycin *D*; ×, CAM strips treated with 4 μg/ml. of actinomycin *D*; △, CAM strips treated with 8 μg/ml. of actinomycin *D*; ○, cells treated with 0·3 μg/ml. of actinomycin *D*

In autoradiographic experiments *HEp*-2 cells were infected with a saturating dose of *PR*8 virus; 0·5 μg/ml. of actinomycin *D* was added just after infection. Every hour after onset of incubation samples of cultures were exposed to 1 μc./ml. of tritiated uridine for 30 min, washed with unlabelled uridine and fixed. The autoradiographic technique used has already been described[6].

All incorporated radioactivity in the control cells was localized in the cell nuclei with heavy labelling of nucleoli. Actinomycin *D* completely inhibited incorporation of tritiated uridine into nuclei of uninfected cells at all times of observation. No differences were observed in grain localization between control cells and infected cells for the first 2 h after infection. 3 h after infection, and later, labelling of nucleoli was inhibited (Table 1 and Fig. 2). Thus redistribution of grains was the only indication of *PR*8 virus infection; decrease in number of grains was in accordance with radioactivity measured (Fig. 1*A*).

Table 1. Incorporation of Tritiated Uridine into *HEp*-2 Cells Infected with *PR*8 Virus (Middle Number of Grains p.c.)

Intervals after infection (h)	Experimental: Control cells *N*	Control cells *n*	Actinomycin *N*	Actinomycin *n*	Infected cells *N*	Infected cells *n*	Actinomycin-treated infected cells *N*	Actinomycin-treated infected cells *n*
1	42±2·2	26±1·7	0	0	46±2·8	22±1·6	0	0
2	55±6·7	36±6·0	0	0	50±2·8	28±2·0	0	0
3	37±2·1	18±0·6	0	0	30±2·3	6±0·9	22±2·6	0
4	—	—	0	0	35±2·6	9±1·0	27±2·6	0
5	53±6·2	33±3·2	0	0	50±3·5	4±0·3	0	0

N, nucleus excluding nucleolus; *n*, nucleolus

There was no labelling of nuclei the first hour after infection of actinomycin-treated cells ; 2 h after infection single cells with labelled nuclei were seen ; 3 and 4 h after infection labelling of nuclei in most actinomycin-treated cells was observed. Grains were distributed over the whole nuclear area without any concentration in nucleoli (Fig. 2). Incorporation was absent at 5 h.

It may be concluded that actinomycin *D* does not prevent the synthesis of RNA of *PR*8 and fowl plague viruses, and, moreover, RNA synthesis starts earlier as well as with Sendai virus[1]. The replication of myxovirus RNA seems to proceed independently of cell DNA function. The inhibition of *HA* and *CF* antigen production of influenza and fowl plague viruses was observed when chicken fibroblasts were treated with actinomycin *D* no later than 2 h after infection and strips of chorioallantoic membranes no later than 3 h. In spite of 10–100 fold decrease in *HA* titre in actinomycin-treated

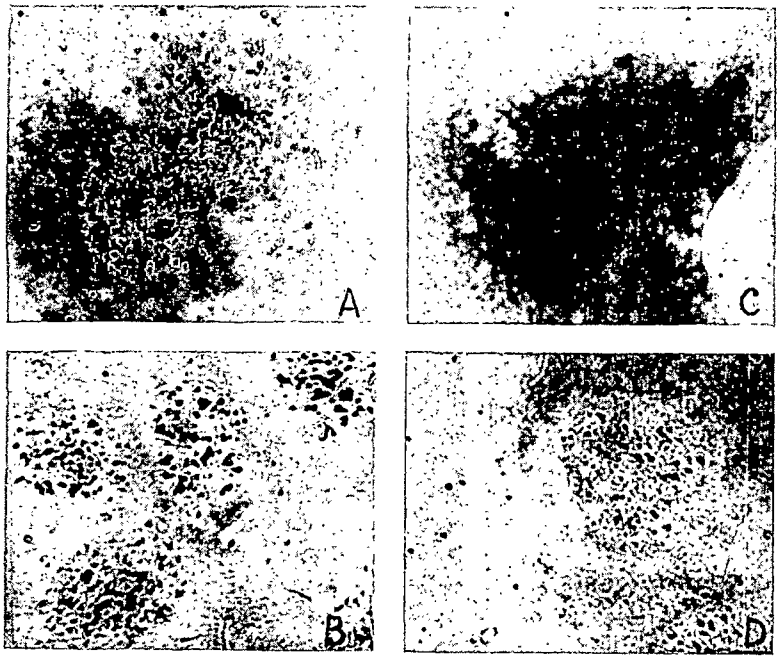


Fig. 2. *HEp*-2 cells: autoradiographs. Unstained (× 100). *A*, control cells; *B*, 3 h after *PR*8 virus infection; *C*, 1 h after actinomycin action, typical 'rosette' around nucleoli; *D*, actinomycin-treated cells 3 h after infection

chicken fibroblasts and chorioallantoic membrane strips, EID_{50} of *PR*8 virus as well as influenza viruses *A*/Asia/59, *B*/*Lih*/59 and *B*/95/61 was the same as in control cells. EID_{50} of fowl plague virus was lowered in accordance with *HA* and *CF* antigen inhibition.

Thus actinomycin *D* seems to interfere with a cystron of a new synthesized viral RNA molecule which is responsible for viral proteins production; other explanations are not excluded.

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VETERINARY SCIENCE

Levels of Coenzyme *A* in the Liver of Normal Cows and of Cows suffering from Primary Ketosis

Highly significant changes have been shown in the levels of four members of the citric acid cycle (pyruvate, citrate, α-oxoglutarate and succinate) in the serum of cows suffering from primary ketosis[1]. It was suggested that there may be interference with the reactions of the citric acid cycle, involving the conversion of pyruvate and α-oxoglutarate to citrate and succinate respectively. Furthermore, it was suggested as a working hypothesis that the cause of such an interference may be a deficiency of a co-factor or co-factors common to both oxidative decarboxylation reactions, such as coenzyme *A*. During the winter of 1962–63 coenzyme *A* levels were estimated in the livers of 7 cows suffering from spontaneous primary ketosis and compared with the corresponding levels in the livers of 7 normal cows at the same stage of lactation.

Cores of liver tissue weighing 500–800 mg were removed by the biopsy technique described by Loosemore and Allcroft[2]. Immediately after removal, the liver core was placed on filter paper to remove surface moisture, then dropped into a test tube standing in a bath of acetone and solid carbon dioxide. Protein was estimated on 100 mg of the frozen tissue by the method of Lowry, Roseborough, Farr and Randall[3], while the rest of the liver core was transferred to a boiling tube containing 5 ml. water standing in a boiling water bath. The liver was boiled for 5 min, cooled, then homogenized in a Potter–Elvehjem homogenizer. The homogenate was made up to a known volume and centrifuged for 20 min at 20,000*g* in a Spinco model *L* ultracentrifuge. The coenzyme *A* content of the supernatant was estimated using a method based on the investigations of Stadtman, Novelli and Lipmann[4], who showed that coenzyme *A* catalyses the interchange of acetyl bound and inorganic phosphate, and the arsenolysis of acetyl phosphate by a phosphotransacetylase enzyme system. The procedure adopted has been described in detail by Reece[5].

It may be seen from Table 1 that there is a highly significant depression of the protein level of the ketosed liver. Therefore, although the level of coenzyme *A* (expressed as coenzyme *A*/g tissue) in the liver of the ketosed cow is lower than that found in the normal animal, no significant difference exists between the two groups when the coenzyme *A* concentration is expressed in terms of the protein content of the normal and ketosed tissue.

Table 1. Levels of Coenzyme *A* and Protein in the Liver of Normal Cows and of Cows suffering from Primary Ketosis

	Normal cases (7) mean ± *S.E.M.*	Pathological cases (7) mean ± *S.E.M.*	*P**
Coenzyme *A* : *LU*†/g tissue	94·6 ± 10·2	59·2 ± 14·8	0·1
Protein: mg/g tissue	185·0 ± 17·6	92·2 ± 8·5	0·001
Coenzyme *A*: *LU*†/100 mg protein	51·5 ± 5·2	62·0 ± 11·5	0·5

* Probabilities calculated by the *t* test (ref. 10).
† Lipmann units.
No. of liver samples estimated given in brackets.

In view of these findings in liver tissue, the hypothesis that the interference in the oxidative decarboxylation of pyruvate and α-oxoglutarate is due primarily to a deficiency of coenzyme *A* appears to be unlikely. It has been suggested[6,7] that this interference may be associated with other factors, such as deranged levels of the pyridine nucleotides, and other plausible arguments have been put forward[8]. However, it must be remembered that even in the normal cow there is a heavy drain on the coenzyme *A* reserves during the first 3 months of lactation due to the excretion of the co-factor in the milk[9].

Until the levels of all the co-factors relevant to oxidative decarboxylation can be thoroughly investigated in the tissues of the ketosed animal, it will be difficult to explain the accumulation of pyruvate and α-oxoglutarate in the blood of cattle suffering from a starvation or a spontaneous primary ketosis.

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, October 12

INSTITUTION OF AGRICULTURAL ENGINEERS (at the Institution of Mechanical Engineers, 1 Birdcage Walk, London, S.W.1), at 10.15 a.m.—Mr. B. M. Camm: "Some Aspects of the Economic Utilization of Farm Machinery"; Mr. H. G. Pryor: "The Development and Application of High Power Units in Agriculture"; Mr. J. D. Savage: "Significant Features of Fuels and Lubricants for the Winter Use of Diesel-Engined Tractors".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. D. E. N. Davies: "Application of Electronic Vector Scanning Techniques Applied to Height-Finding Landing Systems"; Miss E. Killick: "Frequency Scanning Aerial Systems".

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. John Szentágothai (Medical University of Budapest): "The Neuronal Circuits of the Cerebellar Cortex".*

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 6 p.m.—"The Living Pattern" (Colour Films).

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (joint meeting with the Ewell County Technical College Faraday Society, at the Ewell County Technical College, Reigate Road, Ewell, Surrey), at 7 p.m.—Mr. A. D. S. Tantrum: "Energy Production".

Tuesday, October 13

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, Westminster, London, S.W.1), at 5.30 p.m.—Mr. C. M. Johnson and Mr. J. J. Gandy: "Construction of the Maiduguri-Bama Road, Northern Nigeria".

INSTITUTION OF ELECTRICAL ENGINEERS, SCIENCE AND GENERAL DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Prof. M. W. Humphrey Davies: "The Design and Control Revolution" (Chairman's Address).

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 5.30 p.m.—Prof. R. King: "Metals". (Afternoon lectures for Sixth Form Boys and Girls from Schools in London and the Home Counties. Further lectures on October 14, 20 and 21.)

INSTITUTION OF MECHANICAL ENGINEERS, STEAM PLANT GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Problems Involved in the Estimation of Steam Purity".

ROYAL AERONAUTICAL SOCIETY, ASTRONAUTICS AND GUIDED FLIGHT SECTION (at 4 Hamilton Place, London, W.1), at 6 p.m.—Monsieur R. Chevalier: "Some Aspects of Space Launcher Development Problems in France".

Tuesday, October 13—Wednesday, October 14

INSTITUTION OF MECHANICAL ENGINEERS (at the Royal Naval Engineering College, Manadon, Plymouth)—Conference on "Engineering in the Royal Navy".

Wednesday, October 14

SOCIETY OF ENVIRONMENTAL ENGINEERS (in the Mechanical Engineering Department, Imperial College of Science and Technology, London, S.W.7), at 6 p.m.—Mr. B. E. Grant: "Some Electronic Systems Applied to Environmental Problems".

INSTITUTE OF INFORMATION SCIENTISTS (at the Swedenborg Society Hall, 20 Bloomsbury Way, London, W.C.1), at 6.15 p.m.—Mr. H. F. Vessey: "Mechanization of Information Services in T.I.L., at the Ministry of Aviation".

INSTITUTION OF ENGINEERING INSPECTION (at the South-West Essex Technical College, Forest Road, Walthamstow, London, E.17), at 7.30 p.m.—Mr. J. Davey: "Testing Motor Cars for Quality and Reliability".

ROYAL AERONAUTICAL SOCIETY, GRADUATES' AND STUDENTS' SECTION (at 4 Hamilton Place, London, W.1), at 7.30 p.m.—Mr. B. S. Leete: "Commonwealth Air Routes Development".

Thursday, October 15

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p.m.—Prof. H. Grüneberg: "Genetical Research in an Area of High Natural Radioactivity in South India".*

INSTITUTION OF MINING AND METALLURGY (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—General Meeting.

INSTITUTION OF CIVIL ENGINEERS, JOINT BUILDING GROUP (at Great George Street, Westminster, London, S.W.1), at 5.30 p.m.—Informal Discussion on "Problems of Professional Collaboration in the Building Industry" introduced by Mr. W. A. Allen and Mr. C. E. P. Wooster.

CHEMICAL SOCIETY (in the Lecture Theatre, School of Pharmacy, 29–39 Brunswick Square, London, W.C.1), at 6 p.m.—Dr. A. D. Buckingham: "Experiments with Oriented Molecules" (Tilden Lecture).

ROYAL AERONAUTICAL SOCIETY (at 4 Hamilton Place, London, W.1), at 6 p.m.—Mr. J. T. Dyment: "Tools of Airline Management" (Twentieth British Commonwealth Lecture).

SOCIETY OF CHEMICAL INDUSTRY, ROAD AND BUILDING MATERIALS GROUP (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Dr. C. A. Chaplin: "The L.C.C. and Building Materials".

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (at Manson House, 26 Portland Place, London, W.1), at 7.30 p.m.—Dr. P. D. Marsden: "A Longitudinal Study of the Health of Gambian Children from Birth to the Age of Eighteen Months".

Friday, October 16

ASSOCIATION OF APPLIED BIOLOGISTS (in the Lecture Hall of the British Museum (Natural History), Cromwell Road, London, S.W.7), at 10.50 a.m.—Symposium on "The Problem of Persistent Insecticides in British Agriculture".

TELEVISION SOCIETY (at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 7 p.m.—Mr. H. Steele: "Transcoding from SECAM to N.T.S.C.".

Saturday, October 17

LONDON COUNTY COUNCIL (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Miss Alice G. C. Grandison. "Snakes, Lizards and Frogs of Nigeria".*

Monday, October 19

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Dr. J. L. Garraway: "Studies on Certain Organo-Sulphur Compounds as Fungicides".

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (at Enfield Technical College, Queensway, Ponders End, Enfield, Middlesex), at 7 p.m.—Dr. T. R. E. Kressman: "Ion Exchange".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.15 p.m.—Mr. Eric E. Shipton, C.B.E.: "Over the North Patagonian Icecap".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURER/ASSISTANT LECTURER IN MATHEMATICS IN THE DEPARTMENT OF SCIENCE AND MATHEMATICS—The Principal, Rugby College of Engineering Technology, Eastlands, Rugby (October 14).

LECTURERS (3) (with good academic qualifications in physics, preferably including a higher degree) IN PHYSICS, for duties in the University Department of Surgery at the Royal Infirmary, Practice of Medicine at the Western Infirmary and Surgery at the Western Infirmary—The Secretary of University Court, The University, Glasgow (October 15).

ASSISTANT LECTURER (with a special interest in experimental psychology) IN PSYCHOLOGY—The Registrar, The University, Manchester, 13 (October 16).

PRINCIPAL LECTURERS (graduates with suitable teaching, research and/or industrial experience) at West Ham College of Technology, to teach the following subjects to degree students: civil engineering, mechanical engineering, electrical engineering, chemical engineering, organic chemistry and physics—Chief Education Officer, Education Department, 95 The Grove, Stratford, London, E.15 (October 19).

VICE-PRINCIPAL (with high academic qualifications, appropriate teaching experience at degree or diploma in technology level, and some administrative experience, preferably as Head of a Department) of West Ham College of Technology—Chief Education Officer, Education Department, 95 The Grove, Stratford, London, E.15 (October 19).

LIBRARIAN and an ASSISTANT LIBRARIAN (Fellows of the Library Association) at the University College of Science Education, Cape Coast, Ghana—The Assistant Registrar (Overseas), University College of Science Education, 15 Gordon Square, London, W.C.1 (October 20).

SENIOR LECTURER or LECTURER IN VETERINARY SURGERY—The Secretary of University Court, The University, Glasgow (October 25).

SENIOR LECTURER or LECTURER IN ORGANIC CHEMISTRY at Makerere University College, Uganda—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (October 28).

ASSISTANT LIBRARIAN (chartered librarian preferably with some experience in a university or technical college library)—The Clerk to the Governors, Woolwich Polytechnic, London, S.E.18 (October 30).

LECTURER or ASSISTANT LECTURER (preferably specializing in algebra) IN MATHEMATICS at the University of Basutoland, Bechuanaland Protectorate and Swaziland—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (October 30).

SENIOR LECTURER (honours graduate with postgraduate qualifications and research experience in the physiology and genetics of animal breeding) IN ANIMAL BREEDING IN THE DEPARTMENT OF VETERINARY ANIMAL HUSBANDRY, University of Queensland, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, October 30).

ASSISTANT LECTURER IN THE DEPARTMENT OF PSYCHOLOGY—The Registrar, University College of North Wales, Bangor, North Wales (October 31).

CHAIR OF CHEMICAL ENGINEERING at the University of Canterbury, Christchurch, New Zealand—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, October 31).

CHAIR OF PATHOLOGICAL BIOCHEMISTRY—The Secretary of University Court, The University, Glasgow (October 31).

LECTURER (preferably with a Ph.D. or equivalent experience in metallurgy) IN METALLURGY—The Registrar, The University, Sheffield (October 31).

PROFESSOR OF AGRICULTURAL ENGINEERING—The Registrar (Room 22, O.R.B.), The University, Reading (October 31).

BIOCHEMIST or CHEMIST (Scientific Officer grade), initially to work on the action of organo-phosphorus insecticides and causes of resistance—The Secretary, Rothamsted Experimental Station, Harpenden, Hertfordshire (November 1).

CANCER BIOLOGIST (trained in zoology, veterinary science or medicine, and preferably some experience in the maintenance of experimental tumours in mammals, in the pathology of tumours, and in animal tissue culture techniques) IN THE DEPARTMENT OF MICROBIOLOGY, University of Auckland, New Zealand—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, November 2).

ELECTRON MICROSCOPIST (with up-to-date experience in specimen preparation techniques for biological materials) to take charge of electron microscopy in the Department of Microbiology, University of Auckland, New Zealand—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1, New Zealand and London, November 2).

LECTURER (with special qualifications and research interest in some branch of physical geography) IN GEOGRAPHY; a LECTURER (with postgraduate qualifications and research interests in experimental psychology) IN EXPERIMENTAL PSYCHOLOGY; and a LECTURER (with qualifications in entomology, and preferably research experience in ecology) IN ZOOLOGY at the University of Auckland, New Zealand—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, November 2).

LECTURER IN PURE MATHEMATICS at the University of Waikato, Hamilton, New Zealand—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, November 3).

LECTURER, Grade II (preferably with a medical qualification) IN PHARMACOLOGY—The Registrar, The University, Bristol (November 6).

POST-DOCTORAL RESEARCH ASSISTANTS (2) (biochemists or chemists) IN THE DEPARTMENT OF BIOCHEMISTRY, to undertake research in the general

field of sulphate metabolism—Prof. K. S. Dodgson, Department of Biochemistry, University College of South Wales and Monmouthshire, St. Andrew's Place, Cardiff (November 10).

LECTURER (with an honours degree in psychology and preferably a higher degree) IN PSYCHOLOGY at the University of Queensland, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, November 13).

LECTURER IN PHYSICAL CHEMISTRY (Durban); and a LECTURER IN INORGANIC CHEMISTRY (Pietermaritzburg), University of Natal, South Africa—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, November 14).

LECTURER OR JUNIOR LECTURER IN THE BIOCHEMISTRY SECTION OF THE DEPARTMENT OF BOTANY, University of the Witwatersrand, Johannesburg, South Africa—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, November 14).

ANALYTICAL CHEMIST (with experience in analytical chemistry, preferably on silicate materials) IN THE DEPARTMENT OF GEOPHYSICS, for work involving the chemical analysis of silicate materials, rocks, meteorites and minerals, both for major and trace element concentrations—The Bursar, Australian National University, Box 4, G.P.O., Canberra, Australia (November 30).

LECTURER (with a mathematical or physical background, preferably at doctoral level, and capable of teaching meteorology to postgraduates) in the field of meteorology IN THE DEPARTMENT OF GEOGRAPHY, University of Malaya—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Malaya and London, November 30).

ASSISTANT DIRECTOR (with an honours degree in botany, a sound knowledge of two or more modern languages, ability to write good English, and preferably some editorial experience)—The Director, Commonwealth Bureau of Plant Breeding and Genetics, School of Agriculture, Downing Street, Cambridge (January 15).

AGRONOMIST (national of the United Kingdom or the Republic of Ireland, with a degree in agriculture or horticulture and at least two years experience in the field of experimentation or two years postgraduate training) in Tanganyika, to be responsible for a programme of field experimentation, often involving many district trials off the main research centres—The Appointments Officer, Room 301, Department of Technical Co-operation, Eland House, Stag Place, London, S.W.1, quoting Ref. RC.213/173/06.

ASSISTANT OR ASSOCIATE PROFESSOR IN THE DEPARTMENT OF BIOCHEMISTRY—Dr. M. Blanchaer, Department of Biochemistry, Faculty of Medicine, University of Manitoba, Winnipeg 3, Manitoba, Canada.

CHIEF TOXICOLOGIST to take charge of the Toxicology Department; EXPERIMENTAL PATHOLOGISTS (preferably with experience of animal histopathology, tissue culture or immunological techniques); RESEARCH BIOCHEMISTS (preferably with experience in metabolic studies, biochemical pharmacology, enzyme biochemistry or connective tissue biochemistry)—The Secretary, British Industrial Biological Research Association, Woodmansterne Road, Carshalton, Surrey.

ELECTRONIC ENGINEER (preferably with H.N.C. in electronic or electrical engineering) IN THE DEPARTMENT OF PHARMACOLOGY, The Medical College of St. Bartholomew's Hospital, to assist in the design and construction of electronic apparatus used for research into drug action—The Clerk to the Governors, St. Bartholomew's Hospital, London, E.C.1, quoting Ref. PT/697.

GRADUATE RESEARCH STUDENT (recently graduated engineer (honours degree or Dip. Tech.) wishing to work for an M.Sc. or Ph.D. degree) IN THE MATERIALS GROUP of the Department of Civil and Mechanical Engineering to investigate the creep and high temperature properties of metals—The Secretary, Northampton College of Advanced Technology, St. John Street, London, E.C.1.

PASTURE RESEARCH OFFICER (national of the United Kingdom or the Republic of Ireland, with an honours degree in natural science, agriculture or agricultural botany and at least two years postgraduate work in pasture research) in Tanganyika, to study the means whereby annual production can be increased through improvement in pastures and the consideration of how tropical grasslands can be managed in the context of local agriculture systems to give the maximum economic production without deterioration—The Appointments Officer, Department of Technical Co-operation, Eland House, Stag Place, London, S.W.1, quoting Ref. RC.213/173/04/6.

PLANT PATHOLOGISTS (nationals of the United Kingdom or the Republic of Ireland, with a degree in botany or natural science, and preferably specialist training or postgraduate experience in mycology, virology or plant pathology) in Northern Rhodesia, to investigate major diseases of crops of economic importance and to make recommendations based on experimental results—The Appointments Officer, Room 301, Department of Technical Co-operation, Eland House, Stag Place, London, S.W.1, quoting Ref. RC.213/132/07.

RESEARCH FELLOW (with a Ph.D. degree and preferably electrochemical or organic chemical experience) IN THE DEPARTMENT OF CHEMISTRY, for work in either of the fields of molten salt electrochemistry or the chemistry of coal components—The Secretary, Northampton College of Advanced Technology, St. John Street, London, E.C.1.

RESEARCH STUDENT IN GEOGRAPHY—The Registrar, The University, Hull.

SOILS AGRONOMIST (national of the United Kingdom or the Republic of Ireland, with an honours degree in agriculture or horticulture with agricultural chemistry as the main subject, and preferably postgraduate experience in the field, experimental work concerning soil fertility and a knowledge of tropical soils and crops) in Sarawak, to design and conduct field experiments with a variety of tropical crops on selected soil types in order to assess the potentiality of the soils—The Appointments Officer, Room 301, Department of Technical Co-operation, Eland House, Stag Place, London, S.W.1, quoting Ref. RC.213/155/02.

TECHNICIAN (aged 30–40, with experience of routine chemical pathology, and preferably F.I.M.L.T. or equivalent), to take charge of routine laboratory services and to supervise junior staff in the Mineral Metabolism Unit—The Director, Medical Research Council, Mineral Metabolism Research Unit, The General Infirmary, Great George Street, Leeds.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

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Editorial and Publishing Offices of "NATURE"
MACMILLAN (JOURNALS) LIMITED
ST. MARTIN'S STREET, LONDON, W.C.2
Telephone Number: Whitehall 0081. Telegrams: Phusis London W.C.2

Annual subscription: Inland £13, Overseas £13 15s., payable in advance, postage paid to any part of the world.

Advertisements only should be addressed to
T. G. Scott & Son, Ltd., 1 Clement's Inn, London, W.C.2.
Telephone Number: Holborn 4743

Registered as a newspaper at the General Post Office
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U.S. PUBLIC HEALTH SERVICE: NATIONAL INSTITUTES OF HEALTH

STATEMENT

THE National Institutes of Health, U.S. Public Health Service, have recently announced that 14·7 million dollars in biomedical research project grants were awarded to institutions in 57 countries outside the United States during the financial year ending June 30, 1964. The regional distribution of awards was as follows (dollars):

Europe	5·5 million
Latin America	2·0 „
Canada	2·0 „
Middle East	1·3 „
Asia	1·3 „
Australia and New Zealand	0·7 „
Africa	0·4 „
International organizations	1·5 „

During the present financial year, ending June 30, 1965, the funds from the National Institutes of Health available to institutions and scientists outside the United States will total 13·5 million dollars—a reduction of 8 per cent from the previous year's total.

It is anticipated by the Institutes that this reduction in funds, which is dictated by the unfavourable United States balance of payments abroad, will be limited to economically advanced countries. Such countries, which include those of Western Europe, Canada, Australia and New Zealand, may, as a group, anticipate a reduction of approximately 15 per cent in total National Institutes of Health funds available during the present year. The allocation to these countries, which will amount to a total of 7·0 million dollars, will be sufficient to meet all existing commitments but will necessitate a reduction in new grants and in renewal of previously supported projects of approximately 13 per cent during the present year. Approximately 2·2 million dollars will be provided for new and renewal awards.

The funds available to less economically and scientifically advanced countries will be maintained at approximately the same level as in the previous year. A total of 6·5 million dollars will be allocated to such countries (including international organizations), of which 2·4 million dollars will take the form of new and renewal grants.

The National Institutes of Health have stated that they will continue their policy of making awards on a competitive basis, using scientific excellence, uniqueness and programme interests as criteria for approval of applications submitted. Fixed ceilings in individual countries are not contemplated.

Continued support of biomedical research throughout the world is considered by the National Institutes of Health to be an indispensable complement to research by scientists in the United States. Projects supported in other countries represent a very careful selection from among the many opportunities offered to solve specific biological and medical problems of interest to both the United States and foreign investigators by using research skills, materials and resources not found in the United States.

National Institutes of Health fellowship and training programmes, collaborative research projects between American and foreign scientists, and projects supported with American-held local currency abroad, are expected to continue at present levels or to increase.

AMERICAN AID FOR SCIENTIFIC AND MEDICAL RESEARCH

THE grants which the National Institutes of Health of the United States Public Health Service have been making for biomedical research projects outside the United States have been made in the belief that such support is an indispensable complement to research carried out by scientists in the United States. These overseas projects supported from the United States represent a very careful selection from among opportunities offered by the utilization of research skills, materials and reserves not found in the United States to solve specific biological and medical problems of interest to investigators both in the United States and elsewhere. That the unfavourable United States balance of payments abroad has dictated a reduction of some 8 per cent in the funds which the National Institutes of Health are able to make available during the year ending June 30, 1965, compared with the previous year, for institutions and scientists outside the United States does not, however, affect the belief of the Institutes in continued support of such overseas research. It is in fact anticipated that the Institutes' fellowship and training programmes, collaborative research projects with foreign scientists, and projects supported with American-held local currency abroad will continue at their present levels or increase.

If this is the first point that should be noted, the next is to thank the National Institutes of Health of the United States Public Health Service for the very considerable financial and other assistance they have given to scientific research in the medical and related fields all over the world. That, as the Statement itself indicates, such assistance brings at least indirect returns to the donor, because scientific advance is international and indivisible, in no way detracts from the generosity of the United States in giving such aid, to which, moreover, no strings have been attached. The opportunity of paying just tribute to the generosity of the people and Federal Government of the United States is as welcome as the appreciation of scientists in Britain is deep and sincere.

With that word of thanks should go also one of equal appreciation that, when compelled to reduce to some extent the magnitude of this assistance, the United States has so wisely chosen to limit the reduction to the economically advanced countries. The funds available to the countries less advanced economically and scientifically will be maintained at approximately their present level, and of the total of 6·5 million dollars, 2·4 million will be available in the form of new or renewed grants. Even in the economically advanced countries the alloca-

tion will suffice to meet all existing commitments, although involving a reduction of some 13 per cent in new grants and renewal of support for existing projects.

There is thus nothing of the start-and-stop principle about the change in policy, and the unavoidable reduction has been placed where it is most fair and just. A much severer cut should undoubtedly be accepted by the advanced countries before aid to those less advanced is restricted, even when full regard is had to the way in which the benefits of research in the advanced countries flow into those less advanced. There can be no doubt that the United States Public Health Service is fully justified in its action, and that the decision is sound and wise from the scientific point of view.

It should be remembered, moreover, that the aid provided to Great Britain by the United States Public Health Service is only a part of that received from the United States. During the past forty years the Rockefeller Foundation has provided the Medical Research Council with funds for about 200 Rockefeller travelling fellowships for advanced research abroad, and until recent years these were almost the only such appointments available. There are now many other opportunities for highly qualified young investigators, and the Foundation made its final contribution of 25,000 dollars to this programme in 1963. Besides this, however, the Rockefeller Foundation has made other generous grants for research in biophysics and in biochemistry for work at the Universities of Cambridge, London and elsewhere.

The importance of these fields for medical research can scarcely be overstressed any more than the implications for medicine or molecular biology. In the present context it is sufficient to remember that the finance and the organization of medical research are most appropriately considered in relation to the finance and organization of scientific research generally. First, however, it is to be noted that the National Institutes statement does not indicate what proportion of the 5·5 million dollars awarded by the United States Public Health Service to institutions and scientists in Western Europe in 1963–64 went to Great Britain. Even with a high proportion of this total, the 15 per cent reduction for 1964–65 can scarcely be of the order of £100,000, especially as in a survey of the finance of medical research published earlier this year (*Nature*, 203, 999; 1964) the Office of Health Economics did not consider it significant enough in amount to distinguish, although emphasizing that in the United States the Federal Government in 1965 contributed some 205 million dollars to medical research mainly through the National Institutes of Health.

Government expenditure on medical research in Great Britain in 1961–62 is estimated at £14·5 million and in 1962–63 at £15·5 million, and clearly if it were simply a matter of finance, it would be reasonable to expect that the Government should promptly provide a further contribution of the order of £100,000 or even of £200,000 to make good any interruption of research that the reductions now announced in aid from the United States might otherwise necessitate. This, however, is only a part of the issue. Financial support may be an important factor determining the advance of medical and allied research; but it is by no means the only factor determining the pace of medical progress. It is a permissive rather than a direct stimulus, and expansion of funds does not necessarily accelerate progress, which may, for example, be limited by the intractable nature of particular problems, which depend for elucidation or solution on advance in other and possibly unexpected fields.

It is also clear from the Statement that the National Institutes of Health, in making their grants, have sought to do so on the same principles as have guided the Rockefeller Foundation in its choice of projects and individuals for support—scientific excellence, uniqueness and promise of growth. This is the policy which guides also the Carnegie Institution of Washington and the Nuffield Foundation and is increasingly accepted as marking the most appropriate field of work for the independent Foundation. The Office of Health Economics in the survey just mentioned also noted a marked tendency among the charitable trusts and organizations to provide their support for medical research not only along general lines, but also in terms of the investigation of specific diseases.

To the extent, therefore, that the aid given by the National Institutes of Health has been along either of these lines, the replacement of the cut by Government grant is not quite so simple. In the first place, the Foundations have frequently selected for support projects which, from their very nature, while of high promise, were unlikely to attract Government support. The appropriateness, if not the desirability, of continuing such projects, or embarking on new projects of like character, with direct Government grant, is at least open to question. Even if the Treasury agreed to release its control over further considerable sums of public money, the whole trend of recent reports from the Select Committee on Estimates suggests that that Committee is at present far too obsessed with asserting the principle of close accountability to Parliament to appreciate what is involved in the control of speculative research projects. Furthermore, the Medical Research Council has repeatedly expressed the view that in Britain no worth-while projects in medical research are held up by the absence of funds.

In this position, on the financial side the Government would probably do better to offset the reduction in American aid by an increase in the grant to the University Grants Committee. University expenditure on medical research through the University Grants Committee is estimated at something more than £8 million a year, or about the same order as the expenditure by the Medical Research Council. Neither the universities nor the University Grants Committee could well refuse or even question an earmarked grant of a further £200,000 or even £500,000 for medical research in the circumstances: the larger grant would be a more dignified compliment to the United States.

That, however, is only the first step. Finance is only a part of the problem and one that is often misunderstood in the discussions on medical or on pharmaceutical research which, whether in Parliament or elsewhere, have been more often bedevilled by sectional or political, even party political, prejudice than informed by sense and sound judgment. With it should go a fresh appraisal of both the structure and objectives of medical research in relation to scientific research generally, and this should be undertaken by the Minister of Education and Science before and not after the reorganization of civil science at present contemplated is finally enacted. Nor, so far as objectives are concerned, is it a matter for that Ministry alone. At least the Minister of Health is involved, for one of the questions which was raised in *The Finance of Medical Research* is whether the balance of public advantage lies in stimulating medical research or in seeking short-term

economies in expenditure on the National Health Service. There is some evidence that Britain has gone too far in the latter direction, with dangers to the national economy as well as to scientific research that have yet to become apparent. Grateful as all should be to the United States for the generous help she has long given—and is continuing to give—Britain and other countries in the support of medical research, countries outside the United States have reason to be even more grateful if the present slight reduction leads us to think more realistically and imaginatively alike about the objectives of medical research and its finance and organization in the context of public accountability and public benefit.

NUCLEAR OPTICAL MODEL

The Optical Model of Elastic Scattering

By P. E. Hodgson. Pp. viii+211. (Oxford: Clarendon Press; London: Oxford University Press, 1963.) 30*s*. net.

DURING the past ten years or so, the use of the nuclear optical model of elastic scattering has been attended by a steady improvement in the description of experiments throughout wide regions. This model represents the nucleus as a spherically symmetric potential well in which absorption of the bombarding particle can occur, and some of the improvement has come about because theoreticians have indicated logical modifications to the simple form of potential which was first conceived. Account must also be taken of the mass of experimental data accumulated during this period, and a third reason for the success of the model is that the use of computers by analysts, such as Dr. Hodgson, has greatly helped the phenomenological approach of variation of the parameters of the potential. Because most experimentalists now have ready access to computers, it is to their advantage to know how to analyse their own results, and for that reason alone the appearance of *The Optical Model of Elastic Scattering* is justified. In addition, the book provides a description of the model and its history up to the middle of 1962 which it is convenient to have in the same volume, both to remind and to serve as a source of reference to original papers. All newcomers to experimental nuclear physics will find rewarding reading here.

The first chapter describes the introduction of the model (1949–52) to account for the observation of nuclear transparency and very broad resonances (in neutron total cross-sections) which appeared, at the time, to be inexplicable by the compound nucleus model. The more important limitations of the model are explained and the inclusion of a spin-orbit term in the potential is discussed. The student surprised by the sudden introduction of the rotation parameter β on p. 21 will find it defined 20 pages later. He should also note that in equation 1.17 (taken from Schiff's *Quantum Mechanics*) the function $U(r)$ has different dimensions from that used previously in, for example, equations 1.2 and 1.9.

In Chapter 2, the treatment of averaging over sharp resonances is reproduced giving reconciliation with the compound nucleus model and introducing the compound and elastic scattering cross-sections. In equation 2.10, a bar over the symbol for reaction cross-section has crept in unobserved. By p. 30, the author has begun to describe the fundamental process of producing cross-sections by solving the Schrodinger equation for a local optical model potential using numerical methods. At first, the simplest case of bombardment of a spinless nucleus by an uncharged spinless particle is considered. (In fact, the list of properties starting on p. 30 marks the particle as being that rare bird, the spinless neutron.) Later on, the effects of Coulomb field and spin are introduced.

The next chapter gives further extensions to the analysis, dealing separately with particles of unit spin, a non-local potential and an isotopic spin-dependent potential. The inclusion of these refinements has sound theoretical foundation and, indeed, so have two others noted, the spin–spin addition to the potential and the representation of a deformed target nucleus by a spheroidal well. There is experimental evidence, however, that spin–spin forces are small, and difficulties arising out of the distorted potential approach are noted. This chapter is particularly well written and covers a good deal of ground in 16 pages.

Chapter 4 is an account of the fitting process. In one sense, an optical model analysis is irreversible; that is to say, one can use potentials to derive an angular distribution but not vice versa, so fitting involves variation of the parameters of the potential. When a good fit is obtained, the possibility remains that other parameters might give an equally good fit. (The necessity for the variational procedure accounts for the great value of a computer.) The author gives approximate values to start the process and some rules on what variations should be made to achieve the desired result, followed by ways of minimizing the deviations. The chapter ends with a discussion on the uniqueness of the parameters obtained, that is, whether or not they are physically realistic.

The fifth chapter is very properly by far the longest, being concerned with the scattering of nucleons, a diverse subject in which the validity of the model is least questioned. In the discussion of size resonances, both here and in the introduction, it is not emphasized that this property of a potential well was realized long before the optical model, probably because readers are expected to be well grounded on a book such as that by Schiff. The peaks in the neutron strength function and their interpretation by Feshbach, Porter and Weisskopf in terms of the model are described, also the difficulties introduced by the compound elastic scattering when few exit channels are open. This is followed by a discussion of the energy dependence of the depth of local potentials for neutrons. and attempts to account for it by a non-local potential (which depends not only on the neutron position (r) but also on the wave function in the neighbourhood of r). Such a potential is readily shown to be momentum-dependent. Similar considerations are then paid to proton scattering. There is a section on fluctuations of the parameters of the potential as a function of N, Z, and energy, the presence of which was expected and can now be established, and finally a section on the importance of the potential shape, in particular the absorptive part.

The two succeeding chapters deal with the scattering of composite particles (deuterons, helium ions and heavy ions), and mesons, for which the characteristic features are different from those of the nucleon situation, the experimental data are sparser and the model is less well-tried. A return to nucleon scattering is made in Chapter 8 but for high energy collisions. An account is given of the formalism needed because of relativistic effects, and because the short de Broglie wave-length implies better localization of the nucleon but makes high angular momenta important. There is a mistake in equation 8.33, where "imaginary part" should read "real part", as reference to equation 8.28 makes clear.

The ninth chapter concerns itself with the bound state problem and not with elastic scattering at all. The subject is introduced here because at negative energies the optical model goes over into the single particle model for bound states. It is felt that a short account of the Lane, Thomas and Wigner intermediate coupling model would have been valuable for several reasons. The connexions of the optical model with the shell model and the compound nucleus model are emphasized, particularly by the appearance in the formula for the size resonance of the single particle width and the absorptive potential W. Experimentalists might well find this kind of discussion involving other familiar models more to their taste than the account of Brueckner theory in the last chapter. It must be

granted, however, that this is clearly reviewed and is the most fundamental way we would expect to be able to understand the optical model.

Dr. Hodgson's book is a well-balanced presentation, it satisfies a need and is coherent. Proof reading has been generally thorough, notwithstanding the two different spellings of the name Mottelson which were noticed and the incorrect reference date for Wolfenstein (1956 should be 1954) on p. 161. I would make one criticism concerning the symbol k which is used throughout the book both for momentum and wave number. In Appendix C, headed "Numerical Relations", k, said to represent the wave number, is defined as a quantity with the dimensions of momentum. On p. 161 k is used for momentum, but in equation 5.16 it is the wave number again, this time having the correct dimensions. Also in Appendix C the units of E are not stated.

Paper, printing and binding of this book are all of the excellent standard one has come to expect from the publisher and the price is very reasonable.

J. E. Evans

THE 'FORE AND AFT' OF SEDIMENTARY ROCKS

Paleocurrents and Basin Analysis

By Prof. Paul Edwin Potter and Prof. F. J. Pettijohn. Pp. viii + 269 + 30 plates. (Berlin: Springer-Verlag, 1963.) 40 D.M.

In any rapidly advancing subject a valuable contribution can be made by a timely review of existing knowledge. Profs. Potter and Pettijohn have attempted such a survey of one aspect of sedimentology, namely, the usefulness of directional properties in determining current patterns and analysing sedimentary basins.

There are ten chapters. The first outlines the use of palæocurrent investigation, shows how this is related to other aspects of geology, and itemizes the directional properties concerned. A historical review of palæocurrent investigations follows. Chapters 3–8 deal with the directional properties themselves. Arrangement of fabric elements and the geophysical properties resulting from preferred arrangements are examined first. Cross-bedding, ripple marks, other linear structures and deformation structures follow in sequence. Chapter 7 relates internal directional structures to the geometry of the sedimentary bodies containing them. Dispersal patterns and the current systems they reveal are discussed. Chapter 9 covers the more theoretical aspects of basin analysis. The model concept of sedimentary basins is developed and illustrated with examples. A final chapter reviews methods of investigation, including the collection, processing and statistical analysis of data, and presentation of results.

In layout the book is thus sound and logical, and a vast amount of useful information is assembled. The authors' main thesis and their views on integrating various lines of investigation are worth while and sometimes illuminating. Unhappily, however, the book as a whole is disappointing, mainly due to weaknesses in choice and presentation of material. There are many signs of hasty preparation, the standard sometimes falling short of that expected in a scientific treatise.

The most obvious weakness was perhaps self-imposed. In their preface the authors state that "much of what has been written about the environmental significance of sedimentary structures is omitted; that which pertains to their relation to transport direction and to basin analysis is emphasized". Obviously, over-concentration on environments would have detracted from the main topic. But the treatment of environments and the origin of structures is too slender and lacking in rigour. This allows, even encourages, a rule-of-thumb approach. A more exhaustive examination of origins in place of the repetitive and irrelevant parts of the text would make the book twice as useful. Irrelevancies certainly exist. For example, in the section on deformation structures, features having no known or inferred directional significance are described at length. Sometimes the text reads like a manual of sedimentary structures—not a review of a limited topic.

Balance between detail and generalization is uneven. Sometimes there is too much detail: sometimes relatively uncritical generalizations obscure unsolved problems. Surprisingly little reference is made to hydraulics and only a few Recent examples are taken.

Any review must rely heavily on published work. Here, too much of it is presented uncritically and some important references are omitted. In contrast, unpublished theses are referred to—a growing habit these days.

Ease of reading is impaired by the style ('catalogue English') and the profusion of bibliographic references in the text. This is exasperating because, being a reasoned whole, the book should read from cover to cover. Increase in the number of page cross-references and improvement of subheading and paragraph styles would help.

Occasional textual mistakes, such as spelling, can be excused where the intention is obvious. But misspelt names cause confusion (for example, Forgottson and Fergotson for Forgotson) or misunderstanding (for example, Nairn is always rendered as Narin). At least 9 spellings in the text differ from those in the reference list. More serious errors concern publication dates. No fewer than 18 dates in the text disagree with the bibliography. Generally the discrepancy is a few years, but ranges up to a century. At least 8 references in the text do not appear in the bibliography. Such carelessness inevitably casts doubt on the accuracy of the volume and page numbers listed. Blame is not always attributable to the printers. For example, (1) "Old Red Sandstone" appears where "New Red Sandstone" is intended, and (2) a reference to the work of Donner and West in a large area of south-eastern England leads the reader to a publication by Donner and West on a small area in Spitsbergen!

The statistical section in the last chapter is weak. Too much prior knowledge is assumed. Those able to understand will find most of this unnecessary. Obvious slips feature in 3 formulæ. More surprising is the calculation of an arithmetic average where 3 out of 9 numbers are wrong.

Text figures, generally clear and informative, are plentiful. Some are beautifully drawn, but many described as "modified from" previously published illustrations lack indication of the nature or extent of the modification. Some of the inconsistencies could have arisen from this. For example, a map showing cross-bedding directions is accompanied by a rose diagram which gives their frequency distribution but contains a northerly component not on the map. Many of the legends need amplification. Figures and textual statements about them do not always tally.

The plates are good, generally illustrating the points discussed. A few of them, however, lack scales. The method used for indicating current directions is occasionally ambiguous. Currents are variously described as "parallel to top of page", "from lower right to upper left", etc. Confusion inevitably arises when the photographs are mounted sideways on the page. Arrows marked on the pictures would have avoided this.

Intended for "both the student new to the subject and for the experienced geologist whose work leads him to the study of sedimentary basins", the book falls short of the mark. Students new to the subject will find it heavy going and must be warned of the imperfections. Experienced geologists able to detect and avoid such pitfalls will value the compilation while lamenting its deficiencies.

A. Lees

CYANINE CHEMISTRY

The Cyanine Dyes and Related Compounds

By Frances M. Hamer. (The Chemistry of Heterocyclic Compounds: a Series of Monographs.) Pp. xxxvi+790. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, 1964.) 340*s*.

THE ability of certain dyes to make silver halide emulsions sensitive to light of wave-lengths longer than those to which they respond *per se* has made possible the more faithful photographic reproduction of scenes in black and white. Also, without sensitizers colour photography would have been unknown and all film speeds would have been severely limited since no energy derived from light of the longer wave-lengths could have been utilized. Noisy arc lamps in the film studio would certainly have handicapped the production of sound films —sensitizers made possible the change to the yellower but silent filament lamps. Thus photography in many of its technical aspects has been intimately connected, for the past 60 years, with sensitizers, and the useful sensitizers have almost invariably been selected from a class of dyes known generically as the cyanines. Dr. Frances M. Hamer brings out these points in the interesting introduction to her volume in the Interscience series of monographs on heterocyclic compounds. She has been associated perhaps more than anyone else with the chemistry of the cyanines and it is therefore most appropriate that this monograph should be written by her.

With some important exceptions, notably in the merocyanine series, characteristically, cyanine molecules comprise two heterocyclic nuclei, very often quinoline or benzothiazole, one of which bears a quaternized nitrogen atom, joined by an odd number of carbon atoms in such a way that the two nitrogen atoms of the heterocyclic residues are linked in a fully conjugated system. This volume is devoted almost entirely to the synthetic aspects of the subject. Dr. Hamer has collected together an enormous mass of information, culled mostly from the patent literature, and has classified it according to the type of cyanine and the basis of the methods of preparation.

Nomenclature in the cyanine field has often been confusing in the past, and the systematic names of the various classes can be rather cumbersome. Dr. Hamer has chosen to use semi-trivial names in the text but systematic names in the chapter titles. Thus the chapter devoted to "pentamethincyanines" includes sections on "2,2′-dicarbocyanines", and "thia-2′-dicarbocyanines". One can sympathize with the author in her dilemma and congratulate her on her solution.

The sheer bulk of material discussed has obviously made it impossible to include enough structural formulæ to make the text easily readable. For the most part only the structures of reaction products are given and it will be necessary for the reader himself to translate the names of intermediates and starting materials into structures. His task is not made easier by the labelling of formulæ with the cumbersome Roman numerals or by cross-referencing to chapters and sections of chapters rather than to particular pages.

Dr. Hamer has obviously been handicapped by the particular nature of her subject which, being closely connected with technology, is subject to trade secrecy. Thus the constitutions of particular sensitizers in present-day use are not disclosed. The patent literature, of great importance in this field, is of little value in the assessment of the relative values of different products.

The sources of information also make it difficult to select the best method of preparation of a compound, although whenever possible the yield of product has been given.

This book will be invaluable to chemists about to work in the field of cyanine chemistry and will for many years be a standard work of reference. As with other monumental compilations, by the time it finally appeared in print the work recorded was already a few years old.

A. F. CROWTHER

PLANT CHEMISTRY

The Chemistry of Plant Processes

By Prof. C. P. Whittingham. (Methuen's Monographs on Biological Subjects.) Pp. 209. (London: Methuen and Co., Ltd., 1964.) 25*s*.

IN the words of Prof. C. P. Whittingham "this book attempts to present concisely a broad survey of plant physiology and biochemistry suitable for university students . . . who should have some basic knowledge of the physical sciences". There is little doubt that it succeeds admirably in these aims.

The first part, occupying slightly more than half the book, deals with cellular metabolism. Starting with a brief statement of the historical development and the nature of experimental botany, Prof. Whittingham then sets the modern scene with an outline of the fine structure of plant cells and the methods of studying organelles and their functions. This leads naturally to a chapter on enzymes as an introduction to the metabolic meat of the book, the four chapters on fermentation and respiration, energetics of respiration and biological syntheses, photosynthesis and nitrogen metabolism respectively. Together these four chapters make up nearly half the book and are masterpieces of conciseness and clarity. Prof. Whittingham has been absolutely ruthless in the elimination of all unnecessary frills and verbiage, and the result is a highly condensed and, at the same time, completely thorough and readable portrayal of the present-day scene. The reader is brought right up to date on recent advances although there is no neglect of the classical foundations that have made these advances possible.

The second part of the book is entitled "Plant Processes" by which is meant phenomena of plant function which are not susceptible to the same dissection into discrete chemical processes as those of the first part. The author includes the osmotic relations of the cell, the water relations of the whole plant, the transport of solutes and growth. The first of these chapters on osmotic phenomena is as excellent as those preceding it, but thence the quality declines somewhat. Possibly because the relevant phenomena cannot yet be examined with comparable precision, possibly because the systems involved are more complex and difficult to define, the author has not been able to weld the selected material into such a satisfying story. This is particularly true of the chapter on growth, which is incomplete and a little disjointed; but this might have been cured by a more generous allocation of space, since even the masterly conciseness characterizing the first part of the book could not be expected to create a balanced statement of such a complex subject as plant growth in a mere twenty pages.

The book closes with an eleven-page appendix on the chemistry of the constituents of living organisms. This could with profit have been longer.

As was his intention, the author has produced an excellent framework on which the student of plant-physiology and biochemistry can base his further reading. For this there are 167 titles in a list of references in which the inclusion of a few additional critical review articles would have been an improvement.

In spite of these minor criticisms the book excellently fills a very real need and can be strongly recommended to all advanced students of botany.

L. J. AUDUS

SOURCE-BOOK OF LABORATORY INFORMATION

Laboratory Handbook

Edited by N. L. Parr. Pp. xi+1520. (London: George Newnes Ltd., 1963.) 210*s*.

THIS is a remarkable book: within its 1520 pages of well-illustrated text, the editor and 46 other contributors have sought to provide " . . . a useful source of information for those already engaged in any given field to technique and equipment employed elsewhere which may be relevant or capable of adaptation for their particular problems".

The editor has organized the book into twelve parts and in the first very briefly reviews laboratory design, organization and administration—mainly from the public service aspect. Part 2, construction of apparatus, includes, *inter alia*, chapters on electronics, remote-handling, furnaces and a particularly useful introduction to the manipulation of glass. The third part is concerned with measurement —dimensional, optical, electrical and thermal. Part 4 deals with essential techniques such as photography, optical and electron microscopy, X-ray crystallography and vacuum technology, while Part 5 covers the more specialist requirements, for example, low temperatures, distillation, high purity, inductive and dielectric heating; radioisotopes are given a whole part (6) to themselves. Part 7 contains 238 pages and is a comprehensive survey of chemical analysis. Particularly commendable is the chapter on physical methods which furnishes excellent introductions to the principles, instrumentation and procedures of emission spectrography, polarography, flame photometry, X-ray diffraction and fluorescence techniques, electron microscopy and diffraction, radioactivation analysis and ultra-violet, visible and infra-red absorptiometry. Included are comprehensive tables of detection limits, diagnostic wave-lengths and half-wave potentials in a variety of base electrolytes. On the other hand, it is to be regretted that the editor was unable to include sections on such important aids to the analyst as mass spectrometry (dismissed in two lines in Part 5) and atomic absorption; instruments for both techniques are now commercially available. More understandable perhaps is omission from a book of this type of reference to specialist tools of the organic chemist, such as nuclear magnetic resonance, electron spin resonance and optical rotatory dispersion equipment.

Part 8 is equally valuable: five chapters (186 pages) cover mechanical testing, non-destructive testing, industrial radiography, metal corrosion and soil tests. Part 9 is concerned with the work and equipment of specialized laboratories: eighteen widely divergent fields are discussed (from forensic to pharmaceutical, rubber to refractory) and yet the editor apologizes for omissions (for example, forestry and building). Laboratory hazards, including fire, toxicity and radiation, are succinctly reviewed in Part 10, while three chapters in Part 11 briefly consider the recording, analysis and presentation of results. Here the 'non-specialist' is more likely to be deterred by the condensed account, if he is not already familiar with the 'simple statistical procedures' given: this is unfortunate since a wider appreciation of the value of analyses of variance and tests of significance is desirable—not to mention standardization of nomenclature. Finally, Part 12 indicates various information services and methods of publication. The general index contains less than 2,000 entries: in a book of this size and scope this might be considered barely adequate.

In a necessarily superficial work, it is not difficult to make minor criticisms within a given field. Thus there is a lack of correlation between references in various sections to electronic devices: the brief descriptions of primitive valve and transistor circuits in Chapters 3 and 6 serve only to whet the appetite. Only the non-chemist would find useful the summary tables of gravimetric and volumetric procedures in Chapter 32. No distinction is made between a.c. and d.c. polarography, although appropriate instruments are named. Frequent mention is made of 'colour temperature', but no cross-reference is given to the short explanation in Chapter 13—and no mention at all in the index. The scientific application of ciné photography is briefly dismissed and the availability in Britain of magnetic stripe for 8-mm sound films is not mentioned. A few inevitable typographical errors have escaped notice, but they are mainly self-evident.

Notwithstanding these and similar criticisms, this handbook surely achieves the editor's primary aim. While the price will generally preclude purchase by the individual, this work should not be buried in the laboratory central library; copies should be made available on a divisional basis for general browsing by staff at all levels.

G. F. Phillips

STRUCTURE AND FUNCTION OF THE LIVER

The Liver

Morphology, Biochemistry, Physiology. Edited by Ch. Rouiller. Vol. 1: Pp. xiii+683; 26 dollars. Vol. 2: Pp. xiv+674; 171*s*. 6*d*. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1963.)

THIS book in two volumes attempts to correlate hepatic structure, function and biochemistry. Particular emphasis is laid on the subcellular and molecular levels as revealed by electron microscopy, histochemistry, cytochemistry and by isotopic methods. Many authors are concerned, all active workers in their respective fields, mainly derived from Switzerland and the United States with a few representatives from Britain, Canada, France, Germany and Hungary.

The editor, Ch. Rouiller, is an eminent electron microscopist, and this technique is particularly well covered, both in his two very comprehensive chapters and in those of others. Guido Majoro contributes a thought-provoking chapter on liver cell death, and Stanley Hartroft an outstanding account of experimental cirrhosis. K. Alterman uses electron microscopy to describe sinusoids and sinusoidal cells. Hans Elias (p. 44) provides a valuable diagram of the ultrastructure of the liver cells, and A. M. Rappaport (p. 176), in another diagram, attempts to correlate the ultrastructure with the cellular metabolism.

The cyto- and histo-chemistry of the liver are discussed by M. Wachstein. The isolation, morphology and biochemistry of the cell components of the liver are described by Yvonne Moulé and Jean Chauveau. These authors advise caution in the interpretation of the results, for the cell fractions are not in the state they find themselves *in situ* and a mean value is obtained without reference to extremes in the population. Nevertheless, such methods do allow investigation of functional interrelationships within the cell. The importance of nuclear cytoplasmic interrelationships in respect of DNA and RNA is emphasized.

A. M. Du Bois writes a fascinating account of the human embryonic liver. He touches on normal anatomy and physiology, regretting that more is not known of bile secretion in the embryo. The section on abnormalities is rather brief and will be of little help to the clinician anxious to know more of a particular defect in any individual patient.

H. Elias emphasizes the continuous nature of the hepatic parenchyma. Lobules are imaginary areas which can be created arbitrarily around portal canals or hepatic vein roots. A. M. Rappaport prefers to group the liver anatomically into acinar units arranged around an axis of terminal hepatic arterioles, portal venules and bile

ductules, lymph vessels and nerves which grow out together from a small triangular portal field. A concentric zonal relationship is established between these acinar cells and their blood supply. A most interesting interpretation of classical histopathology can be made in terms of the acinar concept.

A. Fischer contributes a comprehensive account of the dynamics of the liver circulation. He raises the question of the purpose of the large portal venous flow in relation to hepatic requirements. He suggests that the large portal circulation is not primarily concerned with the needs of the liver, but that the liver merely acts as a thoroughfare for blood from the splanchnic area.

Hepatic metabolism is covered fairly fully. Amino-acids and proteins are described by H. Tarver, lipids by P. Farager, carbohydrates by L. A. Pesch and Y. J. Topper, biliary excretion by Burton Combes, steroid metabolism by C. B. Cameron, vitamins by A. Wiss and F. Weber, and liver and blood coagulation by C. A. Bouvier and P. A. Maurice. The chapter on liver function tests by G. Milhaud is interesting but patchy. Tests, such as formal gel, sia and cadmium, are included which are quite out of vogue to-day and which have been replaced by more specific indications of hepatic function. On the other hand, the chapter includes a fascinating account of how human liver biopsy material may be incubated in various media so that defects in hepatic metabolism may be understood. This represents an important, advancing field.

Cancer of the liver in man is discussed by H. Elias and its relation to experimental dietary liver disease by E. B. Taft. Unfortunately, no mention is made of the place of contamination of foodstuffs by moulds which can produce the carcinogen, aflotoxin. This important subject will have to wait for the second edition.

These volumes are well produced and profusely illustrated. They provide a comprehensive source of literature. The references include titles of papers and number some 1,200—about one-fifth of the two volumes. The author index is careful and complete. The chief criticism lies in the lack of correlation between one chapter and the next, which is the common fault of the multi-author book. The chapters are of a variable length which does not always depend on the importance of the subject in relation to the whole work. Liver sinusoids, for example, are covered in 76 pages, whereas the basic anatomy of the liver occupies only 19. The practice of numbering the figures chapter by chapter instead of serially throughout the book enhances the isolation of one section from the next. The postulates of one author are often ignored or denied by the next. The volumes must therefore be regarded as a series of monographs rather than as a complete coverage of the subject. A good basic knowledge of the liver is demanded of the reader.

The volumes provide a stimulating, up-to-date account of the morphology, biochemistry and physiology of the liver. They deserve a wide success and should be found in every medical library. SHEILA SHERLOCK

BLOOD ENZYMES

Enzymes in Blood Plasma

By Benno Hess. Translated from the German by Keith S. Henley. Pp. xii+167. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1963.) 64*s*.

THE recent progress in the use of enzyme assays for clinical diagnosis has been reflected in some excellent text-books which deal with the theory of enzyme action and the techniques used in the clinical laboratory. *Enzymes in Blood Plasma*, by Benno Hess, does not concern itself with details of the tests as they are used at the bench, and refers in each case to the relevant original literature. There is a section on methods which describes the general principles of measurement of enzyme activity, reaction velocities, the role of temperature and the advantages of substrate excess.

The main parts of the book are devoted to the biochemistry of enzymes and to their "pathology and clinic". The title of the book does not prevent Prof. Hess from discussing enzymes in urine, cerebrospinal fluid, and fæces. One table lists, with full references for further reading, 47 plasma enzymes. Five are named plasma-specific because they appear regularly in the plasma at a higher level of activity than in the tissues from which they are derived: cæruloplasmin, pseudocholinesterase, the lipase for lipoprotein, and the enzymes which convert, respectively, plasminogen into plasmin, and prothrombin into thrombin. The other 42 are the excretory enzymes such as the phosphatases, and the numerous cellular enzymes, representing virtually all levels of intra-cellular activity.

There is a discussion on the identification of enzymes. This may be done, for example, by determining their primary, secondary, tertiary or even quaternary structure, or by investigating function, be it activity, kinetics, thermodynamics, or biological parameters such as genetic determination and immunological properties. The clinical section discusses the diagnostic value of enzyme estimation for a growing list of diseases of different organs. They include: heart, liver, pancreas, muscle, blood, kidney, bones and the nervous system. Special applications are described for diseases of children and for gynæcological problems. One is surprised to find in a work with such emphasis on theory so many sound rules of thumb and practical guidance for the clinician.

Though the author has attempted to be selective with his quotations of literature, there are 587 references in a book with 141 pages of text. Inevitably there cannot be much detailed discussion and evaluation. Nevertheless, this is a stimulating monograph which brings a new approach to a rapidly expanding section of clinical biochemistry. H. LEHMANN

PATHS OF INFLUENCE IN MEDICAL THOUGHT

The Growth of Medical Thought

By Lester S. King. Pp. xi+254. (Chicago and London: University of Chicago Press, 1963.) 5.50 dollars; 41*s*.

THERE are many ways of writing medical history. For example, the approach may be chronological or biographical, that is, illustrative by the lives of pioneers like Harvey, Simpson, Lister, Ehrlich and Fleming who have advanced knowledge and therapy by their work and discoveries.

The method of approach selected by Dr. King is that of the concepts and ideas that have influenced medicine. In the course of this investigation he also makes use of the chronological and biographical methods, although, as he explains, his particular selection of eras and authors is purely arbitrary. Above all, he urges his readers to remember, first, that these scientists of the past were of equal intelligence to those of to-day, and, secondly, that their doctrines and explanations, however erroneous, must be evaluated in the light of knowledge then available.

The book begins with the religous aspect, instancing the epidemic among the Greek forces besieging Troy which was ascribed to the arrows of Apollo in the *Iliad*. A similar explanation was given to outbreaks of pestilence among the Anglo-Saxons as due to elf-shots, arrows from malignant elves. Indeed the belief that diseases generally are a punishment for sin is held by many up to the present day.

From Homer onwards, through Asclepios, the doctrines of Hippocrates and his School represent the scientific medicine which co-existed for a long time with the religious and magical beliefs of the Greeks. Here there was thoughtful observation and deliberate reflexion. It is not very important, says Dr. King, that much of it was wrong by to-day's standards. The association with stagnant water in marshes and lakes was good observation, although it was not realized that they were the breeding places of the mosquito *Anopheles*. The concept of the four humours—the blood, phlegm, yellow bile and black bile—which endured for many centuries was partly observation, but mainly based on mistaken inference.

Galen is the next exemplar, and he is now recognized as a keen observer and experimentalist. Many of his theories and inferences were wrong, but his voluminous writings advanced and consolidated medical knowledge. Because his work and doctrines were regarded as truths for all time well into the sixteenth century, he should not be blamed for this lack of progress.

For the philosophical approach, Dr. King describes the work of Paracelsus at some length. Yet Paracelsus had little influence on medical thought in his own time and for long afterwards. It is only of recent years—and especially through Pagel's recent investigations—that the work of this physician-philosopher towards medical progress has come to light, for the mists of magic, alchemy and astrology long obscured it. As to Vesalius, Dr. King praises the *De Fabrica* as a work of genius, but observes, as Prof. C. D. O'Malley has shown in his recent biography of the great anatomist, that others had previously developed anatomical investigations and paved the way for his insight and initiative.

In considering William Harvey's work, Dr. King deals solely with the discovery of the circulation of the blood. This has overshadowed Harvey's other original work as a public health administrator and adviser to the State, a pioneer in embryology and neurology, a forerunner of Wallace and Darwin, the founder of scientific midwifery, a planner of the first pharmacopœia, an epidemiologist, an advocate of smoke abatement, one who first showed that contagious and infective disease spread through the blood, and whose prophetic vision extended even to the threshold of bacteriology.

Friedrich Hoffman and his theories are discussed at some length. The last chapter, entitled "The Cell Theory: Key to Modern Medicine", is of special interest, for Dr. King is a pathologist. Introduced by an account of the great Boerhaave, the exemplars here are Schwann, Rokitansky and Virchow. The epilogue to this scholarly book ends with the view that the medical doctrines of to-day will be the discarded theories of to-morrow. This, of course, is true to a large extent, but, as this book reveals, despite much confusion and error throughout the history of medicine, the path of advance to fuller knowledge is unmistakable. ARTHUR MACNALTY

RATIONAL VIROLOGY

Viruses of Vertebrates

By Sir Christopher Andrewes. Pp. xi+401. (London: Baillière, Tindall and Cox, 1964.) 63*s*. net.

BEFORE the Second World War, when the isolation and identification of viruses were still part of the search for the causes of disease, there was no need to give them any names apart from those of the conditions for which they seemed responsible. So accounts of the viruses became books about virus diseases first, and catalogues of related viruses second; the parasites themselves were lumped together as bacterial, plant, or animal viruses according to which department of the kingdom of living organisms they happened to infect, rather than as the expression of any relationship between the viruses assigned to each group. The great expansion of virus research during and after the War, in particular the revolution brought about by the introduction of tissue-culture techniques to virology, has now made such a classification neither convenient nor necessary. Many viruses exist the clinical manifestations of which, if any, are unknown, and many syndromes are known to be the work of more than one virus. So the time is ripe for an attempt to classify viruses on the basis of more fundamental properties than their ability to cause disease. No one could be more fitted to make this attempt than Sir Christopher Andrewes, who has been actively investigating viruses throughout this formative period during which virology has progressed from being a special corner of bacteriology to become a science in its own right which can perhaps shed light on the roots of the whole of biology. So it is not surprising that he has produced a book that will be as important to the virologist as Bergey's *Manual* to the bacteriologist, or Bentham and Hooker to the amateur of wild flowers.

Sir Christopher groups viruses into broad divisions, based mainly on physical and chemical properties. These include the possession of a cubical or helical symmetry of protein sub-units, and whether or not there is an outer envelope enclosing this more rigid structure. This last-mentioned seems to be correlated with a chemical attribute, namely, sensitivity to destruction by ether, bile salts and certain detergents. Another chemical criterion, the possession of a single type of nucleic acid, serves to divide viruses into two main groups containing either DNA or RNA, and also to exclude the psittacosis–lymphogranuloma–trachoma group, which contains both. Pathological properties are considered only in so far as they are necessary to identify a virus, or indicate its economic importance. When applied to viruses infecting vertebrates such criteria reveal the existence of broad groups with a wide similarity in structure, behaviour and habitat. Among them are the myxo- and picorna-viruses, the homogeneity of which invites guesses about a possible underlying unity in the chemistry of the cells they infect; cells may only possess a limited set of tools for processing their environment, and there may be only a few ways in which viruses can be constructed to exploit them.

To generate speculation is, of course, one of the virtues of any scheme that is more than a successful key to the identification of species. Good classifications express patterns the significance of which may not be suspected at the time they are devised. Thus the Linnean system contained the seed of the evolutionary tree, and the Periodic Table of the elements exposes underlying structural patterns among the atoms which comprise it. By these standards, Sir Christopher Andrewes's classification is an exciting one, and it would be petty to carp about details like his acceptance of dubious criteria—such as the results of acridine orange staining—to determine the nucleic acid type of a virus. Where better evidence is not available, we must accept the data we have. This book also shows admirable caution in refusing to assign to a group any virus about which there is doubt, and in admitting that some of the groups may still be artificial collections of unrelated agents. One can only praise the author's refusal to foist on us a binomial system of virus nomenclature such as Holmes and Zhdanov have suggested. But one wishes at times that Sir Christopher would throw caution to the winds. One can see the necessity of restricting the length of this book, but it is a pity that, while attempting a rational classification, he should restrict himself to viruses of vertebrates. This is only a slightly narrower pigeonhole than the old 'animal viruses' and has the same fault of grouping viruses by their hosts. A bold system of classification, like that proposed by Lwoff, Tournier and Plissier, seems at first to bring together seemingly unrelated viruses infecting plant and animal cells, but may actually reveal an unsuspected

truth—that there are indeed only a few ways in which viruses can be constructed. One hopes that Sir Christopher, in his next book, will attempt a broad survey of all macromolecular 'particles' with infective properties. It might show up some interesting resemblances between, say, certain insect viruses and arbo- or plant-viruses able to multiply in their insect vectors. In the meantime, *Viruses of Vertebrates* deserves a place on the bookshelf of every virus laboratory. F. K. SANDERS

BREEDING BETTER RICE

Genetics and Breeding of Rice

By Prof. M. F. Chandraratna. (Tropical Science Series.) Pp. ix+389+12 plates. (London: Longmans, Green and Co., Ltd., 1964.) 70*s*.

RICE is the staple food of more than half the World's population. It so happens that many of the people who depend on it inhabit the newly developing countries and in these parts of the World diets are often inadequate. Compared with other important staple cereals the scope for crop improvement through rice breeding is large, particularly within the tropical *indica* sub-species where the range of variation in morphological and physiological characters is very wide. The *indica* group has been much less thoroughly worked by plant breeders using modern methods than most other important staple cereals, and the large-scale exploitation of the variation derived from *indica* × *japonica* crosses has only recently begun. There is little doubt that big increases in rice production can be made with little capital expenditure by breeding and distributing improved seed.

Apart from any academic interest, therefore, the publication of a new book on rice breeding is an event of potential economic and humanitarian importance. Prof. Chandraratna, in the preface to his new book, acknowledges his debt to other recent books on rice breeding, but there is little doubt that his own work represents an original and considerable advance on these. His attractive and critical approach makes the book very readable; no section is merely a catalogue of information. It is well produced with excellent line-illustrations. Unfortunately, about half the photographs are of poor quality and sometimes rather pointless; it is to be hoped that they could be replaced in a later edition. The bibliography includes papers not referred to in the text. Such a list is of doubtful value unless it is substantially complete, and this list excludes several important recent publications; it might have been better to have restricted it to text references.

The book begins with a succinct account of the origin and taxonomy of rice. This is followed by chapters on morphology, hybridization techniques, cytology and cytogenetics, sterility, and the genetics of morphological characters. Most of the remaining chapters are of immediate practical importance and they discuss the genetics of metric and physiological characters, photoperiodism, linkage groups, hereditability and interrelations of metric characters, principles and procedures of pure line and mass selection, pedigree and bulk-population methods, fertilizer response, breeding for disease resistance and cooking quality, induced-mutation breeding, and seed improvement and certification.

One of the most useful chapters in the early part of the book is a description of techniques of hybridization. This is the best illustrated account which has so far been published. One minor criticism, however, is that it is stated that poor germination is obtained after using emasculating techniques which result in seed deformity. This ignores Carpenter's recent work which shows that excellent germination is obtained with these methods if the seeds are afterwards germinated under sterile conditions.

It is now becoming obvious that photoperiod responses are extremely important in rice agronomy, and it is necessary for rice breeders to pay a great deal of attention to them. The chapter on the photoperiodic control of flowering is well written, as one would expect, since Prof. Chandraratna was the first to emphasize the quantitative nature of the response in rice and show the importance of constructing photoperiodic response curves. However, this close personal contact with the problem seems to have led to insufficient emphasis being placed on later work, particularly that of Best, whose main monograph[1] (not referred to in the bibliography) provides the clearest and most comprehensive description so far available of photoperiodism in rice. Prof. Chandraratna finds it hard to relinquish his original concept of defining response curves in terms of second-degree polynomials, in spite of the fact that Best's recent experimental curves bear little relation to this ideal. Part of the attraction of this concept was the use of an objective criterion (the rate of change of curvature of the second degree polynomial) for the assessment of photoperiod sensitivity. In this chapter the inadequacy of this criterion is quite properly pointed out (it had already been demonstrated by Best). Prof. Chandraratna now suggests that the best measure of photoperiod sensitivity is provided by the linear regression coefficient of that section of the curve which covers the range of variation of natural daylengths. But this would also seem to be a doubtful procedure, since Best's work shows that the curves in this region do not approximate to a straight line except in the case of very sensitive varieties.

The important chapter on fertilizer response includes an interesting discussion of crop yield in terms of growth-analysis concepts. The basis of this discussion is the well-known hypothesis that lower yields are normally experienced in the tropics because the higher temperatures increase respiration ($Q_{10} \approx 2$) but not photosynthesis ($Q_{10} \approx 1$); dry-matter production represents the balance between photosynthetic gain and respiratory loss. This is a neat explanation and, because it is, there is a tendency to accept it too readily as fact. Such acceptance could discourage work in a field where it is urgently required. The hypothesis may be true but it assumes rather too easily that respiration represents total loss without explaining what happens to the energy provided by it (or is it implied that it is all lost as heat?). Might not the energy be used to produce the higher growth rates which Njoku has shown to exist in other plants under tropical conditions? Thus the dry-matter loss due to respiration might be balanced by higher leaf-area indices and thus more photosynthesis. Another possible explanation to account for the differences in yield of rice in the tropics compared with the sub-tropics might be found in the intrinsic differences between the tropical *indica* varieties and the sub-tropical *japonica* varieties. As Prof. Chandraratna points out later on, there is evidence that *indica* varieties have a higher crop-growth rate than *japonicas* but that the *japonicas* convert much more of the dry-matter to grain than the *indicas*. Here, obviously, is the basis of a clearly posed research problem of immense importance. The scientific value of phytotrons in relation to their cost is questioned in some quarters, but the operators of one of the new installations could find ample justification for their facilities in the exploration of this important economic problem.

There is not space here to do full justice to Prof. Chandraratna's book, but the few controversial points raised here indicate the stimulating level at which it is written. It will be a most useful source of information and inspiration to all rice breeders—particularly to those who work under the sometimes trying conditions associated with rapidly developing agricultures.

E. H. ROBERTS

[1] Best, R. (1961). Some Aspects of Photoperiodism in Rice (*Oryza sativa* L.) (Elsevier Publishing Co., Amsterdam).

Advances in Food Research

Vol. 12. Edited by C. O. Chichester and E. M. Mrak. Pp. ix+433. (New York and London: Academic Press, 1963.) 100*s*.

IN this twelfth volume of a valuable series of reports on technological and scientific advances in knowledge of food handling the editors have collected seven contributions from experts of varied experience and nationality. In a paper on chemistry of non-enzymatic browning, T. M. Reynolds of the C.S.I.R.O. Division of Food Preservation, Australia, not only discusses the reactions at present known between aldoses and amines in concentrated and dried foods but also poses a series of important questions which call for further investigation.

Osmophilic yeasts, which cause spoilage of dried fruits, concentrated fruit juice, honey, maple syrup, curing brines and salted meats, are reviewed by Hiroshi Onishi of the Noda Institute for Scientific Research, Chiba-ken, Japan, with special attention to the characteristic tolerance of high concentrations of sugar and salt by such yeasts. It is emphasized that further work on the genetic origin of these high tolerances is needed.

Transport and storage are the subject of two papers from the United Kingdom. W. Hugh Smith, of the Agricultural Research Council Ditton Laboratory, assembles the present information on the use of carbon dioxide in the transport and storage of fruit and vegetables, and K. C. Hales, of the Shipowners Refrigerated Cargo Research Association, Cambridge, reports on designs in refrigerated shipping and on types of cargoes and their particular requirements.

A more recondite problem is set forth by J. S. Pruthi from the Central Food Technological Research Institute, Mysore, India. He discusses the physiology, chemistry and technology of passion fruit. This fruit has had a limited market owing to its relatively short storage life. The paper gives a most interesting survey of improvements in handling and in the preparation and use of passion fruit products for man and for animal feeding.

From the Technical Centre of the U.S. General Foods Corporation, Martin Glicksman contributes a most useful account of the utilization of synthetic gums in the food industry. Advances in the preparation of cellulose derivatives, polyvinyl compounds and other types of synthetic gums and resins have made this an increasingly important field of food technology, and this paper deals with nutritional and technical aspects.

The final paper, by Eiichi Tanikawa of the Laboratory of Marine Food Technology, Hokkaido University, Japan, is concerned with the fish sausage and ham industry in Japan. It is fascinating to discover how fish can be reddened and processed to form 'ham'. The handling and storage of processed fish foods is considered in detail.

There are certainly more components in food than man may dream of, and these papers help to widen the views of all who are interested in the problem.

A. M. Copping

The Production and Uses of Natural Graphite

By A. R. Tron. (Department of Scientific and Industrial Research: Warren Spring Laboratory.) Pp. viii+83. (London: H.M. Stationery Office, 1964.) 5*s*. 6*d*. net.

THE underlying theme of this instructive little book is to present a re-appraisal of the economic value and intrinsic properties of the natural mineral graphite in face of the ever-increasing tendency to use artificially produced material. The versatility of natural graphite in the arts is perhaps not generally recognized. Most of the world's production, about 70 per cent, is consumed by the metallurgical industries, in crucibles, foundry facings, moulds, retorts, pouring nozzles, refractory bricks and other similar articles. In the manufacture of lubricants, brushes for electrical machinery, batteries, pencils, stove polishes, electrodes, explosives and paints, to mention a few other applications, natural graphite plays a prominent part, while one of the most modern uses explored by the West German graphite industry is in the production of graphite blocks for nuclear purposes.

The author's argument is that this great variety of usage of natural graphite, coupled with variations in degree of purity, has tended to a lack of understanding between producers and industrial users of the mineral; this is further accentuated by the absence of any universally accepted methods for testing and classifying such desirable qualities as durability, flakiness, and particle toughness. The conclusion is drawn that in these circumstances many users are over-specifying purity of the raw graphite involved to the detriment of varying grades of the natural mineral which may be potentially satisfactory for the product concerned; in some instances these impurities would have no critical effect on performance.

That there is a serious deadlock in the industrial situation as regards production and consumption of natural graphite at the present time is abundantly clear from the author's findings. Producers want to be assured well in advance of planned exploitation of a long-term market of particular grades satisfactory to users; the latter, however, appear reluctant to adjust formulæ and manufacturing techniques to suit new graphite producers; meanwhile competition resulting from increasing use of less expensive forms and grades of artificial graphite is growing. "If it is to compete effectively with this new trend, the natural graphite industry in all parts of the world will have to be prepared to support a more extensive research effort to discover and develop new outlets for natural graphite, and to improve technological preparedness." This book is commendable for the impartial manner in which the facts are presented, and it merits the close study of producer and user of graphite alike.

H. B. Milner

British Transistor, Diode and Semiconductor Devices Data Annual, 1963–64

Edited by G. W. A. Dummer and J. Mackenzie Robertson. Pp. lxxi+1610. (London and New York: Pergamon Press, 1963.) 200*s*. net.

British Miniature Electronic Components Data Annual, 1963–64

Edited by G. W. A. Dummer and J. Mackenzie Robertson. Pp. xviii+1356. (London and New York: Pergamon Press, 1963.) 140*s*. net.

American Subminiature Electronic Component Parts Data Annual, 1963–1964

Edited by G. W. A. Dummer and J. Mackenzie Robertson. Pp. xiii+679. (London and New York: Pergamon Press, 1963.) 140*s*. net.

British Miniature and Microminiature Electronic Assemblies Data Annual, 1963–64

Edited by G. W. A. Dummer and J. Mackenzie Robertson. Pp. xii+335. (London and New York: Pergamon Press, 1963.) 105*s*. net.

THE increasing importance of electronics in technology is exemplified not only by the number of text-books and manuals dealing with the subject, but also by the number and size of the publications furnishing data on the range of transistors and other semi-conductor devices and electronic components which are available.

In the series of electronic data annuals published by the Pergamon Press, there are two new volumes: the second volume of *British Transistor, Diode and Semiconductor Devices Data Annual* and the third volume of *British Miniature Electronic Components Data Annual*. Under the same editorship, that of G. W. A. Dummer and J. Mackenzie Robertson, are also two companion volumes, *American Subminiature Electronic Component Parts Data Annual* and *British Miniature and Microminiature Electronic Assemblies Data Annual*.

These books collect, into compact individual volumes, manufacturers' data sheets and basic information on electronic components, and in addition provide, where appropriate, sections dealing with operating principles, applications and design methods. They are, in fact, compendia of electronic techniques. The annual dealing with British microminiature electronic components and assemblies is, in fact, a new book, based on the "Assemblies" section which previously formed part of the *British Miniature Electronic Components Data Annual*. The increasing use of thin-film solid-state units and of printed-circuit techniques has made it necessary to devote more space to miniature and microminiature electronic assemblies.

This series of annuals forms a source of technological data and information on electronic techniques which is remarkably comprehensive. The books are well indexed and the information therefore readily accessible. The series forms a most valuable day-to-day reference for research workers and designers concerned with electronic equipment, and should serve a most useful purpose when placed, perhaps, in the research laboratory or workshop rather than in the library. J. GREIG

Controlled-Potential Analysis

By Garry A. Rechnitz. (International Series of Monographs on Analytical Chemistry, Vol. 13.) Pp. 85. (Oxford, London, New York and Paris: Pergamon Press, 1963.) 35*s*.

AN electrolysis that is carried out with the potential of the working electrode fixed at a selected value is a controlled-potential electrolysis. When such an electrolysis is used in analysis it is controlled-potential analysis. Most commonly in analysis, the substance being analysed is determined by weighing the amount deposited at the working electrode, that is, controlled-potential electrogravimetric analysis. Less commonly, the amount is determined by measuring the quantity of electricity that passes during the electrolysis, that is, controlled-potential coulometry.

It is a pity that the author of this monograph has, throughout the book and especially in Chapter 1, "Introduction", used these terms at random. In fact, after reading the book, the reader is left wondering whether the author intended to write about controlled-potential analysis generally or about controlled-potential coulometry in particular.

Chapter 2, "Theory", deals with the elementary theory of controlled-potential coulometry and its application to analysis and kinetic investigations. Secondary reactions are also discussed. In Chapter 3, "Apparatus and Techniques", the ideal characteristics of potentiostats are listed and described, and five commercial instruments are appraised. Current integration by graphical, mechanical and electronic means, and the electrodes and electrolysis system, are discussed. In the final chapter, "Analytical Applications", some 35 elements are treated together with the alkali metals, the rare earths and the halides.

This work is intended for practising analytical chemists. Many would find it, in spite of the confusion of terms, a ready source of information on controlled-potential analysis. J. F. C. TYLER

Teaching Machines and Programming

Edited by K. Austwick. Pp. vii+201. (Oxford, London and New York: Pergamon Press, 1964.) 42*s*.

THIS book deals with a relatively new venture in the field of instruction. Programmed learning is the label now accepted for a group of techniques in which subject matter is presented to the individual in an orderly series of 'steps', with future progress made in some way dependent on past success, and in which the individual can be informed about the success with which he has tackled one step before proceeding to the next. Programme presentation can vary from the traditional live teacher to time-sharing computers, with simple pencil and paper devices and more complex electro-mechanical machines in between. Programmes may be written to cover very small parts or the whole of a syllabus.

By the beginning of 1963, when the six contributors wrote their chapters, industry, schools, services and universities on both sides of the Atlantic had accepted programmed learning as a useful medium in an era where demand for instruction had outstripped supply. The questions now raised are centred primarily on ways and means: Under what conditions, and for what kind of subject-matter, is programmed learning suitable when judged by a number of criteria? *Teaching Machines and Programming* answers some of these questions as well as giving the necessary background information. The writers have all had practical and research experience in programmed learning in Britain, and one common theme is the extent to which laboratory and real life merge in this field.

In Kay's searching introduction no stitches are dropped and no punches pulled. Annet's chapter includes valuable cost-accounting. Sime stresses the use of teaching machines as a research tool. Pask states the case for completely automated teaching. Reid's robust chapter is an enjoyable exposition of Skinner's ideas, pure and applied. The editor discusses the possible uses of automation in schools.

The decision to make the chapters self-contained produces much repetition of general concepts. Some of the plates are poor, and many give little idea of scale. The index and references are good. J. A. LEONARD

Comprehensive Biochemistry

Edited by Prof. Marcel Florkin and Prof. Elmer H. Stotz. Vol. 15: Group-Transfer Reactions. Pp. xii+246. (Amsterdam, London and New York: Elsevier Publishing Company, 1964.) 70*s*.

COMPREHENSIVE Biochemistry is one of a section of five volumes devoted to an examination of enzyme catalysis at the molecular level. The reader must not expect, therefore, to be presented with an integrated account of metabolic processes; this will presumably follow in a later section of this treatise. Six chapters describe the present state of our knowledge of those biochemical reactions in which a group is transferred from one substrate or co-enzyme to another. The transferred group may be as small as a methyl group or as large as an oligosaccharide residue. The reactions which are discussed in this volume include the transfer of formyl, hydroxymethyl and methyl groups (S. Harvey Mudd and G. L. Cantoni); reactions catalysed by transketolase and transaldolase (B. L. Horecker); transfer of acyl groups (P. Goldman and P. R. Vagelos); the transfer of glycosyl groups (L. Glaser); transaminations and decarboxylations in which vitamin B_6 is implicated (B. M. Guirard and E. E. Snell); and the transfer of phosphate groups catalysed by phosphokinases and phosphomutases (R. K. Crane, C. F. Cori and D. H. Brown).

Reading this volume and the others in this series dealing with biochemical reaction mechanisms emphasizes the dearth of our knowledge about the early stages of an enzyme-catalysed reaction. Investigations of pre-steady-state kinetics and the conformational changes induced in an enzyme by the approach of a substrate are virtually unexplored territories. Two features of *Comprehensive Biochemistry* must be commended. In contrast to some of the earlier parts of the treatise, this volume is not badly out of date; there are a few references as recent as 1963. Secondly, several of the authors have included a lot of detailed information in tabular form. This simplifies the reading for both the expert and the person who requires a background of modern enzymology. D. T. ELMORE

SCHIZOPHRENIA AS A GENETIC MORPHISM

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IT now appears clear that schizophrenia, at least in the great majority of cases, is based on a single partially dominant gene with low penetrance[1–3]. We wish to put forward the hypothesis that it involves a genetic morphism, a conclusion independently reached by two of us (J. H. and E. M.).

As is now agreed among evolutionary geneticists (for example, Mayr[4a] and Ford[4b]), all genetic characters which exist in a population at a higher frequency than can be maintained by mutation alone involve morphism[5]; the frequency of a morphic gene is the result of a balance between its selectively favourable and unfavourable properties.

(1) In schizophrenia there is abundant evidence of a strong genetic component.

(*a*) In single families, only certain children become schizophrenic.

(*b*) Monozygotic twin-pairs show a concordance rate of 76–91 per cent, dizygotic pairs only 10–17 per cent (for example, Kallmann[6,7], Shields and Slater[8]).

(*c*) An examination of schizophrenics and normal sibs of the same sex and less than 2 years' difference in age, whose mothers were not schizophrenic[9], showed that the future schizophrenics and their sibs manifested marked personality and temperament differences from an early age, and that these could not be associated with environmental influences, including psychological trauma. The future schizophrenics were more dependent and less able to deal with traumatic events: they were *schizoprone*, apparently from birth.

(*d*) The work of Böök[1,2] and Slater[3] on the morbid risk (likelihood of developing schizophrenia) in different relatives of schizophrenics indicates that the usual genetic basis for schizophrenia is a single dominant gene which we will call Sc, with about 25 per cent penetrance, that is, it only induces manifest schizophrenia in about a quarter of the cases where it is present in the genotype. Non-manifestation of the disease in the 75 per cent of Sc-carriers is due to the effect (1) of the genetic environment—minor genes affecting manifestation; (2) of the external environment—familial, social, cultural and physical.

(2) (*a*) Wherever reasonable statistics are available, the incidence of manifest schizophrenia is at least 1 per cent in all racial and ethnic types[10] and probably in all social classes (some authors maintain that there is an increase in the lower strata; but others have been unable to confirm this).

The only considerable exception is the 3 per cent incidence found by Böök[1] in an area north of the Arctic Circle in Sweden. This high figure is doubtless due partly to inbreeding in an isolated but physically dispersed population. Dr. Eliot Slater has further suggested to us that it is correlated with the nature of the environment. We would add that schizophrenics are more likely to be able to lead a normal life in a socially simple and physically harsh environment of this type, and one where they are necessarily withdrawn from much contact with their fellow human beings, and where husbands will not trouble very much about their wives' mental peculiarities, provided they are physically resistant and capable of elementary domestic skills. In addition, it may be suggested that the high incidence may be wholly or partly due to a higher penetrance (manifestation) of the Sc gene in a harsh environment: further investigations of the degree of penetrance in different types of environment would be of great interest.

(*b*) The high frequency of schizophrenia cannot be maintained by mutation alone, and is evidence of a balanced morphism. The fertility (reproductive fitness) of schizophrenics is only about 70 per cent of that found in socio-economically comparable normals[11]*. The incidence of the disease would therefore be rapidly reduced to the level where it is maintained by mutation alone, unless its selective disadvantages of lower viability and fertility were compensated by some selective advantage.

(*c*) There are two possible types of compensatory advantage:

(1) *Physiological.* Overt schizophrenics are extremely resistant to surgical and wound shock (and recover much more rapidly), to visceral perforation, to high doses of histamine (correlated with fewer mast cells in the skin), insulin, thyroxin and other physiologically active substances, to pain, to arthritis, to many allergies, and probably to many infections†[12–16]. One of us (H. O.) has seen a schizophrenic recover successfully from the most appalling burns which would have killed any normal person in hours or minutes‡. We suggest that crypto-schizophrenic Sc-carriers probably enjoy similar selective advantages, though presumably of lower magnitude.

Dr. Slater has suggested to us that in historic times resistance to mass epidemics of diseases like smallpox and

* Many morphic genes are lethal or semi-lethal when homozygous. In view of the drastic physiological effects of the Sc gene (see following) such lethality may well apply to it.

† But not to tuberculosis, where the schizophrenics' reduced ability to produce fibrous tissue reactions is a disadvantage. Further, since the incidence both of schizophrenia and of tuberculosis is positively correlated with Sheldon's ectomorphic (Kretschmer's leptosomic) type of body-build (see Harrison, Weiner, Tanner and Barnicot[17]), schizophrenics are more likely than normals to suffer from tuberculosis.

‡ Clarke[18] mentions some of these advantages of schizophrenia, and discusses the possibility of its being a genetic morphism, but without reaching a firm conclusion.

bubonic plague may have been a contributory factor. If the high frequency of the schizophrenia gene is in any considerable measure due to resistance to infectious diseases, especially of childhood, we may expect its steady decline in the more hygienic modern world.

(2) *Reproductive.* Male schizophrenics are certainly at a high reproductive disadvantage, through earlier onset, higher suicide rate[18], more frequent hospitalization, and failure to marry. Female schizophrenics, however, not infrequently marry, and seem to seek for affectional satisfaction through children: so their fertility may possibly be above average. In hæmophilia, a moderate increase (22 per cent) in female fertility is able to compensate for a high reproductive disadvantage (36 per cent) in males[20]. Nothing is known about the fertility of the cryptoschizophrenic Sc-carriers. This merits intensive investigation, as it could be of great importance for the frequency of Sc.

Presumably both the penetrance and the expressivity of the Sc gene are largely controlled by the residual gene-complex[21].

It will be of importance to be able to identify carriers of the Sc gene, as has been done with a number of other genes[22,23]. The simplest way would be by biochemical tests; but psychological tests would also be useful.

Many workers consider that the perceptual disorder characteristic of schizophrenics is due to a biochemical abnormality which affects the brain's sensory-perceptual integrative system. Much work is in progress on the subject. Symptoms closely resembling those of schizophrenia can be induced both by certain psychomimetic drugs[24], and by hypnotically induced alterations in perception[25,26].

Hoffer and Osmond[24,27] consider that the specific substance involved is adrenolutin, and claim that the urine of schizophrenics in 75 per cent of cases contains a substance giving a mauve reaction when tested by paper chromatography, while Friedhof and Van Winkle[28] claim a similar high frequency for a 'pink spot' chromatographic reaction produced by a closely related substance. If these claims are confirmed, and the methods improved, it might be possible to detect the responsible substance in the urine of non-manifest Sc-carriers.

Hoffer and Osmond (1961) have devised a battery of psychological tests which they claim are diagnostic of schizophrenia as against other psychoses, neuroses and, of course, normality (see also Lidz *et al.*[29]). If it proves possible to identify schizoprones in childhood by such tests, educational and other methods could be devised to prevent or mitigate the manifestation of the disease.

It is clear that schizophrenia constitutes not a purely medical or psychiatric but a biological problem, with opportunities for a combined attack in many fields—genetics, biochemistry, selection theory, psychology, psychiatry, public health, demography, social science, pathology, and general environmental and reproductive physiology.

In particular, we need further investigations: (1) of the selective advantage and disadvantage, both as regards survival (viability) and reproduction (fertility) of overt schizophrenics, non-manifest carriers of the Sc gene, and normals: and also better biochemical and psychological tests identifying the same types, and if possible applicable in childhood; (2) the heritable and environmental contributions to the manifestation of schizophrenia—the major gene (or genes) involved; its (or their) penetrance and expressivity, as influenced by modifiers and by environment; the possible influence of race, physical and cultural environment; and investigations of twins, sibs and other relatives: a comparison of same-sexed and opposite-sexed dizygotic twins would be especially rewarding. (3) Of the psychological and physiological effects of schizophrenia, overt and non-manifest, of psychomimetic drugs, and of induced alterations of perception.

Summary and Conclusions

(1) Schizophrenia probably depends primarily on a major dominant gene, Sc, with about 25 per cent penetrance.

(2) The overt schizophrenia-rate in all countries investigated is at least 1 per cent, much too high to be maintained by mutation alone. The Sc gene must therefore be in morphic balance, and must confer certain selective advantages to compensate for its obvious disadvantages, including a 30 per cent reduction of fertility.

(3) The physiological advantages are high resistance to surgical and wound shock, to otherwise dangerous concentrations of insulin and other hormones, histamine, etc., and to various allergies and infections. Cryptoschizophrenic Sc-carriers (and some overt female schizophrenics) may possibly have a higher than average fertility. Known or presumed Sc-carriers should be tested to ascertain whether they possess any degree of these advantages.

(4) The Sc gene appears to cause an error of metabolism, resulting in the formation of some substance interfering with the normal integration of perception.

(5) The temperamental and personality traits of future schizophrenics in most cases show, from early childhood onwards, distinct differences from those of permanently normal sibs of the same sex and similar age.

(6) Chromatographic tests are beginning to provide evidence of detectable 'mauve' and 'pink' chemical factors in the urine of a large majority of schizophrenics, and suitable psychological tests promise to differentiate schizophrenics from other psychotics, neurotics and normals.

(7) If such tests can be further improved, schizoprones and future schizophrenics might be diagnosed in childhood and appropriate educational and possibly medical measures taken to prevent or ameliorate overt manifestation.

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GENETICAL RESEARCH IN AN AREA OF HIGH NATURAL RADIOACTIVITY IN SOUTH INDIA*

By Prof. H. GRÜNEBERG, F.R.S.

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In wild populations of animals and plants, new genetic variance constantly comes into being by the process of 'spontaneous' mutation. Nearly all mutations upset the smooth working of the organism to a greater or lesser extent. They thus become the target for natural selection which sets a limit to their spread. An equilibrium is thus established between mutation and natural selection, and the genetic variance of a population is in a steady state.

Since the pioneering work of Muller in 1927, it has been known that mutations can be induced in all animals and plants by means of X-rays, and it has since been shown that other radiations like γ-rays emitted by radioactive substances or the cosmic rays from outer space have a similar effect. Generally speaking, the mutagenic effect is proportional to the dose administered. Whereas this relationship is comparatively easy to verify for medium and large doses up to a few 1,000 r. units, it becomes very difficult for small doses of radiation. However, by a heroic effort which involved the scoring of well over a million individuals, the linear relation between dose and mutation rate has been verified down to 5 r. units[1] in *Drosophila melanogaster* and down to even smaller doses in micro-organisms. A dose of 5 r. units is roughly what a human being, in a Western country, accumulates in the thirty years of his reproductive life due to natural radioactivity, cosmic radiation and the activities of the diagnostic radiologist. It is thus a dose which has to be taken seriously.

The question of genetic damage by radiation is essentially a medical problem. From the medical point of view, we are less interested in the occasional accident with a large acute dose of radiation. On the other hand, if the horrors of a thermonuclear war should ever be let loose on humanity, its genetical aftermath would probably be rather trivial by comparison with its immediate effects. The real danger which is with us now and likely to remain with us indefinitely is the exposure to small doses of radiation over long periods. This may happen as the result of contamination of our environment with radioactive substances and thus involve whole populations over many generations; similarly, large numbers of people may be exposed to increasing industrial risks in our changing atomic technology.

In the laboratory, it is comparatively simple to investigate the immediate genetic effects of large doses of radiation administered acutely. The long-term effects of small doses of radiation administered at a low dose-rate over many generations are far too complex for mathematical treatment and virtually beyond the scope of laboratory experiments, at least with mammals. What we want to know is this: If the mutation rate is increased as the result of radiation, will the genetic variance of a population be raised to a higher level? Or will natural selection be able to take care of the extra mutations so that the equilibrium stays where it normally is? For medical purposes, data on mammals are essential. Though all animals behave alike in principle, there may be great quantitative differences: for example, it is known that the mouse is many times more sensitive to the genetic effects of radiation than *Drosophila*[2]. Now the establishment of a new equilibrium is a slow process which takes a great many generations, and for the mouse it would take at the very least fifty years, and more probably a century or more.

Fortunately, Nature herself has carried out such an experiment for us. There exist a few areas in the world which have a high natural radioactivity. One of these areas is on the Malabar Coast of India, in the State of Kerala, and about 250 km south of Calicut where Vasco da Gama made his landfall in the year 1498 (Fig. 1). North of the small town of Quilon, there exists a narrow coastal strip some 14 miles long which is highly radioactive. The carrier of the radioactivity is black monazite sand. By a kind of 'panning' action, as in panning for gold, the waves of the Arabian Sea have here thrown up a mixture of heavy minerals which are ultimately derived from igneous rocks inland. One of these minerals is thorium phosphate; thorium is a radioactive element with a very long half-life ($1{\cdot}39 \times 10^{10}$ years). Monazite sand is found in varying quantities from Cape Comorin for about 200 km north. Its greatest concentration occurs in Manavalakurichi, just west of Cape Comorin, in the State of Madras, and on the strip north of Quilon in both of which places it is mined (opencast mining). The strip north of Quilon is an island which is separated from the mainland by backwaters which are brackish and which form the main avenue of communications between Trivandrum in the south and Cochin and beyond in the north. Whereas coastal navigation is interrupted during the monsoon seasons, the backwaters can carry traffic all the year round.

The scenery of the strip with the lagoon behind it is most attractive. Except for the beach, the strip is largely covered by coconut groves. Under the trees stand the huts of the natives, each in its own little compound. Many of the huts are very primitive and are constructed entirely of mats made of palm fronds: some stand directly on the sand, others have a base of laterite or of concrete. There are about 60,000 inhabitants of the strip, most of them fishermen and coconut growers, along with some workers in the monazite sand factories. The occupation of the strip is almost continuous from one end to the other.

Now, if a suitable mammal other than man lives on this strip, if it has lived there long enough for the establishment of genetic equilibrium between mutation and natural selection, and if the strip has been ecologically isolated long enough so that our animal—whatever it may be—was prevented from 'commuting' between strip and mainland, if these conditions are fulfilled, the strip north of Quilon would be a natural laboratory to answer the questions which have been posed.

In India, where there is man, there is the rat (the black rat, *Rattus rattus* L.). So a suitable mammal was at hand. The only other mammal which is present on the strip in any numbers is the mole rat or bandicoot (*Bandicota indica* Bechstein), a large rat-like animal which may weigh as much as 2 lb.; but we did not get it in numbers big enough for our purposes.

The field work was carried out by myself, two of my colleagues, Dr. R. J. Berry and Mr. R. A. Weiss, together with Mr. G. S. Bains, a young biologist attached to us by the Indian Atomic Energy Establishment Trombay. During the winter 1961–62, we collected in eight localities on the strip and eight control localities inland altogether nearly 900 rats, rather more than 50 per population. Measurements were afterwards carried out by members

* Substance of a lunch-hour lecture delivered in University College, London, on October 15 1964.

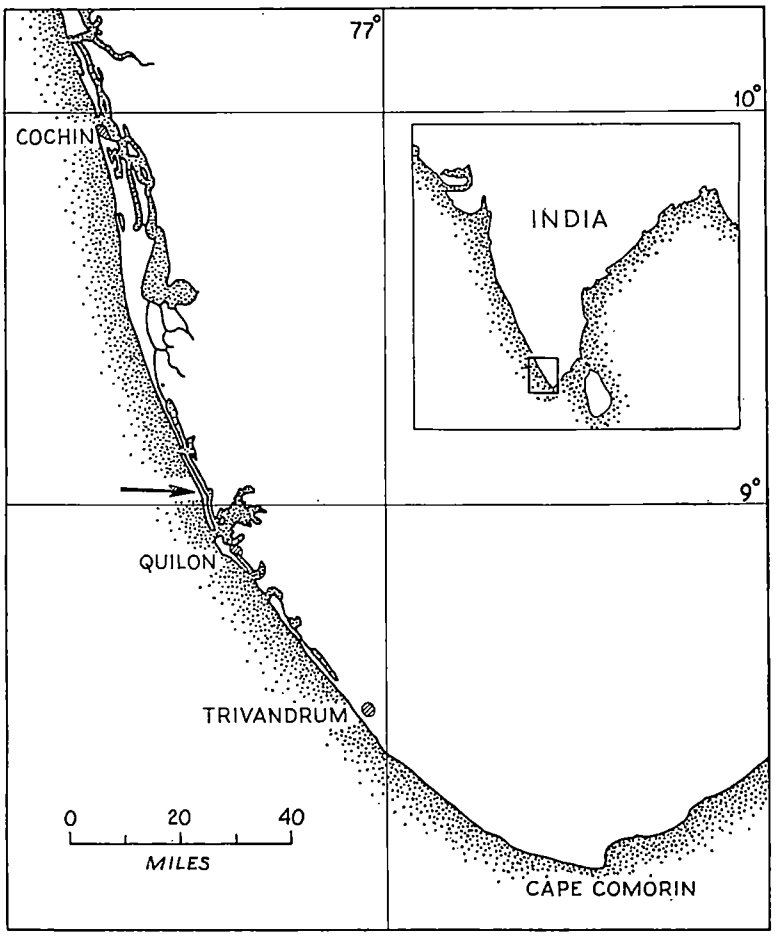


Fig. 1. Map of the Malabar Coast of India. The arrow points to the radioactive strip where the work described here was carried out

of that team, together with Mrs. Linda E. Riles. The statistical evaluation has been in charge of Prof. C. A. B. Smith. The investigation has thus been carried out by a team of six people, under the auspices of the Medical Research Council. The analysis has been completed this summer, and our report[3] has been submitted to the Medical Research Council for publication.

Returning to the question of since when the strip has been completely surrounded by water, the descriptions of the early Portuguese travellers, including Vasco da Gama himself who visited Quilon on his second journey in 1502, leave no doubt that this was so some 460 years ago. Even earlier, the great Moroccan traveller, Abū Abdullah Muhammad, commonly known as Ibn Battūta, found the same situation in 1343 when he travelled in those parts as Ambassador of the Sultan of Delhi, Muhammad bin Tughluq. Testimony to the antiquity of human settlements on this part of the Malabar Coast goes much farther back, and possibly they are already referred to by the Elder Pliny (who wrote about A.D. 75), though here the identity of the places mentioned is open to doubt. It is thus quite clear that the strip has been occupied by man, and by implication by the rat, for many centuries, and that the strip has been an island for at least 600 years or something like 800–1,000 rat generations. Clearly, our rats cannot be far from genetic equilibrium.

Next, we have to get an estimate as to how radioactive the strip really is. The distribution of the monazite sand is far from uniform. Rats can easily walk from hut to hut, and over the centuries they must have occupied the whole strip at random. As no global values for the overall radioactivity of the strip were available, the Indian Atomic Energy Establishment Trombay, at our request, carried out a very complete set of radiation measurements, with some 130 traverses from the Arabian Sea to the backwaters from one end of the strip to the other. There are two main gradients, one from the sea to the backwaters, the other from north to south. If these and local irregularities are smoothed out, the average radioactivity of the strip is about 7·5 times that of the control areas inland which were monitored at the same time. This is not as good as one could have wished. But the only area with a higher radioactivity, at Manavalakurichi near Cape Comorin, has no natural frontiers and the rats can walk to and fro as they please.

In absolute terms, individual rats on the strip, during the whole of their reproductive lives, accumulate about 1 r. unit of γ-radiation. That may seem rather a modest dose. However, the cumulative dose to which, say, 500 consecutive rat generations, in perhaps 300 years, have been exposed is of the order of 500 r. units, whereas the controls, during the same time, have accumulated a dose of only about 67 r. We set out to discover whether the chronic exposure to 7·5 times the normal amount of background radiation has any detectable genetic effects on the population.

Now one might expect that a search for genetic effects of radiation would involve breeding experiments. However, the black rat is not a laboratory animal, and in any event it is not easy to see how critical data could have been thus obtained. We therefore had to take the animals as we found them, comparing a series of 8 populations from the strip with a similar series of control populations. Selecting suitable characters known to be under genetical control, we had to find out whether there exist systematic differences between these two groups of populations. The skeleton and the teeth may be regarded as representative of the animal as a whole, and large numbers of preparations can be made rapidly by the papain digestion method worked out in this laboratory some fifteen years ago. We were very fortunate to enjoy the hospitality of the Sree Narayana College in Quilon, where a temporary laboratory was set up, complete with deep-freeze, incubator and all other equipment shipped from Great Britain.

In work of this kind, if too few measurements are taken, the material is used wastefully, but if too many are taken, there is a law of diminishing returns as biological measurements tend to be correlated with each other. One also has to take into account time and manpower available. In the end, we settled for six dental and 15 skeletal measurements; if the outcome had been ambiguous, we could always have made additional measurements on our skeletons. In addition, there was a group of some 26 minor variations of the skeleton which are mainly of the all-or-none kind such as whether or not a certain foramen in the skull is double rather than single, or whether there are fusions between cervical vertebræ; such variants are counted rather than measured, and their incidence may differ from population to population. Finally, the pregnant females which we trapped provided data on the number of living embryos, the number of dead implantations and the number of corpora lutea in the ovaries; from these can be calculated the rate of embryonic mortality before and after implantation in the uterus.

Now, given this kind of material, what would one be looking for to discover genetic effects of radiation, if any? Metrical variables are usually approximately normally distributed around a mean value which is characteristic for a given population. Populations may differ both in their means and in their variances, that is, the degree of scatter around the mean. If the genetic variance is increased as the result of a higher mutation rate, one may expect to find a higher proportion of individuals with more extreme deviations from the mean value, and correspondingly fewer near the middle of the distribution. In the case of the non-metrical minor skeletal variations, populations may differ as regards the incidence of any particular character; but if the data on all these variants are combined, a measure for the total divergence between any two populations, or groups of populations, can be obtained. On the other hand, we did not expect to find major skeletal abnormalities; these are much too rare to be found in samples of manageable size and in any event such animals tend to die before reaching adult life. None was in fact encountered.

The full results will be found in the report submitted to the Medical Research Council. Here we give a brief summary of the main findings, which is easy as all the results tell essentially the same story.

In the case of the dental measurements, there are significant differences between populations both on the strip and in the controls. Whereas the means may differ, the variances are essentially the same in all the populations. Genetical differences between rat populations from five grain shops in Delhi[4] had previously been discovered, and it was thus not surprising that similar differences between populations should also be found in Kerala. The reason for these differences is probably mainly if not entirely genetic drift (Sewall Wright effect) in the form of the 'founder effect'; that is, individual populations often trace back to small numbers of founder animals whose gene frequencies are the more subject to accidents of sampling the smaller their number. The important point is that although there are differences between populations both on the strip and among the controls, there is no sign whatever of systematic differences between these two groups of populations.

Much the same situation is found for the skeletal measurements. Again, there is a good deal of heterogeneity between the means of populations; but no sign that the two groups of populations differ systematically from each other. In this case, there is also some evidence of heterogeneity of variances though this is much less than that between means. However, there is no consistent pattern in these differences. For example, for one measurement, there is an increased variance in the control ♀♀ which is opposite to expectation. In another case, the increased variance occurs in one sex but not in the other, and in the third instance the effect on the sexes is in opposite directions. Clearly, then, neither as regards the means nor as regards the variances do strip and control populations differ systematically from each other.

Much the same situation emerges from the analysis of the minor skeletal variants. Again, there are highly significant differences between populations, both on the strip and in the controls, but no sign of systematic differences between the two groups of populations.

Finally, there are the data on fertility and embryonic mortality. Once again, there are differences between populations, but the overall fertility and embryonic mortality is just about the same in both groups of populations.

Naturally, there is the possibility that a systematic difference between strip and control rats as regards the characters investigated does, in fact, exist, but that we have failed to discover it as the result of accidents of sampling. If our results in the least pointed in that direction, one might think that larger samples are required to prove the point. But in the complete absence of even straws in the wind, it looks as if our negative results should be accepted at their face value. If so, we now have to discuss how that is to be interpreted.

The rats on the strip are exposed to the extremely low dose-rate of 0·18 mr./h. The lowest dose-rate which has been investigated experimentally (in the mouse[5]), and which is known to produce mutations, is 60 mr./h (= 1 mr./min) and thus some 300 times as high. It is at least conceivable that γ-rays, at the extremely low dose-rate to which the rats on the strip are exposed, do not produce mutations. In view of what is known about the mutagenic mechanism of ionizing radiations, this is not a probable assumption.

If, then, we assume that mutations have, in fact, been induced in the rats on the strip, our negative findings can be interpreted in two different ways which are not mutually exclusive. We may conclude that the production of mutations has been accurately counterbalanced by natural selection so that the level of genetic variance (as inferred from the phenotypic variance) has remained constant. Though our data on fertility and embryonic mortality do not, in themselves, suggest the operation of selective processes, they certainly do not exclude the low level of selection required to achieve equilibrium.

Alternatively, we may assume that the genetic variance has, in fact, increased on account of the radiation, but that this increase has been accurately counterbalanced by a decrease of the non-genetic variance, the total (phenotypic) variance remaining constant. It is a well-known fact that in inbred strains of animals from which the genetic variance has been virtually completely eliminated by inbreeding, the phenotypic variance for metrical characters is sometimes as big, or nearly as big, as in genetically mixed strains in which an appreciable part of the phenotypic variance is genetic in origin. Evidently, as the genetic variance is reduced, the environmental variance increases so that the total phenotypic variance changes little or not at all; in fact, it may increase. Whether, in a genetically mixed population like that of the rats on the strip, an accurate replacement of environmental by genotypic variance is possible such that the phenotypic variance remains constant is a question which is easier asked than answered. If *Rattus rattus* were a laboratory animal amenable to experimentation, the level of genetic variance in strip and control rats could be assessed by their response to selection. This being impracticable, it may be doubted whether with the means at our disposal a decision between these two hypotheses can be obtained. They are, in any event, not mutually exclusive.

Taking our findings at their face value, it might be thought that it makes little difference whether the strip rats show no detectable effects of radiation because the radiation is genetically ineffective at very low dose-rates, or because its effects are completely counterbalanced by natural selection, or because an increase in genetic variance is completely absorbed by a corresponding decrease in environmental variance. So far as the parameters which we have used are concerned, that is certainly so. But we have used these entities to probe a wider problem. If it were true that radiation-induced genetic variance has been added cryptically, below the surface, to the iceberg called phenotypic variance, the same presumably would have happened with genes with major pathological effects which could not have been detected by our method. Whereas our findings thus give no positive indication of genetic damage to the rats living on the strip, they do not rule out the possibility of induced mutations lurking beyond the reach of our method.

[1] Glass, H. B., and Ritterhoff, R. K., *Science*, 133, 1366 (1961).
[2] Russell, W. L., *Amer. Nat.*, 90, 69 (1956).
[3] Grüneberg, H., Bains, G. S., Berry, R. J., Riles, L. E., Smith, C. A. B., and Weiss, R. A., Report submitted to the Medical Research Council.
[4] Grüneberg, H., *Evolution*, 15, 259 (1961).
[5] Russell, W. L., *Repair from Genetic Radiation*, 205 (Pergamon Press, 1963).

GENERALIZATION OF EPIDEMIC THEORY

AN APPLICATION TO THE TRANSMISSION OF IDEAS

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ONE of the most fundamental problems in the field of information retrieval is that of determining the circumstances under which it might be necessary to introduce an information retrieval system as an aid to a given population of scientists. It is proposed that this problem be examined in terms of the transmission and development of ideas within a population. Specifically, the transmission of ideas within a population will be treated as if it were the transmission of an infectious disease, that is, in terms of an epidemic process. An attempt will be made to indicate the role of information retrieval in the development of such a process.

The Epidemic Model

Since the spread of disease in a population is to be our model for the transmission of ideas, it is appropriate to discuss the essential principles pertinent to this issue. These principles are a part of epidemiology. The necessary elements involved in the process of the spread of an infectious disease are those of : (1) a specified population ; (2) an exposure to infectious material. The members of the population belong to 1 of 3 basic classes at a given instant in time : (a) *infectives*, those members who are host to the infectious material ; (b) *susceptibles*, those who can become infectives given contact with infectious material ; (c) *removals*, those who have been removed for one of a variety of reasons such as death, immunity, hospitalization, etc. These latter members may have been either susceptibles or infectives at the time of their removal.

The entire process is time-dependent, that is, a sequence of events occurs which describes the process. An individual is exposed to infectious material either by direct contact with an infective or through some intermediary host or vector. The exposed person may either be resistant to the incoming organism, in which case the organism is rejected, or may be infected by it, in which case the invading organism proceeds on a course of development. The time interval in which the development takes place is called the 'latency period' (or incubation period), that is, the interval of time necessary for a susceptible to be transformed into an infective. In other words, the latency period is the time between the receipt of infectious material by a susceptible and the time at which he is in position to transmit infectious material to another susceptible, thus repeating the process. An epidemic occurs when the process of susceptibles being transformed to infectives crosses a certain threshold. (The American Public Health Association (1960) defined an epidemic as "the occurrence in a community ... of a group of illnesses of similar nature, clearly in excess of normal expectation.")

The process already described here does not take into account the almost endless number of complexities which actually arise. It is a model, that is, an idealization of the real situation in which the complex process is reduced to its essential properties.

Transmission of Ideas as an 'Epidemic' Process

In general, the 'epidemic' process can be characterized as one of transition from one state (susceptible) to another (infective) where the transition is caused by exposure to some phenomenon (infectious material). The process need not be restricted to infectious disease but is a more general abstract process that might be applied to many situations. All that is needed is the appropriate interpretation of the process elements, that is, susceptibles, infectives, removals, infectious material, intermediary host, latency period, disease, etc.

People are susceptible to certain ideas and resistant to others. Once an individual is infected with an idea he may in turn, after some period of time, transmit it to others. Such a process can result in an intellectual 'epidemic' (Table 1). For example, consider the development of psychoanalysis in the early part of this century. Freud was no less host to the infectious material of the 'disease' of psychoanalysis than the person carrying the organism capable of transmitting a cold, nor is his writing less of a 'vector' carrying the 'infectious material' than the mosquito as a carrier of malaria.

Moreover, Abraham, Ferenczi, Jung and Jones were no less 'susceptibles' who were infected by the ideas of Freud and who, after a certain latency period, themselves became 'infectives' than are those individuals infected by the droplets expressed by a cold carrier. Jung might represent an example of acquired resistance to the disease while the resistance of the medical community of Vienna could represent innate immunity. The development of the psychoanalytic movement in the early part of the twentieth century was in its way no less an 'epidemic' than was the outbreak of influenza in 1917 and 1918.

One can argue similarly that Darwin and evolution, Cantor and set theory, Newton and mechanics, and so on, were examples of 'epidemics' in the world of scientific thought which were instigated by the introduction of a single infective into a population. The analogy is not restricted to science; for examples such as Christ, Buddha, Moses and Mohammed can be cited in the

Table 1. Analogy between Infectious Disease and Intellectual Epidemics

Elements of the epidemic process	Elements interpreted in terms of Infectious disease epidemic	Intellectual epidemic
Host		
Agent	Infectious material	Idea
Infective	Case of disease	Author of paper
Susceptible	Person who will be infected given effective contact	Reader of paper who will be infected given effective contact
Removal	Death or immunity	Death or loss of interest
Vector		
Agent	Infectious material (as for host)	Idea (as for host)
Infective	Vector harbouring the agent	Paper containing useful ideas
Susceptible	Vector not harbouring the agent	All papers containing potentially useful ideas
Removal	Death	Deletion or loss

religious field and other examples can be given almost endlessly.

Although biological and intellectual epidemics can be considered as special cases of a general process, there are important differences. Intellectual epidemics are often desirable while biological ones usually are not. This difference, however, does not pertain to the structure of the processes but only to external factors which might be introduced. In one case, one usually wishes to enhance the process, while in the other to eliminate it.

Another important difference relates to the infectious material. In the biological case the infective individual produces infectious material that closely resembles that which started the process ; there has been little mutation or change. Such is not the case with the intellectual epidemic, for here some mutation or change of the original idea (infectious material) is prerequisite to placing it in print.

Ideas are transmitted by personal communication or by publication. An epidemic cannot develop within a given population unless there is effective contact between the susceptibles and infectious material. The purpose of an information retrieval system is to provide such effective contact where it does not already exist. If such is the case, an information retrieval system might be introduced even though such systems are limited to the transmission of ideas contained in published material.

The essential question is : when is it desirable to introduce a formal information retrieval system into the intellectual activity of a population of scientists? Here the epidemic model may be of value for it permits the rephrasing of the question in terms of epidemiological problems such as : (1) the transmission and spread of the disease ; (2) the prediction of the epidemic course ; (3) the discovery of the threshold densities of the population that might be passed before an epidemic can develop. The answer to these epidemiological questions might help to decide when an information retrieval system should be introduced.

Mathematical Models

There has been extensive development of abstract mathematical models in the theory of epidemics which can be put to general use. There are two types of models, namely, deterministic and stochastic. The deterministic approach represents the process as a system of differential equations while the stochastic approach describes the process as a finite state Markov process of either a discrete or continuous parameter (the discrete parameter is applicable where the latency period is constant, in which case the infectives occur in generations) depending on the physical situation. The stochastic representation, though generally more realistic, is mathematically more sophisticated. An excellent detailed treatment of this subject as related to infectious disease can be found in Bailey[1].

A Deterministic Model

The most natural avenue for exploring the process of the transmission of ideas within a population would seem to be by way of the literature produced by the members of that population. Although the published article is not the only way in which ideas can be transmitted, it does play an important part. We are interested in studying this role, for it is only in this respect that an information retrieval system can be justified at present. In terms of the epidemic process we are therefore concerned with the transference of infectious material, that is, ideas, between human hosts by means of an intermediary host or vector, namely, the written article.

We wish to study the spread of the 'disease' D within the population N of the discipline F. Consider a certain field (F) such as medicine and a certain subfield (D of F) such as medical epidemiology. The field (F) need not be as broad as medicine, but might be epidemiology, and mathematical epidemiology might be the subfield (D). N must be specified because D might develop into an 'epidemic' in some population N_1 but not in some other N_2. For example, the 'disease' of mathematical epidemiology will probably follow different courses in the fields of epidemiology, where it might become an 'epidemic', and in the field of mathematics, where it probably will never become an 'epidemic'. An additional example from the field of medicine might be the present epidemic of cancer of the lung among smokers that is certainly different from the pattern of development of the disease among non-smokers.

Let N_0 be the set of authors who have written papers in F at time t_0, where t_0 represents the starting time of the process. Let I_0 be the set of authors who have written papers in D at time t_0. Then I_0 represents the infective population at the beginning of the process. If it is assumed that there are no removals at time t_0, then $S_0 = N_0 - I_0$ represents the susceptible population at time t_0. (Removals constitute those members of N who for some reason or other are no longer producing papers in F.)

Let I'_0 be the set of articles produced in D by the members of I_0 at time t_0 and S'_0 be the set of articles produced by the members of S_0 at t_0. Then $N'_0 = S'_0 + I'_0$ constitutes the vector population at time t_0.

In general as the process develops we have $N = S + I + R$ and $N' = S' + I' + R'$, where R and R' represent removals. At time t_0, $R_0 = 0$ and $R'_0 = 0$.

The process might be described as follows : A member s of S is infected by a member of I'. After a certain length of time (latency period) s becomes an infective and gives birth to new infections in N' which in turn may infect other members of S and so forth. In other words s produces a paper in D in which he cites a paper in I'. (The member of I' might contain more than one idea (more than one type of 'infectious material') with respect to D. This same member of I' may contain ideas that could also infect members of a sub-field other than D. Our only interest in the member of I' is as a carrier of the 'infectious material' of D.) By producing this paper he has introduced a new infective in N'. He may also have cited papers in S' as well, so that he has infected or associated members of S' with the 'disease' D. The appearance of the paper written by s in D indicates that he has made the transition from the susceptible to the infective state.

Let β be the rate at which the members of S become members of I. Hence, β is the rate of infection in the population N. Let β' be the rate of infection in the population N'. Then β' represents the rate at which papers in S' are being cited by members of N who are producing papers in D.

As the process develops with time, a certain number of susceptibles and infectives in the host and vector populations are removed. In the case of the host population this removal might be loss of interest, death, etc. In the case of the vector population it might be lack of citation due to deletion, inaccessibility, etc. Let γ (γ') be the rate of removal of infectives I (I') and let δ (δ') be the rate of removal of susceptibles S (S').

Another aspect of the process as it develops is that new supplies of susceptibles and infectives are introduced into the populations N and N'. Let μ (μ') be the rate at which new susceptibles are introduced and let υ (υ') be the rate at which new infectives are introduced. That is, μ represents the rate at which new authors are introduced in F but not in D, while μ' is the rate at which new papers are introduced in F but not in D. υ represents the rate at which new authors are introduced in D and υ' the rate at which new papers are introduced into D.

The mathematical model to be presented includes the following states of transition of the host and vector populations within a short time interval Δt : the susceptible can remain a susceptible or become an infective

Table 2. States of Transition of the Host and Vector Populations*

State to which an individual belongs	
At time t	At time $t+\Delta t$
(1) Susceptible	Susceptible, infective or removal
(2) Infective	Infective or removal
(3) Removal	Removal

* In general the host and vector populations need not follow the same transitional patterns as in this case.

or removal, the infective can remain an infective or become a removal, and the removal must remain a removal (Table 2). (To keep the mathematical model simple in this presentation, we are not permitting immunes to lose their immunity and to return to the susceptible population.) The latency period is assumed to be zero so that infectives occur continuously with time. If we assume homogeneous mixing among the members of the populations, the total process can be deterministically described by the following systems of differential equations :

$$\frac{dS}{dt} = -\beta SI' - \delta S + \mu \quad : \quad \frac{dS'}{dt} = -\beta' S'I - \delta S' + \mu'$$

$$\frac{dI}{dt} = \beta SI' - \gamma I + \upsilon \quad : \quad \frac{dI'}{dt} = \beta' S'I - \gamma' I' + \upsilon'$$

$$\frac{dR}{dt} = \gamma I + \delta S \quad : \quad \frac{dR'}{dt} = \gamma' I' + \delta' S'$$

A threshold density of susceptibles can be obtained if we assume initial conditions of a single infective introduced into the host (N_0) and vector (N'_0) populations at time t_0, that is, $N_0 = (S_0, 1, 0)$ and $N'_0 = (S'_0, 1, 0)$. For an epidemic to develop from time t_0, the change in the number of infectives in both the host and vector populations must be positive. Hence, from the above differential equations, we obtain the threshold $\rho\rho'$ as follows :

$$\beta SI' > \gamma I - \upsilon$$

$$\beta' S'I' > \gamma' I' - \upsilon'$$

$$\text{or } \beta\beta' S_0 S'_0 > \gamma\gamma' - \gamma\upsilon' - \gamma'\upsilon + \upsilon\upsilon'$$

$$\text{and } S_0 S'_0 > \left(\frac{(\gamma - \upsilon)}{\beta}\right)\left(\frac{(\gamma' - \upsilon')}{\beta'}\right) = \rho\rho'$$

$$\text{where } \frac{\gamma - \upsilon}{\beta} = \rho \; ; \text{ and } \frac{\gamma' - \upsilon'}{\beta'} = \rho'$$

The epidemic curve which traces the development of the epidemic in time is given by the differential equation $\frac{dI}{dt} = F(t)$ with peaks at the points in time where $\frac{d^2I}{dt^2} = 0$. The size of the epidemic is given by the limit of I as t goes to infinity, that is, the size of the infective population I after a long period of time. This discussion indicates that an epidemic can only develop from time t_0 provided that the product of the host and vector susceptible population exceeds the threshold $\rho\rho'$. When a potential epidemic condition is present, the quantity of scientists and information is sufficiently large that given effective contact an epidemic can develop. It is at this time that an information retrieval system might be introduced.

If the population is already in an epidemic state, an information retrieval system can be introduced in order to maintain or increase the frequency of effective contacts between susceptibles and infectives. Hence, the points at which an information retrieval system might be usefully introduced into a population are either at the initial point or the peak of the epidemic process or at a time when intensification of the epidemic process is desired.

Other models different from the one we have presented could be constructed that would interpret the epidemic in a different manner. For example, a model can be elaborated by introducing two interacting populations with rates of migration from one population to the other. One population might consist of biostatisticians and the other of epidemiologists. The 'disease' could be statistical epidemiology. Then one might study the development of the field in terms of the interacting host and vector populations rather than in terms of single populations.

The Stochastic Approach

In a stochastic model of the process described above the actual number of new infectives occurring in a short time-interval would be replaced with the probability of a new case occurring in that interval. This type of mathematical treatment is particularly applicable when dealing with small populations.

An epidemic process within a large population can be characterized as a collection of epidemic processes within sub-populations. For example, the outbreak of an infectious disease in a community might more specifically be studied in terms of the household populations rather than the community as a whole. Similarly the intellectual epidemic process within the population of a certain discipline could be studied in terms of its sub-populations, that is, the 'disease' D of field F might be studied in terms of the sub-fields of F, indicating, from the point of view of information retrieval, where intellectual epidemics are developing in a given discipline.

Thus, a stochastic treatment of the epidemic processes would in general be more desirable because the size of the populations being studied has been reduced. Unfortunately a stochastic model of an epidemic process with a vector population does not exist as far as we know. Such a model is in need of development and attention is being given to this problem by the authors.

Deterministic models, however, can be very useful, particularly in dealing with large populations, and experiments have been designed for the purpose of testing the model presented above.

Discussion

The construction of a mathematical model of a complex physical situation involves a certain amount of simplification. This is because the purpose of the mathematical model is the description and prediction of the essential patterns of the physical process and not the achievement of its complete analysis.

A total understanding of the process requires studies complementary to the mathematical ones. In the case of the infectious diseases this means biological, ecological, and clinical investigations of the process as well as mathematical ; in the transmission of ideas, psychological, sociological, and linguistic studies.

Mathematics cannot indicate why exposure to a certain virus will lead to infection or why a certain article will infect the reader with an idea. These important questions must be answered elsewhere. However, mathematics can make important contributions toward an understanding of the epidemic process if one keeps its limitations in mind.

Of course, the usefulness of a mathematical model depends on : (1) being able to solve the model ; (2) obtaining reliable measurements in order to test it. These are by no means negligible problems, since even the most simple representations present difficulties in obtaining algebraic solutions. This is particularly true of the stochastic case. Additional problems are encountered in relation to defining populations and obtaining reliable estimates of the parameters of the process.

Although implementation of the epidemic model presents certain difficulties, much useful information concerning the transmission of ideas within a population might be obtained from the mathematical approach. It could help in answering several questions that are basic for the design and operation of information retrieval systems. These questions include : (1) Where and when is activity within a given discipline developing into 'epidemic' proportions ? (2) What is the expected

duration of this 'epidemic' activity? (3) What is the intensity of the 'epidemic'? (4) What are the classic papers of a certain discipline, that is, the initial infectives of an outbreak?

Information retrieval systems must be dynamic and should reflect the interactions between the researcher and the literature. It seems that the 'epidemic' approach is a workable method for describing and predicting certain fundamental aspects of this interaction.

Since the process of transmitting ideas, as in the case of an infectious disease, is not a single process within a population but a collection of interacting processes within sub-populations, it would seem that the notion of an all-encompassing information retrieval system spanning the totality of knowledge should be replaced by the notion of small dynamic interrelated systems that appear when needed and disappear when not needed.

'Epidemic' theory could have a wide range of applications other than to the transmission of ideas, for example, to the study of certain chronic diseases, divorces, accidents, etc., but these will not be elaborated on in this report.

This work was supported by AFOSR grant 403–64 and U.S. Public Health Service grant *AM* 06399–03.

[1] Bailey, N. T. J., *The Mathematical Theory of Epidemics* (Griffin, 1960).

NEWS and VIEWS

Director of the Rowett Research Institute, Aberdeen: Dr. D. P. Cuthbertson, C.B.E.

Dr. D. P. Cuthbertson, who will be retiring from the directorship of the Rowett Research Institute, Bucksburn, Aberdeen, on September 30, 1965, obtained his medical qualification in Glasgow and was for nearly twenty years lecturer in pathological chemistry and physiological chemistry in the Royal Infirmary, Glasgow, and in the University of Glasgow. He became an authority on the biochemical effects of injury, and particularly on the acute depletion of protein following injury. His work on the prevention and treatment of these changes became of especial importance during the Second World War. Cuthbertson worked for two years on the headquarters staff of the Medical Research Council before he was appointed director of the Rowett Research Institute, Aberdeen, in 1945. Under his influence the Institute grew rapidly in size and importance, and it is remarkable how Cuthbertson was able to direct so much work and produce an interesting report each year, summarizing work that had been published in 100 papers or more. He managed, however, to keep his own laboratory going and to give a number of lectures, which were published. He is an accomplished artist in water colours and a keen golfer, and will clearly be able to find something to do when he retires.

Dr. K. L. Blaxter

Dr. K. L. Blaxter, at present head of the Nutrition Department, Hannah Dairy Research Institute, has been appointed to succeed Dr. Cuthbertson as director of the Rowett Research Institute. Dr. Blaxter, who is forty-five, is a graduate of the University of Reading. He has been with the Hannah Dairy Research Institute since 1948. Before that he was a research officer at the Veterinary Laboratory of the Ministry of Agriculture, Weybridge. During 1946–47 he was a Commonwealth Fund Fellow at the University of Illinois, Division of Nutrition, under Prof. H. H. Mitchell. With the exception of a period of war service with the Royal Artillery, he was a research assistant at the National Institute for Research in Dairying from his first graduation until going to Weybridge. Dr. Blaxter was Clive Behrens Lecturer in the Faculty of Agriculture, University of Leeds, during 1958–61; 1960 Thomas Baxter Prizeman and Gold Medallist (for research on the nutrition of dairy cattle); National Research Council of Canada visiting lecturer to the Universities of British Columbia, Manitoba, Saskatchewan and Alberta in 1964; 1964 Royal Agricultural Society Gold Medallist (for research on the nutrition of farm livestock). He also gave the Fernhurst Lecture to the Royal Society of Arts in 1962, lectures to the Professors Council of the University, Uppsala, and to the Berlin Academy of Sciences, D.D.R., in 1962, and the third Samuel Brody Memorial Lecturer in the University of Missouri in 1964. Dr. Blaxter's work has been concerned with the nutritive value of home-grown foods for dairy cattle, food intake and food utilization, protein requirements and protein metabolism of dairy cattle and calves, hyperthyroidism in the ruminant and the use of iodinated protein, hypomagnesæmic tetany, vitamin E metabolism and muscular dystrophy, energy metabolism and the energy requirements of cattle, and their relation to the environment. In 1962 his monograph entitled *The Energy Metabolism of Ruminants* was published. Dr. Blaxter is a member of numerous learned societies and is a member of the joint Agricultural Research Council–Medical Research Council Development Commission sub-committee on the monitoring of radioactive fall-out and of the Working Party on Ruminants of the Agricultural Research Council's Technical Committee on Nutrient Requirements of Farm Livestock.

Ministry of Aviation: Director of Engine Research and Development (1): Mr. Dennis H. Mallinson

Mr. D. H. Mallinson has been appointed director of engine research and development (1), Ministry of Aviation, in succession to Dr. J. Remfry, who has retired from the Public Service. Born in 1921, Mr. Mallinson was educated at Prince Henry's Grammar School, Otley, before going to the University of Leeds in 1939. He graduated with honours in applied mathematics in 1942 and was posted as a junior scientific officer to the Engine Department of the Royal Aircraft Establishment. As a member of the small but growing turbine division under Hayne Constant and W. R. Hawthorne, he was employed on engine performance investigations and particularly on the analysis of results from the flight tests of the pioneer jet aircraft. Transferring with the Royal Aircraft Establishment's Turbine Division to Power Jets, Research and Development, Ltd., he remained on engine performance work and was in charge of the Performance Section when the National Gas Turbine Establishment came into being. In 1949, following a period of about a year in which he acted as technical and administrative assistant to the deputy director (Mr. Peter Lloyd, the present director-general of engine research and development), Mr. Mallinson became a member of the National Gas Turbine Establishment's ramjet research team under Mr. R. P. Probert, concentrating first on thermodynamics but later encompassing fuel control systems and combustion development and finally assuming responsibility for the full-scale ramjet test vehicle programme. This programme was successfully completed in 1957 and from then until early 1963 he was superintendent of the Engine Research Division at the National Gas Turbine Establishment in charge of work on propelling nozzles, control systems and high-speed ramjets. He transferred to Ministry of Aviation Headquarters, London, in February 1963 as assistant director responsible for engine project assessment and continued in this post until receiving his present appointment. Dennis Mallinson is a district commissioner of the

Boy Scouts Association and is responsible for scouting in the Odiham District of Hampshire.

Counsellor, Defence Research and Supply, British High Commission, Ottawa: Mr. G. L. Hunt

Mr. G. L. Hunt, who has been appointed Counsellor, Defence Research and Supply, British High Commission, Ottawa, received his training at H.M. Dockyard School, Portsmouth, and the Imperial College of Science and Technology, where he graduated with honours in electrical engineering. After some time in industry, where he worked first on the development of carrier telephone systems, and later on thermionic valves, he joined the Royal Aircraft Establishment Radio Department, to continue in the field of valve research and development. In 1945 he was appointed assistant-director, electronics research and development, Ministry of Aircraft Production, where he was responsible for valves, components and test equipment. Later he changed to electronic systems work to be responsible for aircraft navigation systems and electronic warfare systems. In 1957 he returned to the Royal Aircraft Establishment as senior principal scientific officer to take charge of a division of the Radio Department which has embraced groups working on air–sea warfare, passive electronic warfare and radar techniques. He will be taking up his new appointment some time in October 1964.

U.S. National Academy of Sciences Advisory Group

An *ad hoc* committee of scientists and engineers has been formed by the U.S. National Academy of Sciences to advise Congress on certain national problems pertaining to scientific research. This is the first time in its 101 years of existence that the Academy has been called on to provide continuing advice to Congress. The group will be under the chairmanship of Dr. G. B. Kistiakowsky (professor of chemistry, Harvard University, and chairman of the Academy Committee on Science and Public Policy). An initial survey is being undertaken jointly by the Academy Committee on Science and Public Policy and the House Sub-committee on Science, Research and Development, under the chairmanship of Congressman Emilio Q. Daddario, to determine the areas in which the Academy is best equipped to give advice.

The *ad hoc* group has been asked to look into the following questions: (1) What level of Federal support is required for the United States to maintain a position of leadership through basic research in the advancement of science and technology and their economic, cultural and military applications? (2) What judgment can be reached on the balance of support now being given by the Federal Government to various fields of scientific endeavour, and on adjustments that should be considered, either within existing levels of over-all support or under conditions of increased or decreased over-all support? Although the Academy is composed entirely of Americans who have gained distinction in the natural sciences and engineering, membership in the present advisory group has been broadened to include social scientists in the fields of business administration, economics and public administration. Because of the breadth of the questions as well as the diversity of the views of the membership, the final report of the group will present individually signed statements rather than seek a formal consensus. In addition, the report will summarize the views of the individual contributors and tally their responses to specific questions. It will be delivered to the Daddario subcommittee before the end of the year.

Academic Forecast for the United States, 1965–75

A science resources planning study by Dr. W. A. Consolagio, entitled "Sustaining Academic Science, 1965–75", and reprinted from *The Educational Record*, estimates that during this period 2·6 million bachelor's degrees will be awarded in the United States in science, engineering and mathematics, together with 480,000 master's degrees and 130,000 doctor's degrees. For this output, a full-time science and engineering faculty of 173,000 will be required, 95,000 of whom hold doctorates, and, in academic science and engineering, man-power at trainer and trainee level will approximately double between 1965 and 1975. Instructional costs for the decade are put at 28,000 million dollars, with 15,000 million dollars for research, 7,000 million dollars for assistance to Fellows and Scholars, and 2,500 million dollars for information services, and a like sum for improving education in science and mathematics below this level. Dr. Consolagio suggests that the vital question is not the national need for such man-power, but whether the nation has the resources to train all who seek such training; whether it can, in fact, afford this effort, conservatively estimated at 65,000 million dollars.

Science and Technology in Relation to Politics

The third issue (Summer 1964) of *The Technologist* is devoted to a series of articles on science and technology in relation to politics, and an editorial article, "The Politics of Maturity", suggests that the type of society created by science and technology demands a policy of maturity. Mr. Denzil Freeth writes on the Conservative and Mr. A. Albu on the Labour attitude. Dr. S. Toulmin contributes a shortened version of his paper, "The Foundations of Scientific Policy", and Lord Bowden is interviewed on the technologist in politics. Drs. F. E. Nord and M. K. Malhotra describe the pattern of the organization of scientific research in the United States, Dr. D. W. Hill discusses technology as a vocation, Mr. E. Minshall deals with research and the atmosphere of the colleges of advanced technology, and Dr. R. A. Buchanan contributes a rather circumscribed bibliographical review on science and Government. Despite an occasional naïveté, the number gives a useful conspectus of present debate on the topic.

Diploma in Technology

The report of the National Council for Technological Awards for the period April 1963–March 1964 records 8,718 students attending 122 courses leading to the diploma in technology; 3,211 were first-year students. The diploma in technology conferred on 1,073 students during the year brought the total conferred since 1958 to 3,092, while there are now 106 registered candidates for membership of the College of Technologists, an award which was gained by four students during the year. Of 4,318 industry-based students in engineering, 61 per cent were wholly supported by industry and 39 per cent assisted by public funds for the academic part of the course; for 1,058 students in other technologies the corresponding figures are 65 and 35 per cent. The overall failure rate for entrants to courses in 1959–60 was 31 per cent (32 per cent in engineering and 28 per cent in other technologies). Of 933 entrants to colleges of advanced technology, 68 per cent gained the diploma of technology; for 228 entrants to regional and other colleges the figure was 71 per cent. A list of 131 courses at 31 colleges now recognized by the Council as leading to the diploma in technology is appended, as well as lists of industrial organizations which have provided industrial training and of approved external examiners.

Industrial Archæology

Much has been written of the various industries of the Sheffield area but, for one reason or another, the history of the South Yorkshire glass industry has been neglected. A small excavation was recently organized by the Sheffield Museum at the Catcliffe glasshouse, when it was threatened with destruction. As a result of the interest then aroused, and the discovery of a number of pieces of authenticated local glass in private hands, an exhibition of South Yorkshire glass was arranged at the Sheffield Museum in March and April 1963. The Museum

has a collection of glass, assembled by the late Joseph Kenworthy and attributed to the Bolsterstone glasshouse. An account of the history of the industry has been prepared by the deputy director, Mr. G. D. Lewis] (*The South Yorkshire Glass Industry*. Pp. 4. Sheffield: The City Museum, 1964. 6*d*.). The author directs attention to an increasing awareness of the lack of knowledge concerning many of the fast-disappearing industrial monuments which played an important part in Britain's technological development. Of this destruction, the Catcliffe glass-cone speaks eloquently. It is the last survivor of at least six such cones known to have been built in the area and is the oldest in Europe; only five others remain in Britain.

Archæology in Northern Rhodesia and Turkey

In the journal *Man* for May–June 1964, besides a number of interesting anthropological articles, there are two of more archæological importance. Prof. Desmond Clark directs attention to a small number of special-shaped stone vessels from Northern Rhodesia which he equates with some already known from South-West Africa (Waterbeg) and Tanganyika. He further suggests an affinity with others from further north in East Africa, and postulates a small migration of a pastoral people passing south and south-west. The article is illustrated. No dates can be given. There is also an account of some crude and primitive animal paintings in a small cave site in the Taurus mountains in Turkey. The site is not far from the town of Seydişiher. The cave is visible from miles away in the east-facing side of a hill called Dolmus Tokadi. There are well-worn tracks up to the site. Prof. Solecki, of Columbia University, does not suggest a date earlier than the Neolithic, and compares the new finds with those from Çatal Hüyük, some 85 km to the east, over the mountains, on the Konya plain described a few years ago by James Mellaat. Another somewhat similar find of a painted ibex has been cited by Y. Bostanci from near Beldibi, 140 km to the south-west over the Taurus Mountains. All these rock-shelter sites form an interesting group.

Commercial Oil Production in Australia

A few months ago a brief account was given of the Moonie oilfield, Queensland, the successful results of exploratory drilling there, the potentialities of the area, and particularly the start of a pipe-line from Moonie to Brisbane, 187 miles long (*Nature*, 201, 1080; 1964). On March 25, 1964, the Queensland State Minister for Mines, Mr. Evans, unveiled a plaque at the Moonie field to commemorate a great occasion: the beginning of commercial oil production in Australia. It is recorded that full-scale pumping of the oil through the 187-mile, 10-in. pipe-line then officially commenced, and that production installations are fully automated in the field, all operations being monitored from a central control station at Moonie (*The Australian Mineral Industry Quarterly Review*, 16, No. 4; June 1964). The list of failures in the search for petroleum resources in Australia during half a century or more is depressingly long, but enterprise, determination and patience have at last deservedly met with commercial success. Fully backed by the Commonwealth Government, implementing the Petroleum Search Subsidy Act, 1959, the Moonie strike, though not spectacular by world standards, is an obvious incentive to the vigorous pursuit of more as yet undefined oil-pools in Queensland, if not elsewhere in the Australian continent. In the section on petroleum in this number of the *Review* there is a summary of the present exploratory operations, from which it is clear that Queensland has a strong lead over the other territories in Australia in the matter of drilling activities, judged by the number of wells and total footage completed. It is a measure of progress that drilling activity in Australia in 1963 broke all records and showed an increase of more than 60 per cent over the records for 1962 in both the number of wells and annual footage.

Range Action in Table Bay Harbour

Range action in Table Bay Harbour is well known and is of frequent occurrence. It consists of regular oscillations of the water surface which are initiated by oscillations of similar period in the sea outside the harbour rather than directly by wind or barometric pressure changes. The spectrum of such long waves, recorded at a point on the west coast of the Cape Peninsula, has been examined by Prof. J. and Dr. Mollie Darbyshire (*South African Journal of Science*, 60, No. 6, June 1964). The natural periods of oscillation of the Duncan Dock have been calculated and the selection, by the dock itself, of certain periods from the broad band of the external spectrum is indicated. The amplitude of the shorter oscillations of period 1–6 min is dependent on the amplitude of the ordinary surface waves, whereas the longer ones, of period 15–60 min, are associated with oscillations of the water mass on the continental shelf caused by atmospheric pressure disturbances. Examples of both types of range action, taken from the tidal records in the Duncan Dock, are described.

Urea as a Fertilizer

Urea has held promise as a fertilizer for many years because it contains much more nitrogen than the popular nitrogenous fertilizers, is readily soluble in water and undergoes rapid hydrolysis to liberate ammonia. It is, in fact, an intermediate in the decomposition of calcium cyanamide, which was the first nitrogen compound to be synthesized from atmospheric nitrogen on a commercial scale, and has been used in large quantities as a fertilizer. The commercial production of urea was rather slow to develop and it has been manufactured in the main to satisfy the demand for urea–formaldehyde resin. Some of the earlier agricultural experiments with urea were discouraging since biuret could be present in amounts that were toxic; furthermore, under certain conditions the ammonia arising from hydrolysis of urea could impair the germination of seeds and adversely affect young seedlings; and finally, loss of ammonia from the soil reduced the efficiency of urea as a source of nitrogen in comparison with ammonium salts or nitrates. A fully documented account of the advantages and disadvantages of urea as a fertilizer, of its behaviour in different soils and of the results from field experiments with a variety of crops is given by J. K. R. Gasser in *Soils and Fertilizers* (27, No. 3. Pp. 175–180. Commonwealth Bureau of Soils, Rothamsted, 1964). The uncertainties regarding its value are cleared up and the precautions necessary in order to achieve parity with other nitrogen fertilizers are described. This is a timely review of the position and with the collected references on the subject will be a valuable guide to anyone interested in this particular fertilizer, which is now produced industrially from ammonia and carbon dioxide on a very large scale and sold at a price comparable with other nitrogenous compounds.

Fellowships in Medicine

The following appointments have been made by the Medical Research Council to fellowships in medicine to be held in the United Kingdom, the United States, Canada and Taiwan for the academic year 1964–65.

Clinical Research Fellowships

Dr. P. A. Murphy, registrar, Radcliffe Infirmary, Oxford, to the Department of the Regius professor of Medicine, Oxford; Dr. P. Richards, registrar, St. George's Hospital, London, to the Department of Medicine, Post-graduate Medical School, London; Dr. R. Summerly, registrar, St. Thomas's Hospital, London, to the Medical Research Council Unit for Research on the Experimental

Pathology of the Skin, Birmingham; Mr. P. Vig, Institute of Dental Surgery, Eastman Dental Hospital, London, to the Department of Orthodontics, Institute of Dental Surgery, Eastman Dental Hospital, London; Dr. J. L. Wilkins, registrar, St. Mary's Hospital, London, to the Pædiatric Research Unit, Guy's Hospital Medical School, London.

Medical Research Council Travelling Fellowships

These fellowships replace the Rockefeller travelling fellowships. Dr. M. C. Brain, senior registrar, Department of Medicine, Hammersmith Hospital, London, to the Hæmatology Division, Johns Hopkins Hospital, Baltimore, Maryland; Dr. C. D. Holdsworth, registrar, Medical Unit, Royal Free Hospital, London, to the Department of Physiology, Harvard Medical School, Boston, Massachusetts; Dr. A. H. G. Love, lecturer in medicine, Queen's University of Belfast, to the United States Naval Medical Research Unit, Taipei, Taiwan, and the Gastroenterology Unit, Massachusetts Memorial Hospital, Boston, Massachusetts; Dr. H. Newey, J. J. Graves Medical Research Fellow, Department of Physiology, University of Sheffield, to the Biophysical Laboratory, University of Harvard, Boston, Massachusetts; Dr. V. Parsons, senior registrar in general medicine, Royal Sussex Hospital, Brighton, to the Arthritis Unit, Massachusetts General Hospital, Boston, Massachusetts; Dr. T. H. Williams, lecturer in experimental neurology, Department of Anatomy, University of Manchester, to the Department of Anatomy, Harvard Medical School, Boston, Massachusetts.

Sir Henry Wellcome Travelling Fellowships

Miss M. R. Daniel, cell physiologist, Strangeways Research Laboratory, Cambridge, to the Division of Histology, Ontario Veterinary College, Guelph, Canada, and the Department of Microbiology, the University of Michigan, Ann Arbor, Michigan; Mr. R. P. Gould, senior lecturer in histology, Middlesex Hospital Medical School, London, to the Department of Neuroanatomy, Harvard Medical School, Boston, Massachusetts; Mr. N. Maclean, assistant lecturer, Department of Zoology, University of Edinburgh, to the Rockefeller Institute, New York; Dr. G. Meachim, lecturer in pathology, University of Sheffield, to the Department of Animal Biology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia; Mr. J. L. Provan, senior lecturer, Surgical Unit, University College Hospital Medical School, London, to the Cardiovascular Laboratories, Massachusetts General Hospital, Boston, Massachusetts.

U.S. Public Health Service Fellowships

Dr. M. H. Evans, senior lecturer, Sherrington School of Physiology, St. Thomas's Hospital Medical School, London, to the Department of Physiology, University of Utah, Salt Lake City; Dr. N. L. Jones, senior registrar in respiratory research and senior tutor, Postgraduate Medical School, London, and honorary senior registrar, Hammersmith Hospital, London, to the Cardiovascular Research Institute, University of California Medical Center, San Francisco, California; Dr. J. R. Ledsome, lecturer in physiology, University of Leeds, to the Cardiovascular Research Institute, University of California Medical Center, San Francisco, California; Mr. J. H. P. Main, lecturer in oral pathology, University of Edinburgh, to the National Institute of Dental Research, Bethesda, Maryland.

Alexander Pigott Wernher Memorial Trust Fellowships in Ophthalmology and Otology

Dr. A. J. Bron, junior registrar in ophthalmology, Guy's Hospital, London, to the Wilmer Institute, Johns Hopkins Hospital, Baltimore, Maryland; Dr. P. P. Hakim, registrar, Dudley Road Hospital, Birmingham, to King's College Hospital and the National Hospital for Nervous Diseases, London.

The Royal Society: Research Fellowships

THE Committee representing the Royal Society and the Armourers and Brasiers' Company has appointed Dr. G. A. Chadwick, of the Department of Metallurgy University of Cambridge, to the Armourers and Brasiers' Company research fellowship in metallurgy to work on the effect of the interfacial energy term on the microstructure of eutectic alloys at the Department of Metallurgy, Cambridge. The Committee representing the Royal Society and the University of Sheffield has appointed Dr. R. N. Dixon, of the Department of Chemistry, University of Sheffield, to the Sorby research fellowship to continue experimental and theoretical investigations of the electronic spectra of small molecules at the Department of Chemistry, Sheffield.

Announcements

PROF. A. R. J. P. UBBELOHDE, professor of thermodynamics and head of the Department of Chemical Engineering and Chemical Technology in the Imperial College of Science and Technology, London, and director of the Salters' Institute of Industrial Chemistry, has been elected a corresponding member of the Göttingen Akademie der Wissenschaften.

IN recognition of the centenary of the Cleveland Institution of Engineers, Middlesbrough, which is an Affiliated Local Society of the Iron and Steel Institute, the Institution has been awarded the Medal of the Iron and Steel Institute. This Medal, which was established in 1960, is presented on special occasions to Societies which have, in the opinion of the Council of the Institute, rendered exceptional service to science or industry over a long period. The Medal, which is of gold, has previously been awarded to the Royal Society (1960), the Verein Deutscher Eisenhüttenleute, Düsseldorf, Germany (1960), and Jernkontoret, Stockholm, Sweden (1962).

THE Arthur Stanley Eddington Memorial Lecture for 1964 on "I believe . . ." will be delivered by Dame Kathleen Lonsdale in Cambridge on November 6. Further information can be obtained from the Eddington Memorial Trust, Sedgwick Museum, Cambridge.

A SYMPOSIUM on "Space Environment Simulators", sponsored jointly by the British Interplanetary Society and the Society of Environmental Engineers, will be held at Northampton College of Advanced Technology on November 17. Further information can be obtained from the British Interplanetary Society, 12 Bessborough Gardens, London, S.W.1.

THE first inaugural presidential address of the Institute of Mathematics and its Applications will be delivered by Prof. M. J. Lighthill (Imperial College of Science and Technology, University of London) at a meeting of the Institute to be held at King's College London on November 25. Further information can be obtained from the Institute of Mathematics and its Applications, 29 Gordon Square, London, W.C.1.

CORRIGENDUM. It is regretted that due to a delay in receiving proofs the following corrections could not be made to the communication entitled "Instability of Temperature Ice-sheets owing to a Feed-back Mechanism" by M. L. Lliboutry (*Nature*, 203, 627; 1964): throughout the communication "temperature ice-sheets" should read "temperate ice-sheets", and also "C_0" should be "c_0". Lines 33–35 of the left-hand column on p. 629 should read: ". . . In fact, it has been proved[6] that E must be equal to 1 near the bergschrund and to δ near the front".

THE JOHN INNES INSTITUTE

DR. K. S. DODDS, director of the John Innes Institute, in his annual report* of the work of the Institute, anticipates that with the removal of the Institute to a site near the University of East Anglia it will be appropriate to re-fashion its research activities in the light of modern requirements of agricultural research. Meanwhile, the contents of the report suggest that the Institute maintains its high traditions in genetical research.

The work of the Institute is concerned both with fundamental genetical research and with plant breeding. The Department of Genetics is concerned mainly with the former and work has continued on genetical control of the enzyme NADP-linked glutamate dehydrogenase in *Neurospora* and new mutants and new complementation relationships are reported. Work has continued on the genetics of *Ustilago* with special reference to the effect of inhibitors of DNA synthesis on mitotic crossing-over. The enzyme nitrate reductase has also been investigated in *Ustilago* mutants some of which are unable to utilize nitrate or nitrite. The results suggest that the ammonium ion acts as an inhibitor of nitrate reductase rather than by repressing the formation of the enzyme. The work also suggests that induction of nitrite reductase lags behind that of nitrate reductase. These findings have implications for nitrate metabolism of higher plants. Investigations on *Antirrhinum majus* show that changing the environmental conditions during growth affects the germinal as well as the somatic mutation rate.

Further work in the Department of Cell Biology confirms that much of the rapidly labelled ribonucleic acid in the cell nucleus undergoes breakdown within the cell, largely in the nucleus, to acid-soluble end-products, and it is concluded that much of this short-lived nuclear RNA does not serve as a template for synthesis of protein either in the nucleus or cytoplasm and its function remains obscure. Little is known concerning the relative amounts of DNA and RNA in different parts of the cell nucleus, and attempts are being made to elucidate this on isolated nuclei of different species of higher plants of varying chromosome size. A new technique for isolating nucleoli will help in separating RNA fractions of the nucleus. In cultures of plant callus cells it has been discovered that chlorophyll formation is more adversely affected by certain synthetic auxins than carotenoid formation, but prolonged treatment with high levels of auxins did not impair the ability of the cells to synthesize chlorophyll and on restoration to a lower level they resumed synthesis. In all the callus cultures investigated the rate of carotenoids to chlorophylls was consistently higher than in leaves.

The possibility of devising mathematical models to interpret responses to selection is being continued by the Department of Applied Genetics. Other topics under investigation in this Department include the relative distribution of tomato mosaic virus in susceptible and resistant strains and the genetics of the tomato and the garden pea. The prevalence of the tendency for English garden peas to throw rogues is attributed to the narrow genetic base on which English garden peas are established, many varieties having common ancestors.

The Department of Potato Genetics is responsible for maintaining the Commonwealth Potato Collection and it is providing material for countries interested in potato breeding. Within the Department it provides material for studying the genetics of cultivated diploids, a necessary preliminary to the establishment of useful commercial varieties. Disease resistance and tuber dormancy are also under investigation. The discovery that seed and tuber dormancy may be under a common biochemical control will help to establish stocks of known dormancy.

With Mr. W. J. C. Lawrence's retirement from the Department of Physiology and Plant Culture the results of his work on the rogue tomato are summarized. Investigations into the effects of day and night temperatures on growth of tomato seedlings indicate that optimum temperature régime is a constant temperature close to 25° C but is dependent on the light intensity. Day temperatures affect growth more than night temperatures. The developing leaves respond to temperature more than the cotyledons. Contrary to general belief, it is found that respiratory loss is unaffected by night temperature and in fact a high night temperature is beneficial in stimulating leaf growth by increased cell multiplication. In glasshouse experiments, relatively high night temperatures are found to be beneficial to young plants even under conditions which are at variance with normal commercial practice.

E. C. HUMPHRIES

* John Innes Institute. Fifty-fourth Annual Report, 1963. Pp. 58. (Bayfordbury, Hertford: John Innes Institute, 1964.) 3*s*.

MEDICAL RESEARCH AND THE WORLD HEALTH ORGANIZATION

*THE Medical Research Programme of the World Health Organization, 1958–1963** should be read by all who wish to know what the World Health Organization is doing and has done in the field of medical research. It is a comprehensive survey of the intensified programme of medical research on which the Organization first embarked in 1958. Before that year the promotion of research had, of course, been an integral part of the work of the World Health Organization. In this volume, the director-general, Dr. M. G. Candau, gives for the first time in print a comprehensive report of the medical research programme of the Organization.

The report begins with a general account of the origin, objectives, nature and methods of the programme and the promotion and co-ordination of the work done by co-operative effort in many countries. The programme is directed essentially at the solution of problems that are better tackled by co-operative effort than by local groups, but it is not merely concerned with those projects which the World Health Organization helps financially. Some of the work helped by the Organization involves the collaboration of experts in several scientific disciplines, such as chemistry, pharmacology, biology and epidemiology. Others are carried out in clinical departments in various countries.

A wide range of subjects is discussed in the report and they are grouped under the following five headings: (1) "Communicable Diseases", that is, virus diseases, enteric and diarrhœal diseases, tuberculosis, leprosy, malaria, bilharziasis, diseases caused by spirochætes and filarial nematode worms, other diseases caused by para-

* World Health Organization. *The Medical Research Programme of the World Health Organization, 1958–1963: Report by the Director-General.* Pp. vii+293. (Geneva: World Health Organization; London: H.M. Stationery Office, 1964.) 18 Sw. francs; 30*s*.; 6 dollars.

sites and zoonoses. (2) "Non-communicable Diseases", that is, cancer, cardio-vascular, intestinal and rheumatic diseases. (3) "Public Health Practice". (4) "Human Biology and Pharmacology", including sections on immunology; the biological standardization, efficacy, and safety of drugs; human reproduction and genetics (the last-mentioned being the subjects of two recently published issues of the *Technical Report Series*, No. 280† and No. 282‡). (5) "Environmental Health", under which are included the control and resistance of insect and molluscal vectors and their resistance to insecticides and molluscicides.

Annexes to the report by the director-general give the names of the members of the Organization's International Advisory Committee on Medical Research; details of the meetings of the Organization's Scientific Groups and other research meetings; information about its co-operative research projects and about grants awarded to workers in various countries and the subjects for which the grants were given; as well as information about the Organization's Reference centres and collaborating laboratories in various countries. G. LAPAGE

† *Technical Report Series*. No. 280: *Biology of Human Reproduction*—Report of a WHO Scientific Group. Pp. 30. (Geneva: World Health Organization; London: H.M. Stationery Office, 1964.) 1 Sw. franc; 1*s*. 9*d*.; 0·30 dollar.

‡ *Technical Report Series*. No. 282: *Human Genetics and Public Health*—Second Report of the WHO Expert Committee on Human Genetics. Pp. 38. (Geneva: World Health Organization; London: H.M. Stationery Office, 1964.) 3 Sw. francs; 5*s*.; 1 dollar.

THE WORLD FEDERATION OF SCIENTIFIC WORKERS

THE *Charter for Scientific Workers and the Constitution of the World Federation of Scientific Workers*, which was formulated soon after the foundation of the Federation in 1946, has now been re-issued in order to make the work of the Federation more widely known*.

The Federation claims to be the only considerable organization promoting co-operation between representatives of both the East and the West which has survived the rigours of the 'cold war'; it now represents more than 200,000 scientific workers in some 30 States.

The *Charter* first affirms the responsibilities of scientific workers: (1) to science, in promoting the integrity of scientific work and resisting the suppression or distortion of scientific knowledge, by full publication of results, co-operation with other scientific workers regardless of racial or national barriers, and by securing the growth of science with due regard to the balance between fundamental and applied science; (2) to the community, by studying the implications of science, by endeavouring to ensure that all knowledge is widely understood and used, and by searching for new ways of utilizing scientific knowledge, especially in fighting famine and disease and in improving conditions of life and work, as well as by studying all aspects of public administration so as to ensure that scientific methods are fully used; (3) to the world, by maintaining the international character of science, by studying the underlying causes of war, by assisting agencies seeking to prevent war, by building stable bases for peace, by working against the diversion of scientific effort in preparation for war, and by resisting movements inspired by anti-scientific ideas. Secondly the *Charter* is concerned with the state of science and scientific workers in securing that science is adequately financed, that results of research are rapidly developed and applied, that research is planned to take into account the intrinsic developments of fundamental science, that the needs of communities are scientifically assessed, and that it is ensured that scientific workers participate actively in the formulation of policy at all levels. Thirdly, the *Charter* is concerned with the opportunities of becoming a scientific worker, including secondary education and university education, and opportunities for part-time studies. Fourthly, it is concerned with facilities for employment of scientists; fifthly, their conditions of work; sixthly, the organization of scientific work, and, finally, the special needs for science in under-developed countries.

Accordingly, the *Constitution* details the aims of the Federation as being to promote understanding and co-operative action between member organizations in assisting it and its constituent members: (*a*) to work towards the fullest utilization of science in promoting peace and welfare of mankind and to ensure especially that it is applied to help solve the urgent problems of the time; (*b*) to promote international co-operation in science and technology, particularly in close collaboration with the United Nations Educational, Scientific, and Cultural Organization; (*c*) to encourage the international exchange of scientific knowledge, and of scientific workers; (*d*) to preserve and encourage the freedom and co-ordination of scientific work both nationally and internationally; (*e*) to encourage improvements in teaching science and to spread as far afield as possible the knowledge of science and its social implications; (*f*) to achieve a closer integration between the natural and social sciences; (*g*) to improve the professional, social, and economic status of scientific workers; (*h*) to encourage scientific workers to take an active part in public affairs, and to make them more conscious of, and more responsive to, the progressive forces at work within society. Membership of the Federation is open to any organization of scientific workers or any group of such organizations in any country, subject to meeting the definitions of scientific workers and qualifications given in the appropriate clause of the *Constitution*.

* *Charter for Scientific Workers and Constitution of the World Federation of Scientific Workers*. Pp. 15. (London: World Federation of Scientific Workers, 1964.)

PROGRESS IN CONCRETE RESEARCH

REFERRING to the activities of the Cement and Concrete Association in his introduction to the report for 1963*, the Hon. Leo Russell, director-general of the Association, stresses particularly the decision of its member companies to increase the income of the Association during that year, made necessary not only by rising costs but also to allow for considered expansion of important projects in the field of concrete research. "Apart from enabling the Association to carry out more effectively the work to which it is already committed, the increased resources will make it possible to undertake some much needed new research and development work".

The report is impressive in its survey of the many research problems and development schemes present in the 1963 programme. In the research section, the subjects include: the constitution of anhydrous cements and cement minerals; reactions in the system CaO—SiO_2—H_2O; cement hydration; problems in chemical analysis;

* Cement and Concrete Association. Report for the year 1963. Pp. 108. (London: Cement and Concrete Association, 1964.)

damping capacity of concrete; concrete strength testing; problems associated with excessively high water/cement ratios; high-strength concrete; fineness of cement; methods of measuring workability. The section on research on the design of structures is noteworthy for its treatment of model testing of structures such as bridges (Manchester Skyway Bridge) and buildings (Metropolitan Cathedral at Liverpool). A further section of the report deals with research on methods of construction. In this connexion: "One of the most interesting experiments recorded . . . is the construction of a length of *B* 6247 in Lancashire as a trial to examine the effects of the omission of expansion joints. Observations and measurements on this project are continuing, but early results suggest that the development of stress in the concrete indicates little difference between sections with and without expansion joints".

The section of the report on information services provided by the Association is fully comprehensive. These include: education; library facilities; publications; liaison with Press, radio and television; films; exhibitions; organized visits; lectures and discussion meetings. In the matter of library facilities, there has been recently issued a *Catalogue of Publications of the Cement and Concrete Association currently available for Distribution in the United Kingdom*†. This is an informative and valuable publication and should be in the possession of all concerned with the Association's literature on cement and concrete. It lists the following titles (among others): advisory booklets and leaflets; prestressed concrete; roads and road bridges; concrete as a material; technical memoranda; library abstracts; translations; Cement and Concrete Association reprints; bound volumes (for sale); research reports. Many of these publications are available for free distribution; those to be purchased are clearly so marked.

Recently, there has also been published an important booklet entitled *The Flexural Strength of Plain Concrete: Its Measurement and Use in Designing Concrete Mixes*, by P. J. F. Wright‡. Fundamentally the thesis here presented is that usually the quality of concrete is judged by its crushing strength, less attention being directed to the functions of tensile and flexural strength. Where failures in concrete roads occur, it is argued, these generally arise from tensile stresses. A critical examination of the flexural strength of concrete has accordingly been made, based on a great deal of small- and large-scale experimental work. Writing in the foreword to this publication, Sir William Glanville, director of road research, says: "A study was first made of methods of testing. This led to the introduction of a British Standard method for flexural testing. Using this standard test, the strength of a range of concrete mixes was investigated. The type of aggregate proved to be important. A section of the paper describes the way in which the data obtained may be used in designing concrete mixes and compares mixes based on compressive and flexural strength requirements". It is pointed out that the use of flexural strength of concrete for specification and control purposes has not as yet been widely adopted in road construction in Britain, but there is little doubt that the data recorded in this paper will be closely examined by highway engineers and others concerned with design and durability, and failures, of concrete roads.

H. B. Milner

† *Catalogue of Publications of the Cement and Concrete Association currently available for Distribution in the United Kingdom*. Pp. 40. (London: Cement and Concrete Association, 1964.)

‡ Department of Scientific and Industrial Research: Road Research Laboratory. Road Research Technical Paper No. 67: *The Flexural Strength of Plain Concrete—Its Measurement and Use in Designing Concrete Mixes*. By P. J. F. Wright. Pp. iv+52+4 plates. (London: H.M.S.O., 1964.) 4*s*. 6*d*. net.

SONIC INVESTIGATIONS ON INTERNAL DAMPING IN SOLIDS

A SYMPOSIUM entitled "Sonic Investigation on Internal Damping in Solids" was held at Imperial College, South Kensington, during August 6–8, 1964. It was organized by the Acoustics Group of the Institute of Physics and the Physical Society, and sixteen speakers contributed papers.

It is some thirteen years since the last symposium on this subject was organized jointly by the Physical Society and the Institute of Metals[1]. The gap of thirteen years, far from representing lack of interest in the subject, rather indicates that papers concerning it are often relevant in other contexts. For example, the International Conference[2] on Crystal Lattice Defects, held in Tokyo in 1962, included seventeen papers on internal damping. In the present symposium, besides measuring techniques, such matters as the influence of third-order elastic constants on apparent internal damping, the effects of microcracks and structural changes on the damping of ceramics and the choice of rubbers for highly efficient shock absorbers were discussed.

In the thirteen years since the last internal damping conference in London, interest has widened in non-metals like quartz, spinels and sapphire, in polymers, and in semiconductors, which at that time were academic curiosities. At that time the role of dislocations in internal damping was only beginning to be understood although it was predicted at that meeting that such damping should possess strongly directional properties and experiment has since verified this. At that time, too, little had been done with frequencies over 20 Mc/s, but now frequencies of 70,000 Mc/s have been used in damping work. These high-frequency waves can now propagate because of the modern development of crystals of very high purity and perfection, such as silicon, germanium and sapphire, which possess so few irregularities that little scattering occurs (except from thermal phonons) even with wavelengths as small as 800 Å. Moreover, in modern high-purity metals, the mean free path of the electrons may be comparable with that of ultrasonic wave-lengths, so that interaction can occur and Fermi surfaces can be delineated. Phonon–phonon interaction, and the interaction of waves with nuclear spin and with electron spin, have only been investigated within the past dozen years. Another development of great potential importance concerns semi-conductors, such as cadmium sulphide, which also possess piezoelectric properties. In single crystals of this substance electrons can be mobilized either by an electric field or by bright light and can increase attenuation, often dramatically. When, however, the electron drift field velocity exceeds that of the ultrasonic waves, in place of attenuation, useful amplification of the ultrasonic wave can occur (see Fig. 47, p. 251, of ref. 8).

A broad outline of the subject was given in the introductory address by Dr. R. W. B. Stephens (Imperial College) and continued by Mr. G. Bradfield (National Physical Laboratory) with emphasis on experimental techniques covering a wide range of frequency, temperature and pressure—from 1/1,000 c/s to many megacycles, from absolute zero to 1,100° C and at pressures up to 1,000 bars. It was stressed that the techniques were diversified not only by these wide conditions but also because of the enormous spread of loss angle values from around unity down to 3×10^{-6} so that an embarrassing variety of equipment is involved. Prof. J. F. W. Bell (Royal Naval College, Greenwich) described a magnetostrictive method of test which would give at least qualitative information at temperatures as high as 2,300° C for both longitudinal and torsional modes.

Internal friction becomes manifest in solids subjected to homogeneous cyclic stress, as in torsional pendulum devices, in standing wave phenomena (as in the resonance of rods or disks) and in travelling wave packets, wide in relation to a wave-length, which progress with only a slight divergence limited by diffraction effects. Joint friction between lumped elements limits accuracy in the first manifestation but, using the second, that is, resonance techniques, a useful degree of accuracy can be obtained even for Q values (π/decrement) as high as 30 million or more, a value for a good crystal of silicon. With travelling waves, however, accuracy is difficult to obtain in such a material for modest frequencies up to, say, 100 Mc/s, for relatively great corrections have to be made for the wave packet divergence and, because multiple end reflexions are used, further large corrections must be made for imperfect specimen-transducer joints and for mode changing at encounter with lateral boundaries. These difficulties (see also ref. 3) were discussed by Dr. M. Redwood (Queen Mary College) together with the consequences of practical imperfections in transmitting and receiving electronics; he also touched on the pulse broadening effects due to frequency-dependent internal damping.

Nevertheless, using these travelling wave techniques, relative values are often useful enough and, as Prof. R. Truell pointed out, some of the difficulties soon disappear at higher frequencies, say, at 1,000 Mc/s where, even in single crystals, the attenuation per centimetre is several decibels so that specimens a few centimetres long are adequate. At these high frequencies (*c.* 4,000 Mc/s) and low temperatures this attenuation constant starts off as a high power (*c.* 4) of the absolute temperature but only increases as a low power (*c.* $\frac{1}{2}$) of the frequency.

Waves in Heavily Absorbing Media

Travelling waves, however, present considerable difficulties with high decrements, around 0·13 or more as in polymers and, for example, in polycrystalline lead. Prof. H. Kolsky (Brown University) described the propagation of a sharp pulse into a viscoelastic solid where both the phase velocity and the attenuation are highly frequency-dependent. A pulse, initially very narrow, soon develops a characteristic shape which broadens linearly with range. He had found[4,5] that waves in polycrystalline lead rods behave somewhat like this, but in these rods not only is the anelasticity frequency-dependent but the stress–strain relation is non-linear. In such materials, in general, the bulk modulus loss angle is only a fraction of the shear modulus loss angle. Prof. Kolsky pointed out that, besides the progressive wave shape change, when a sharp pulse encounters an interface between two bodies matched at one frequency but differing largely in the frequency-dependence of their complex characteristic impedances, very curious wave shapes are reflected.

Mr. M. F. Markham (National Physical Laboratory) pointed out that geometrical path length corrections must be applied to the Kono[6] method of measuring polymer internal damping, using travelling waves encountering a slab obliquely when immersed in a liquid. He agreed with Prof. Kolsky's separation of the real and imaginary parts of the bulk modulus K', K'' from the real and imaginary parts

$$(K' + 4/3G') + i(K'' + 4/3G'')$$

of the modulus involved in travelling longitudinal wave propagation, with the help of the corresponding rigidity modulus components G' and G'', derived from shear wave propagation. He supported Mr. G. Bradfield's suggestion that the partition of waves at interfaces when decrements were in the region of unity or more might have to be calculated by introducing complex characteristic impedances into the Knott equations. However, he avoided such difficulties by using two polymer samples of different thicknesses.

Interaction with Conduction Electrons

Dr. K. Mackinnon described measurements in which electron movement in metals caused absorption of the sound wave; frequencies from 10 to 500 Mc/s were used. In order to achieve adequate interaction the mean free path of the electrons must be of similar magnitude to the sonic wave-length. Single crystals of great purity (*c.* 2 p.p.m.) were used, and the experiments were made at low temperatures to avoid scatter from thermally-induced singularities. Two techniques were used, with and without a magnetic field. In the latter, because the Fermi velocity of the electrons is about 300 times the sound-wave velocity, only electrons moving nearly parallel with the sonic wave-front interacted. Much more interaction occurs using a magnetic field to cause the electrons to orbit with a diameter equal to an integral number of sonic wave-lengths for peak absorption. Thus these maxima appear at equal intervals in the value of the reciprocal field. The absolute value of the absorption need not be known. The orbit properties define the Fermi vector in k space and adjustment of the attitude of the magnetic field vector relative to the crystal axes permits scanning of the Fermi surface and its delineation. Metals such as Cu, Ag, Au and Sn have been thoroughly examined and some features of the zinc and cadmium[7] surfaces are also known. With H. V. Böhm, Dr. Mackinnon had investigated Landau levels in, for example, zinc. The ultrasonic method is often as convenient as the Haas–van Alpen magnetic susceptibility method for obtaining information concerning Fermi surfaces.

Lattice Anharmonicity Effects

Because the non-linearity of lattice forces is related to harmonic generation, and, via phonon–phonon interaction, to attenuation measurements, the extent of this non-linearity can yield information on apparent internal damping. Mr. R. T. Smith (Imperial College) described his method of measurement of the travel times of shear and longitudinal waves passing transversely through a bar under axial compression. The changes in velocity were linearly related to this stress and permitted determination of these third-order elastic constants. For an isotropic polycrystalline solid, only three such constants were needed so that the variation of c_{44}, c_{66} and c_{22} sufficed.

Prof. R. Truell (Brown University), well known for his work on internal damping and wave propagation[8], discussed one of the most spectacular of modern sonic investigations concerning non-linearity of the lattice forces and its influence on the generation of a second harmonic wave. This can build up in amplitude linearly with range as the original wave travels along. The energy in this double-frequency wave must be drawn from the fundamental wave and results in its attenuation, that is, there is apparent, but not necessarily real, internal damping. Indeed, it is possible that thermal loss does not necessarily occur. He described carefully designed electronics at 10–20 Mc/s which permitted the accurate measurement of this second harmonic production. Lattice anharmonicity results in thermal expansion because of thermal waves, and consequent static stress produces second harmonics. Indeed, dislocations can give rise to second harmonics because of their stress fields. Thirdly, the presence of charge carriers can cause second harmonic generation in strongly illuminated materials with both photoelectric and piezoelectric properties, such as cadmium sulphide. In this, in single crystal form, the relative attitudes of the sonic wave vector, the direction of the light and the crystallographic axes markedly influence attenuation. In discussion it was pointed out that subharmonic production was mathematically possible; it had not yet been sought experimentally. It had, however, been demonstrated that lattice anharmonicity could lead to beams at sum and at difference frequencies

originating from the intersection of two strong sonic beams.

Interaction with Charge Carriers in Piezoelectric Solids

Dr. P. D. Southgate (Illinois Institute of Technology) described other investigations on cadmium sulphide at about 225 Mc/s using a drift field to cause sonic wave amplification. He had found that the mobility of charge carriers in it was much lessened by trapping mechanisms, about half of the carriers being affected. The presence of a travelling sonic wave could result in a drift field voltage, and noise modulation could arise on the sonic wave due to the electronic processes which were occurring.

Interaction with Dislocations

Internal damping measurements offer information concerning the nature of dislocation movements, which may be stress-dependent or otherwise. This damping can be at least partially explained in terms of the Granato and Lücke theory (ref. 8, p. 178), while the Bordoni peaks in f.c.c. solids behave broadly in accordance with the built-in kink theory of Brailsford (ref. 8, p. 207), a refinement of the theory of Mason, Seeger *et al.* The Granato–Lücke theory was investigated in work reported by Mr. T. Hinton and Dr. J. G. Rider (Battersea College of Technology), who made damping measurements on a series of lead–indium alloy single crystals (chiefly of 0·05, 0·15, 0·25 and 0·50 per cent In) and on lead–tin alloys, using longitudinal resonance at about 4 kc/s either at room temperature or at about 300° C. Various strain amplitudes were used and the damping was separated into strain-dependent and strain-independent components. The former could be interpreted, over a limited range of composition, to vary as the fourth power, as predicted by the Granato–Lücke theory. The break-away stress on these f.c.c. alloys appears to be 5–100 times below that found by static tests. This is in line with the findings of Hasiguti *et al.*[10] on b.c.c. iron (see, for example, equation 23.11 of ref. 8). The solute-dislocation binding energy was four times that deduced from Cottrell's expression.

Dr. P. D. Southgate (Illinois Institute of Technology) reported on an extension of his previous work[9] on Bordoni peaks in hard crystals of silicon and of magnesium oxide. One set of crystals was cut as tuning forks (100 c/s–600 c/s) and another as bars (100 kc/s in longitudinal resonance). Dislocations were introduced systematically. With these two frequency ranges and damping data up to 1,400° C, the activation energy of the silicon Bordoni processes was established as about 1·6 eV. When the calculated core widths of the kinked dislocation were plotted for copper and silicon at various temperatures, normalized against melting point, silicon had only about a third of the core width of copper although the general shapes of the curves were similar. Comparing activation energies for silicon and indium antimonide, it was interesting to note that these were in the ratio of 2·6 which was roughly the ratio of their electron energy gaps. Magnesium oxide behaved markedly differently from silicon, its decrement being distinctly dependent on strain amplitude. For this (and as found also by Hinton and Rider) the plot of log (strain × decrement) as ordinate against reciprocal strain was not the straight line predicted by Granato and Lücke (see equation 22.1 of ref. 8) but was distinctly concave upwards. α-Quartz is an example of a common single crystal the damping of which has been examined up to 70,000 Mc/s. It is suggested now that this damping is not primarily due to dislocations; much is due to phonon viscosity but some is contributed by ionic impurities trapped during growth. Natural crystals have remarkably uniform damping, but Dr. A. Seed (Standard Telephones and Cables, Ltd.) reported comparisons of synthetic crystals showing that the damping increased about ten-fold with doubling of growth-rate ($\frac{1}{8}$ mm to $\frac{1}{4}$ mm/day). A large damping peak existed at about 60° K which was about 10 times smaller at the lower rate. It was much reduced by X-ray irradiation and was split into two (*c.* 30° K and *c.* 80° K) suggesting that paired ions were responsible. The magnitude was strain-independent. Working in the range 5–100 Mc/s he had established that in accordance with theory, damping should vary in the ratio of shear moduli for various cuts; the BT cut had about twice the damping of AT cut. At room temperature the decrement of both dropped roughly linearly with frequency (*c.* 10^{-6} at 10 Mc/s on AT cut).

Prof. Truell remarked that α-quartz had far more damping than artificial sapphire when the latter is really pure. In discussion it was pointed out that though these were both trigonal crystals, the c_{14} constant of quartz was proportionately greater and this cross-term might affect mode distribution and hence increase phonon–phonon interaction.

Damping in Composite Bodies

Of less fundamental significance, though of great technological importance, were three papers by Dr. J. C. Snowdon, Mr. A. R. Payne and Dr. N. F. Astbury. Dr. Snowdon (Pennsylvania State University) had carefully analysed available damping and elasticity data on natural and synthetic rubbers and allied polymers by the reduced variable method over temperatures mainly from 5° to 35° C, and at frequencies from 1 to 10,000 c/s, and had applied this information in the first instance to the calculation of the efficacy of shock absorbers consisting, for example, of a mass supported by a rubber spring[11]. This exercise appears to show that the interaction between damping peak width and the inevitable relation of modulus drop to temperature imposed an upper limit on shock absorber efficacy whatever composition of rubber was tried; some slight improvements could be obtained with paralleled springs of differing composition, and with more complex mechanical structures. Mr. Payne (Natural Rubber Producers Research Association) reported on rubber damping and elasticity. He had found that, with a certain proportion of filler, such as carbon black, the damping was little affected by the nature of the rubber. Indeed, in certain circumstances, the rubber could be replaced by a paraffin liquid, the system retaining similar dynamic damping, and apparent elasticity and anelasticity, and some similarity of strain dependence. He thought, as did Dr. Snowdon, that the need was now not so much for new polymers for such things as tyres and shock absorbers, as for improved methods of dispersing filler in binder and achieving chemical bonding.

Dr. Astbury (British Ceramic Research Association) reported on work carried out at the British Ceramic Research Association[12]. The investigation of the sintering process covered widely different materials—brick, domestic and bone 'china', porcelain (including electrical insulation) and sintered alumina; the loss angles varied from 0·01 to 10^{-5}. The damping varies nearly linearly with pore volume but is nearly invariant with frequency. One type of ceramic which is clay-based acts like a crystalline phase in a glassy phase with pores up to 20 per cent of volume; devitrified glass is similar but without the pores, and fine 'china' porcelain would be mainly vitreous with perhaps 5 per cent of closed pores. There seem to be fine cracks, especially around the pores, and these seem to be responsible for a great deal of the damping; structural changes occurring during damping–temperature runs, however, such as the α-β-cristobalite change at 100°–200° C and the α-quartz change at *c.* 570° C, are reflected in fluctuations in both damping and elasticity. In discussion it was suggested that the great volume change in the former might react with the micro-crack system to give damping peaks. An important problem concerns the desirability of internal damping in ceramics to reduce the risk of fracture from shock waves.

In the symposium as a whole the damping of rocks to earthquake waves (< 1 c/s) and to seismic exploration waves (10–150 c/s) was not discussed, and little was said about frequencies of 5,000 Mc/s and upwards. Little emerged in discussion concerning equipment design and techniques, although no doubt useful interchanges of information occurred privately. Little common ground is shared between workers on metals and hard single crystals on one hand, dealing with loss angles down to 3×10^{-8}, and workers on polymers dealing with loss angles of 0·1–1·2 radians. Even so, an occasional symposium, in which such widely diverse fields of interest are included, serves a useful purpose. G. BRADFIELD

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TENTATIVE CLASSIFICATION OF FOOD IRRADIATION PROCESSES WITH MICROBIOLOGICAL OBJECTIVES

WE were recently asked, as members of an expert group, to define the main types of irradiation process applied to foods for microbiological reasons, and to suggest names for the types recognized. This article briefly records our joint conclusions.

With any kind of process for the preservation of food, two different broad microbiological objectives can be distinguished. In the first, the aim is to kill all micro-organisms of significance in the food, and, where achieved by heating, the process is commonly called sterilization, though a few micro-organisms may remain alive but quiescent; this is the condition well known to food technologists as 'commercial sterility'. For this type of heating process, the French have a special name *appertization*, commemorating Nicolas Appert, who invented it. The second broad objective is to kill many, but by no means all, of the micro-organisms. When achieved by heating, this is called pasteurization; it may be done either to kill pathogenic organisms in particular, or to reduce the number of viable micro-organisms generally and so improve keeping quality.

The same broad division of objectives applies to irradiation, so that hitherto radiation processes have commonly been separated, from the microbiological point of view, into radiation sterilization and radiation pasteurization. However, we did not regard this classification as adequate, or these terms as very suitable.

Sterilization is at best an abstract concept, which cannot be verified experimentally over any sizable number of packages, and special difficulties arise with an irradiation process. While viruses are comparatively easily killed by heat, such killing cannot be guaranteed with any process of irradiation alone, under conditions practicable with foods. Consequently sterilization, in the commonly understood sense of producing almost complete absence of viable micro-organisms, cannot be reliably achieved with radiation at present.

Pasteurization, again, is normally understood to kill most viruses, but this certainly cannot be achieved with the relevant doses of radiation. Moreover, as was pointed out at the European Meeting on the Microbiology of Irradiated Foods (F.A.O./I.A.M.S., Paris 1960 (ref. 1)), in the case of radiation treatment the term pasteurization is undesirably ambiguous, being used to describe processes with two distinct aims, one aim concerned with public health and the other not, which in some countries would require different legislative treatments. Even with heat processing, a different term is needed: for example, milk for cheese-making is sometimes heated to diminish its population of micro-organisms, but the heat treatment is less severe than that defined as pasteurization; the need for a term to describe this procedure has been plain for ten years, and was recognized by the International Dairy Federation, which provisionally proposed the word *thermization*.

We therefore suggest the following working definitions for the main types of irradiation treatment intended to kill micro-organisms in foods:

Sterilization by irradiation would be the application to foods of doses of ionizing radiation which produce sterility, that is, total absence of all viable micro-organisms as determined by any known method. This state cannot at present be guaranteed with any radiation process practicable with foods.

Among the attainable microbiological objectives, we recognized three types distinguished according to objective, and defined as follows:

Type I is the application to foods of doses of ionizing radiation sufficient to reduce the number and/or activity of viable organisms to such an extent that very few, if any, are detectable in the treated food by any recognized method (viruses being excepted) while no spoilage or toxicity of microbial origin is detectable no matter how long or under what conditions the food is stored in the absence of recontamination.

Type II is the application to foods of doses of ionizing radiation sufficient to reduce the number of viable specific non-spore-forming pathogenic micro-organisms (other than viruses) so that none is detectable in the treated food by any standard method.

Type III is the application to foods of doses of ionizing radiation sufficient to enhance keeping quality by causing substantial reduction in the numbers of viable specific spoilage micro-organisms.

It will be evident that *sterilization by irradiation* and *Type I* treatments have both been indiscriminately covered by the term radiation sterilization, while radiation pasteurization also has covered treatments of both *Types II* and *III*.

The following are the names we suggest for these types of treatment: *Type I, radappertization*; *Type II, radicidation*; *Type III, radurization*; and the reasons for these suggestions are given as follows.

The prefix *rad-* or *radi-* stands for the radiation used. (Lat. *radiare*, to shine). We could not think of any term which would indicate more precisely what kinds of

radiation are intended. The prefix *gamma* is too restrictive, excluding types of radiation which might be used. The form *radio-* promotes confusion with Hertzian waves. Terms like *cold pasteurization* may lead to confusion with different means, other than heat, of attaining the same objectives.

The aim of *radappertization* is just the same as that of appertization, to produce the condition of *commercial sterility*. With the prefix *rad-*, there is an almost exact description of the *Type I* process. Appertization has always been understood to imply a heat treatment, but the prefix *rad-* clearly discounts this. There is the inconvenience that English-speaking bacteriologists, not aware of the work of Nicolas Appert, are not yet accustomed to use the word appertization. We consider, nevertheless, that the aptness of the term radappertization far outweighs these objections.

The term *radicidation* derives from Lat. *radiare*, to shine, and *occidere*, to kill. The common use of the root *-cide* in words like germicide and bactericide has given it a more general connotation than would be desirable here, where the implication is towards some particular species. Hence, the specificity of aim implied in *Type II* treatments is not apparent in the term radicidation; and the best solution seems to be the usage, for example *Salmonella* radicidation, as one might say *Salmonella* disinfection. Further, radicidation bears misleading resemblance to radical, radish, etc., derived from Lat. *radix*, root. An alternative variation, radioccidation, is rejected because of written resemblance to radio and spoken resemblance to oxidation, besides possible confusion with Occident. Another alternative, irracidation, derived from *irradiation* and the root *-cide*, does not evidently contain the root *rad-*; and has the further inconvenience of seeming to entail an unnecessary prefix of ir- to the corresponding terms radappertization and radurization.

Radurization, based on *rad-* plus Lat. *durare*, to last or endure, expresses well the aim of the *Type III* process, to make the food more stable by means of radiation. The obvious defect of this term is that it does not indicate any connexion with microbiology. Further, it seems to verge on barbarism when rendered into French.

We submit that the foregoing classification of types of radiation treatment is logical; and adequate, for the present, because it seems premature to try to deal now with conceivable but as yet ill-defined combinations of radiation with, for example, heat or chemical preservatives.

As regards the terms we have suggested to designate the main types of treatment, however, we do not claim that they are anything more than the best we can suggest. We shall be interested to hear further suggestions.

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VARIABILITY OF THE STRONTIUM-90 AND CÆSIUM-137 BURDEN OF NATIVE PLANTS AND ANIMALS

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KNOWLEDGE of the inherent variability of radionuclide burdens of plants and animals is obviously of considerable importance in planning any kind of radiological survey of such populations, whether for research or health-protection purposes. A potentially important additional feature of such knowledge is the prospect of making some inferences about the manner in which radionuclides may be distributed over the landscape, which in turn may have a direct bearing on the course of such materials through a food web and, in extreme circumstances, on the radiation exposure of individual items of the biota.

The work recorded here is primarily concerned with materials originating in world-wide fall-out, and is restricted to the biologically important, long-lived nuclides strontium-90 and cæsium-137 (half-life approximately 30 years). Two sources of data, the Nevada Test Site and National Reactor Test Site (Idaho), receive radionuclides of local origin, but no attempt will be made here to evaluate the relative importance of contributions of local and world-wide origin. Only data on native plants and animals have been used here, principally to avoid uncertainties about origin of domestic animal food supplies, and 'pooling' of samples from large areas.

Where possible, I have used only records obtained over relatively short-time intervals on areas of restricted size.

It is generally accepted that the bulk of world-wide fall-out is brought to the ground by precipitation, and it thus seems logical that variation in the local distribution of precipitation will contribute heavily to the variability of radionuclide deposition. The intensity of rainfall may influence radionuclide deposition[1], and presumably features of topography and wind direction and force will have important local effects on physical deposition.

Radionuclide burden of vegetation may be influenced by plant phenology, in regard to the season and duration of exposure relative to periods of maximum precipitation, and by surface characteristics of leaves and stems[2]. Very likely density of the stand of vegetation and the prospects for overlayering or stratification may also have a bearing

on local variation in exposure to radionuclides, but these factors do not seem to have been investigated in any detail so far.

Animal food habits are clearly important in determining uptake of radionuclides. Seasonal changes in food sources can also be expected to interact with the factors determining radionuclide burden of vegetation to influence both levels and variability. On a local scale, evidently the size of 'home range' or 'cruising radius' of individual animals may be of importance in the sense that accumulation of biologically active (that is, metabolized) radionuclides by animals may be regarded as some sort of averaging or integrating process in time.

Many more factors presumably affecting variation in radionuclide burden may have been demonstrated, or might be proposed, but the purpose of the present report is to present a measure of variability. Much additional attention to details of mechanism and form seems important, but largely beyond the scope of presently available data and knowledge.

The coefficient of variation (standard deviation divided by mean) is used as a measure of variability here. Choice of this measure has been predicated in part on the results, that is, on the fact that the coefficient of variation seems to hold constant over a relatively wide range of mean concentrations of radionuclides, and in part on the form of the available records. Nearly all the data examined here are limited to very small samples in any one area or time-period, usually ranging between three and ten radionuclide determinations per set. Such small samples pose technical statistical difficulties in characterizing variability (for example, form of the distribution function, utility of the higher sample moments, weighting, etc.). I have arbitrarily elected to compute a coefficient of variation for each sample, and to combine the coefficients by weighting by sample size. Justification for the procedure lies largely in the empirical observation that plots of individual coefficients against the means show no evidence of trend in the data used here.

The utility of the coefficient of variation lies in its being a 'dimensionless' measure (expressed as a ratio or percentage), thus permitting comparisons over a wide range of materials, and the ready calculation of sample sizes required for relative precision in designing a sampling survey.

Coefficients of variation for burdens of strontium-90 and cæsium-137 (Table 1) are surprisingly consistent over a wide range of plants and animals. Values for strontium-90 range from about 0·30 to 0·45, while those for cæsium-137 appear somewhat lower, ranging roughly from 0·25 to 0·35. Various factors possibly affecting individual sets of data are indicated in notes appended to Table 1. With one exception (Maryland white-tailed deer), the bone–strontium-90 values were calculated in terms of picocuries (10^{-12} c.) per g calcium (strontium units). Analysis of the Alaskan caribou data in terms of picocuries per g standard dry weight yields virtually the same coefficient of variation. The plant data were calculated on the standard dry weight basis, while soil-levels were on a unit area basis. All cæsium-137 values were expressed in units per g dry weight.

The influence of analytical technique on these values is difficult to appraise, since the data come from a number of different sources. Presumably sample mass, length of counts, detection system, chemical processing, and other factors involved in analysis for radionuclides could contribute substantially to the observed variability. Appraisal of variation due to analytical technique for the caribou muscle content of cæsium-137 is possible, since most of the samples were run in duplicate or triplicate, starting with nearly equal masses (about 1,200 g) of muscle tissue from individual animals. Average 'within-sample' coefficients of variation for 34 duplicate and 32 triplicate caribou muscle samples were 0·04 (in both sets), suggesting a relatively small contribution due to technique for our data. A similar calculation on 42 duplicate strontium-90 in soil[3] yields a coefficient of variation of 0·05, again indicating a minor contribution from technique. Five 'blind duplicates' on strontium-90 in deer bones[4] yield a coefficient of variation of 0·06.

Table 1. COEFFICIENTS OF VARIATION FOR STRONTIUM-90 AND CAESIUM-137

Area	Organism	Total sample size	Coefficient of variation	Sampling period	Notes	Ref.
		Strontium-90 in bone				
Alaska	Caribou	57	0·47	Month	*a*	6
Idaho	Jackrabbits	87	0·30	Year	*b*	5
	Antelope	41	0·41	Year	*b*	5
	Deer and elk	28	0·44	Year	*b*	5
	Coyote	42	0·44	Year	*b*	5
Nevada and Utah	Jackrabbits	43	0·45	Month	*c*	7
	Jackrabbits	57	0·30	Day	*d*	7
	Jackrabbits	222	0·32	Year	*e*	7
California	Black-tailed deer	36	0·34	Year	*f*	4
Maryland	White-tailed deer	67	0·26	Month	*g*	8
		Strontium-90 in plants				
Alaska	Lichens	18	0·34	Month	*a*	6
	Sedges	25	0·25	Month	*a*	6
	Willows	10	0·30	Month	*a*	6
	Caribou rumen contents	53	0·28	Month	*a*	6
		Strontium-90 in soil				
Nevada		367	0·20	—	*h*	7
'World-wide'		32	0·24	—	*i*	3
		Cæsium-137 in muscle tissue				
Alaska	Caribou	59	0·36	Two weeks	*j*	9
		Cæsium-137 in plants				
Alaska	Lichens	56	0·22	Day	*k*	10
	Sedges	66	0·30	Day	*k*	10
	Willow	35	0·26	Day	*k*	10
	Caribou rumen content	55	0·24	Two weeks	*k*	9

a, Caribou were collected in the general area bounded by Kotzebue, Point Hope, and Anaktuvuk Pass (in the Brooks Range), all above the Arctic Circle, in 1960 and 1961. Plants were collected in the Cape Thompson area in summers of 1959, 1960, and 1961. *b*, Data on jackrabbits used here are those given for the Taber area (Table 2 of the reference), which is cited (p. 457) as an 'offsite control station'. Data for the other species are that of Table 1 in the reference, and source of the samples is described on p. 455. *c*, Data are from Table 1 of the reference, and are for an area 13 miles from the Nevada Test Site, established as a 'close-in fall-out area' (p. 45). *d*, Data are from Table 2 of the reference, and are for an area '99 miles from Ground Zero at the Nevada Test Site'. Only those collections indicated as having been made on a single day are used here. Inclusion of two additional samples (from Table 2), identified as 'May, 1960' and 'May, 1961' increases the average coefficient of variation to 0·46 for this area. *e*, Data are from Table 3 of the reference, and are for a sequence of distances from the Nevada Test Site, ranging from 0 to 432 miles. Dates are given only as '1958' and '1959'; but the small collections at each of the 18 sites may well have been made on single days. There seems to be no indication of a trend in magnitude of the coefficient of variation with distance from the Test Site. *f*, Data are those of Table 1 of the reference, grouped into periods approximately one year in length, starting with 1954. Individual animals ranged in age from 13 to 24 months. *g*, Coefficients of variation were computed from data arranged by age-class and would presumably be somewhat larger if age (to the year) had not thus been excluded from consideration. The value for the 13–20 month class is 0·32, very nearly that given for black-tailed deer of the same age-group. *h*, Data from Tables 1, 3, and 4 of the reference cited. *i*, Data from Tables *A* and *B* of the reference cited. Coefficients of variation were calculated for the replicate locations labelled '*A*' and '*B*' at each site, thus pertaining to 'within-site' variability. Weighted value multiplied by $\sqrt{2}$ to adjust for use of replicate samples at each subsite. *j*, Data were retabulated for two-week collecting periods within a given river drainage or other restricted area. All caribou records used in this article are from essentially the same set of animals. *k*, Data are essentially those of the references cited, but were regrouped in varying degree in the computations used here, and stem from an analysis for design of later samplings.

The results reported here should not be interpreted as meaning that the coefficient of variation for levels of these two radionuclides is everywhere roughly constant. Calculations covering large areas yield appreciably higher values, as would be expected on the basis of real differences in radionuclide levels over large areas or time-periods. Relative variability increases rapidly as levels approach 'detection limits', or 'background' counts. Further, extensive data on jackrabbit bone strontium-90 levels in the vicinity of chemical processing plants[5] yield many coefficients of variation well over unity.

I have been unable to locate data on cæsium-137 analysis of sizable samples of native plants and animals other than the Alaskan data reported here. Limited data from the state of Washington obtained in a present investigation of fall-out in natural ecosystems suggest very much the same values as those obtained from the Alaskan data.

Rather larger samples than are now available appear desirable for making satisfactory inferences about the actual form of the distribution of radionuclide burdens in individual plant and animal samples. The observed relative constancy of the coefficient of variation does, however, suggest that the logarithmic transformation may be suitable for statistical analysis of such data.

This work was performed under contract *AT*(45–1)–1350 between the U.S. Atomic Energy Commission and the General Electric Co.

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RADIUM-226 IN DIET AND HUMAN BONE FROM SAN JUAN, PUERTO RICO

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THE relationship reported here between radium-226 in the diet and radium-226 appearing in the bone of Puerto Ricans is particularly interesting, since the daily radium intake is a factor of three lower and the calcium intake is a factor of two lower than that of a standard Western diet.

For the past few years the Health and Safety Laboratory has been performing measurements to enlarge our knowledge of the natural activity levels in the biosphere. This programme is pertinent because man is most likely in equilibrium with this environmental activity, whereas this is not true of the artificially produced radionuclides, such as strontium-90, from radioactive fall-out. An understanding of the equilibrium situation may very well shed considerable light on non-equilibrium situations, for example, where the relationship between diet and bone levels of strontium-90 is sought.

In 1963 this Laboratory performed analyses for radium-226 on samples of diet and human bone collected in our tri-city diet programme[1]. The average dietary intakes in New York, Chicago and San Francisco were found to be 2·3, 2·1 and 1·7 pc. ^{226}Ra/day. Since the average calcium intake in these cities is 1·05 g/day[2] the radium intake may be expressed as 2·2, 2·0 and 1·6 pc. ^{226}Ra/g calcium, respectively. The corresponding human bone levels were found to be 0·032, 0·031 and 0·026 pc. ^{226}Ra/g calcium. This implies a rather uniform observed ratio[3] between the radium to calcium ratio in bone to that in the diet of about 0·015.

In this present work, two Puerto Rican diet samplings, spaced one year apart, were obtained for the Health and Safety Laboratory's ^{90}Sr programme. Samples of human vertebræ were also obtained from Puerto Rico as part of the same programme.

In all cases, the individuals were living in San Juan for a few years before their deaths and are known to have been lifetime residents of Puerto Rico. Their ages at death ranged from 15 to 60 years. The individuals in this investigation were all in an extremely low income group. The calcium intake is approximately a factor of two lower than that typical of the continental United States. Also, the calcium contribution from milk is about 35 per cent as contrasted to 60 per cent for the urban United States.

The radiochemical determination of ^{226}Ra in human bone and in vegetation samples has been reported in detail elsewhere[4,5]. In brief, for human bone, 10 g of ashed vertebræ, barium carrier, and ^{133}Ba tracer for recovery were dissolved in hydrochloric acid, and $RaBaSO_4$ was precipitated in buffered medium. After centrifuging and decanting, the $RaBaSO_4$ precipitate was dissolved in alkaline tetra-sodium EDTA. Radium was determined by an emanation technique[5], where the gaseous daughter ^{222}Rn was counted overnight in a 2-l. ion chamber. In these chambers 1 pc. ^{222}Rn yields 225 c.p.h. and the backgrounds for eight different chambers range from 6 to 10 c.p.h.

The radium blank for reagents used in this bone procedure is 0·02 pc. The average recovery of the twenty-seven Puerto Rican bone samples based on the ^{133}Ba tracer yield was 97 per cent, while eighteen test samples with added radium gave 94 per cent recovery.

Radium and barium in the Puerto Rican vegetation samples were isolated as chromates as part of the procedure for determination of ^{90}Sr in these same samples. The barium chromate precipitate was dissolved in dilute perchloric acid and radium again measured by the emanation technique. Recovery of radium from these Puerto Rican vegetation samples was determined radiometrically from ^{85}Sr tracer yield data on each sample. The ratio of ^{85}Sr and ^{133}Ba yields from twelve experimental vegetation samples was 1·00 ± 0·08, so the ^{85}Sr is an adequate tracer.

The results for the two Puerto Rican diet surveys are shown in Table 1 in terms of pc. ^{226}Ra/kg of original material. Based on average consumption figures compiled by

Table 1. Radium-226 Content of Puerto Rican Diet Samples

Category	Items in composite	Dietary intake (kg/yr.)	Content of original material (pc. ^{226}Ra/kg)			
			g Ca/yr.	Survey I (Jan. 1962)	Survey II (Jan. 1963)	Average ^{226}Ra consumed per year (pc.)
Dairy products	Liquid milk	68	66·6	0·15	0·08	7
Rice	Rice	65	52·6	—	0·28	18
Beans	Beans	22	22·2	2·74	0·83	40
Codfish	Codfish	10	9·1	0·31	0·61	5
Viands	Plantains, bananas, yautia	55	9·9	1·81	2·46	118
Other vegetables	Pumpkins, onions	6	2·6	0·76	1·22	6
Fruits	Mangos, oranges, guanabana, mamey	25	5·8	0·83	0·67	19
Bakery products	Bread	20	16·2	1·86	1·49	34
Water		400 litres	—	0·007	0·007	3
Total			185 g Ca/yr.			250 pc./yr.

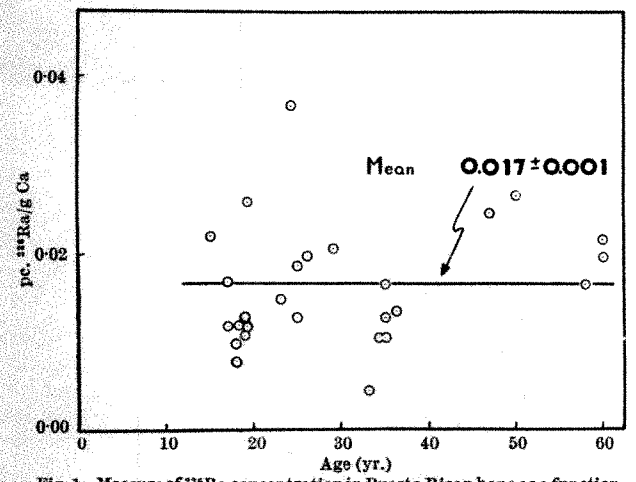


Fig. 1. Measure of ^{226}Ra concentration in Puerto Rican bone as a function of age. The solid line is drawn through the mean of all data

J. Rivera of this Laboratory, after consultation with scientists from the University of Puerto Rico and the Department of Agriculture of the Commonwealth of Puerto Rico, these results may be converted to quantity of ^{226}Ra consumed per year. This is also tabulated in Table 1. The calcium intake of people living on this diet was estimated from calcium measurements made on the ash from these samples[6] and is 185 g per year.

The results for the human bone samples are plotted in Fig. 1 as a function of age. In order to compute the value pc. ^{226}Ra/g calcium from the measured value pc. ^{226}Ra/g of bone ash, the mean value 0·037 ± 0·02 g calcium per g of ash was used. It has been determined in this Laboratory from some 115 samples that this factor is constant with age and the error in using this mean value is slight.

The mean ^{226}Ra content of Puerto Rican human bone and the standard deviation of the mean is seen from these data to be 0·017 ± 0·001 pc./g of calcium. This value is to be compared with the dietary intake computed from Table 1 of 1·35 pc. ^{226}Ra/g calcium. From these measured bone and diet values an observed ratio can be computed for Puerto Rico of 0·013, which compares quite well with observed ratios already noted in New York, Chicago and San Francisco of 0·015, 0·016 and 0·016.

This study shows that individuals on a low-milk, low-calcium diet exhibit the same bone to diet radium–calcium ratio (*O.R.*) as those on a 'normal' Western diet. The maintenance of the *O.R.* over such widely differing radium and calcium intakes indicates that the uptake of radium in the human skeleton may be controlled by the dietary calcium intake.

We thank Isabel M. Fisenne and Salvatore Garafalo for performing the chemical separations and Helen W. Keller for the radon measurements.

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ROTATIONAL STRUCTURE IN THE CHEMILUMINESCENCE SPECTRUM OF MOLECULAR OXYGEN IN AQUEOUS SYSTEMS

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MOLECULAR oxygen was identified, in a previous communication[1], as the origin of the red chemiluminescence observed in the reaction of hydrogen peroxide with OCl^- (or Cl^2) in alkaline solution, following earlier work of Seliger[2] and others. Our observations of a second very strong narrow band in the chemiluminescence spectrum permitted the correlation of the observed 1567 cm^{-1} spacing with the vibrational frequency of ground state oxygen. Considering two alternative interpretations, we tentatively assigned the bands to the $^1\Sigma_g^+ \rightarrow {}^3\Sigma_g^-$ transition of O_2, displaced to higher frequency by solvent interaction. Several additional investigations have been published in the meantime, broadening the observations and their possible interpretation[3–6].

Because this chemiluminescence reaction is a vigorous, heterogeneous reaction, and especially because, as Ogryzlo *et al.*[4] have shown, a whole series of different O_2 electronic transitions is involved in the chemiluminescence spectrum, it is of interest to learn which transitions could possibly originate from solvated oxygen species, and which from gaseous oxygen. The hydrogen peroxide reactant is solvated, the reaction intermediate[7] O—O—Cl^- is also solvated, and yet ultimately the end-product is ground-state O_2 in the gaseous state. We have undertaken spectroscopic investigations at higher resolution in order to gain more detailed information on this problem.

In our new experiments a special externally silvered flow cell was used, permitting continuous bubbling of tank chlorine, through a vertically oriented sintered glass filter, mounted behind a 'Pyrex' observation window, into flowing 0° C alkaline 10 per cent hydrogen peroxide. Since we were determined to investigate the fine structure of extremely faint bands at moderate spectroscopic resolution, precautions were taken for very long periods of operation (3–5 h). The Steinheil 'Universal' spectrograph *GH*, with 3 large glass prisms, and with 640/650 mm collimator/camera *f*/10 optics in an infra-red setting was used, yielding a dispersion of approximately 41 Å per mm in the region of 7000 Å. This arrangement, which was adequate to reveal rotational fine structure in glow discharge spectra of O_2 gas, was used in all the experiments reported. Eastman *I–N* water hypersensitized infra-red spectroscopic plates, sensitive to approximately 9000 Å, were used throughout.

The $^1\Sigma_g^+ \rightarrow {}^3\Sigma_g^-$ emission in gaseous O_2, first observed by Kaplan[8], was examined (0–0 band) at high resolution by Branscomb[9]. His analysis indicated that, although the pure Q branch is missing as expected for this case, nevertheless R_Q and P_Q branches accompany the R and P branches, respectively, in a fashion characteristic of spin-intercombinations. A definite gap in each vibration–rotation band can then be expected as a prominent feature at moderate resolution, between the R, R_Q, and P, P_Q band systems. Krishna and Llewellyn in this laboratory have investigated the *RF* electrodeless glow discharge spectrum of gaseous oxygen at moderate resolution[10], and have observed the strong 0–0 band and the much weaker 0–1 band of the $^1\Sigma_g^+ \rightarrow {}^3\Sigma_g^-$ transition. Their

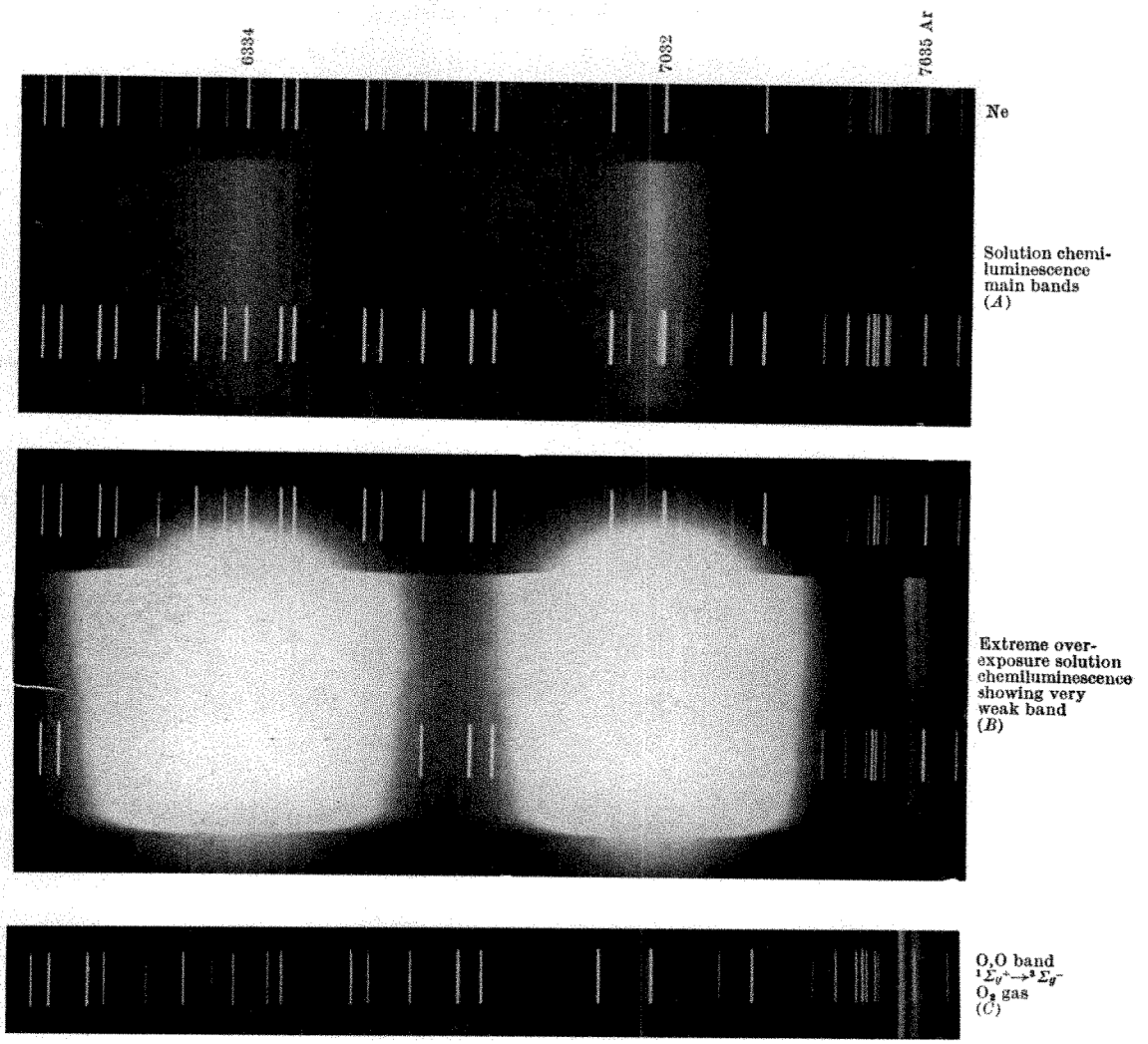


Fig. 1. Chemiluminescence spectra at higher spectral resolution for the reaction of alkaline hydrogen peroxide with chlorine, compared with *RF* electrodeless discharge spectrum of gaseous oxygen (after Krishna and Llewellyn[16]). *A*, slit 0·25 mm, exposure 5 min; *B*, slit 0·50 mm, exposure 5 h; *C*, slit 0·25 mm

plates show partially resolved rotational structure (rotational band flutings are clearly visible on the original plates), with a pronounced gap in each band. A print of one of their plates for the 0–0 band is shown in Fig. 1*C*.

The solution chemiluminescence main bands (H_2O_2 + Cl_2 reaction), which we previously reported[1] at 6334 Å and 7032 Å at low resolution, are shown in a weak exposure (5 min) in Fig. 1*A* at moderate resolution. There is no suggestion of any fine structure or unsymmetrical band contour in the observed diffuse bands; at 50 per cent plate blackening the bands appear much broader and represent their actual half-width more accurately, because of this more nearly linear response region of the plate. The diffuseness and anomalous relative intensity of these bands require a special interpretation, which we shall discuss later.

In a few somewhat strenuous experiments, extreme over-exposures of the chemiluminescence spectrum were photographed. This required 5 h of continuous bubbling of chlorine, wider spectrograph slits, and careful maximization of the optical arrangement. The typical spectrum is shown in Fig. 1*B*. The main chemiluminescence bands are now seen as extremely broad regions of emission. But the (strongest) weak band previously noted by us, and observed more extensively by Browne and Ogryzlo[4], is now seen to reveal definite rotational structure, and to coincide perfectly with the 0–0 band of the $^1\Sigma_g^+ \rightarrow {}^3\Sigma_g^-$ transition of gaseous O_2, investigated by Krishna and Llewellyn[16]. (As noted in the legend of Fig. 1, the slit used for the solution chemiluminescence was twice that used in the gas discharge experiment, but the rotational gap is still clear; curvature of the slit image at the top end of the band is the result of our collimator focus setting.) The results described require the unique interpretation that the band system observed near 7635 Å originates from $^1\Sigma_g^+$ excited O_2 trapped inside a gas bubble. That a very small fraction of the O_2 molecules produced could emit light as gaseous O_2 is not surprising. Browne and Ogryzlo reported 0–0, 1–1 and 0–1 bands for the $^1\Sigma_g^+ \rightarrow {}^3\Sigma_g^-$ transition and 1–0 and 0–0 bands for the $^1\Delta_g \rightarrow {}^3\Sigma_g^-$ transition of oxygen; we believe that all these extremely weak bands originate from gaseous oxygen from within the bubbles during (or after) the chemiluminescence reaction (also assumed by Browne and Ogryzlo). Higher resolution investigations should reveal structure in all of these bands.

The observed diffuseness of the main chemiluminescence bands, and their occurrence at 6334 Å and 7032 Å, requires consideration of several alternative interpretations:

(1) The bands may represent the $^1\Sigma_g^+ \rightarrow {}^3\Sigma_g^-$ transition in hydrated O_2, shifted by 2,593 cm^{-1} to higher frequency by the solvent interaction.

(2) The bands may represent the superposition of quantum states[11] transition $2(^1\Delta_g) \rightarrow 2(^3\Sigma_g^-)$ brought

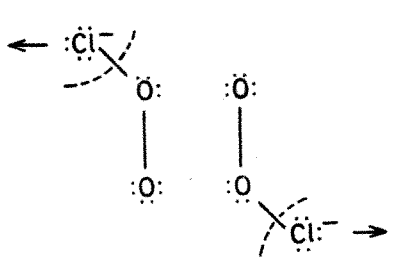
about directly by the interaction of two $^1\Delta_g$ excited O_2 molecules in the gas phase.

(3) The bands may represent the superposition of quantum states transition $2(^1\Delta_g) \rightarrow 2(^3\Sigma_g^-)$ brought about indirectly by the interaction of two Cl—O—O⁻ intermediates in solution, with emission from the solvated O_2 collision pair, and assuming negligible solvation shift.

Concerning case (1), our present experiment leaves room for a solvent-displaced $^1\Sigma_g^+ \rightarrow {}^3\Sigma_g^-$ transition to occur, since the bands reported by Ogryzlo *et al.*[4] for this transition are clearly for excited O_2 gas in the bubbles. The fact that Arnold, Ogryzlo and Witzke were able to observe very weak bands at 6340 Å and 7030 Å in electrical discharge also in gaseous oxygen still leaves some doubt because of the possibility of minute traces of water in such systems[12]. Griffith's crystal field calculations[6] for hydration of oxygen indicate that exactly such a high frequency shift could be found as suggested by this interpretation case; moreover, the $^1\Delta_g$ state is shifted downward in energy by the crystal field interaction, which could account for its non-observation as a hydrated species. A decisive experiment needed here is to observe the $^1\Sigma_g^+ \leftarrow {}^3\Sigma_g^-$ absorption of O_2 in water solution. Because of the extreme forbiddenness of this transition, a high-pressure, long-path experiment is essential.

Concerning case (2), it was proposed by Stauff and Schmidkunz[13] that the combination or superposition transition proposed for certain absorption bands in liquid oxygen by Ellis and Kneser[11] could account for the chemiluminescence band at 6334 Å because of the obvious coincidence of wave-length with the 0–0 band of the $2(^1\Delta_g) \rightarrow 2(^3\Sigma_g^-)$ absorption in liquid oxygen. Ogryzlo *et al.*[4] also favour this interpretation. Although these superposition bands have been referred to as "O_4 bands" no stable complex is required by the transition mechanism. We are inclined to reject this interpretation involving the direct interaction of two singlet excited gaseous oxygen molecules, liberated by the primary chemiluminescence reaction and emitting from within the gas bubbles. We would expect more single-molecule emission. (The relative yield of $^1\Delta_g \rightarrow {}^3\Sigma_g^-$ is undetermined, but may be large[4].) However, the $^1\Sigma_g^+$ and $^1\Delta_g$ states are highly metastable, and the enhanced probability of the superposition transitions could account for the dominating observability of the proposed $2(^1\Delta_g) \rightarrow 2(^3\Sigma_g^-)$ transition. An absolute quantum yield determination for the overall chemiluminescent reaction could assist in securing this interpretation. We are not certain what rotational structure should be expected for O_2 pairs, or weak O_4 complexes, but the diffuseness and broadness of the two main chemiluminscence bands argues against a gas phase emission.

Case (3) is a modification of case (2) which we believe is more in accord with the mechanism of the primary reaction[7]. We consider that the (ground-state) intermediate Cl—O—O⁻ may be relatively stable toward solvent chemical interaction, but is highly unstable toward reaction with another Cl—O—O⁻. We next picture the vigorous elimination, on collision of intermediates, of two Cl ions simultaneously, leaving (perforce) two singlet oxygens in collisional proximity:



Thus, emission of combination or superposition transition would be favoured over single molecule singlet → triplet emission. This could account for the apparent dominance of the superposition transitions, but then one would expect not only the emission from the $2(^1\Delta_g)$ states, but also from the $2(^1\Sigma_g^+)$ and $(^1\Sigma_g^+ + {}^1\Delta_g)$ states[11,14,15]. Perhaps the failure to observe these additional combination or superposition bands is an argument against case 3 (and case 2) as possibilities. Ogryzlo and co-workers have assumed that the greater 'quenching' of the $^1\Sigma_g^+$ state of gaseous O_2 by H_2O molecules favoured the formation of $2(^1\Delta_g)$ states of collision pairs of O_2 over other combination states. This suggestion could apply equally well to case 3, especially because we assume all events take place in the liquid phase. Case 3 assumes that Griffith's crystal field hypothesis[6] is invalid, and that the solvent shift of the transition is negligible.

The general character of the main chemiluminescence bands for the reaction calls for additional comment. Investigations[14,15] of the absorption spectrum of O_2 in the liquid and solid states, and at high pressures in the gas, show band contours and relative band intensities which differ markedly from those observed and expected for unperturbed gaseous O_2. The Franck–Condon intensities for the transition in the latter case are of the order 10/0·4/0·001 for the 0–0, 0–1 and 0–2 bands, respectively. Like the condensed phase and high pressure spectra, the main chemiluminescence bands also show anomalous 0–1 intensity relative to the 0–0 band, whereas the 0–0 and 0–1 weak bands reported by Browne and Ogryzlo[4] suggest apparently normal intensity ratios, confirming the assignment of these weak bands to gaseous oxygen.

At this stage we do not find it possible to decide unequivocally between case (1) and cases (2) or (3), until we have high resolution investigations of the gas phase $2(^1\Delta_g) \rightarrow 2(^3\Sigma_g^-)$ bands (with assurance of no hydration), understand the rotational structure expected for these superposition transitions, or have direct absorption data for the $^1\Sigma_g^+ \leftarrow {}^3\Sigma_g^-$ transition for O_2 dissolved in water.

The main purpose of these investigations is to gain information on the metastable electronically excited states of molecular oxygen originating from inorganic and organic peroxide breakdown. If, as we believe, the metastable $^1\Sigma_g^+$ or $2(^1\Delta_g)$ states can be generated for oxygen dissolved in water, such a metastable species could play a very important part in reaction mechanisms. In the gaseous state the $^1\Sigma_g^+$ state has a mean lifetime of about 7 sec; in water solution this could be diminished several orders of magnitude, but even such a resulting lifetime would be a long one in terms of reactions in the liquid phase.

We acknowledge the use of a plate obtained by Drs. V. G. Krishna and A. Llewellyn for the electrodeless discharge of gaseous O_2. This work was supported by contracts with the Physics Branch, U.S. Office of Naval Research, and with the Division of Biology and Medicine, U.S. Atomic Energy Commission. A special grant from the Charles F. Kettering Foundation to one of us (M. K.) is acknowledged with thanks.

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ORIENTATION OF PYROPHYLLITE SYNTHESIZED IN SLOWLY STRAINED MATERIALS

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A DISTINGUISHING feature of slates is parallel orientation of minute platy grains of mica and other phyllosilicates. The familiar splitting property called slaty cleavage arises from this grain orientation pattern, or fabric. It is generally recognized that the mica in slates grows during metamorphism and that its orientation results from anisotropic stress conditions or consequent flow in rock bodies, but there is no general agreement on the geometric relation between slaty cleavage and directions of stress or flow[1]. Experiments with model materials[2] have shown that random platy particles can be made parallel by deformation of an enclosing plastic matrix.

The following describes exploratory experiments in which the phyllosilicate, pyrophyllite, was synthesized hydrothermally in slowly strained materials. Pyrophyllite fabrics were obtained that are broadly similar to those of micas in slates.

Experiments were run at 425°–475° C and 1,000 atm. water pressure, conditions under which pyrophyllite had been grown by previous workers[3]. Three starting materials were used: a quartz–kaolin claystone and 4 : 1 molecular mixtures of amorphous silica–chi-alumina and amorphous silica–amorphous alumina. The reactions were:

$$\underset{\text{kaolin}}{Al_2Si_2O_5(OH)_4} + 2SiO_2 \rightarrow \underset{\text{pyrophyllite}}{Al_2Si_4O_{10}(OH)_2} + H_2O$$
$$\underset{\text{quartz}}{}$$

$$4SiO_2 + Al_2O_3 + H_2O \rightarrow Al_2Si_4O_{10}(OH)_2$$

The claystone, from the Gore Lignite Measures, Southland, New Zealand (*S*171/220461), contained some illite and had the appearance and consistency of blackboard chalk. X-ray diffractograms, electron micrographs and differential thermal analysis indicated a platy variety of kaolin. The claystone lacked visible stratification in hand specimens but contained kaolin and illite grains with basal planes preferentially parallel to bedding. Cylinders were cored normal and parallel to bedding and fitted in open-ended brass jackets 15 mm long, with internal diameters of 10 mm and external diameters of 12·7 mm.

The oxide mixtures provided homogeneous starting materials with minimum orientation. Chi-alumina is a poorly ordered alumina[4], and that used (Merck chromatographic) gave typical diffuse X-ray reflexions. The amorphous alumina (prepared by heating May and Baker aluminium nitrate to 350° C under reduced pressure) and the silica ('Mallinckrodt' analytical reagent silicic acid, 12 per cent loss on ignition), gave no X-ray reflexions and were optically isotropic. Cylinders of the oxide mixtures were formed by pressing the powder into brass jackets with internal diameters of 10 mm or 12·7 mm, depending on whether the piston was intended to bear on the specimen and jacket together—or on the specimen alone (Fig. 1). The larger type of jacket and specimen were supported by a thick steel cylinder to limit radial expansion.

The specimens were compressed axially at constant strain rates (3–13 per cent/day) by a stainless-steel piston (12·7 mm diam.) in a stainless-steel pressure bomb 36 cm high which was held in a press. The lower 15 cm of the bomb was surrounded by a furnace and the upper third was cooled by an air jet to prevent leakage of water pressure through the piston seal (a graphite–asbestos chevron packing). Water vapour filled the interior of the bomb and had access to the specimens.

Fabrics of the experimental products were assessed by examination of thin sections and by comparison of the X-ray diffractometer peak heights obtained from a surface cut parallel to the cylinder end (1–3 mm from an end) with those from a surface normal to the cylinder end. Exterior surfaces were usually not mineralogically representative of the interior (for example, rich in kaolin, cristobalite). The approximate amounts of pyrophyllite lying parallel and sub-normal to the surface irradiated were indicated by the heights of the basal peaks and of the *hk*0 peak at about 20° 2θ (Cu $K\alpha$ radiation).

After more than 2 days at 450° C and 1,000 atm. water pressure abundant pyrophyllite was found in unstrained claystone cylinders which had not been deformed. Pyrophyllite grew more slowly in unstrained cylinders of amorphous silica and chi-alumina under these conditions. Reaction occurred in three days, but unreacted material remained, especially in the centre of the cylinder, after eleven days. Thin sections and X-rays showed pyrophyllite with basal planes preferentially parallel to bedding in unstrained cylinders of claystone. By contrast the pyrophyllite in an unstrained chi-alumina–silica cylinder showed no detectable preferred orientation in thin section and only weak orientation was suggested by X-rays.

The deformation experiments were of two types determined by the arrangements of piston, etc., shown in Fig. 1: (*a*) shortening accompanied by radial expansion; (*b*) shortening with little or no radial expansion. The chief observations from experiments of type (*a*) were:

(1) Claystone cylinders compressed normal to bedding and shortened less than 50 per cent had pyrophyllite fabrics with no marked differences from those of unstrained cylinders.

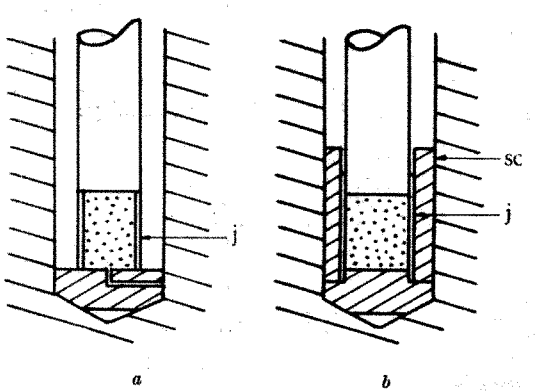


Fig. 1. Arrangement of piston, specimen, and brass jacket (*j*) for (*a*) deformation with radial expansion, (*b*) deformation with radial constraint provided by thick steel cylinder (*sc*)

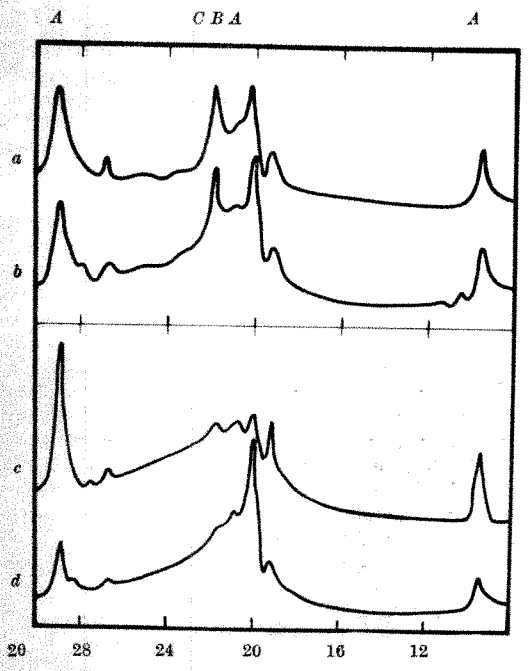


Fig. 2. Diffractometer traces from unstrained and strained chi-alumina–silica cylinders. All surfaces of comparable size. Copper $K\alpha$ radiation. *a*, Surface parallel to and 3 mm from end of cylinder held without strain for 11 days at 1,000 atmospheres water pressure and 450° C. *b*, Surface normal to end of cylinder *a*. *c*, Surface parallel to piston face and 1·5 mm from end of cylinder shortened 42 per cent in eight days at 1,000 atmospheres water pressure and about 475° C. *d*, Surface normal to piston face in cylinder *c*. *A*, 00*l* reflexions from pyrophyllite. *B*, *hk*0 reflexions from pyrophyllite. *C*, 101 reflexions from cristobalite

(2) A claystone cylinder compressed parallel to bedding and shortened 65 per cent in six days at 1,000 atm. water pressure and about 475° C contained pyrophyllite and mica crystals predominantly oriented with basal planes parallel or sub-parallel to the piston face, and thus at a high angle to the initial orientation of bedding. In this experiment the jacketed cylinder expanded radially and met the walls of the bomb at about 60 per cent strain.

(3) A chi-alumina–silica cylinder shortened 75 per cent in eleven days at 1,000 atm. water pressure and about 425° C contained pyrophyllite predominantly with basal planes parallel or sub-parallel to the piston face. This cylinder also expanded to meet the walls of the bomb at about 60 per cent strain. Pyrophyllite grains comprised about 20 per cent of the specimen except at its base, where there was a 0·3 mm oriented layer of pure pyrophyllite.

When radial expansion was restricted in experiments of type (*b*), oriented pyrophyllite was also obtained with oxide specimens as follows.

(4) A chi-alumina–silica cylinder shortened 42 per cent in eight days at 1,000 atm. water pressure and about 475° C contained about 50 per cent of pyrophyllite, again mainly with basal planes parallel or sub-parallel to the piston face. Diffractometer traces from this material are shown in Fig. 2 along with traces from an unstrained cylinder for comparison.

(5) An amorphous alumina–silica cylinder shortened 60 per cent in 4·5 days at about 475° C and water pressures fluctuating between 500 and 1,000 atm. had a pyrophyllite fabric similar to (4). A representative part of this specimen is shown in thin section in Fig. 3.

In all claystone cylinders shortened more than a few per cent some irregular slickensided fracture surfaces were characteristic. These made no regular angle with the cylinder axis and were typically at least a millimetre apart. In the claystone cylinder shortened 65 per cent there were fracture-free blocks up to 2 mm across. The deformed oxide cylinders showed very few fractures in thin section and were more coherent than deformed claystone cylinders. The cylinders deformed 42 per cent and 75 per cent had hardnesses of 3 and 4 on Moh's scale. Claystone specimens changed colour from white to grey with increasing deformation. Oxide specimens remained white.

The optical and X-ray methods of examining products detected marked preferred orientation but did not allow

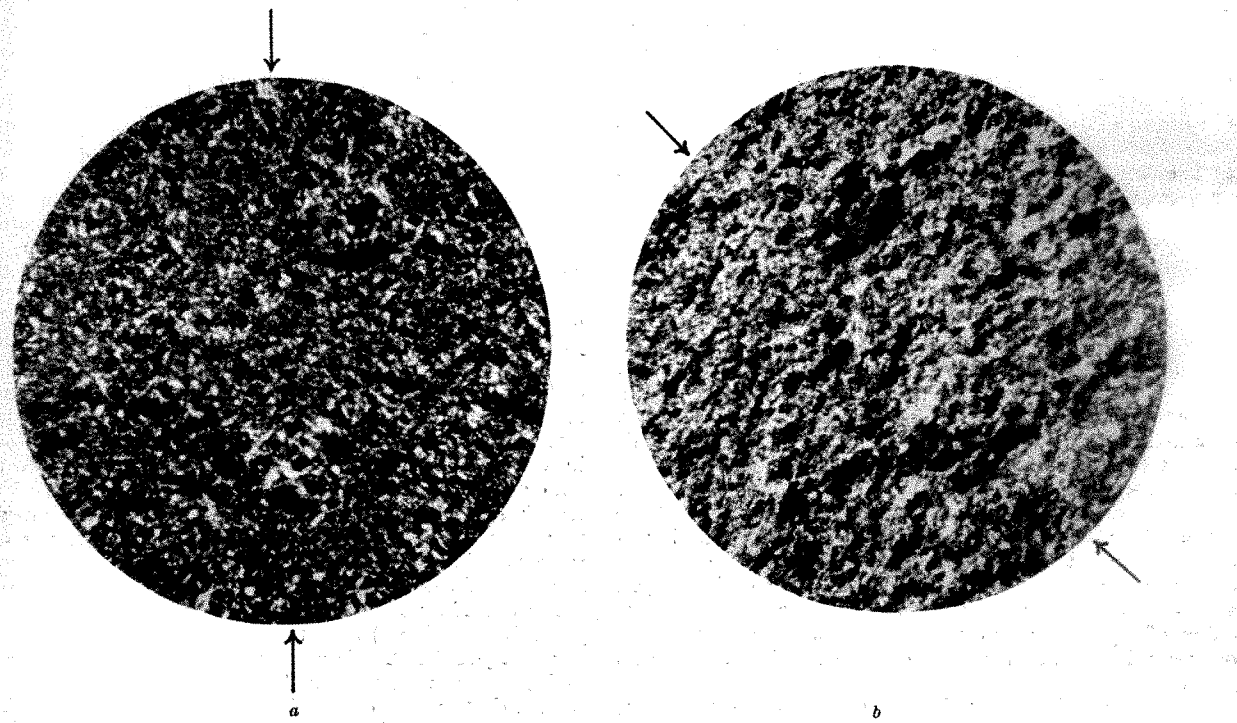


Fig. 3. Thin section normal to piston face from amorphous alumina–silica cylinder shortened 60 per cent. Field diameter 0·5 mm. Crossed nicols. *a*, Trace of piston face E–W; *b*, trace of piston face in 45° position. Most grains illuminated in *b*; but dark in *a* are pyrophyllite oriented with trace of (001) parallel or subparallel to trace of the piston face

detailed description of the fabrics, for example, whether more pyrophyllite lay parallel to the piston face or at 5° or 10° to it. There were indications in thin sections of some deformed oxide cylinders that most of the pyrophyllite lay at some such small angle to the piston face and that it was concentrated in poorly defined symmetrically oriented zones that made a moderate angle (30°–40°) with the piston face.

Strain and grain orientation mechanisms in these experiments are incompletely known. Strain in claystone cylinders was accomplished at least in part by rupture and relative movement of blocks. In oxide cylinders intergranular slip on a fine scale almost certainly occurred. In all experiments there were movements on an ionic or molecular scale during chemical reaction and these probably contributed to total strain. Oriented overgrowth on kaolin and illite grains may have been responsible for mimetic orientation of pyrophyllite in unstrained claystone cylinders. In the deformation experiments orientation may have resulted from rotation of crystals, from nucleation or growth of crystals in certain orientations, or from a combination of these mechanisms.

Pyrophyllite was the main phyllosilicate grown in most experiments with the claystone. Specimens containing muscovite as the only phyllosilicate were prepared by adding potassium carbonate to the bomb. This was not done regularly, however, because the strongest basal X-ray reflexion of muscovite overlaps with the strongest quartz reflexion and because of the difficulty of distinguishing reflexion intensity changes from recrystallization of illite from those marking synthesis of muscovite.

Temperatures, strain rates and growth rates were probably higher in the experiments than in natural slate-producing environments; effective confining pressures may have been lower at least in the experiments involving radial expansion. The experiments demonstrate that an oriented fabric can form while a phyllosilicate is synthesized hydrothermally from material undergoing slow strain.

The experiments were performed in the high pressure-temperature laboratory of the Chemistry Department, University of Otago. The assistance of Mr. V. G. Tyrrell and Mr. E. McKenzie in making the apparatus, and also the preparation of electron micrographs of the claystone by the Physics and Engineering Laboratory, Department of Scientific and Industrial Research, is gratefully acknowledged. The claystone was collected by Mr. D. G. Bishop, and Mr. N. Miller calibrated the strain gauges. We also thank Mr. B. W. Collins, Prof. D. S. Coombs and Dr. M. S. Paterson for their advice.

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FACTORS INFLUENCING THE RADIO-PROTECTION OF TISSUE CULTURE CELLS BY SULPHYDRYL COMPOUNDS

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In previous publications[1–3] results were described indicating that the radio-protective action of cysteamine at the cellular level increases with higher concentrations of this sulphydryl compound. Révész and Bergstrand[4] demonstrated that higher concentrations of cysteamine in the medium resulted in an increased intracellular non-protein sulphydryl content. At equimolar concentrations of the compound this increase appeared to be of a closely similar magnitude as the amount of radio-protection found in our tissue-culture system. These results suggest that radiation protection is dependent on the concentration of the non-protein-bound sulphydryl content effected in the protected cell. Vos *et al.*[2] reported that cysteamine provided a better radio-protective effect than cysteine at equimolar concentrations. Since the dissociation constant of the –SH group of cysteamine (pK $8{\cdot}50 \pm 0{\cdot}14$) is different from that of cysteine (pK $8{\cdot}9 \pm 0{\cdot}2$) at the physiological pH of 7·3, this will result in important differences in the $-S^-$ ion concentration at equimolar concentrations of these compounds. This suggested that not the concentration of –SH groups but that of their dissociated form, namely, the $-S^-$ ions, might be responsible for the protection against radiation. Two types of experiments were performed to test this hypothesis. First the radio-protective effect of equimolar concentrations of sulphydryl compounds of different pK values was determined and secondly the radio-protective effect of equimolar concentrations of a given sulphydryl compound was measured in culture media at different pH values.

The amount of radiation damage caused by X-irradiation (physical constants: 250 kV, 15 m.amp, half-value layer 1·9 mm copper, filtration 1·5 mm copper corrected, dose rate 200 r./min, maximal back-scatter) was assayed by the loss of reproductive integrity of human kidney cells[1–3]. The cells were cultured at 37° C during 13 days, using Puck's feeder layer technique[5], in modified Petri dishes, consisting of glass rings fitted with a 'Melinex' bottom. The culture medium consisted of 0·5 per cent lactalbumin hydrolysate in Hanks's salt solution and 5 per cent calf serum, supplemented with 100 I.U. of penicillin and 0·1 mg of streptomycin/ml. For the experiments it was essential that the pH of the solution remained constant during the whole prophylactic incubation period before and during irradiation. Therefore another culture medium[6], wherein Hanks's solution was replaced by a phosphate buffered salt solution, was used to dissolve the sulphydryl compounds. The pH of this culture medium was 7·3 and could be varied between pH 6·3 and pH 8·3. This medium was also used before and during the irradiation of non-treated cells and of the cells made anoxic by flushing with oxygen-free nitrogen[1,3]. All solutions were freshly prepared, added to the cells 10 min before irradiation and removed directly afterwards by washing three times with the standard culture medium. The dose-reduction factors (DRF) were calculated by drawing the best fitting curve through the experimental points and dividing the X-ray dose giving 10 per cent survival of the treated cells by the dose giving the same survival of the non-treated cells.

First, different sulphydryl compounds with diverging pK values (Table 1) were tested in equimolar concentrations at pH 7·3. Their radio-protective effects expressed in DRFs are presented in Table 2. Since it is not excluded that anoxia caused by autoxidation of the sulphydryl compounds is partly responsible for the radio-protective action, the experiments were repeated under anoxic conditions. Anoxia was established by flushing

Table 1. *p*K VALUES AND PERCENTAGES OF DISSOCIATED –SH GROUPS OF THE TESTED SULPHYDRYL COMPOUNDS AT *p*H 7·3

Sulphydryl compound	*p*K	Percentage –S⁻ at *p*H 7·3
Cysteamine	8·50 ± 0·14	4–8
d- and *l*-Cysteine	8·9 ± 0·20	1–2·5
l-Glutathione	9·28 ± 0·07	1·0
Thioglycol	9·60 ± 0·03	0·5

Table 2. DOSE REDUCTION FACTORS (DRF) OBTAINED WITH DIFFERENT CONCENTRATIONS OF SULPHYDRYL COMPOUNDS (Incubation before irradiation: 10 min at room temp.)

Sulphydryl compound	DRF at *p*H 7·3				
	8 mM	16 mM	40 mM	100 mM	200 mM
Cysteamine	2·96	3·5	—	—	—
l-Cysteine	1·7	3·2	—	—	—
d-Cysteine	1·4	2·8	—	—	—
l-Glutathione	1·1	1·2	1·6	—	2·3
Thioglycol	—	1·5	—	2·9	4·0

Table 3. DRF OBTAINED WITH DIFFERENT CONCENTRATIONS OF SULPHYDRYL COMPOUNDS UNDER ANOXIC CONDITIONS (Incubation: see Table 2)

Sulphydryl compound	DRF at *p*H 7·3 under anoxic conditions			
	0 mM	8 mM	16 mM	32 mM
Cysteamine	2·5	4·08	4·5	—
l-Cysteine	2·5	3·45	3·65	4·5
d-Cysteine	2·5	3·2	3·25	4·0
l-Glutathione	2·5	—	2·5	—
Thioglycol	2·5	2·9	3·3	3·6

Table 4. DRF OBTAINED WITH CONSTANT CONCENTRATIONS OF A SULPHYDRYL COMPOUND AT DIFFERENT *p*H VALUES (Incubation: see Table 2)

Sulphydryl compound	Concentration (mM)	DRF at *p*H		
		6·3	7·3	8·3
Cysteamine	16	2·3	3·5	4·8
l-Cysteine	32	3·0	—	4·4
Thioglycol	100	2·8	2·9	3·8
Thioglycol	200	—	4·0	4·7

Table 5. DRF OBTAINED WITH CONSTANT CONCENTRATIONS OF CYSTEAMINE UNDER ANOXIC CONDITIONS AND AT DIFFERENT *p*H VALUES (Incubation: see Table 2)

Pretreatment (10 min)	DRF at *p*H		
	6·3	7·3	8·3
Nitrogen	2·4	2·5	2·5
Nitrogen + 8 mM cysteamine	3·7	4·1	4·7
Nitrogen + 16 mM cysteamine	4·1	4·5	4·9

with oxygen-free nitrogen during the incubation with a sulphydryl compound in a modified closed culture vessel[3]. After 10 min of this treatment less than 15 p.p.m. of oxygen could be demonstrated with a Hersch cell[7] connected with the outlet of the culture vessel. The DRF values obtained after this combined pretreatment at *p*H 7·3 are presented in Table 3. It will be seen that with the exception of thioglycol the radio-protective potency of the sulphydryl compounds increases with decreasing *p*K value. The differences in effect between *d*- and *l*-cysteine in equimolar concentrations are relatively small but might indicate that the steric configuration near the –S⁻ group is of some importance for the mechanism of radio-protection. Thioglycol gave a better radio-protection than could be expected from its low –S⁻ concentration at *p*H 7·3. Dewey[8] postulated on account of his experiments with *Serratia marcescens* that at higher concentrations the –OH group of thioglycol is responsible for a radio-protective effect in addition to the effect of the –SH group. High concentrations of various alcohols also provided in our tissue-culture system a considerable radio-protective effect (Vos, to be published). For this reason it seems reasonable to explain the deviating protection of thioglycol on account of its –OH group.

Next we tested our hypothesis by changing the *p*H of the solution and keeping the concentration of the sulphydryl compound constant. It could be shown that the cells could withstand a 30-min exposure to culture media at a *p*H varying between 6·3 and 8·3 without loss of plating efficiency. The results of pretreatment with cysteamine, *l*-cysteine and thioglycol at different *p*H values are presented in Table 4. Variation of the *p*H of the medium did not influence the radiosensitivity of the otherwise untreated cells. These experiments were repeated under anoxic conditions to exclude the possible influence of variation in the degree of hypoxia at the different *p*H values. The results are presented in Table 5. From both groups of experiments it follows that the radio-protective effect of equimolar concentrations of a given sulphydryl compound increases with increasing *p*H.

The results presented here indicate a correlation between the radio-protective action of the sulphydryl compounds and the extent of their dissociation in the medium. The concentration of the –S⁻ ion thus depends on the *p*K value of the sulphydryl compound, the concentration of the compound and the *p*H of the medium. Kohn and Gunter[9] earlier reported that the radio-protection of *E. coli B* and *B/r* by *l*-cysteine was less at *p*H 5·3 than at *p*H 7·8 ; this result was confirmed by Dewey[10] in similar experiments with *Serratia marcescens*. More experimental evidence will be required to prove our hypothesis. At present our work is extended to include more thiol compounds and their penetration into the cells. The present hypothesis might be of value in the search for more powerful radiation protectors.

We thank Dr. O. Vos for his advice and Dr. A. W. Kaandorp, Chemical Laboratory of this Organization, for determining *p*K values.

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DEVELOPMENT OF RIBONUCLEIC ACID POLYMERASE IN CELLS INFECTED WITH ENCEPHALOMYOCARDITIS VIRUS AND THE SYNTHESIS OF SINGLE- AND DOUBLE-STRANDED RNA BY THE ISOLATED POLYMERASE

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THE replication of viral RNA in cells infected with the small RNA-containing viruses is thought to be catalysed by an RNA-dependent RNA polymerase the synthesis of which is induced by the invading viral genome. Franklin *et al.* have described the presence of such an enzyme in HeLa cells infected with poliovirus[1] and in *L* cells infected with mengovirus[2]. In both instances, the polymerase catalyses the incorporation of

nucleoside triphosphates into RNA in the presence of actinomycin *D*, in contrast to the normal cellular DNA-dependent RNA polymerase. The enzyme could not be detected in uninfected cells, and its activity increased concurrently with the increase in intracellular titre of virus.

The RNA polymerases of Krebs II ascites tumour cells infected with murine encephalomyocarditis (EMC) virus have been examined by Cline, Eason and Smellie[3]. They found a polymerase which differed markedly from that described by Franklin's group. This enzyme was present in uninfected cells and its activity increased during infection in a manner unrelated to the growth of the virus. It was present in both nuclear and cytoplasmic extracts and was sensitive to ribonuclease. The enzyme reaction product was not characterized other than by demonstrating its lability to alkali.

The methods for extraction and assay of polymerase used by Cline *et al.* differed from those used by Franklin *et al.*, and the possibility therefore arises that more than one polymerase may be induced during infection. As part of an examination designed to locate the site of synthesis of viral RNA in Krebs cells infected with EMC virus, we have re-examined the situation as far as the virus-specific RNA polymerase is concerned. We have found that two types of polymerase are present in the infected cell. One of the enzymes is similar to that found in polio- and mengo-infected cells and it is probably concerned in catalysing the synthesis of progeny viral RNA.

Preparation of RNA polymerase from infected cells. Krebs II ascites tumour cells were prepared as a suspension ($1{\cdot}5 \times 10^7$ cells/ml.) in Earle's medium containing 3 μg/ml. of actinomycin *D*. EMC virus (3–5 plaque forming units/cell) was added and the suspensions left overnight at 4°. Virus growth was initiated by raising the temperature to 37°. Details of the incubation of cells and assay of virus are given in ref. 4. Samples of about 10^9 infected or control cells were removed 5·5 h after initiation of infection, washed with buffered saline, then with 0·25 M sucrose–1 mM $MgCl_2$ and then suspended in 20 times their packed cell volume of water. The cells were disrupted with a Dounce homogenizer[5] and *tris*-hydrochloric acid buffer, *p*H 8·3, and $MgCl_2$ were added to a final concentration of 0·1 M and 5 mM respectively. Sodium chloride was added to a final concentration of 0·5 M and, 10 min later, the homogenate was diluted with an equal volume of water. The suspension was centrifuged at 600*g* for 10 min, and the supernatant centrifuged for a further 60 min at 100,000*g*. The pellet from this spin was suspended in 0·25 M sucrose–1 mM $MgCl_2$ and assayed for its ability to catalyse the incorporation of ^{14}C–ATP into acid precipitable products in the presence of actinomycin. The assay method was based on that described by Baltimore and Franklin[2]. The results are summarized in Table 1.

Table 1. RNA Polymerase Activities of Infected Cell Homogenates

	Incubation mixture	AMP incorporated (μμmoles/mg protein)
Complete	none	135
,,	uninfected cells	10
,,	omit CTP, GTP, UTP	103
,,	omit $MgCl_2$	45
,,	omit phosphoenol pyruvate	33
,,	add ribonuclease	97
,,	add deoxyribonuclease	150

Homogenates from cells infected with EMC virus for 5·5 h were prepared as described in text. The assay mixture contained *tris*-HCl *p*H 8·3, 35 μmoles; triphosphates of cytidine, guanosine and uridine, 90 mμmoles each; ^{14}C–ATP (spec. act. 1·55 c./mole), 90 mμmoles; phosphoenol pyruvate, 5 μmoles; pyruvate kinase (C. F. Boehringer and Soehne), 10 μg; $MgCl_2$, 5 μmoles; actinomycin *D*, 1 μg and 0·1 ml. of enzyme preparation containing about 1 mg protein. The final volume was 0·4 ml. 50 μg ribonuclease (C. F. Boehringer and Soehne) and 10 μg deoxyribonuclease (Sigma Chemical Co.) were added where indicated. Incubation was for 15 min at 37°. All activities were corrected for radioactivity in a control incubated at 0°.

Preparations from cells infected for 5·5 h with EMC virus showed appreciable incorporation of ^{14}C–ATP and therefore contained an actinomycin-resistant polymerase. Incorporation, which was negligible in preparations from uninfected cells, required Mg^{++} and an ATP-generating system. The reaction was only partly dependent on the presence of all four nucleoside triphosphates. Deoxyribonuclease did not reduce the incorporation, and it was only partially inhibited by the addition of 50 μg of ribonuclease. The properties of this enzyme preparation from EMC virus-infected cells, therefore, are very similar to those described by Baltimore and Franklin[2] and by Baltimore[6] for their mengovirus-infected L-cells and poliovirus-infected HeLa cells.

Localization of polymerase activity. The sedimentation behaviour of the polymerase from EMC-infected cells was investigated by differential centrifugation. Krebs cells were infected with EMC virus for 5·5 h, washed and disrupted as before. The homogenate was treated at 0° for 10 min with 0·5 M NaCl and diluted with an equal volume of water. This suspension was then fractionated into 'nuclear', large particle and small particle fractions by centrifugation at 600*g* for 10 min, 40,000*g* for 15 min and 100,000*g* for 60 min, respectively. Each fraction was suspended in a small volume of sucrose–$MgCl_2$ solution and assayed for its ability to incorporate ^{14}C–ATP into acid-insoluble products in the presence of actinomycin.

Table 2. Distribution of RNA Polymerase Activities among Subcellular Fractions of Infected Cells

Fraction (*g*-min)	Virus growth when collected (% of final)	Incubation mixture	AMP incorporated (μμmoles/mg protein)
Nuclei (6×10^5)	80	Complete	35
	80	omit CTP, GTP, UTP	20
Large particle (6×10^5)	50	Complete	690
	50	omit CTP, GTP, UTP	300
	50	Complete plus ribonuclease	363
Small particle (6×10^6)	40	Complete	151
	40	omit CTP GTP, UTP	350
	40	Complete plus ribonuclease	26

Assay conditions as for Table 1

The results (Table 2) showed that most of the activity was localized in the large-particle fraction and here all four triphosphates were required for maximum activity. It was unaffected by the addition of mercaptoethanol. Added EMC viral RNA caused only a 20 per cent stimulation of synthesis. The optimum *p*H for polymerase activity in the large particle fraction was 8·0–8·4. In all these respects the enzyme preparation resembles that found by Baltimore and Franklin[2] in polio- and mengo-infected tissue cultures. The large particle fraction does, however, contain some enzyme activity which could be assayed by the addition of ATP alone. This latter 'homo-polymerase' activity was more concentrated in the small-particle fraction and the presence of all four triphosphates inhibited ATP incorporation by the second enzyme. The homo-polymerase activity of the small-particle fraction explains the apparent lack of a specific requirement for all four triphosphates in the assay of the total particle fraction (Table 1). The nuclei contained negligible amounts of actinomycin-insensitive polymerase activity.

Time course of appearance of polymerase activity. Cell suspensions incubated with 5 μg/ml. of actinomycin were taken at various intervals after the initiation of infection with EMC virus. The large-particle fraction was isolated and assayed for actinomycin-insensitive RNA polymerase activity. Samples were also taken for assay of intracellular virus by the hæmagglutinin assay[4]. The results are summarized in Fig. 1. It can be seen that the polymerase activity, which is negligible during the first 3 h, rises to a maximum at about 5 h after infection, and thereafter falls off. The rise in enzyme activity parallels

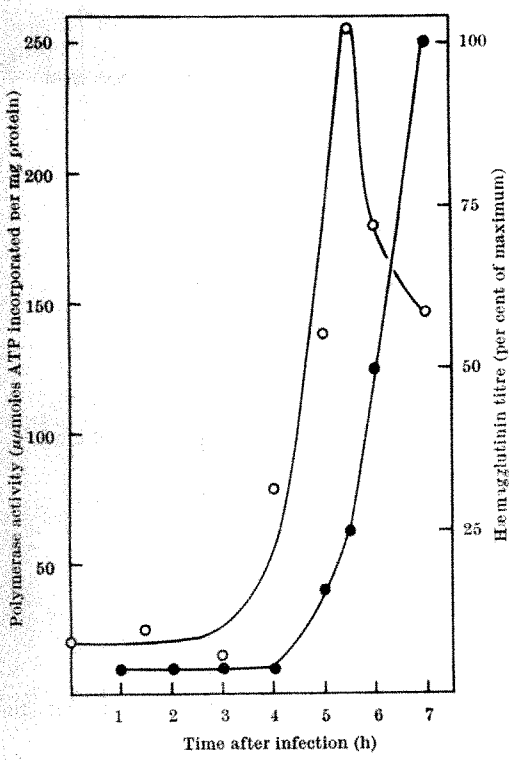


Fig. 1. Time-course of appearance of RNA polymerase activity during infection. ○, RNA polymerase of the large particle fraction from cell homogenates; ●, titre of intracellular virus

the rise in virus titre, but takes place about 1·5 h beforehand. As we have shown previously that the synthesis of the RNA of the mature progeny virus takes place about 1·5 h before the appearance of an equivalent amount of whole virus[7], it is probable that the formation of the enzyme assayed in these experiments was coincident in time with, and responsible for, the appearance of progeny viral RNA.

Nature of the product. The product formed on incubation of the large-particle fraction from infected cells with ^{14}C–ATP was hydrolysed with 0·3 N KOH at 35° for 18 h. It was found that 87 per cent of the radioactivity was rendered soluble in 0·5 N perchloric acid. In one experiment, uridine triphosphate labelled in the α-position with phosphorus-32 was included in the reaction mixture and the product was hydrolysed with alkali and the nucleoside monophosphates separated by electrophoresis[8]. The distribution of radioactivity between the monophosphates of cytidine, adenosine, guanosine and uridine was 27·3, 24·6, 16·7 and 31·4 per cent, respectively. It is evident from these results that the RNA synthesized by the large-particle enzyme was a heteropolymer containing all four nucleotides in normal proportions.

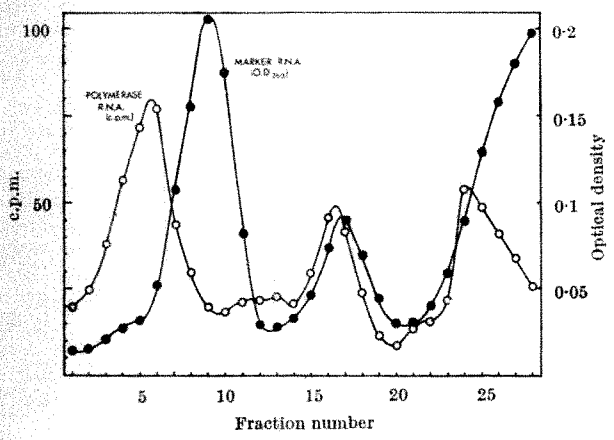


Fig. 2. Sedimentation of the enzyme reaction product on a sucrose gradient. The incubation mixture (0·45 ml.) was thawed with bentonite (2·5 mg) and Krebs cell ribosomes (1 mg) added. Five per cent sodium dodecyl sulphate in 0·05 M potassium phosphate buffer, *p*H 7·4 (3 ml.), was then added and the mixture stirred for 3 min at 37°. It was cooled and 3 ml. layered directly on to a 5–20 per cent sucrose gradient containing 0·1 M NaCl, 10 mM *tris*-HCl *p*H 7·0 and 1 mM EDTA and centrifuged at 0° for 16 h at 24,000 r.p.m. ○, Radioactivity of RNA synthesized by polymerase; ●, optical density of marker RNA

To characterize the product further, its sedimentation behaviour on a sucrose gradient was investigated. After incubating the large-particle fraction with ^{14}C–ATP, the reaction product was mixed with Krebs cells ribosomes, treated with 5 per cent sodium dodecyl sulphate and centrifuged through a sucrose gradient. The gradient was sampled in the usual way and examined for distribution of acid-insoluble radioactivity and optical density at 260 mμ. As shown in Fig. 2 the radioactivity sedimented in three distinct peaks: (1) a fast peak sedimenting well ahead of the 30 *S* ribosomal RNA marker; (2) an intermediate peak of about 20 *S*; (3) a slowly sedimenting peak of 4–6 *S*. When the reaction product was run on a sucrose gradient together with RNA extracted from purified EMC virus, the fastest peak corresponded with the viral RNA marker ($S_{20,w}$ = 37 *S* (ref. 9)). The molecular weight of one of the products of the enzyme reaction is therefore similar to that of the RNA of the mature virus particle.

Montagnier and Sanders[10] have reported the isolation from EMC-infected Krebs cells of a double-stranded RNA (20 *S*) which is resistant to digestion with pancreatic ribonuclease. The product obtained from the incubation of the large particle fraction with ^{14}C–ATP was therefore treated with 1 μg/ml. of ribonuclease at 20° for 30 min before detergent treatment and centrifugation on a sucrose gradient. The 37 *S* peak of radioactivity was abolished, but the intermediate 20 *S* peak remained. Hence, this latter material may be identical with the double-stranded RNA reported by Montagnier and Sanders.

When analysed by the sucrose gradient technique the product obtained by incubation of the small-particle enzyme, either with ATP alone or with all four triphosphates, showed only a single major component, which, when compared directly, sedimented in a similar manner to the 4–6 *S* component obtained with the large-particle enzyme and with *E. coli* transfer RNA, the latter used as an optical density marker. The small-particle enzyme appears therefore to catalyse an actinomycin-insensitive incorporation of ATP into transfer RNA, probably into its terminal position, and is similar to that described by Franklin[11].

From the results presented here we deduce that the infecting RNA of EMC virus directs the synthesis of RNA polymerase which catalyses the incorporation of nucleoside triphosphates into two types of RNA—a single-stranded molecule, probably the RNA of the mature virus, and a ribonuclease-resistant form which is most likely double stranded. The relationship of these enzyme products to each other and their involvement in the replicative process is at present being investigated.

Cline *et al.*[4] described an actinomycin-insensitive RNA-dependent polymerase from EMC-infected Krebs cells which was stimulated by the addition of yeast RNA, and was not localized in any particular cell fraction. It was present in uninfected cells. The properties of this polymerase differ greatly from those of the large-particle enzyme described here. It is possible that Cline *et al.* have assayed an enzyme of the type we have observed in the small-particle fraction. This enzyme may not be directly concerned with the synthesis of viral RNA.

Baltimore[3] has reported the formation of reaction products similar to ours when an enzyme from polio-infected HeLa cells was incubated with ^{32}P-labelled GTP. This polymerase was mainly localized in a cytoplasmic fraction sedimenting at 600,000*g*-min. The polymerase of mengo-

infected *L* cells was only partially sensitive to ribonuclease and showed a marked dependence on the presence of all four nucleoside triphosphates and Mg^{++} (ref. 2). In all these respects, and in optimum *p*H and time course of appearance during infection, the polymerase found in EMC-infected Krebs II ascites cells is remarkably similar to the polymerase of mengo- and polio-infected cells.

We thank Dr. R. M. S. Smellie for a gift of ^{32}P–UTP, and Mr. H. D. Brown of Merck, Sharp and Dohme, New Jersey, for a gift of actinomycin *D*. This work was carried out during the tenure of a Damon Runyon Foundation fellowship by E. H. and of a Melbourne University travelling scholarship by L. D.

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APPLICATIONS OF ISO-IMMUNE CYTOLYSIS USING RADIOLABELLED TARGET CELLS

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MANY investigations of iso-immunity have benefited from the use of inbred (isohistogenic) strains of animals, and, of the few species available, the mouse is the most common choice, because of the large background of genetic information available, together with rapidity of breeding and relatively easy economic maintenance of a defined colony. Assays of humoral iso-immunity in mice, which are usually based on agglutination or damage of cellular targets, have a number of drawbacks. Erythrocytes may not possess all the antigens characteristic of their strain of origin[1], while dissociated nucleated white cells may be difficult to prepare in a homogeneous form. Furthermore, the estimation of a humoral effect may be imprecise, as in agglutination, or unduly prolonged, as in cytotoxic tests judged by counting of stained cells. Nevertheless, a great deal of semi-quantitative information about strain specific antigens has been gained by the use of hæmagglutination[2], hæmolysis[3,4], and cytotoxicity[5]; recent modifications[6,7] have been aimed at the development of more objective and quantitative assays.

For stricter quantitation and more definitive analysis of humoral iso-antibodies and iso-antigens in cytotoxic studies, the principal requirements are an easily obtained homogeneous suspension of cells which have a complete spectrum of strain-specific antigens and which release quantifiable substances on lysis. These requirements have been closely approached by the use of mouse lymphocytes which are labelled with chromium-51, and the release of radioisotope effected by specific antisera and guinea-pig complement (*C′*) is used to determine the extent of lysis. Chromium-51 is frequently used in hæmolysis studies, where Weinrach, Lai and Talmage[8] first pointed out that the smaller quantity of cells required enabled a higher *C′*: cell ratio and allowed more precise kinetic determinations. Goodman[9] first suggested the use of chromium-51 as a label for other kinds of cells and used a rabbit anti-Ehrlich (mouse) tumour heteroimmune system, while Wigzell[10] found that the mouse (*C57BL*) tumour, *EL*4, similarly labelled, served as a satisfactory target cell in iso-immune studies. *In vivo* labelled (^{3}H-thymidine; ^{14}C-amino-acids) mouse tumour cells have also been used[11,12] in iso-immune systems.

In the work recorded here normal mouse lymphocytes were used in preference to tumour cells in order to avoid possible complications due to antigenic disparity between a tumour and its specific host, be these differences qualitative or quantitative[4]. The amenability of the assay to precise microanalysis has been studied and several applications are recorded.

The cytolytic assay. The test system used is as follows: lymph nodes from a normal mouse are gently teased apart in Medium 199 (ref. 13) containing 0·1 per cent bovine plasma albumin (Armour Co. Ltd., Eastbourne, Sussex), which is used throughout as diluent. (Manipulation of the small quantities of material involved is achieved by the use of Lang-Levy constriction micropipettes[14] manufactured in the laboratory[15].) A coarse sinter (porosity 0) is used to remove gross debris and clumps of cells, while allowing the passage of dispersed lymphocytes, which are collected by centrifugation and resuspended in a small volume of medium. An equal volume of $Na_2{}^{51}CrO_4$ solution (Radiochemical Centre, Amersham, supplied as 1 mc. samples in isotonic saline, *p*H 7), containing 50–100 μc./μg, is added and the cells are labelled by incubation for 2 h at 37° C. Excess label is removed by washing several times with medium and the cells are finally suspended at $5–6 \times 10^6$/ml.; dead cells rarely exceed 4 per cent. Dilutions (to 0·2 ml. final volume in small tubes, 100 mm × 75 mm) of antiserum are made in medium which contains *C′*. Appropriate controls containing heat-inactivated *C′* and normal mouse serum are always included. The concentration of *C′* used is the maximum amount which will cause less than 5 per cent lysis per h of labelled lymphocytes in the presence of normal mouse serum. An aliquot of labelled, washed, lymphocytes is added to each tube, which is then sealed with a rubber stopper and rolled in an inclined rack, to ensure adequate mixing, at room temperature. After an appropriate time, depending on the purpose of the experiment, reaction is terminated by addition of 0·3 ml. ice-cold versene in isotonic saline at *p*H 7·5 to each tube over the same staggered intervals as was used to add the cells. The tube contents are mixed, centrifuged, and a convenient portion of supernatant solution is removed for estimation of the radioisotope released. The total radioactivity present in an aliquot of lymphocytes is also measured.

Parameters affecting the assay. Using the quantities of radioisotope mentioned above, between 15 and 40 per cent of the available label is incorporated, dependent on the time, temperature and cell concentration during incubation. Bunting, Kiely and Owen[16] have shown that up to 50 per cent of available chromium-51 is incorporated into rat lymphocytes under similar conditions and on osmotic lysis is released in some bound

form not re-incorporated on incubation with further unlabelled cells. Microscopic examination of lymphocytes before and after labelling did not reveal any difference in form or motility, although the dead cell (trypan blue) count decreased, presumably due to fragmentation and solubilization during incubation at 37° C. The 50 per cent titre of an antiserum measured using cells which had been labelled for 2 h was indistinguishable from that obtained using cells labelled for 4 h, showing that labelling of cells did not increase their fragility. Furthermore, it has been reported[17] that tumour cells labelled with chromium-51 under very similar conditions to the above show no evidence of impaired viability. These considerations suggest that for the duration of labelling and subsequent lysis the presence of radioisotope does not alter the lytic characteristics of the lymphocytes, a conclusion which is supported by an accumulation of evidence from erythrocyte labelling[18,19].

Careful attention to reaction time and temperature was necessary to ensure reproducibility. The guinea-pig complement used was from a pool of animals: it required absorption with mouse cells (spleen) before use in order to reduce blanks, and proved stable for several weeks when stored sealed and frozen at −20° C. Not all the incorporated radioisotope is released from cells on complete lysis, although all cells are dead as judged by trypan blue staining: a maximum of 60–70 per cent of the label incorporated is usually released. For reasons to be discussed later it is believed that the measurable extent of label release is dependent on the degree of damage suffered by the strain of lymphocytes in question together with the amount of insoluble bound isotope removed by centrifugation, and is not necessarily a reflexion of heterogeneity in the cell population with respect to fragility or antigenic content.

After subtraction of control values, which for acceptance should be less than 5 per cent of the maximum release, the degree of lysis caused by antiserum may be calculated. Maximum isotope release is taken to be 100 per cent lysis and is usually arranged to occur in several tubes at the higher concentrations of antiserum. Occasional prozones are seen which are presumably due to an anti-complementary effect of mouse serum or the presence of some other inhibitor[20] of immune lysis effective at high mouse serum concentrations.

When the foregoing factors are taken into consideration, the assay as described yields results which are as precise as those obtained in the classical rabbit antisheep hæmolysis[21]. When analysed by the method of von Krogh[22], which uses an empirical expression to relate lysis to antiserum dilution, results such as those shown in Fig. 1 were obtained.

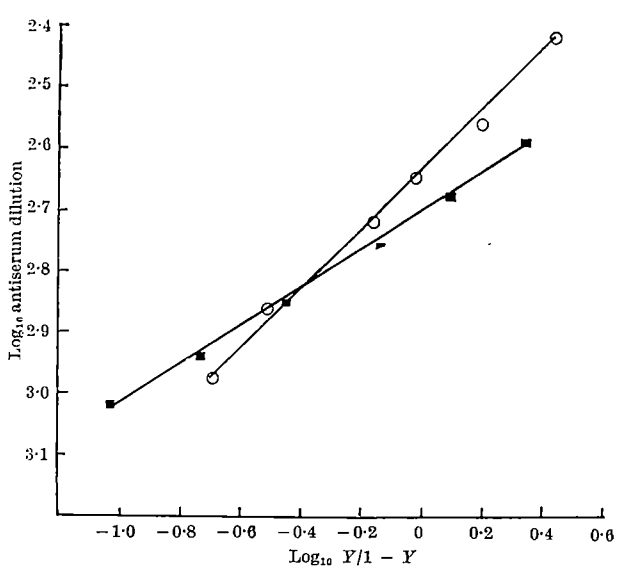


Fig. 1. Von Krogh analysis of cytolysis. Y is the fraction of cells lysed. ○, Lysis of *C3H* lymphocytes with *DBA*/2 anti-*C3H* serum; ■, lysis of *DBA*/2 lymphocytes with *C3H* anti-*DBA*/2 serum

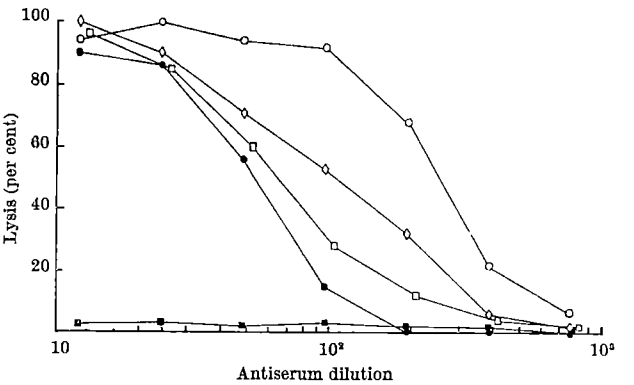


Fig. 2. Titration of antisera with *A/Jax* lymphocytes. Known *H*-2 specificities participating in the interactions are in parentheses. *BALB/c* and *DBA/2* are identical at the *H*-2 locus. ○, *BALB/c* anti-*A/Jax* (*AEKY*); ◇, *C57BL* anti-*CBA* (*AKCHY*); □, *C57BL* anti-*BALB/c* (*DMCHJ*); ●, *BALB/c* anti-*C57BL* (*E*); ■, *BALB/c* anti-*DBA/2*

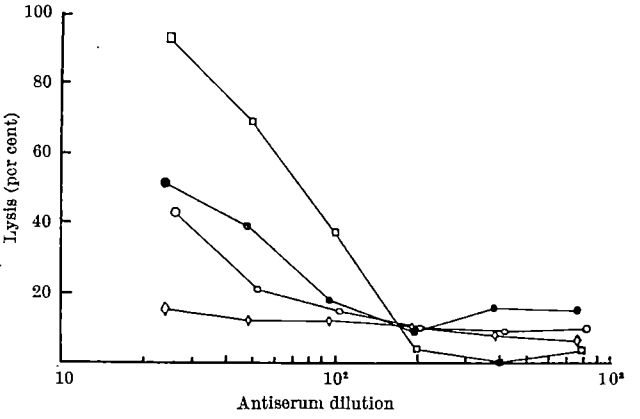


Fig. 3. Titration of antisera with *DBA/2* lymphocytes: demonstration of non-*H*-2 interaction. □, *CBA* anti-*DBA/2*; ●, *BALB/c* anti-*C3H*; ○, *BALB/c* anti-*CBA*; ◇, *BALB/c* anti-*DBA/2*

Antiserum	Potential *H*-2 specificities in antiserum	Potential *H*-2 specificities expected to react with *DBA/2* target
CBA anti-*DBA/2*	*DE^{d}FMJNRA'B'C'*	*DE^{d}FMJNRA'B'C*
BALB/c anti-*C3H*	*AEKY*	None
BALB/c anti-*CBA*	*AEKY*	None
BALB/c anti-*DBA/2*	None	None

Similar data from other combinations of iso-immune sera have established that the slope of a von Krogh analysis of C' titration is very close to 0·2, while kinetic studies under conditions of limited C' or limited antibody behaved as in the rabbit antisheep system[21]. When analysed by the method of Weinrach *et al.*[8] many sera showed an order of reaction close to 2 with respect to antiserum. The lag period in 'complement first' studies was 17–19 min, similar to that found by these authors[23], while kinetic examination of the lysis of pre-sensitized ('antiserum first') lymphocytes showed no such lag, and proceeded at a steeper initial lytic rate. The similarity of the iso-immune systems mentioned here and the rabbit antisheep hæmolysis which has been so carefully studied make it seem likely that the molecular events causing lysis in the two cases are the same. The mouse situation possesses one advantage, that of a defined antigenic and uniform genetic background, and further studies could prove fruitful. In the foregoing work, the meaning of C' units and antiserum units, as well as target cell concentrations and lytic conditions, have been operationally defined for relevance to the mouse system, and are considerably different from those used in sheep erythrocyte immune lysis[21].

Applications of the assay. The pattern of iso-antigenic specificities in mice which are controlled by the *H*-2 genetic locus has been elaborated[24] using skin grafting, tumour grafting and hæmagglutination. The applicability of the present assay to the same problem was tested as shown below. Each of 6 strains of mice (*A/Jax*; *C57BL*; *C3H/He*; *CBA*; *BALB/c*; *DBA/2*) was separately immunized against each of the other five, by weekly (five) injections of spleen suspensions. The 30 antisera produced were separately tested for cytolytic activity in the presence of *C′*, against panels of labelled lymphocytes derived from each strain of mouse. The 180 results thereby included specificity controls, since each antiserum was tested against lymphocytes of the strain from which it was produced: no abnormal results were recorded in such controls. The pattern of titres so obtained not only included every reaction predicted from the known distribution of *H*-2 specificities but also contained several unpredicted results; this is presumably a reflexion of the more complete or more dense representation of *H*-2, and possibly non-*H*-2, specificities on lymphocytes than on erythrocytes. Titres were found to be reproducible within a few per cent when performed by different investigators on different days using different samples of the same batch of antiserum, and *C′*. Examples of such titres are presented in Fig. 2. 100 per cent lysis in this figure represents 65 per cent release of radioisotope.

Antigenic heterogeneity in the target cell population is unlikely to be the reason for incomplete release, because of the identical maximum liberation caused by several antisera of known widely different specificities, a result which has been noted many times in the combinations of sera and targets used, most strikingly in the parent/hybrid case mentioned later.

Table 1. 50 PER CENT TITRES FOR ANTISERA DIRECTED AGAINST PARENT AND HYBRID CELLS: THE 'GENE-DOSE' EFFECT

Antisera	Target lymphocytes *C3H*	*BALB/c*	(*C3H* × *BALB/c*) *F*1 hybrid
C3H anti-*BALB/c*	—	222	117
BALB/c anti-*C3H*	256	—	135
C3H anti-*BALB/c* + *BALB/c* anti-*C3H*	270	231	232

It is worth noting that although no examples of interaction were noted between targets and antisera when the latter were produced between mice differing only at non-*H*-2 loci, nevertheless non-*H*-2 interaction could be demonstrated as shown in Fig. 3. This confirms the findings of others[25] that the normally weak response to non-*H*-2 differences between mice may be considerably augmented when it is accompanied by an *H*-2 incompatibility: Fig. 3 represents the serological demonstration of this effect.

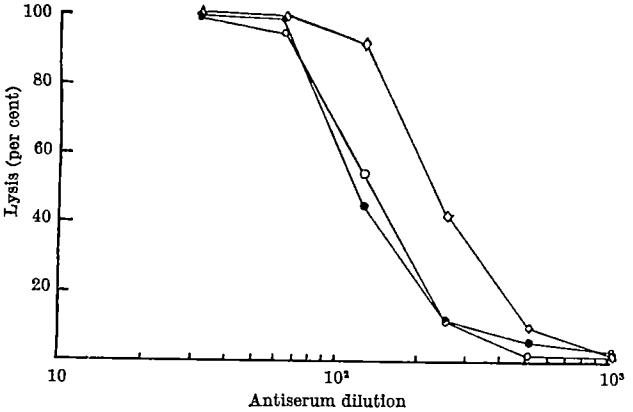


Fig. 4. Titrations of antisera with (*C3H* × *BALB/c*) *F*1 hybrid lymphocytes: demonstration of the additive effects of different specificities against the same target cell. ●, *C3H* anti-*BALB/c*; ○, *BALB/c* anti-*C3H*; ◇, *C3H* anti-*BALB/c*, *BALB/c* anti-*C3H*

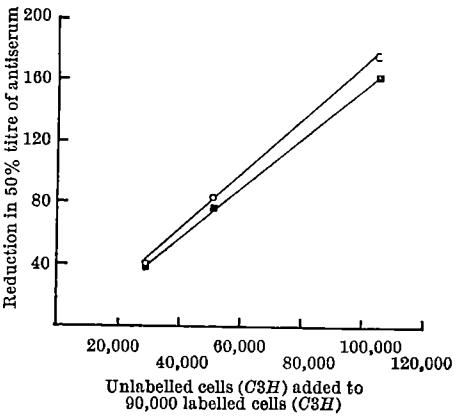


Fig. 5. Relationship of antigen added, in the form of lymphocytes (*C3H*), to the reduction in 50 per cent titre of *DBA/2* anti-*C3H*. ○, Calculated from von Krogh analysis of lysis; ■, calculated from Weinrach *et al.* analysis of lysis

An interesting application of the cytolytic technique throws quantitative light on two problems which have recently received attention, namely: (*a*) the additive cytotoxic effect of antisera directed against different antigenic specificities of the same target cell[4,26]; (*b*) the so-called 'gene-dose'[4,7,27,28] effect. The latter refers to the change in the phenotypic expression of a genetically controlled characteristic when the amount of genetic material is altered. This can conveniently be examined using mouse histocompatibility specificities as indicators. The homozygous genes controlling mouse *H*-2 antigenic specificities are believed to be co-dominant, that is, the heterozygous *F*1 hybrid will qualitatively exhibit all the specificities present in its parents. That this relationship is quantitative is shown in Table 1, and Fig. 4 shows the additive effect of different specificities in antisera directed against the same target.

The sensitivity of a hybrid lymphocyte to an antiserum directed from either parent against the other is almost exactly half that of either appropriate parental cell. Furthermore, the sensitivity of the hybrid cell to a combination of the antisera is the same as the respective parental cells to their appropriate antisera, showing that the inherited antigenic specificities which dominate the lysis of the cells concerned are present at half the density on the hybrid cell at which they occur on the respective parental genetic donor. It is, of course, fortuitous (and fortunate for the demonstration of this experiment) that the titres of the antisera against the hybrid target shown in Fig. 4 are so close, but, had they not been, appropriate dilution with normal mouse serum would have enabled the same effect to be revealed. Möller[26] was able to show a qualitative additive effect by combining antisera raised between different strains of mice which would be expected to react against antigens present in the chosen tumour cell targets, but it would be difficult in his experimental design to separate the effects of addition of different antibody specificities from the cumulative effects of the same antibody specificities in different antisera. The use of parental and hybrid targets precludes this possibility. The present test offers quantitation and could have potential use in following the appearance of new genetically controlled specificities, for example, in induced or spontaneous tumour formation.

A further application of the test is in the determination of iso-antigens by inhibition of the lytic reaction. Methods similar in principle have been used before[27,29], but all these depended on inhibition of a visually estimated effect, with concomitant lack of precision. By choosing amounts of antiserum which, in the absence of inhibitor, will give greater than and less than 50 per cent lysis, the effect of increasing amounts of inhibitor on the 50 per cent titre of an antiserum can be determined by the use

of von Krogh analysis or the log log plot of Weinrach *et al.* The 50 per cent titre is used because this is the region of the sigmoid lytic response most sensitive to small changes in antiserum concentration. Fig. 5 illustrates the inhibition of lysis of labelled lymphocytes caused by the addition of unlabelled, but otherwise identical, lymphocytes. Inhibition by lymphocytes from the strain of mouse providing the antiserum is only 2 per cent that of lymphocytes from the strain of mouse providing the target cells, thereby demonstrating the specificity of the system.

It should be borne in mind that tissue transplantation is not always accompanied by the occurrence of detectable circulating antibodies, and for sensitivity and specificity the biological assays such as skin grafting and tumour grafting cannot, as yet, be improved on, but they are imprecise, and the present assay possesses sufficient advantages to merit its use in cases where histocompatibility differences give a humoral response. Preliminary studies of iso-immunity in dogs[30] and chickens[31] with a similar assay using peripheral blood leucocytes as target cells appear promising.

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IDENTITY OF *Pseudomonas* CYTOCHROME OXIDASE WITH *Pseudomonas* NITRITE REDUCTASE

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IT is well known that, in the living cell, cytochrome oxidase catalyses the reaction of molecular oxygen with electrons liberated from various substrates. In most animal tissues, in many plant tissues and in some micro-organisms, cytochrome *a* is known to function as the cytochrome oxidase[1]. On the other hand it is generally assumed[2] that cytochrome a_2 functions as a cytochrome oxidase in many micro-organisms which do not possess cytochrome *a*. Indeed, from several bacteria, all of which contain cytochrome a_2, Barrett[3] extracted and partially purified a green hæmin, which he designated as hæmin a_2. However, cytochrome a_2 itself has not been isolated from bacteria which are thought to possess it.

It is known that nitrite reductases are present in micro-organisms and that they are metal enzymes[4–7]. However, they have not been exhaustively purified so their chemical entities are unknown.

In this laboratory we have purified and crystallized the cytochrome from *Pseudomonas aeruginosa* which was formerly called *Pseudomonas* cytochrome$_{GB}$ and later named *Pseudomonas* cytochrome oxidase[8–15]. Although the cytochrome a_2 with only hæm a_2 as its prosthetic group has not been isolated, *Pseudomonas* cytochrome oxidase has hæm a_2 and hæm *c* in the molecule. *Pseudomonas* cytochrome oxidase not only possesses the general properties of cytochrome oxidase but also acts as a nitrite reductase[16,17], and is apparently the same entity as *Pseudomonas* nitrite reductase[18]. This cytochrome oxidase is, therefore, interesting both from physiological and enzymological points of view.

Enzymatic Properties

Pseudomonas cytochrome oxidase shows the general properties of a cytochrome oxidase, that is, it oxidizes ascorbate, hydroquinone, the reduced form of *Pseudomonas* blue protein (a copper protein)[19,20] and the reduced form of *Pseudomonas* cytochrome *c*–551 (ref. 21) which is known to function in the organism just as cytochrome *c* does in animal tissues[22]. These reactions are strongly inhibited in the presence of cyanide and carbon monoxide. The enzyme is extremely labile in the presence of a trace of hydrogen peroxide[23].

One mole of the enzyme can oxidize 2,400 moles of the reduced *Pseudomonas* cytochrome *c*–551 per minute under aerobic conditions at 37° C[13]. This turnover number corresponds to 600 moles of oxygen consumption, and consequently to 13,440 litres oxygen consumption per mole of the enzyme per min at 37° C. Although the reduced form of *Pseudomonas* cytochrome *c*–551 is rapidly oxidized by the enzyme, that of mammalian cytochrome *c* is scarcely oxidized[13]. This biological specificity of the enzyme is useful for detecting delicate differences in the molecular structures of various forms of cytochrome *c*.

Pseudomonas cytochrome oxidase acts as a nitrite reductase[16,17]; it reduces nitrite to nitric oxide with reduced *Pseudomonas* cytochrome *c*–551 as the electron donor, under anaerobic conditions.

$$\text{cytochrome } c\text{–}551\,(Fe^{++}) + NO_2^- + H^+ \xrightarrow{\text{Pseudomonas cytochrome oxidase}} \text{cytochrome } c\text{–}551\,(Fe^{+++}) + NO + OH^-$$

One mole of the enzyme reduces 4,000 moles of nitrite to nitric oxide per min at 37° C under anaerobic conditions. This reaction is strongly inhibited by cyanide but not by carbon monoxide. The behaviour of the enzyme with these inhibitors is the same as that of whole cells of *Pseudomonas aeruginosa*[18]. Thus, *Pseudomonas* cytochrome oxidase seems to be the same entity as the nitrite

reductase functioning in this organism. This will be discussed further in this contribution. *Pseudomonas* cytochrome oxidase is to be named *Pseudomonas* cytochrome *c*–551: nitrite, O_2 oxidoreductase (*EC* 1·9 group)[18].

Spectral Properties

As described already, *Pseudomonas* cytochrome oxidase has two hæms in the one molecule. Therefore, it shows a very complicated absorption spectrum[13,14]. This spectrum has peaks at 280 mμ, 412 mμ, 525 mμ and 635 mμ in the oxidized form, and at 420 mμ, 523 mμ, 549 mμ and 554 mμ in the reduced form. In the latter form, there is a bump at 460 mμ which is the γ-band due to hæm a_2, and at *p*H 7·0 the peak at 635 mμ of the oxidized form changes to two peaks at 629 mμ and 652 mμ. The ratio of $A_{\gamma}^{\text{reduced}}/A_{\alpha}^{\text{oxidized}}$ is 2·28 at *p*H 7·0 (A = absorbancy). Therefore, the γ-band of cytochrome a_2 has a very low absorbancy compared with α-band, as reported by other workers[24]. The spectrum of the dithionite-reduced enzyme in the red region varied considerably with the *p*H (Table 1). When ascorbate was used in place of dithionite to reduce the enzyme, an asymmetrical peak at 645 mμ appeared, whereas the corresponding γ-band was at 460 mμ. With ascorbate, there is no such variation in the position of the α-band of hæm a_2 with *p*H as seen with dithionite. From the above facts it is concluded that there is no parallelism between the positions of the peaks at 460 mμ and in the red region, although it is evident, as described here, that the two peaks are caused by hæm a_2. In the presence of cyanide, carbon monoxide and nitric oxide, the spectrum of the reduced enzyme in the red region is strongly affected, as shown in Table 1.

Table 1. Effect of *p*H, Cyanide, Carbon Monoxide and Nitric Oxide on the Absorption Spectrum of *Pseudomonas* Cytochrome Oxidase

Reagent added	*p*H	Position of peak (mμ) γ-band*	α-band*
None	7·6	460	655
	7·0	460	629, 652
	5·6	460	625
CN^-	7·0	443, 472	627
CO	7·0	—	622, 658
NO	7·0	—	665

*Peaks due to hæm a_2.

Prosthetic Group

When *Pseudomonas* cytochrome oxidase is treated with acid acetone, hæm a_2 is extracted into the acetone whereas hæm *c* is precipitated with the protein moiety[15]. The hæm a_2 is easily separated as aqueous solution from the acetone by adding sodium hydroxide. The aqueous solution of hæm a_2 has absorption peaks at 405 mμ and 684 mμ in the oxidized form (that is, hæmatin a_2) and at 453 mμ and 626 mμ in the reduced form. These peaks change greatly on the addition of various reagents (Table 2). From the protein fraction precipitated by acid acetone treatment, hæm *c* can be split off as hæmatohæm by the silver sulphate method[25].

Reconstitution of the Enzyme

When hæm a_2 and the protein fraction described above are mixed, an enzyme which is very similar to the original enzyme can be reconstituted[26,27]. The reconstituted enzyme has both cytochrome oxidase and nitrite reductase activities, and these activities are both about 50 per cent of those of the original enzyme. Although the reconstituted enzyme is not purified, as it has not been obtained in sufficient amounts to be subjected to chromatography, it can be predicted that it will show the same activity as the original enzyme. It seems likely that in the 50 per cent of the reconstituted enzyme hæm a_2 combines with the protein fraction in abnormal positions, perhaps inverted in position.

Table 2. Absorption Peaks of Hæm a_2 and its Derivatives

Reagent	State	Position of peak (mμ) γ-band	α-band
None	Oxidized	405	684
None	Reduced	453	626
Pyridine	Reduced	432	620
CN^-	Reduced	449, 480	632
NO	Oxidized	407	645
NO	Reduced	408, 436	625
CO	Reduced	413, 440	644

Table 3. Relative Activities of the Reconstituted Enzyme and Various Enzyme Models

	Cytochrome oxidase activity (%)	Nitrite reductase activity (%)
Hæm a_2	100	100
Hæm a_2-derivative*	12	24
Fe-chlorophyllin	22	46
Protohæm	22	50
Hæmatohæm	13	24
Hæm *a*	4·9	7·4

*A hæm derived from hæm a_2 by removing extra hydrogen atoms at positions 7 and 8.

The protein fraction which is made by splitting off the hæm a_2 is insoluble at neutral *p*H. When the protein fraction is combined with the hæm a_2 it becomes soluble. Thus, the hæm a_2 in this enzyme plays an important part both in solubilizing the protein fraction and in activating it. When various hæms are used in place of hæm a_2, various enzyme models can be constructed (Table 3). From the activities of these enzyme models, it is concluded that two extra hydrogen atoms at positions 7 and 8, and the vinyl group at position 2 of hæm a_2, are essential for enzymatic activity. *Pseudomonas* cytochrome oxidase is the first hæmoprotein with hæm a_2 which has been reconstituted from its hæm and protein fractions.

Biological Significance

As described here, *Pseudomonas* cytochrome oxidase has two enzymatic activities, as cytochrome oxidase and as nitrite reductase, and it seems that this enzyme is responsible for the nitrite reductase activity in the cells of *P. aeruginosa*, because it is obtained only from cells cultivated in the presence of nitrate[18]. Cells cultivated in the absence of nitrate lack nitrite-reducing activity whereas they reduce nitrate to nitrite[22]. Although this enzyme contains no copper[11], its biosynthesis is greatly stimulated by addition of copper sulphate to the culture medium. The idea that *Pseudomonas* cytochrome oxidase is the same entity as the nitrite reductase functioning in the living cell is supported by the observation by Walker and Nicholas[29] that nitrite reductase of

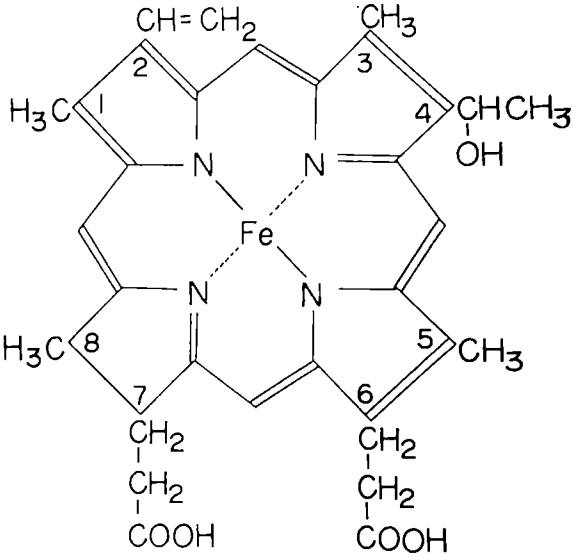
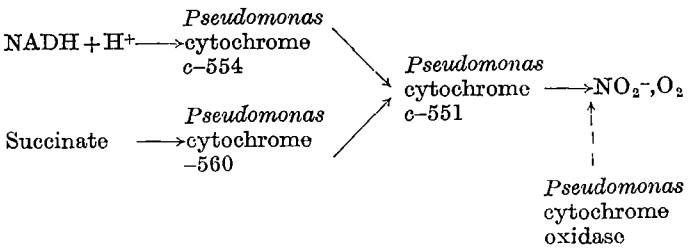


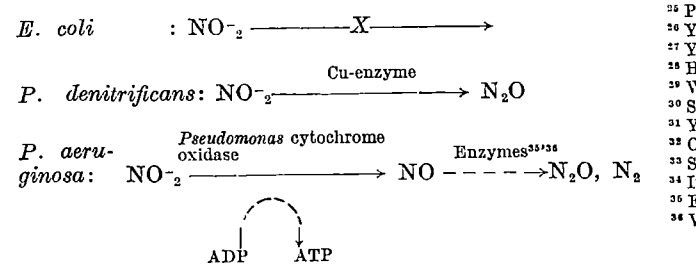
Fig. 1. The structural formula of hæm a_2 (ref. 3)

P. aeruginosa which has been partially purified shows an absorption spectrum very similar to that of *Pseudomonas* cytochrome oxidase. Nevertheless, it cannot be denied that this enzyme participates in the oxygen consumption of cells cultivated in the presence of nitrate, because it possesses the general properties of cytochrome oxidase. The nitrite-reducing system of *P. aeruginosa* seems to be as follows[17,22]:



Evolution of the Nitrite-reducing System

In denitrifying bacteria, it is known that nitrate can act as the ultimate electron acceptor instead of oxygen under anaerobic conditions. This phenomenon is called nitrate respiration[30]. Indeed, it is demonstrated in cell-free extracts of pseudomonads that oxidative phosphorylation occurs, coupled with anaerobic nitrate reduction[31,32]. Nitrate respiration seems to be a more primitive respiration mechanism than oxygen respiration (that is, common respiration). But as the nitrite produced by reduction of nitrate is very poisonous to the organism, it has to develop a mechanism to eliminate nitrite. Thus the nitrite-reducing system might have appeared after the nitrate-reducing one. For example, we can consider three kinds of nitrite-reducing systems of dissimilar types, namely, those of *Escherichia coli*, *P. denitrificans* and *P. aeruginosa*. *E. coli* can scarcely reduce nitrite, whereas, from *P. denitrificans*, a copper enzyme has been purified[33,34] which reduces nitrite to nitrous oxide. It seems very likely that this nitrite reductase is more primitive than that of *P. aeruginosa*, namely, *Pseudomonas* cytochrome oxidase, because it is not a hæmoprotein. It may be that the nitrite-reducing system of *P. denitrificans* is effective only in elimination of nitrite, whereas that of *P. aeruginosa* participates in ATP formation by utilizing the energy obtainable in consuming nitrite. As the nitrite-reducing system of *P. aeruginosa* comprises a highly developed cytochrome system, as described here, it may have been able to participate in oxygen respiration as soon as molecular oxygen appeared on the Earth.



Pseudomonas cytochrome oxidase is the same entity as the nitrite reductase functioning in *P. aeruginosa*. Thus, *Pseudomonas* nitrite reductase is a cytochrome which has two hæms, namely, hæm a_2 and hæm c, in the one molecule. It has been obtained in a crystalline state. It possesses no copper, but copper salts are necessary for its biosynthesis. It reduces nitrite to nitric oxide with reduced *Pseudomonas* cytochrome c–551 as the electron donor. The nitrite-reducing system of *P. aeruginosa* is composed of a highly developed cytochrome system, and this fact suggests that ATP formation coupled with nitrite reduction occurs in this organism. Thus, the nitrite-reducing system of *P. aeruginosa* is very similar to the typical electron transport system which participates in normal respiration in higher organisms. The fact that *Pseudomonas* nitrite reductase possesses the general properties of cytochrome oxidase suggests strongly that oxygen respiration may have evolved from a nitrate-respiration system such as that seen in *P. aeuroginosa*. It is also very interesting that the prosthetic group of *Pseudomonas* cytochrome oxidase, hæm a_2, is a derivative of chlorin, that is, that the structure of hæm a_2 is similar to that of chlorophyll.

I thank Prof. K. Okunuki for advice.

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ENZYMATIC BASIS OF THE RESPIRATORY STIMULATION DURING PHAGOCYTOSIS

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SEVERAL metabolic concomitants of phagocytosis have been observed[1]. One striking phenomenon in polymorphonuclear leucocytes (PMN) is an increased respiration during particle uptake[2,3] which has been shown to be insensitive to 1 mM cyanide. It was concluded that this increase in respiration is not cytochrome-linked[3]. Accompanying the respiratory increase in PMN is a 7-fold, cyanide-insensitive stimulation of the con-

version of glucose carbon-1 to carbon dioxide. The energy for phagocytosis in PMN appears to come from glycolysis rather than from oxidative phosphorylation, judging from results obtained anaerobically and with various metabolic inhibitors[1,3].

To explain the respiratory stimulation in PMN that is insensitive to cyanide, it has been postulated[4] that two key enzymes, a cyanide-insensitive NADH oxidase and NADPH-linked lactate dehydrogenase, are involved. The following hypothesis was offered[4]: during uptake of particles, the cyanide-insensitive NADH oxidase is activated, which accounts for the increased oxygen uptake. Some pyruvate from glycolysis is then available in excess of the NADH available to convert it to lactate. An NADPH-linked lactate dehydrogenase catalyses the conversion of this pyruvate to lactate and this reaction increases the available NADP. NADP has been shown to be a rate-limiting factor for the hexosemonophosphate shunt in leucocytes[5], and the rate of reoxidation of NADPH is therefore important in the stimulation of this pathway. Postulation of the rather indirect mechanism above was necessary because the oxidase for reduced pyridine nucleotides that was found in PMN was about 10-fold more active with respect to NADH than toward NADPH, and transhydrogenase, measured by conventional means, was not found[4,6].

Quastel *et al.*, who used dialysed homogenates of PMN, have reported that these cells contain an NADPH oxidase rather than an NADH oxidase, and that it is the former enzyme that participates in changing the metabolic pattern during phagocytosis[7,8]. It should be emphasized that their experiments were conducted in the absence of cyanide. Recently, however, Roberts and Quastel[9] reported that the enzyme they had been examining (NADPH oxidase) is inhibited by 1 mM cyanide, and is probably, in fact, peroxidase.

It appears that two different enzymatic reactions are under discussion by Quastel's laboratory and our own: (*a*) A cyanide-sensitive, manganese-stimulated oxidation of reduced pyridine nucleotides catalysed by peroxidase. It is well known that peroxidase is capable of such activity[10,11]. As shown here and by others[12,13], peroxidase is localized in the granules of leucocytes. (*b*) A cyanide-insensitive oxidation of reduced pyridine nucleotides catalysed by an enzyme is under study in our laboratory.

We shall attempt in this article to clarify two matters: (1) that of the characteristics of oxidases for reduced pyridine nucleotides in PMN; (2) that of the cytological localization of the cyanide-insensitive enzyme (NADH oxidase).

The abilities of several different types of preparation from guinea-pig PMN to catalyse the oxidation of reduced pyridine nucleotides are summarized in Table 1, and it may be seen that homogenates or the supernatant fraction therefrom have the ability to oxidize NADH faster than NADPH, in contrast to the findings of Quastel *et al.*[7,8]. In the absence of cyanide, Mn^{++} or ethanol were stimulatory as has been pointed out by Iyer and Quastel[8]. These effects were absent in the presence of cyanide, under which conditions there was little or no activity toward NADPH. On the other hand, the activity of the enzyme in the supernatant fraction was virtually unaffected by cyanide.

None of the preparations we have used, in either the absence or presence of cyanide, showed the specificity reported by Quastel *et al.*[7,8]. We have tested several different lots of the reduced pyridine nucleotides (Sigma Chemical Co. and Pabst Laboratories) using freshly prepared solutions. Contamination of the cell preparations with monocytes was considered, since the period (30 h)[7] used by the Canadian group for eliciting the sterile peritoneal abscess could conceivably yield preparations with many monocytes[15]. However, a homogenate of monocytes did not show the preference for NADPH reported by Quastel *et al.*[7,8]. Possibly the NADH they used contained the inhibitor reported by Fawcett *et al.*[16].

Table 1. NADH and NADPH Oxidase Activities in Preparations from PMN Homogenate

Exp. No.	Preparation*	Substrate	Addition or treatment*	Activity† −KCN	Activity† +KCN
1	Whole homogenate	NADH	—	—	3·0
		NADH	*FT*	—	2·9
		NADPH	—	—	0·9
		NADPH	*FT*	—	0·9
2	Whole homogenate dialysed	NADH	—	2·4	2·6
		NADH	Mn^{++}	5·4	2·8
		NADH	Ethanol	6·2	2·1
		NADPH	—	0·8	0·0
		NADPH	Mn^{++}	4·5	0·0
3*A*	Homogenate dialysed	NADH	—	7·0	—
		NADPH	—	0·0	0·0
		NADPH	Mn^{++}	7·1	0·0
B	Homogenate dialysed	NADH	—	5·0	2·7
		NADH	Mn^{++}	10·4	1·7
4	Supernatant fraction	NADH	—	1·31	1·14
		NADPH	—	0·35	0·31

* Experimental conditions. Exp. 1. Cells (20 per cent suspension) homogenized 3 min in alkaline isotonic KCl (ref. 14) with a motor-driven 'Teflon' pestle in a glass tube. *FT* denotes freezing (−14 to −16° C) and thawing (37° C) twice prior to assay. Cells were PMN (ref. 15). Exp. 2. Whole homogenate as in Exp. 1, also dialysed 17 h according to Iyer and Quastel (ref. 8). Exp. 3. Homogenate prepared according to Quastel *et al.* (refs. 7 and 8). This included freezing and thawing, and dialysis. 3*A* and 3*B* run on subsequent days with enzyme stored overnight at 5° C. Exp. 4. Supernatant fraction after centrifuging homogenate at 30,000*g* for 1 h.

† Results of Exps. 1, 2 and 3 expressed as μl. O_2 consumed per 30 min and mg protein. Those of Exp. 4 expressed as mμmole reduced pyridine nucleotide oxidized per minute and mg protein. Enzyme assays: Exps. 1 and 2. Manometric assay, 37° C, *p*H 7 (0·03 M phosphate buffer) with air as gas phase. NADH or NADPH at $2{\cdot}5 \times 10^{-3}$ M. $MnCl_2$ at $5{\cdot}0 \times 10^{-4}$ M and ethanol at $4{\cdot}5 \times 10^{-3}$ M. Exp. 3. Manometric assay, 37° C, *p*H 5·5 according to Iyer and Quastel (ref. 8). Exp. 4. Spectrophotometric assay, by decrease in absorbance at 340 mμ; 25° C, *p*H 7 (0·03 M phosphate buffer), NADH or NADPH at $1{\cdot}7 \times 10^{-4}$ M. Where KCN was used, it was present at 1 mM

The enzyme from the supernatant fraction was purified about 10-fold by precipitation with ammonium sulphate (30–60 per cent saturation), dialysis and column chromatography on DEAE-cellulose. Such preparations are essentially free of lactate dehydrogenases and show no stimulation of either NADH or NADPH oxidase activity by Mn^{++}. At this stage the enzyme is virtually inactive toward NADPH. Its *p*H optimum is about *p*H 5, at which it has roughly double the activity shown at *p*H 7. Preliminary experiments with this enzyme show that in the presence of 1 mM potassium cyanide, 1 mole of oxygen is consumed per mole of NADH oxidized, and hydrogen peroxide is presumably formed. Evidence from fluorimetric investigations[17] of the enzyme preparation at various stages of purity suggests that it contains FAD. Preliminary experiments with polyacrylamide gel electrophoresis indicate that the enzyme may be separated from other proteins and it can be located on the gel by a diaphorase reaction with nitro blue tetrazolium and NADH[18]. Elution of the enzyme from such gels has been achieved with a further increase in purity, but as yet in low yields.

The distribution of the oxidase among subcellular fractions was compared with that of a number of known enzymes of the characteristic granules of PMN. As shown in Table 2, the hydrolytic enzymes and peroxidase are found predominantly in the granule fraction, in agreement with previous reports[12–14]. Cyanide-sensitive NADH oxidase follows the same pattern. On the other hand, the cyanide-insensitive NADH oxidase, measured either spectrophotometrically or manometrically, is found largely in the supernatant fraction. The possibility that this enzyme was released during the fractionation is remote, in view of the results obtained with the five other enzymes examined.

As a result of these findings, we must modify our previous hypothesis for the mechanism underlying the increase in respiration and direct oxidation of glucose-6-P in PMN when they phagocytose. Previously we suggested[4] that the cyanide-insensitive NADH oxidase is associated with the granule fraction. This stemmed from the fact that some of the enzyme adheres to the granules, from which it may be partially removed by washing, thus giving rise to the impression that it was liberated by freezing

Table 2. DISTRIBUTION OF TOTAL ENZYMATIC ACTIVITIES OF PMN

Component	Fraction* Debris	Granule	Post-granule	Supernatant
Acid phosphatase†	29	57	9	5
β-Glucuronidase	35	47	1	17
β-Galactosidase	8	68	3	21
Peroxidase	23	74	1	1
NADH oxidase				
CN-sensitive‡	20	64	9	7
CN-insensitive‡	27	18	5	51
CN-insensitive§	11	5	11	73
Protein¶	24	24	4	47

* Results are based on recovered enzyme or protein as 100 per cent. Cells homogenized as in Table 1 (Exp. 1). Homogenate spun at 240*g* (10 min) and the pellet resuspended and rehomogenized. This homogenate spun at 240*g* (10 min) to obtain the debris fraction. The combined supernatant fractions were spun at 30,000*g* (15 min) to obtain the granule fraction, and then at 100,000*g* (1 h) to obtain the post-granule fraction. The fraction that remained was termed 'supernatant fraction'.

† Hydrolytic enzymes and peroxidase were assayed by standard methods (refs. 19–21) with some modifications.

‡ Spectrophotometric assays as in Table 1. Cyanide-sensitive activity obtained from difference between total and cyanide-insensitive (1 mM KCN) activities.

§ Manometric assays (37° C, *p*H 7) as in Table 1.

¶ Protein determined by the method of Lowry *et al.* (ref. 22).

and thawing[4]. Our previous failure to detect it in the supernatant fraction was due to excessive dilution of this fraction with washings in the course of the fractionation. In the work recorded here we were careful to keep the supernatant fraction as concentrated as possible. Our hypothesis[4] previously involved the release of the oxidase from the granules during phagocytosis, as had been established for the lytic enzymes[23], and its activation. Postulation of such a release of the oxidase is no longer necessary in view of the foregoing distribution investigations. Activation may be the result of a transient increase in intracellular H^+ concentration[1,24], since an increase in lactate occurs during phagocytosis[3,6] and the enzyme has a low *p*H optimum.

In accord with the foregoing results, Rossi and Zatti[25] have reported that, whereas acid phosphatase is increased in the supernatant fraction from phagocytosing cells (indicating release from the granules), no such increase of the NADH oxidase in the supernatant fraction occurs—suggesting that the latter enzyme is not released from granules during phagocytosis. The supernatant fraction in their experiments also exhibited no activity toward NADPH. Their investigations of granule-associated oxidases are not clear, however, since no cyanide was present.

The level of activity of the cyanide-insensitive NADH oxidase determined manometrically at 37° C on whole homogenates is of the order of 5–6 μl. oxygen/h and mg protein at *p*H 7. The respiratory stimulation due to phagocytosis by intact PMN amounts to 244 per cent of the resting level of 2·5 μl. oxygen/h and mg protein[15]. The net increment in respiration during phagocytosis of 3·6 μl. oxygen/h and mg protein is thus readily accounted for by the activity of the cyanide-insensitive NADH oxidase. If the optimal *p*H for this enzyme is taken into account, there appears to be about 3 times as much enzyme present as is required to account for the respiratory increase during phagocytosis.

Our original concept that a cyanide-insensitive NADH oxidase is responsible for the bulk of the respiratory stimulation during phagocytosis by PMN seems tenable in the light of the observations reported. Our first impression that this enzyme is localized in the granules of these cells has been corrected ; it is recovered mainly in the supernatant fraction from cell-homogenates. The existence of the NADH oxidase, its cyanide-insensitivity, and its occurrence in amounts adequate to account for the respiratory stimulation in intact cells, make it unnecessary to invoke the granule-bound peroxidase which is cyanide-sensitive. Roberts and Quastel expressed the opinion that the very large content of peroxidase in PMN might permit its involvement, by release from the granules, even in the presence of cyanide[9] ; but there is no experimental basis for this idea. Further, the work of Hirsch[26] indicates that when degranulation occurs during phagocytosis, the granule contents are discharged into the phagocytic vacuoles. This would raise an additional problem of a morphological nature as to how peroxidase, a component of the granules, might react with reduced pyridine nucleotides in the cytoplasm. This problem was also, of course, implicit in our previous hypothesis[4], and the findings outlined above obviate this consideration.

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ATTEMPTS TO DEMONSTRATE A TRANSMISSIBLE AGENT IN KURU, AMYOTROPHIC LATERAL SCLEROSIS, AND OTHER SUB-ACUTE AND CHRONIC NERVOUS SYSTEM DEGENERATIONS OF MAN

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THIS article is a summary of our programme of work on the possibly infectious ætiology of chronic human central nervous system diseases. It presents negative results, after the first year of observation, of attempts at virus isolation and experimental transmission, particularly in primates, of kuru and amyotrophic lateral sclerosis. Moreover, it briefly describes recently initiated experiments, after only one to two months observation, on sub-acute inclusion encephalitis of the Dawson type and passage of central nervous system tissues from a monkey infected in the U.S.S.R. with human amyotrophic lateral sclerosis material. Because of the increasingly frequent suggestions that a transmissible agent may be involved in the pathogenesis of these diseases[1–10], this progress report on experiments which will not be terminated before five years is in order.

At the time of medical discovery of the kuru focus among the Fore people and their neighbours in the high-

lands of eastern New Guinea, the possibilities of acute infectious, post-infectious, and of chronic persistent infectious ætiology, as well as of an infection-stimulated hypersensitivity state, were all considered[11,12]. The cause of this unremitting and unaltered plague on some 30,000 Melanesian natives, still causing to-day more than half the mortality in the regions of highest incidence, has remained unsolved.

Early in 1957 small laboratory animals, including chick embryos, mice, rats, guinea-pigs and rabbits, were inoculated with suspensions of brain tissue from kuru patients in an attempt to transmit the disease experimentally, or to propagate a micro-organism associated with it. Neuropathological investigations of patients had failed to elicit any definitive histopathological lesions in the central nervous system, such as were commonly associated with viral infections. Furthermore, clinical laboratory investigations of cerebrospinal fluid specimens from kuru patients did not elicit findings such as pleocytosis or elevated protein values common, at least in a high percentage of cases, to all known central nervous system viral infections of man. Patients failed to show febrile reactions at any stage of the disease, no consistent change in erythrocyte sedimentation rate or in the peripheral blood picture was found, and no episode of contact infection was recorded epidemiologically. Thus, acute infectious ætiology seemed unlikely. Nevertheless, brain tissue obtained from kuru patients taken shortly after death was preserved in buffered glycerin or by ice refrigeration and flown to Melbourne, Australia, where isolation attempts were initiated. Some of these specimens were afterwards shipped under dry ice refrigeration to the National Institutes of Health, Bethesda, Maryland, where additional isolation investigations were conducted utilizing small laboratory animals, embryonated chicken eggs, and a variety of tissue and cell cultures prepared from human, animal and avian origins. These early investigations failed to elicit any recognizable or recoverable pathogenic micro-organisms during the three months that the animals remained under observation.

More recently, however, Hadlow[3] has reported that a similarity exists between the neuropathological lesions in sheep with scrapie disease and human beings with kuru. These observations led us to re-evaluate our isolation procedures and laboratory techniques and to reconsider the possibility that an ætiological agent for kuru might be a slow or latent virus with, perhaps, a genetically determined susceptibility, or perhaps, even more speculatively, a temperate virus actually genetically transmitted, acting somewhat like a lysogenic bacteriophage. Indeed, because of the unusual heat stability and alleged genetic transmission in flocks of sheep, the suggestion had often been made that the scrapie agent might be denuded nucleic acids originating from the host genetic apparatus itself, and not a complete virus particle as commonly encountered in virology[13]. Although such a postulate would help to explain the lack of detectable antibody in scrapie disease in sheep, more detailed investigation of heat inactivation of the agent and its enzyme sensitivity, including the failure to inactivate its infectivity with deoxyribonuclease and ribonuclease, and the rather conventional virus behaviour of the pathogen, have convinced most workers that the scrapie agent is a more orthodox animal virus[14,15].

Beginning in 1962, long-term investigations aimed at observing primates for five years or more, and smaller animals and tissue cultures for as lengthy periods as possible, were initiated in our laboratories. Table 1 summarizes the isolation investigations now in progress on specimens of central nervous system tissues from patients who had died of kuru, as well as experiments on amyotrophic lateral sclerosis and Parkinsonism-dementia syndrome associated with amyotrophic lateral sclerosis on Guam. Although only one chimpanzee (*Pan satyrus*) has, in general, been inoculated with the material from a single patient, other primates, rhesus (*Macaca rhesus mulatta*), cynomolgus (*Macaca cynomolgus irus*), and African green (*Cercopithecus aethiops*) monkeys, have been inoculated in groups varying from a few to a dozen with inoculum from a single patient. In addition, groups of large numbers of new-born and weanling mice of several strains, hamsters and guinea-pigs, and primary and stable tissue culture lines have been inoculated with serially diluted brain suspension (in phosphate buffered physiological saline, *p*H 7·4) prepared immediately before inoculation from freshly thawed tissue. Most primates have received inocula of 0·2 ml. intracerebrally and 0·2 ml. intravenously, while smaller animals have been injected with varying amounts of diluted and undiluted materials in a variety of routes.

The earliest of these long-term investigations were started in small laboratory animals inoculated in October 1962. In January 1963 a 10 per cent suspension of brain tissue from the right temporal region of the brain, obtained 4·5 h after death from a patient who had died of kuru, was inoculated intracerebrally and intravenously into a young (less than 6 months of age) rhesus monkey; this suspension was also inoculated into small laboratory animals and tissue cultures. After 20 months of close observation none of the animals have developed any evidence of neurological disorders, nor has a viable agent been isolated in them; tissue culture investigations likewise revealed no cytopathological effect or evidence of chronic carrier state.

As the scope of our programme became enlarged and intensified in early 1963, greater emphasis was placed on the selection and preservation of specimens, on the range of experimental hosts used, and on improved virological and tissue culture procedures for successful isolations. Thus, for example, brain and visceral tissue specimens have been obtained from kuru patients in New Guinea within 2 h of death. These tissues were immediately frozen in liquid nitrogen and shipped by air in liquid nitrogen to the National Institutes of Health, where portions were rapidly thawed, ground to 20 per cent suspensions in phosphate buffered saline solution (*p*H 7·4) and inoculated into test animals, primarily primates. Specifically, 6 chimpanzees, 32 rhesus, 25 cynomolgus and 10 African green monkeys have been observed for 2–19 months after inoculation with kuru brain material without developing signs of disease. In all, brain from seven different kuru patients has been used in these primate inoculations. These inocula have also been put into the following tissue culture lines: routinely into primary and stable rhesus kidney, *BS-C*-1 green monkey kidney, human amnion, Chang liver, chick embryo, mouse embryo, *Hep*-2, *L*-cells and HeLa; on occasion into human embryo kidney, primary pig kidney and pig choroid plexus. No cytopathogenic effect has been observed. Attempts are also being made to culture viable cells *in vitro* from liquid nitrogen preserved tissue for the purpose of determining cell morphology and abnormality, as well as for isolations of viruses.

We have, furthermore, also been interested in the possibility that transmissible agents may be involved in the pathogenesis of a wide range of chronic progressive neurological diseases. Among these the foci of amyotrophic lateral sclerosis and the related Parkinsonism-dementia syndrome in extremely high incidence in the Chamorros population on the Island of Guam is of particular interest[16,17]. In these indigenous Micronesians amyotrophic lateral sclerosis attains a prevalence some one hundred times greater than that observed in the United States, Europe, or Japan. We are, thus, searching for a possible transmissible agent in these diseases; the brain tissue from Guamanian amyotrophic lateral sclerosis and Parkinsonism-dementia patients has been collected, preserved, and processed, as has been described for kuru. So far we have inoculated a total of 6 chimpanzees and 46 monkeys with brain material from five Guamanian patients with amyotrophic lateral sclerosis

Table 1. SUMMARY OF ISOLATION STUDIES ON SUB-ACUTE AND CHRONIC DISEASES OF THE NERVOUS SYSTEM*
Negative results as of August 1964

Disease and patient	Preservation†	Experimental hosts by date of inoculation							Tissue culture
		Primates				Other mammals			
		Chimpanzee	*M. rhesus*	*M. cynomolgus*	*C. aethiops*	Mouse	Guinea-pig	Hamster	
Kuru:									
Moba	CO_2	Sept. '63		Sept. '63 Jan. '64	Sept. '63 Jan. '64	Oct. '62	Oct. '62	Oct. '62	Nov. '62
Eiro	CO_2	Sept. '63	Jan. '63	Sept. '63 Jan. '64	Sept. '63 Jan. '64	Oct. '62	Oct. '62	Oct. '62	Nov. '62
Kigea‡	CO_2	Aug. '63	Aug. '63	Aug. '63 Sept. '63	Sept. '63 Jan. '64	Aug. '63	Aug. '63	Aug. '63	Aug. '63
Enage	CO_2	Aug. '63	Aug. '63	Sept. '63	Sept. '63 Jan. '64	Aug. '63	Aug. '63	Aug. '63	Aug. '63
Kariwani	N_2	Nov. '63	Nov. '63			Feb. '64			Feb. '64
Igierakaba	N_2	Feb. '64	Feb. '64			Feb. '64			Feb. '64
Kabuinampa	N_2	Feb. '64	Feb. '64			Feb. '64			Feb. '64
Amyotrophic lateral sclerosis:									
Naganta	CO_2		Sept. '63	Sept. '63 Jan. '64	Sept. '63	Feb. '64			Feb. '64
Taitingfong	CO_2			Sept. '63 Jan. '64	Sept. '63	Feb. '64			Feb. '64
Santos	CO_2			Sept. '63 Jan. '64	Sept. '63 Jan. '64	Feb. '64			Feb. '64
Parkinsonism-dementia§:									
Domingo	CO_2			Sept. '63 Jan. '64	Sept. '63	Feb. '64			Feb. '64
Toves	CO_2	Aug. '63		Aug. '63 Jan. '64	Aug. '63 Jan. '64	Feb. '64			

* Brain tissue was used as inoculum in all cases; it was obtained at autopsy within a few hours of death. † CO_2 = dry ice; N_2 = liquid nitrogen; −70° C = electric refrigeration. ‡ Acute blood specimens from Kigea and two other kuru patients inoculated into mice in 1962 and 1963. § Parkinsonism-dementia syndrome on Guam associated with amyotrophic lateral sclerosis.

and from three with the Parkinsonism-dementia syndrome. These animals have been observed for from 2 to 14 months and no illness attributable to the inoculations has appeared among them.

In addition, brain tissue from a surgical biopsy of one patient with sub-acute inclusion encephalitis of Dawson type has recently been inoculated into one chimpanzee and four monkeys. Finally, a 20 per cent (w/v) suspension of brain and cord material from a second passage rhesus monkey suffering from experimentally induced motor neurone disease has been inoculated intracerebrally and intraperitoneally into 10 rhesus monkeys in our laboratory. This material, supplied by Dr. L. A. Zilber of Moscow, is from one of the second passage monkeys that developed motor neurone disease resembling amyotrophic lateral sclerosis clinically and pathologically following primary inoculation with human brain tissue from a patient with amyotrophic lateral sclerosis[6]. Disease first appeared three years after primary inoculation. Of course, confirmation of this reputed transmission of amyotrophic lateral sclerosis from man to primate is necessary and, furthermore, there is as yet no way of establishing that a transmissible agent causing motor disease in primates is the ætiological agent of motor neurone disease in man.

In spite of the negative findings in the past in search for micro-organisms, especially viruses, as the causative agents of chronic neurological diseases, the possibility of infectious ætiology must now be re-evaluated in the light of newer concepts of latency and masking of viral activity. Viruses known to cause chronic degenerative diseases of the central nervous system in animals, such as scrapie and visna of sheep, have not, as yet, been clearly implicated in any disease in humans, though neutralizing substances to visna virus have been reported in human sera[10], and in at least two instances reports have been made suggesting scrapie-like illness in sheep inoculated with tissues of the central nervous system from patients with sub-acute encephalitis[7] and acute disseminated sclerosis[9]. Furthermore, the virus causing Aleutian disease in mink has recently been suggested as the cause of a case of encephalitis in man[8]. Chumakov has reported on one occasion the isolation of Russian spring-summer encephalitis virus from the brain of a patient suffering from Kozhevnikov's epilepsy, a chronic convulsive syndrome which has often been suggested to be a late effect of this virus infection[2].

The Russian workers have also suggested the possibility that Vilyiusk encephalitis, a chronic degenerative central nervous system disorder of Iakutsk people in Siberia, is of viral ætiology[4,18,19]. In neither of these cases is a viral ætiology established, however. In fact, the relationship of the known scrapie, visna, and Aleutian disease in mink viruses to any of the chronic degenerative nervous system diseases of man, as well as the relationship of these human diseases one to another, as, for example, between amyotrophic lateral sclerosis and Parkinsonism-dementia on Guam, and between these syndromes and similar syndromes in America and Europe, cannot be established even if a transmissible virus is located from them, without a specific diagnostic test for the agents. Thus, we are also at present working on the production of hyperimmune animal sera to various particulate and macromolecular tissue fractions of human brain tissue from the diseases under investigation, as well as of tissue from scrapie-infected animals. These antisera may eventually serve as disease specific reagents in neutralization, complement fixation, fluorescent antibody, hæmagglutination-inhibition, and other serological tests which will be essential in establishing a micro-organism as a cause of any of these illnesses.

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HETEROGENEITY IN SEGREGATION DATA FROM WHEAT CROSSES

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In Soviet papers on plant genetics the claim is frequently made that heritability of characters is greatly influenced by external conditions. Aizenstat[1] found that the amount of pollen, the age of the pollen and the physiological status of the florets were important. Pollination of the first and last floret led to more patroclinous progeny, and, as a rule, with increasing age of the gametes their ability to transmit the characters they carried diminished. More recently, Allard[2] in the United States has found that recombination values in the lima bean, *Phaseolus lunatus* L., varied according to the time of sowing, the time of gathering, and the direction in which selection was practised. Certain aspects of the work recorded here are very similar to the aforementioned and the most significant one is the heterogeneity of data.

In an earlier publication[3] it was shown that differential transmission of gametes in wheat occurs, and it was suggested that the Gabo-type resistance to wheat stem rust, *Puccinia graminis* Pers. var. *tritici* (Eriks. and Henn.), previously thought to be due to the action of two linked dominant complementary factors, and the 'Mentana'-type resistance to wheat leaf rust, *Puccinia recondita* Rob. ex Desm., were due to single dominant factors. A similar conclusion was reached by Sears and Loegering[4]. Both resistances are carried on chromosome 6*B* (*X*) and were found to be very closely linked[3].

A main feature of the previous report[3] was the heterogeneity in segregation ratios obtained in different crosses involving the two factors. This article extends observations on heterogeneity between crosses and shows that heterogeneous data are obtained within crosses when examining different F_2 families, and also within single F_1 plants when testing seed of individual ears.

In this work a second factor besides that from 'Mentana' for resistance to leaf rust is also introduced, namely, the factor which was transferred from *Aegilops umbellulata* to 'Chinese Spring' wheat by Sears, the derivative being named 'Transfer'. Like the other two factors it is located on chromosome 6*B*. In Table 1 are listed results of several crosses (in many instances reciprocal) which did not fit a single factor segregation.

The aberrant F_2 results shown in Table 1 undoubtedly represent a whole range of different ratios. Moreover, when the F_3 generation was examined, ratios within crosses differed even more widely and ranged from a 15 : 1 to a 0·4 : 1. F_3 segregation ratios which went beyond this range were found to be the result of either chromosomal aberrations or outcrossing.

In the cross ('Transfer' × 'Kenya 1483') the transmission ratios for the factor for leaf rust resistance and the factor *Sr*11 were similar (Table 1) and cross-over types were rare, but there was a significant deficiency in number in the heterozygous class. The high fertility of the ears tested combined with the high germinability of the F_2 seeds did not suggest differential zygotic viability. A hypothesis which assumes on the male side pollen abortion coupled with recombination and on the female side differential transmission proves insufficient to explain these results. Selective conjugation during fertilization, however, could account for the data.

Further, a highly significant intra-plant heterogeneity (P = 0·01–0·001) in both crosses involving 'Kenya 1483' was found when separate ear progenies of individual F_1 plants were tested. Again, this was mainly due to variation in number in the heterozygous class.

In the crosses ('Chinese Spring' × 'Gabo') and ('Chinese Spring' × 'Yalta') and their reciprocals, data from different F_2 families were doubtfully homogeneous. However, in results presented by Driscoll[5] involving similar crosses using monosomic lines of 'Chinese Spring', heterogeneity is apparent if the very small families are removed.

In 1961 two different explanations for the distorted ratios were suggested. Sears and Loegering[4] invoked a pollen-killing gene, *Ki*, on chromosome 6*B* in 'Chinese Spring', which they claimed was responsible for a complete removal of all gametes carrying the recessive allele, *ki*. I myself[6], on the other hand, found a high degree of lagging of chromosomes in F_1 plants of crosses which gave distorted segregation ratios. A detailed study of meiosis in F_2 plants of the cross ('Mentana' × 'Yalta') revealed that the univalent frequency was greatest (more than 20 per cent tetrads with micronuclei) in plants heterozygous for the factors for stem-rust and leaf-rust resistance. Since no cross-over types combining the two resistances were obtained among 750 F_3 lines, and since Heyne and Livers[7] have reported a recombination value of 21·7 ± 7·8 per cent for these factors, it could be assumed that chromosome 6*B* was partially asynaptic. Several leaf chimeras involving loss of the dominant allele for rust resistance were found and they could have been the result of somatic elimination of misdivided univalents. Aneuploid segregants occurred so rarely that they would have had no significant effect on the F_2 ratio. Pollen abortion was noticed in several F_1 and F_2 plants, but it was not possible to correlate pollen fertility in the F_2 with subsequent F_3 behaviour to rust. Recently, I have made a detailed study of pollen abortion in F_2 plants of the cross ('Gabo' × 'Mentana'). Approximately 37,500 pollen grains were classified and the percentage of aborted pollen ranged from 0·3 to 93·2 per cent. As in the case of chromosome lagging, plants heterozygous for the factors for rust resistance were far more often found to exhibit a high degree of pollen abortion. However, in several F_3 plants which later gave rise to progeny which segregated in greatly distorted ratios of less than 5 per cent aborted pollen was found. As this article is not concerned with small differences but with gross aberrations, such a small amount of pollen abortion should have been of no consequence.

It was also found that pollen abortion was differentially inherited when seed from individual ears of the same F_1 plant was grown. Thus in three F_2 plants heterozygous for the factors for rust resistance and which originated from the second ear of an F_1 plant, 452 aborted pollen grains were counted among a total of 1,980 (average percentage = 23·9), while seven heterozygous F_2 plants from the seventh ear of the same plant showed only 120 aborted pollen in a total of 2,239 (average percentage = 4·6). Somatic segregation of a cytoplasmic factor, nevertheless, seems unlikely as reciprocal crosses gave the same results when the data of several F_2 families were compared[6].

In a more recent publication[8], Loegering and Sears suggested about 9 per cent recombination between the pollen-killing gene *Ki* and *Sr*11 in the cross ('Chinese

Table 1

Cross: Resistant parent	Susceptible parent	No. of F_2 plants tested	Ratio res./sus.	Heterogeneity between F_2 families	Heterogeneity between Ear samples
(a) Crosses involving 'Gabo'-type stem-rust resistance (*Sr*11)					
Gabo	Transfer	1,271	2·6 : 1	Het.*	?
Gabo	Mentana	1,354	2·2 : 1	Hom.	?
Gabo	Chinese Spring	1,002	1·9 : 1	Hom.	?
Gabo	*W* 2691	305	1·8 : 1	—	—
Charter	Mentana	726	2·2 : 1	Hom.	—
Yalta	Federation	1,544	3·5 : 1	?	—
Yalta	Chinese Spring	1,930	1·4 : 1	?	?
Yalta	Mentana	3,092	1·3 : 1	Het.	Het.
Kenya 1483	Mentana	2,030	2·9 : 1	Het.	Het.
Kenya 1483	Transfer	1,282	2·6 : 1	Hom.	Het.
(b) Crosses involving leaf rust resistance of 'Mentana'					
Mentana	Chinese Spring	1,143	4·6 : 1	Het.	—
Mentana	Federation	1,061	6·4 : 1	Hom.	—
Mentana	Yalta	1,311	7·9 : 1	Het.	Het.
Mentana	Kenya 1483	2,223	4·6 : 1	Het.	Het.
(c) Crosses involving leaf rust resistance of 'Transfer'					
Transfer	Chinese Spring	1,111	2·4 : 1	Hom.	—
Transfer	Kenya 1483	1,196	2·9 : 1	Hom.	Het.
Transfer	Mentana†	1,837	2·1 : 1	Hom.	Hom.
Transfer	Federation‡	1,461	4·1 : 1	Het.	—
Transfer	Gabo	1,273	3·9 : 1	Het.	?

* Het., heterogeneity; Hom., homogeneity; ?, for low probability, and —, indicates that no tests were made.

† This cross was tested with strain 162–1, 2, 3, 4 to which 'Mentana' is susceptible.

‡ McIntosh and Luig (unpublished results).

Spring' × 'Timstein'). Variation in pollen abortion in different samples from the same plant, with a tendency for later samples to approach or reach 50 per cent abortion, was also noticed. In the work recorded here an analysis of the segregation data collected from testing seed from individual ears of F_1 plants of crosses ('Chinese Spring' × 'Gabo') and ('Yalta' × 'Chinese Spring') failed to indicate a tendency for later emerging ears to produce more susceptible F_2 hybrids.

Loegering and Sears[8] also reported that certain varieties showed virtually no pollen abortion in crosses with either 'Chinese Spring' (*KiKi*) or 'Thatcher 6*B*' (*kiki*). In the present work the crosses ('Chinese Spring' × 'Mentana') and ('Gabo' × 'Kenya 1483') gave 43·7 and 40·4 per cent pollen abortion, respectively, although on the assumption of a simple system for pollen abortion these hybrids should have been fully fertile. On the other hand, in crosses in which 50 per cent pollen abortion was expected the actual percentages ranged from 7·7 to 46·2. These results, as well as those of Loegering and Sears[8], clearly demonstrate something more complex than a single dominant allele for pollen abortion.

The character of differential transmission of gametes was inherited from generation to generation. Sometimes the degree of differential transmission was also inherited, especially when the transmission rate for one of the alleles was very low, that is, when female as well as male gametes were involved.

The hypothesis of a pollen-killing gene as the sole cause of differential transmission is rejected because it fails to explain differential transmission of female gametes, the absence of crossing-over between the factors involved, the failure to recover true 3 : 1 segregation ratios resulting from recombination between *Ki* and genes for rust resistance, the various segregation ratios varying from 15 : 1 to 0·4 : 1, the heterogeneity of data, differential transmission in crosses which should have involved only one of the alleles, *Ki* or *ki*, lack of pollen abortion in plants which gave rise to progeny which segregated in greatly distorted ratios, the deficiency in number of heterozygous plants in crosses involving 'Kenya 1483', and various cytological abnormalities mentioned earlier.

Differential transmission of gametes in wheat does not seem to be restricted to chromosome 6*B*. Nyquist[9] has shown that stem-rust resistance in the variety *C.I.* 12633, previously thought to be due to the action of duplicate dominant factors, is controlled by a single factor. This resistance is carried on chromosome 2*B* (XIII), and Nyquist explained the heterogeneous ratios obtained on the basis of differential fertilization. It should be noted, however, that *C.I.* 12633 has 'Chinese Spring' in its pedigree and that percentages of pollen abortion approximating to 50 per cent were observed by Nyquist. As in the present case, the allele from the 'Chinese Spring' background was transmitted at a favourable rate, and for all such reasons a single explanation for the mode of inheritance of factors on chromosomes X and XIII would be more justified.

The work recorded here suggests a complex system operating in wheat which determines the transmission of gametes.

Another as yet unexplained finding is variation for rust reaction in the variety 'Mentana' itself. Genetic and cytological investigations have failed to detect a possible explanation for this, although tripolar spindle formation in the second meiotic division was found with approximately 15 per cent of the tetrads having more than the four nuclei.

This work was supported by a grant from the Wheat Industry Research Council.

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PROGESTERONE AND 6-CHLORO-Δ^6-17-ACETOXYPROGESTERONE AS INHIBITORS OF SPERMATOGENESIS IN THE RABBIT

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THE effect of progesterone and related synthetic compounds on ovulation has been extensively investigated. Evidence from the female indicates that progesterone prevents ovulation by inhibiting release of the gonadotrophic hormone complex[1–3]. Information relative to the action of progesterone on the male reproductive system is limited[4]. Administration of progesterone or compounds with similar biological activity to the male

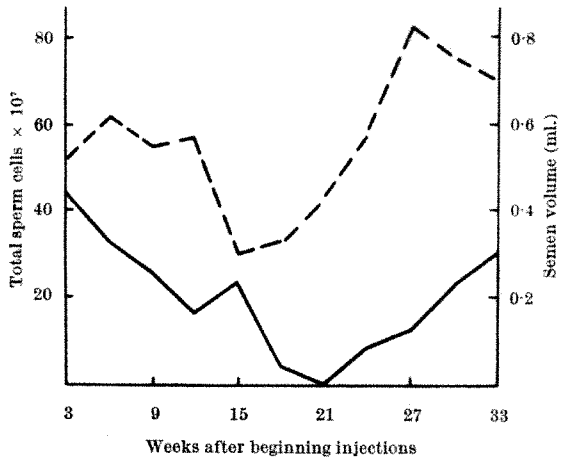


Fig. 1. Changes in semen volume and total number of sperm cells ejaculated by rabbits after progesterone injections. – – –, volume; —, total sperm cells

should result in impairment of spermatogenesis and accessory gland function. Experiments were conducted to determine the effect of progesterone and 6-chloro-Δ^6-17-acetoxyprogesterone (CAP) on semen production and libido in the male rabbit.

Two mature male rabbits were injected subcutaneously with 10 mg of crystalline progesterone in propylene glycol solution every other day for 14 days. Semen for laboratory examination was collected weekly in an artificial vagina. Total volume, volume minus gelatinous material, percentage motile cells, sperm cell concentration and percentage abnormal cells were determined. The only change in any semen characteristics during an 18-week collection period was an increase in the frequency of spermatozoa with protoplasmic droplets. These droplets are not a permanent structure of the mature sperm cell, and when present on ejaculated sperm are considered to indicate immaturity[5]. It is possible that the discharge of stored sperm from the epididymis would allow sufficient numbers in the ejaculate to obscure any interference of spermatogenesis which might result from the 2-week hormone treatment. However, the appearance of protoplasmic droplets indicates an arresting effect on maturation of the sperm cells in the epididymis.

Two additional male rabbits were injected with 10 mg of progesterone every other day for 28 days. One of the rabbits showed reduced libido shortly after beginning of the treatment and refused to serve the artificial vagina unless placed in a breeding position with a teaser doe. Libido in this rabbit gradually returned to normal 8 weeks after beginning of treatment. The remaining male showed no decrease in sex drive during a 33-week collection period. Average total sperm cells and semen volume produced by the two males are shown in Fig. 1. Semen volume and total sperm cells were significantly lower. The lower semen volume in treated males was partially due to a lack of gelatinous material. This gel-like substance, usually present in the semen of the rabbit, was absent for the entire collection period. In two control males, gelatinous material was present in 73 per cent of the ejaculates and constituted 21 per cent of semen volume. The normal function of the glandulæ vesiculares, the glands which are responsible for production of the gelatinous material in rabbit semen[6], was apparently impaired by the progesterone injections. Semen volume of the treated males was significantly lower ($P < 0{\cdot}01$) than controls from the 8th to the 32nd week of the experiment.

Spermatogenesis was also suppressed by the progesterone injections. The treated males produced significantly lower ($P < 0{\cdot}01$) total number of sperm cells than control males from the 15th through the 27th week. By the 19th week of treatment both males were azoospermic, and this condition continued through the 21st week. The lowest numbers of sperm cells occurred in the semen six weeks later than the low point in semen volume (Fig. 1). A delay in deletion of sperm would be expected since cells from the epididymis would serve as a source for a period following inhibition of spermatogenesis.

Testicular biopsies were not taken from the above males due to possible interference with semen collections. However, histological sections prepared from the testis of a male injected with 20 mg of progesterone every other day for 60 days and a control male are shown in Fig. 2. The testis was removed from the treated male 10 days after the last injection of hormone. In contrast to the compact and orderly arrangement of cells in the seminiferous tubules of the control male, the germinal elements in the tubule from the treated male were disorganized and the presence of sloughed cells and debris in the lumen indicated degenerative processes. It was apparent that normal spermatogenic activity in the treated male had been disrupted.

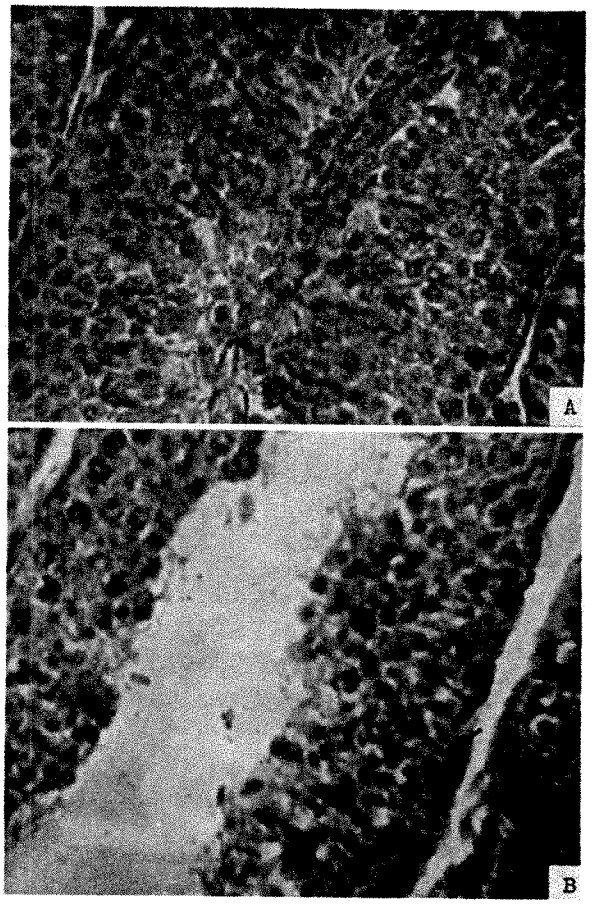


Fig. 2. Photomicrograph of histological sections of rabbit testes (× *c.* 330). *A*, section of seminiferous tubule from control; *B*, section of seminiferous tubule from male injected with 20 mg of progesterone every other day for 60 days

In order to determine the effects on semen production of injecting an orally active progestin, 10 mg of 6-chloro-Δ^6-17-acetoxyprogesterone were injected every other day for 30 days into two male rabbits. Libido was lowered in both males shortly after the injections were started, and continued up to the sixth week, when one male refused to serve the artificial vagina. This male was unilaterally

castrated 11 weeks after the last injection, and histological sections were prepared from the testis. As in the progesterone-treated male, the seminiferous tubules contained cellular debris but no sperm, and the germinal elements were degenerate. The other male continued to serve the artificial vagina, and a few weeks after the end of injections showed a gradual increase in sexual desire. Total sperm cells decreased in the male that continued to produce semen, and from the 19th to the 22nd week his ejaculates were azoospermic. Thereafter, there was a gradual increase in sperm cell numbers. The percentage of motile cells was not significantly affected.

Results from these investigations show that progesterone and certain related compounds which are orally effective as inhibitors of ovulation in the female also interfere with spermatogenesis and accessory gland function in the male rabbit. When progesterone was injected into male rabbits for a short period (2 weeks), the only apparent effect was an increased frequency of protoplasmic droplets on ejaculated sperm. However, when injections of progesterone or CAP were continued for longer periods, the characteristic gelatinous fraction of the ejaculate was not present, and both semen volume and sperm numbers decreased.

The significant lowering of total sperm in semen and the histological appearance of the seminiferous tubules following the longer periods of treatment suggest that spermatogenic activity has been curtailed. The inhibition of spermatogenesis is apparently reversible, since sperm numbers eventually returned to normal level. Further investigations with these or similar compounds on inhibition of spermatogenesis and interference with the normal maturation process of sperm are warranted as potential means of birth control.

We thank the Eli Lilly Co., Indianapolis, Indiana, for supplying the 6-chloro-Δ^6-17-acetoxyprogesterone.

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PURIFICATION OF POLIOVIRUS BY DEAE 'SEPHADEX A-25'

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NUMEROUS methods have been used for the separation of viruses from extraneous protein attributable to the host. These techniques have primarily involved a combination of ion-exchange chromatography and centrifugation[1,2]. Other techniques have involved the adsorption and subsequent elution of the agent from ion-exchange columns by the gradual change of *p*H or eluate[3]. We wish to report on a rapid method for the purification of Sabin type 1 poliovirus from extraneous tissue culture proteins. This procedure requires only a single manipulation prior to virus purification.

The HeLa cell monolayers were frozen and thawed three times, the lysate centrifuged at 1,000*g* for 10 min and the supernate then purified by ion-exchange chromatography. Utilizing similar elution procedures, three types of ion exchangers were compared as to their effectiveness in purifying virus. The columns consisted of 'ECTEOLA-*SF*' ('Cellex-*E*'), 'Sephadex *G*-25', or DEAE 'Sephadex *A*-25'.

The 'Cellex-*E*' was suspended in M/15 phosphate buffer at *p*H 7·5. The fines were removed and 9 cm × 1·2 cm columns were packed under a pressure of 10 lb./in.^{2}. 'Sephadex *G*-25' was packed as 14 cm × 1·2 cm columns, utilizing M/15 phosphate at 7·5 *p*H. The DEAE 'Sephadex *A*-25' was allowed to swell in water. After removal of the fines, the '*A*-25' was washed in sequence on filter paper by 0·5 N hydrochloric acid; water; 0·5 N sodium hydroxide; and water. It was finally neutralized with 0·5 N hydrochloric acid, suspended in M/15 phosphate buffer at *p*H 7·5, and packed in 14 cm × 1·2 cm columns.

Five ml. of stock poliovirus, prepared as indicated above and having a titre of $4{\cdot}8 \times 10^7$ P.F.U./ml. in HeLa cells, were added to the 'Cellex-*E*' column. Two ml. of this virus suspension were added to the 'Sephadex' columns.

The elution for all columns was carried out at room temperature with M/15 phosphate buffer at *p*H 7·5. The first eluate consisted of 3 ml. Subsequent fractions were collected in 1 ml. quantities at a rate of 1 ml./min. Protein content of the fractions was determined by the Lowry method[4] and virus yield was determined in HeLa cell plaque bottles. Our results indicate that the 'Cellex-*E*'

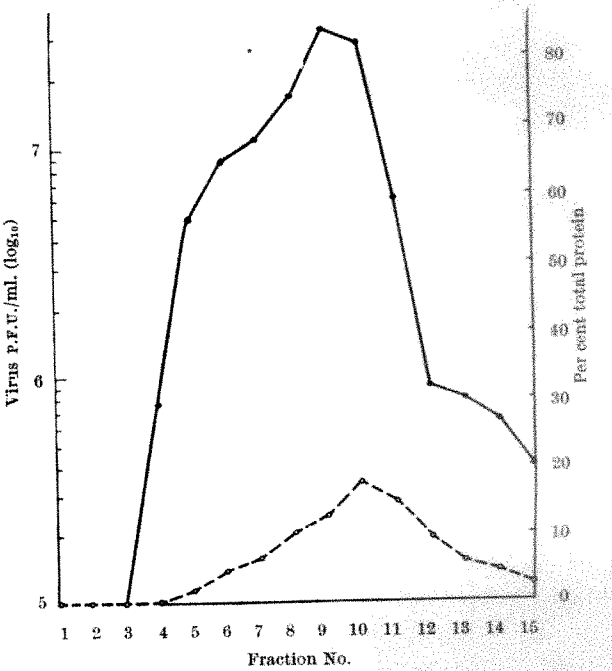


Fig. 1. Poliovirus (—) and protein contents of eluate (———) obtained with 'Cellex-*E*' columns

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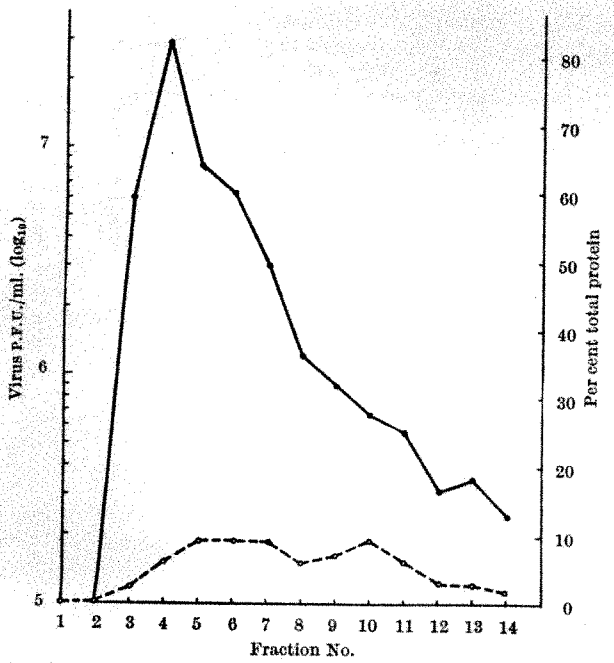


Fig. 2. Poliovirus (—) and protein contents of eluate (- - - -) obtained with 'Sephadex *G*-25' columns

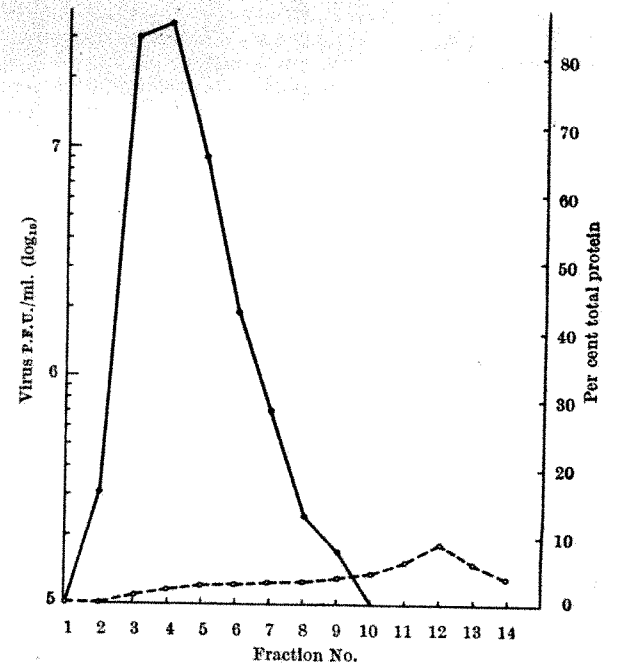


Fig. 3. Poliovirus (—) and protein contents of eluate (- - - -) obtained with DEAE 'Sephadex *A*-25' columns

column gave an 87·5 per cent protein and a 46·7 per cent viral recovery in a total of 17 ml. of eluate. As seen in Fig. 1, 'Cellex-*E*' resin could not separate extraneous protein and virus adequately. High virus titres were accompanied by relatively high protein content. Similarly, Fig. 2 depicts the efficacy of 'Sephadex *G*-25' columns in separating poliovirus from non-viral protein. It is noted that high virus titres are again associated with high protein content.

DEAE 'Sephadex *A*-25', it is noted in Fig. 3, allows recovery of approximately 30 per cent of the virus prior to appreciable evidence of protein. Once more, when a total of 89 per cent of virus is recovered in the 12 ml. of eluate, only 20 per cent of the total protein in the original lysate is eluted.

When fractions 4, 5 and 6 of the poliovirus preparation from the *A*-25 column were passed again through the DEAE 'Sephadex' column, no further detectable protein was noted though approximately one log of the virus titre was lost in this repassage.

Electron microscopic evidence as seen in Fig. 4 indicates that the viral preparation is quite pure considering that this represents the '*A*-25' eluate 4 and 5 with a titre of 2×10^7 P.F.U. concentrated to 1×10^8 P.F.U. It is evident that this procedure is rapid and efficient in the purification of viral preparations. The poliovirus preparation was originally genetically heterogeneous as indicated by plaque morphology. There was no apparent separation by these resins of genetically heterogeneous polioviruses present in the original crude preparation.

We thank Dr. J. Ghidoni for preparing the electron micrographs.

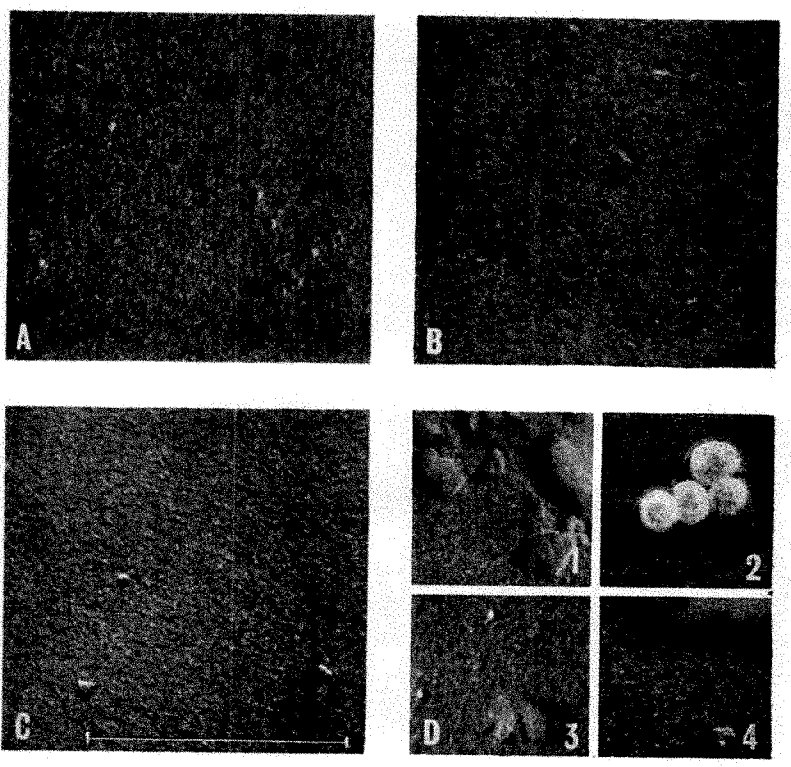


Fig. 4. Electron micrograph of Sabin type I poliovirus, purified by DEAE 'Sephadex *A*-25'. *A*, *B*, *C*, viral preparations of representative locations on one grid, shadowed with 80 per cent platinum and 20 per cent palladium at a 45° angle. *D*, 1, an electron micrograph of the fines from DEAE 'Sephadex'. *D*, 2, 3, 4, extraneous material encountered infrequently during electron microscopic examination of the preparation

The avirulent Sabin type I poliovirus was obtained in 1962 through the courtesy of Dr. J. L. Melnick and, in this laboratory, has gone through four HeLa cell passages.

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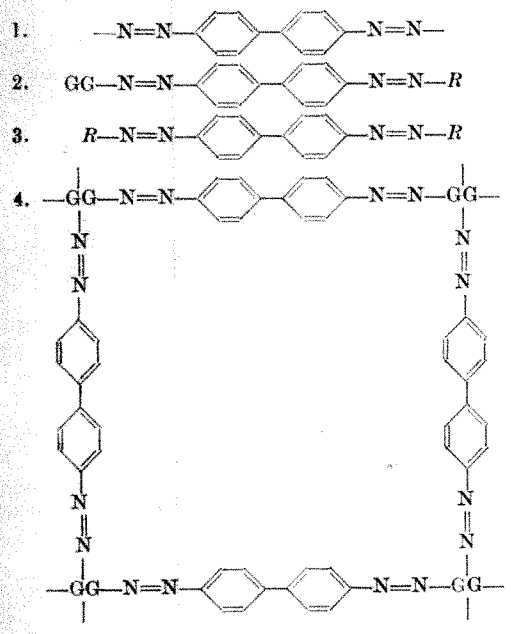
IMMUNOCHROMATOGRAPHIC PARTITION OF SOLUBLE ANTIGENS ON COLUMNS OF INSOLUBLE DIAZO-GAMMA-GLOBULINS

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ATTEMPTS to render antigens insoluble for absorption of specific antibodies from mixtures have been described[1–7]. The present method represents a reverse approach resulting in insolubilization of antibodies for absorption of specific antigens. Aggregation of whole γ-globulin and of Porter's fraction III by *bis*-diazotized benzidine (BDB) with conservation of C'F, HA and skin-sensitizing antibody properties is described by Ishizaka[7a,b].

During attempts to couple cyclic therapeutic compounds to immune γ-globulins[8], it was found that the reaction of bifunctional tetrazobenzidine groups with γ-globulins induced gelation of the protein. This finding was explored with the view of the utilization of the three-dimensional polyglobulin as an insoluble absorbent for specific antigens.

Preparation of Polyglobulin Column. (1) Diazotization of the benzidine was accomplished as described before[8]. (2) Copolymerization of immune γ-globulin: in a 1,000-ml. beaker place 600 ml. of a solution of 1·6 per cent γ-globulin in 1/15 M Sørensen phosphate buffered saline *p*H 7·2 (PBS 7·2). This solution is kept to 8° C during the operations. In a period of 30 min add 720·0 mg of the tetrazobenzidine with slow stirring to avoid foaming. Wait 7 min and then add 10 ml. of 8 N potassium carbonate. Continue to stir for another 10 min. Stirring during coupling prevents massive gelation of the globulin. The final product has the following probable composition:



The mixture is then filtered in a Buchner and washed with PBS 7·2. The slurry is resuspended in 60 ml. of PBS 7.2. It consisted of particles of uniform size (0·5 mm) with a rubbery consistency which on microscopic examination revealed arrangement in bundles of anastomotic fibres with crystalline features under polarized light. The resuspended slurry is placed in the column all at once and allowed to pack by gravity. The antigen solution is added to the packed columns and allowed to soak the column thoroughly. The flow is then stopped and the column containing the soaked antigen is incubated for 1 h at 4° C. After this time, the stopcock is turned and the collection of the adsorbate started. The flow rate in this column averages 120 drops/min at the point of collection. The column and vessel in which the absorbate is collected are surrounded by a polyethylene bag containing crushed ice to maintain the temperature of the column and the absorbate at 4° C. The following antigen–antibody systems were tested on these columns.

System I: Mixtures of protein antigens from pooled human tumours through columns of equine anti-normal human γ-globulin.

System II: Mixtures of protein antigens from pooled human leukæmic tissues through columns of equine anti-normal human γ-globulin.

System III: A mixture of tetanus toxoid fluid and rabbit serum through columns of equine anti-tetanus immune γ-globulin (tetanus antitoxin, TAT).

Results in Systems I and II. A column of equine anti-normal human γ-globulin (Lot $NH_{2,4}$ *GG21*)[9] was prepared by placing 9·6 g of polyglobulin in a 30 cm × 1·25 cm glass column in 50 ml. of PBS 7·2 at 4° C. To the packed column, 50 ml. containing 4·3 g of pooled leukæmia protein, Lot *LK26B*[9] in 1/10 M glycine buffered saline, *p*H 10·4 (GBS 10·4), were added. Absorbate 1 was sampled and re-run twice more, giving absorbates 2 and 3. After this, 50 ml. aliquots of PBS 7·2 were added and washings 1–16 obtained (Table 1).

Table 1. Precipitin Titres in a Two-dimensional Titration System

Fraction	Reaction against: Equine anti-human normal γ-globulin	Equine anti-leukæmia γ-globulin	Rabbit anti-horse γ-globulin
Pooled normal protein	2^{-7}	2^{-2}	0
Pooled leukæmia protein *LK26B*	2^{-3}	2^{-5}	0
Absorbate 1 of *LK26B*	2^{-1}	2^{-4}	0
Absorbate 2 of *LK26B*	0	2^{-5}	0
Absorbate 3 of *LK26B*	0	2^{-6}	0
First washing	0	2^{-4}	0
Sixth washing	2^{-3}	0	0
Sixteenth washing	0	0	0

The different fractions were tested by the precipitin methods described before, namely, single and double gel-diffusion. Single gel-diffusion is performed either as serial dilutions of antibody versus constant antigen concentration[10], here referred to as single unidimensional gel-diffusion, or a checkerboard titration of dilutions of the antigen versus dilutions of the antibody[11], here referred to as single two-dimensional gel-diffusion, or the double gel-diffusion method of Ouchterlony as modified by Korngold[12]. A similar column in the same conditions was tested with pooled tumour protein, Lot *TU25A*[9]. The results are shown in Table 2 and Fig. 1.

Results in System III. Reagents: (*a*) Tetanus toxoid (Lilly) containing 15 L_f units of tetanus toxoid/ml. (*b*) Rabbit serum (Microbiological Associates, Lot No. 1294)

Table 2. Precipitin Titres in a Two-dimensional Titration System

Fraction	Reaction against: Equine anti-normal human γ-globulin	Equine anti-tumour γ-globulin
Pooled normal protein	2^{-7}	2^{-3}
Pooled tumour protein *TU25A*	2^{-3}	2^{-1}
Absorbate 1 of *TU25A*	2^{-1}	2^{-3}
Absorbate 2 of *TU25A*	0	2^{-4}
Absorbate 3 of *TU25A*	0	2^{-4}
First washing	0	2^{-4}
Sixth washing	2^{-3}	0
Sixteenth washing	0	0

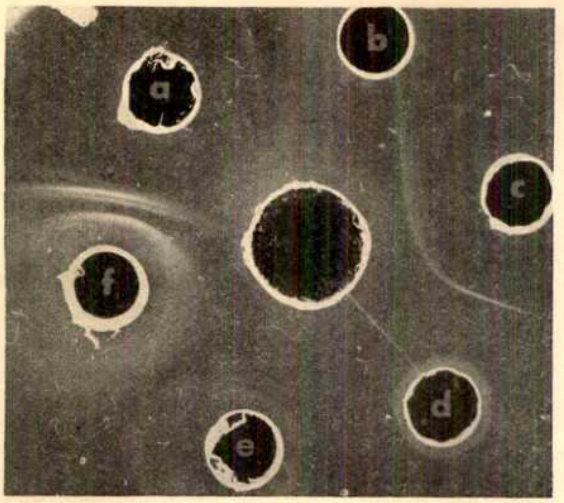


Fig. 1. Double gel-diffusion plate showing results in experimental System I. In the centre and in *e* absorbate 3 from a tumour protein mixture absorbed on a column of polymerized equine anti-normal human γ-globulin. In *a*, the anti-normal horse γ-globulin used in the column showing no reaction against the absorbate of tumour proteins but reacting against *b*, a rabbit anti-horse γ-globulin which does not react with the tumour absorbate. In *c*, equine anti-tumour γ-globulin showing reaction of identity against the unabsorbed (*d*) and the absorbed tumour proteins (centre and *e*). An additional antigen appears in the absorbed material. In *f*, pooled human γ-globulin showing strong reaction against anti-normal equine γ-globulin (*a*) but none against absorbed tumour proteins (centre and *e*)

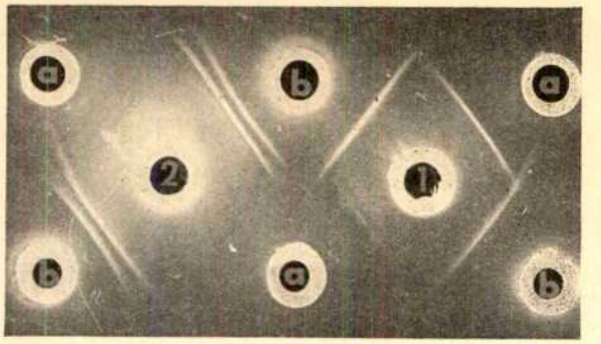


Fig. 2. Double gel-diffusion plate showing results in System II. In *a*, tetanus antitoxin (TAT). In *b*, sheep anti-rabbit γ-globulin. In 1, mixture of tetanus toxoid rabbit serum reacting as expected with the corresponding antibodies which are otherwise unidentical. In 2, absorbate of the antigenic mixture through a column of poly-TAT showing disappearance of the tetanus toxoid and persistence, with further antigenic enrichment, of rabbit serum

containing 4·6 g protein/100 ml. (*c*) Equine tetanus antitoxin, TAT (Lilly, Lot No. 080–823880), as a globulin concentrate containing 3,000 neutralizing units and 0·22 mg protein/ml. This corresponded to a precipitin titre of 2^{-9} against the tetanus toxoid. (*d*) Sheep anti-rabbit-serum globulin (Colorado Serum Co., *CS* 1466). A sheep immune globulin with a precipitin titre of 2^{-3} against pooled rabbit γ-globulin.

A column of 1 cm × 0·5 cm was made with 13 mg of poly-TAT. Preliminary determination of the precipitin titre and zone of equivalence of the TAT toxoid system provided the estimation of the amount of toxoid not in excess of the total precipitating capacity of all the TAT in the column. Accordingly, 0·3 ml. of toxoid was diluted in 0·6 ml. of rabbit serum and the mixture brought to 10 ml. volume with PBS 7·2. The mixed antigen dilution was then run twice through the column and samples of the absorbates tested in precipitin systems. Table 3 gives the results and Fig. 2 shows the disappearance of tetanus toxoid from its mixture with rabbit serum after absorption through a column of diazo-antitoxin.

The results of the foregoing test seem to indicate that:

(1) The polymerized equine γ-globulin is totally insoluble since no traces were detected in absorbates or washings by reaction with a high-titred rabbit anti-horse γ-globulin.

(2) The polyglobulin possesses full and specific antibody activity since it retains all normal antigens from the pooled tumour protein after two absorptions without retaining all the anti-tumour activity as tested by reaction of the absorbates against an equine immune γ-globulin possessing both anti-normal and anti-tumour activity.

(3) There is no non-specific adsorption of non-specific antigens as shown by the fact that leukæmia or tumour antigens react to higher titres after absorption through the column than before in their mixture with normal antigens. The absorption which seems to occur in the column has the characteristics of an immune reaction. As in any other precipitin reactions in gels of soluble antigens and antibodies, an excess of antigen prevented absorption in the column.

Thus, when three times the amount of antigen in the zone of equivalence predicted was added to the column, no absorption of antigens was detected. Instead, quantitatively, mg/mg, they were all recovered in the absorbates and washings. It is indispensable to conduct pilot tests of the system under examination by single two-dimensional diffusion (checkerboard titration of dilutions of the antigen versus dilutions of the antibody), to find the ranges of the zone of equivalence, and thus to avoid a pro-zonal system in which no absorption would take place in the column since a large excess of antigen will dissociate antigen already bound. This may be the reason why even in well-known systems of predetermined conditions complete absorption might not occur before the second or third run through the column.

(4) Washing the columns after absorption with a buffer of composition, molarity and *p*H different from the buffer used as solvent for the antigen results in: (*a*) dissociation of the specifically absorbed antigens; (*b*) recovery of a clean column that can be re-used for absorption of further material. The column used to absorb an aliquot of tumour antigens was re-used four times to absorb three more aliquots of the same pooled tumour antigen, yielding all four times the same results in terms of precipitin titres.

Tight packing in the lower segments of the columns may end up greatly reducing the flow rate. Inverted flow and recycling principles such as proposed by Porath may obviate this problem. The use of tyrosine to stop the polymerization process and grade the particle is also under study.

Further work is under way to demonstrate the 'cleanliness' of antigens absorbed by polyglobulins, including immunization of animals and other immunochemical analyses.

Table 3. Precipitin Titres in a Two-dimensional Precipitation System

Fraction	Reaction against: Sheep anti-rabbit γ-globulin	TAT	Rabbit anti-horse γ-globulin
Mixture of tetanus toxoid and rabbit serum	2^{-3}	2^{-9}	0
Absorbate 1	2^{-3}	2^{-7}	0
Absorbate 2	2^{-3}	0	0
First washing	2^{-3}	0	0
Third washing with PBS *p*H 7·2	2	2^{-3}	0
Sixteenth washing	0	0	0

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'UNREWARDED' EXPLORATION AND LEARNING OF COMPLEX MAZES BY WILD AND DOMESTIC MICE

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DOMESTIC rodents tested for brief periods in simple mazes in the absence of extrinsic rewards may show 'latent learning', but their maze running performance fails to improve[1]. The limitations imposed by the experimental design of such investigations have not always been recognized[2,3], leading to an over-emphasis of the role of extrinsic rewards in general theories of learning. This article describes the learning of highly complex mazes by wild and domestic mice in the absence of extrinsic rewards (that is, 'unrewarded').

Mazes[3] were constructed as follows: The *TGPS* maze consisted of passageways made of glass tubes (80 ft. long), plastic strips (30 ft.), and aluminium partitions fastened to a plywood board mounted at a 45° angle (112 ft.). The vertical transparent maze (*VT*) consisted of aluminium partitions fastened between upright layers of acetate film (245 ft.). The vertical dark maze (*VD*) was similar to the *VT* maze, but plywood replaced the acetate film (224 ft.). All passageways were 1·5 in. wide. Each maze contained approximately 2,000 1·5 in. cubic units of space. The average parameters (one way) for the mazes were: shortest path, 96 ft.; required climbs, 20 ft.; 90° turns, 313; blind alleys, 148 (58 per cent of the total space).

Equal access was provided to the mazes via empty cages and connecting passageways. In addition, the mazes were connected to a series of cages containing food and water, dirt, an activity wheel, and a nest[3]. Gross movements were recorded automatically by electromagnetic counters and an event recorder that were triggered as an animal passed under each of 20 hanging gates placed along the shortest routes. Visual monitoring by television in the deep red and infra-red supplemented these records[3].

In complexity and extensiveness the maze systems were roughly intermediate between conventional laboratory mazes and the habitat of a wild mouse. Accordingly, it was anticipated that exploration and learning of them might lead to the establishment of movement patterns similar to those observed in the field[4]. To test this hypothesis, mice of different environmental and genetic histories were examined. They are referred to by a hyphenated pair of capital letters; the first letter designates their environmental background and the second their genetic background, as follows: (1) 6 *L-W* (laboratory-wild) canyon mice, *Peromyscus crinitus stephensi*, that is, wild animals kept for long periods of time in the laboratory (including F_1 and F_2 generations); (2) 6 *L-W* deer-mice, *P. maniculatus sonoriensis*; (3) A *W-W* (wild-wild) group including 2 *P. c. stephensi*, 2 *P. m. gambelii*, and 3 *P. m. clementis*, that is, freshly captured animals; (4) 1 *W-W* and 3 *L-D* (laboratory-domestic, Swiss strain) *Mus musculus*.

The nest cage of each mouse was connected to the enclosure for 4–12 days (average 7·5). The activity wheel was unlocked for 2–5 days (average 3·3) during this period. Groups were used approximately in the order listed except that *W-W Peromyscus* were used as they became available.

Of the 23 mice, 12 completely explored only one maze (11 *TGPS*, 1 *VT*), 7 completely explored both the *TGPS* and *VT* mazes, while 2 completely explored all three mazes. Once an animal began exploring a maze it usually completed the exploration on the same day and did not explore another maze for several days, resulting in step-like increases in exploration (Fig. 1). In some cases mice failed to return to explored regions, much as wild animals appear to abandon portions of their habitat[4].

The three mice that failed to explore more than a small portion of any maze (2 *L-D Mus* and 1 *W-W P. m. clementis*) evidenced what might be described as highly fearful behaviour. Such behaviour also appeared to be responsible for the low level of exploration found to be typical of all *W-W Peromyscus*, a characteristic which led to the nearly uniform results from this genetically diverse group, as shown in Fig. 1*C*. Apparently low activity-levels reduce exploration, whether they are a product of domestication or are due to fear or conservativeness. Only 7 mice (4 *W-W* and 3 *L-W*) were away from food and water long enough (24 h) to be considered lost. Three of these, the *W-W Mus* and 2 *W-W Peromyscus*, were returned to the food and water cage after being lost for 33–48 h; the remainder found their way back.

All the *L-W* mice explored at least one maze (Fig. 1*A* and 1*B*). Comparisons between species, sexes, parental and filial generations, etc., were complicated by the fact that successive inhabitants tended to explore more extensively, apparently because of the accumulation

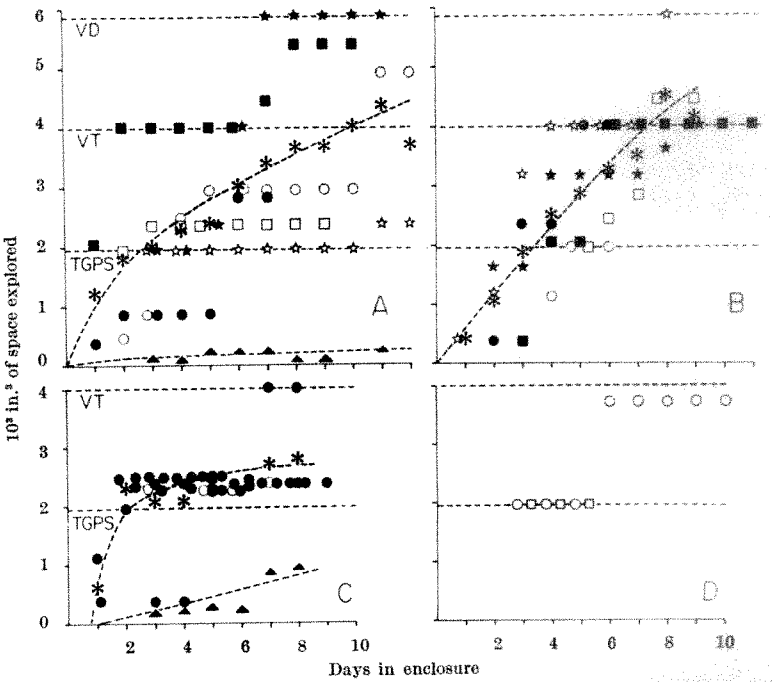


Fig. 1. Exploration of the *TGPS*, *VT*, and *VD* mazes by: (*A*) 6 *L-W P. crinitus*; (*B*) 6 *L-W P. maniculatus*; (*C*) 6 *W-W Peromyscus* of both species; (*D*) a *W-W* (circles) and *L-D* (squares) *M. musculus*. Open symbols represent females and closed symbols males. Individual males are not identified in *C* because of the uniformity of their behaviour. Asterisks, average values of space explored; solid triangles, average values of space 'abandoned'. The number of 1·5 in. cubical units of space in each of the mazes is indicated by the distances between the horizontal lines. Not shown in appropriate sequence is the fact that 4 animals explored *VT* before *TGPS*

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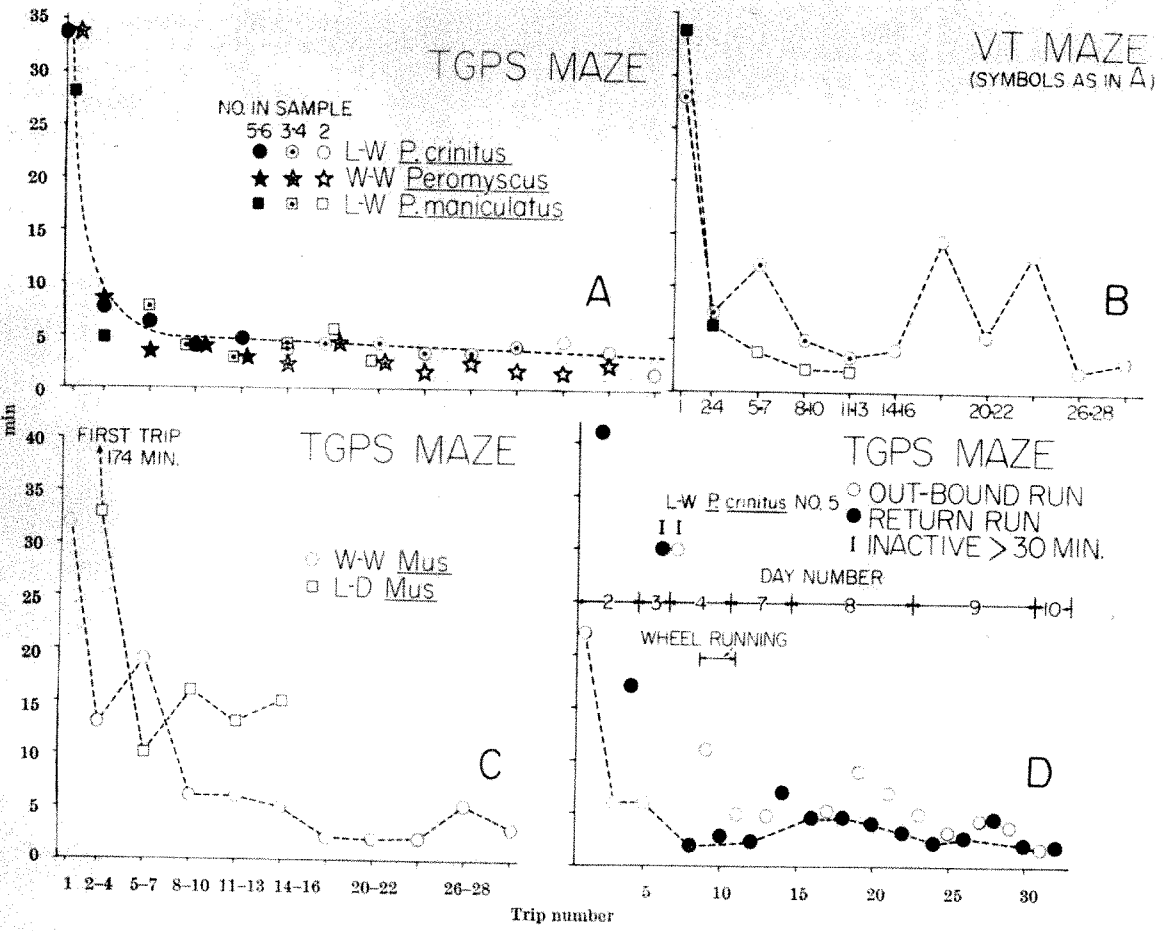


Fig. 2. Time to complete one-way trips through the *TGPS* and *VT* mazes. The average durations of the first trip and of the fastest of each succeeding triad of trips are plotted. The curve in *A* gives the times for the *L-W P. crinitus* (circles); the curves for *L-W P. maniculatus* (squares) and *W-W Peromyscus* (stars) are not plotted but are very similar. One *W-W Peromyscus* also ran the *VT* maze; its times are not shown but they lie roughly between the values for the other two groups in *B*. In *D* the durations of all trips through the *TGPS* maze are recorded for a representative *P. crinitus* (solid circles in Fig. 1*A*). The values for the fastest trip in each triad are connected by the straight broken lines. Note that this *P. crinitus* made only two trips in the maze during the period when the activity wheel was unlocked (days 4–6)

of excreta and other odoriferous substances. Accordingly, the curves of exploration for the 12 *L-W Peromyscus* (Fig. 1*A* and 1*B*) do not necessarily reflect species differences, although the sex differences may be significant.

The first round trip through a maze was completed after an average of 2·8, 4·4, and 2·6 days in the enclosure by 6 *L-W P. crinitus*, 6 *L-W P. maniculatus*, and 7 *W-W Peromyscus*, respectively. The corresponding averages for the amount of active time spent in a maze before reaching its end for the first time were 325, 297, and 239 min, respectively. The proportions of the latter times spent in previously unexplored sections of the maze were 22, 39, and 77 per cent. These figures reflect the fact that the *W-W Peromyscus* tended to explore a maze completely during a single sustained period, whereas the *L-W Peromyscus* usually left a maze and returned to it several times before completely exploring it. Only one *L-D Mus* and the *W-W Mus* did any exploring (Fig. 1*D*).

Confining an animal in a maze greatly reduced the time required for complete exploration. Thus, a *L-W* and a *W-W P. crinitus* placed in the far end of the *VD* maze (with food and water) traversed it completely within 220 min, even though both had failed to explore it 'voluntarily' from the other end.

The average duration of the first completed outbound trips for each of the three groups of *Peromyscus* and the one *W-W Mus* ranged between 29·6 and 32·0 min, but the one *L-D Mus* that explored a maze took 171 min. The return trips for both the *L-W Peromyscus* groups not only averaged 36 per cent faster than the outbound trips (7·5 versus 11·4 min) but also were faster than the preceding outbound trips in a significant number of cases (138 out of 210, $P < 1$ per cent). On the other hand, the outbound and return trips of the *W-W Peromyscus* were of about equal duration (7·0 and 6·9 min), with 32 of the 79 return trips being faster than the preceding outbound trips. The one *L-D Mus* that learned a maze and the *W-W Mus* made slower outbound than return trips (42·7 versus 25·1 and 16·0 versus 6·0 min, respectively), and they did so in the great majority of cases (21 out of 27, $P < 1$ per cent). Slower outbound than return trips apparently reflected a tendency to 're-explore' blind alleys on outbound trips. The brevity of these trips (22 min or less for all except the *L-D Mus*), apparently unrelated to a build-up of hunger or thirst, suggests that exploration, learning, and the subsequent running of complex mazes are self-rewarding.

Fig. 2*D* shows the time distribution of successive outbound and return trips in the *TGPS* maze for a representative *L-W Peromyscus*. Note that the time for one-way trips decreased progressively and that the animal spent very little time in the maze when the activity wheel was unlocked. To obtain a measure of the rate of learning, successive one-way trips after the first were divided into groups of three. The times for the fastest trips in each triad are connected by the broken line in Fig. 2*D*.

Since average values for the fastest *Peromyscus* trips (in each triad) through the *TGPS* maze were much the same, only the curve for the *L-W P. crinitus* is given in Fig. 2*A*. The major differences between the mice studied were: (1) *W-W Peromyscus* ran the *TGPS* maze

faster than either of the *L-W* groups (Fig. 2*A*); (2) *L-W P. crinitus* ran more slowly than the *L-W P. maniculatus* in the *VT* maze (Fig. 2*B*); (3) the single *W-W Mus* (Fig. 2*C*) and all *Peromyscus* (Fig. 2*A*) learned to run the *TGPS* maze at about the same rate, whereas the *L-D Mus* learned much more slowly and its fastest trips took about 5 times as long. Notable also was the fact that the running times of *L-W P. crinitus* were more erratic in the *VT* maze than in the *TGPS* maze (Fig. 2*A* and 2*B*); but only two of these animals completely explored the *VT* maze.

The most maze-running during a 24-h period was done by the *L-W P. crinitus* that did not run the wheel. During its 10th day in the enclosure it made 13 round trips (4 *TGPS*, 7 *VT*, and 2 *VD*) totalling 2,370 ft. and including climbs of 610 ft. The inclusion of movements in the remainder of the enclosure and deviations from the shortest pathways would, of course, increase these totals. The mice doubtless would have run the mazes even more had no activity wheel been present and had the periods of observation been longer.

The amount of exploration, learning, running, and climbing which occurred in the enclosure probably would bring a mouse in many wild habitats into contact with all its 'needs' (food, water, mates, etc.). Since these activities persisted even though 'unrewarded', they would appear to be self-rewarding, a finding which casts doubt on the postulate that wide-ranging locomotor movements are motivated by the reduction of hunger, thirst, and sex-drives[3]. Thus, the investigations of learning in simple mazes by domestic animals given extrinsic rewards[1] may have only limited significance for understanding the learning process in wild and relatively unconfined animals.

This work was supported by research grants *MH*-05001-03 from the National Institute of Mental Health, U.S. Public Health Service, and *G*-14533 from the National Science Foundation.

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A NEW HÆMOGLOBIN—$J_{\alpha\ \text{OXFORD}}$ FOUND DURING A SURVEY OF AN ENGLISH POPULATION

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ABNORMAL hæmoglobins are known to exist in purely English families. Two were discovered by chance: hæmoglobin Norfolk, a new variant[1], and hæmoglobin $J_{\text{Baltimore}}$ (ref. 2), already known to exist in the United States[3]. Two were found as a result of a deliberate survey of 1,000 non-related inhabitants of the County of Norfolk[4]. One was the already known hæmoglobin $D_{\beta\ \text{Punjab}}$ (ref. 5), and the other the hitherto not described hæmoglobin $G_{\alpha\ \text{Norfolk}}$ (ref. 4). In a further survey of 1,971 non-related out-patients in Oxford and Peterborough, again one person has now been found to be heterozygous for a not yet described hæmoglobin variant. The family came from Gloucestershire, but there was also some Irish ancestry. In addition to the propositus, a seventy-year-old woman, the variant was found in six of her siblings and one of her two children. No hæmatological abnormality and no morbidity were associated with this hæmoglobin. As it had the electrophoretic properties of hæmoglobin J, and could, on hybridization experiments[6,7], be shown to carry its abnormality in the α-chain, the new variant is named hæmoglobin $J_{\alpha\ \text{Oxford}}$.

The hæmoglobin was isolated[8] and a peptide chromatogram ('finger-print') was prepared[9,10]. Peptide αTpIII was absent. This peptide contains five amino-acid residues of the α-chain, namely, alanyl-alanyl-trytophanyl-glycyl-lysyl, which are in position 12–16 of the 141 residues composing this chain[11,12]. Two new peptides could be demonstrated instead which were in the neutral area and which both contained tryptophan[13]. These findings resemble those described for hæmoglobin $J_{\text{Baltimore}}$ (ref. 14), which differs from hæmoglobin A in its β-chain. In hæmoglobin Baltimore βTpII which in the β^A-chain contains residues 9–17 (seryl-alanyl-valyl-threonyl-alanyl-leucyl-tryptophanyl-glycyl-lysyl) is replaced by two new peptides. One differs only by a replacement of glycyl in position 16 by an aspartyl, and the other is the same peptide, but the following peptide of the β-chain (βTpIII) has not been split off on digestion, because of the partial resistance of the lysyl bond in position 17 to tryptic hydrolysis when it is next to the acidic aspartyl of the β^J-chain.

The position was similar in hæmoglobin J_{Oxford}. One of the two new peptides was isolated by utilizing the fact that the neighbouring peptides in the neutral area contain histidyl, and can therefore be made to move towards the negative pole on electrophoresis at *p*H 3·5, whereas this peptide remains in the neutral area. It could then be finally purified from the remaining peptides in the neutral area by 20-h chromatography[10]. Table 1 shows the results of the amino-acid analysis[15] for $\alpha^A Tp$III and the peptide which can be taken to be $\alpha^J Tp$III. It will be seen that the glycyl of $\alpha^A Tp$III is replaced by an aspartyl in $\alpha^J Tp$III. The formula of hæmoglobin J_{Oxford} is therefore $\alpha_2{}^{15}\text{-Asp}\beta_2$. For the other new tryptophan containing peptide, staining reactions showed that histidine, tyrosine and arginine were also present[13]. The only peptide of hæmoglobin A which contains all three—histidyl, tyrosyl and arginyl—is αTpIV. The additional finding of tryptophan makes it virtually certain that this second new peptide arises from the incomplete tryptic separation of $\alpha^J Tp$III and αTpIV. A proportion of both peptides had failed to separate on tryptic digestion because of resistance to the enzyme arising from the acidic aspartic residue

Table 1. Molar Ratio of Amino-acids formed after Acid Hydrolysis of Tryptic Peptides III from Hæmoglobin A and Hæmoglobin J_{Oxford}

	$\alpha^A Tp$III	$\alpha^J Tp$III
Aspartic acid	<0·1	1·0
Glycine	0·9	<0·1
Alanine	2·0	2·2
Lysine	1·1	0·9
Tryptophan	+	+

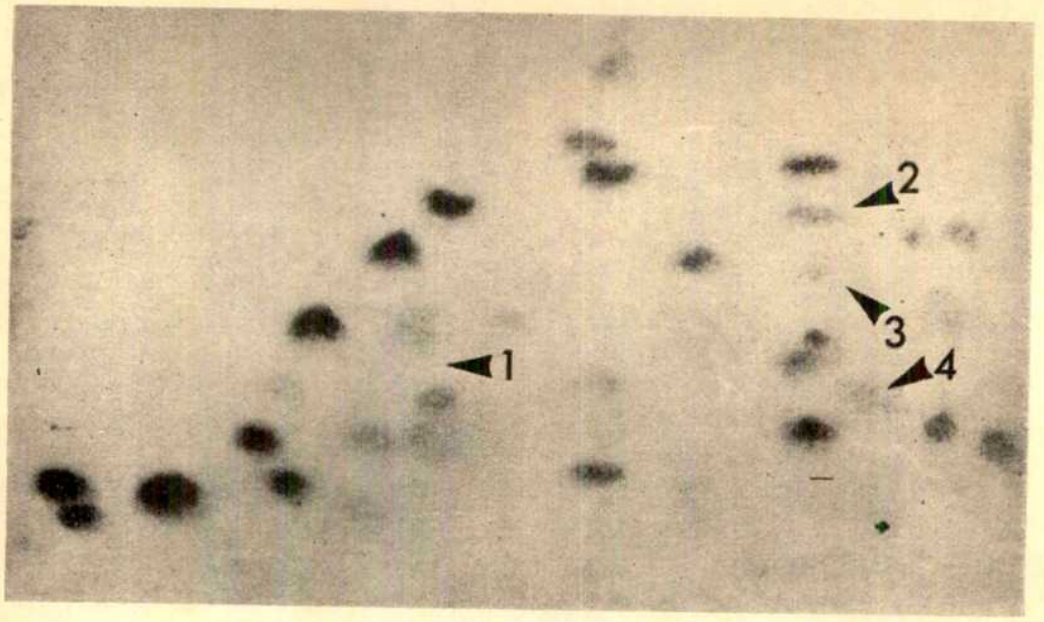


Fig. 1. 'Fingerprint' of hæmoglobin J_{Oxford}. 1 indicates where $\alpha^A Tp$III is missing; 2 is $\alpha^J Tp$III; 3 is $\alpha^J Tp$III—αTpIV; 4 shows the diminished amount of αTpIV

next to lysyl in position 17. This interpretation is further supported by the fact that the peptide corresponding to free αTpIV was much fainter in the finger-print of hæmoglobin J_{Oxford} than in that of hæmoglobin A.

The α- and β-chains of human hæmoglobin A are basically similar[16]. Fig 2 shows that although the amino-acid replacement in the two hæmoglobins $J_{\alpha\ Oxford}$ and $J_{\beta\ Baltimore}$ are comparable, the proportion of the abnormal hæmoglobin is 20 per cent in the first and 50 per cent in the second. One possible explanation is that the coding for glycyl is degenerate and differs for the α- and β-chain. This would result in different messenger RNA for the two aspartyl substitutions. However, it is noteworthy that, as a rule, α-chain abnormal hæmoglobins are present in a lower proportion in the heterozygote than are β-chain variants. It is of interest to be able to compare two hæmoglobins with the same amino-acid substitution in the corresponding position of the α- and the β-chain: $\alpha_2^1{}^5\text{-Asp}\beta_2^A$ and $\alpha_2^A\beta_2^1{}^6\text{-Asp}$ (Table 2). They are identical in their electrophoretic properties, except that the β-variant

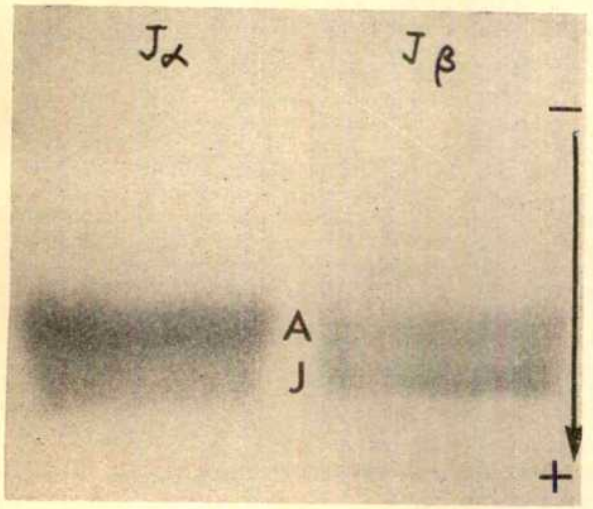


Fig. 2. Paper electrophoresis at pH 8·9 of hæmoglobin A + J_{Oxford} and A + $J_{Baltimore}$. It will be seen that the α-chain variant is present at a much lower proportion in the heterozygote than the β-chain variant

Table 2. The Two Homologous Peptides βTpII and αTpIII
Amino-acid sequences for $\beta^A Tp$II and $\alpha^A Tp$III (ref. 11) and the suggested substitutions for $\beta^J Tp$II (ref. 14) and $\alpha^J Tp$III

βTpII	9	10	11	12	13	14	15	16	17
Hb-A	ser-	ala-	val-	thr-	ala-	leu-	try-	GLY-	lys
Hb-$J_{Baltimore}$	ser-	ala-	val-	thr-	ala-	leu-	try-	ASP-	lys
αTpIII				12	13	14	15	16	
Hb-A				ala-	ala-	try-	GLY-	lys	
Hb-J_{Oxford}				ala-	ala-	try-	ASP-	lys	

just separates from hæmoglobin A at pH 6·5, whereas the α-variant fails to do so. This difference could be shown to be due to the higher proportion of $J_{\beta\ Baltimore}$ in the heterozygote.

The amino-acid substitution in hæmoglobin Norfolk is also a replacement in the α-chain of glycyl by aspartyl, but it occurs in position 57 (ref. 17). The electrophoretic properties of hæmoglobin J_{Oxford} and hæmoglobin Norfolk, however, are clearly different. The present observation is a good example of the importance of the steric position of the amino-acid substitution in the α-chain in determining the physico-chemical properties of the hæmoglobin molecule.

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LETTERS TO THE EDITOR

COSMOLOGY

Conservation of Protons in a Stable Nickel Nucleus

THE conservation laws of Nature (for example, charge, energy and baryon number) are considered to be true, since there is no experimental evidence available which proves otherwise. However, there are some theoretical speculations available which consider violations of these conservation laws. Bondi and Gold[1], in their steady-state theory of the cosmos, consider a net production of protons out of 'emptiness', in order to keep the average mass density in an expanding universe constant. Feinberg and Goldhaber[2] remind us that all conservation laws rest only on experimental evidence, and that it is a useful question to search for the limits in which they hold. In this spirit, Backenstoss *et al.*[3] and Giamati and Reines[4] investigated the conservation of baryon number. Alväger *et al.*[5] recently tested the conservation of neutrons in copper nuclei.

Now, it can also be asked : How accurately is it known that in a perfectly closed system the number of protons is constant with time? Obviously it is difficult to perform the experiment with free protons, but protons, assembled in a nucleus, are a rather good 'closed system'. So the question now is : How accurately is the number of protons in a nucleus conserved ? If the number of protons in a nucleus were not conserved, but in fact increased by one, the nucleus would become isotopic with the next heaviest element. Such a process would clearly violate quite a number of conservation laws and would have some consequences for any theory of knowledge.

As an experimental test for the existence of this process, the following hypothetical reaction was studied:

$$^{60}\mathrm{Ni} \xrightarrow{\text{picking up a proton out of the 'emptiness'}} {}^{61}\mathrm{Cu} \qquad (1)$$

The isotope copper-61 is radioactive with a half-life of 3·3 h and decays with emission of positrons. Twenty kg of pure $NiSO_4 + 7H_2O$ were dissolved in 1/10 N HCl, and 1 g $CuSO_4 + 10$ g $Hg(NO_3)_2$ were added. Copper and mercury were recovered from the solution by precipitation with hydrogen sulphide and the copper then separated from the mixed sulphides by heating in an airstream. This yielded CuO, which was placed between two sodium iodide scintillation crystals arranged to detect positron annihilation quanta in coincidence. No activity above the background of about two coincidences per hour was detected in two experiments. Assigning a half-life $t_{1/2}$ for reaction (1), one can state the result with 80 per cent confidence, as follows :

$$t_{1/2}\,[\text{reaction of type (1)}] > 6 \times 10^{18}\ \text{years}$$

Alväger *et al.*[5] give the limit for the destruction of neutrons in a copper nucleus under violation of baryon conservation as $> 10^{21}$ years. They discuss their result in the light of the steady-state theory of Bondi and Gold[1] and assume that the average rate of destruction of neutrons is of the same order of magnitude as the rate of creation of matter (10^{-15} nucleons per cm^3 per year). They further assume that the actual rate of destruction of neutrons, which is investigated, depends only on the number of nucleons present. They calculate then a half-life of a nucleus against destruction of one of its neutrons with 10^{12} years and conclude that the observed stability of nucleons is much greater than 'predicted' by Bondi and Gold[1].

However, it is possible to interpret the limit for the observed stability of a nucleus against spontaneous creation of a proton in such a way as to show that the observed limit for the stability is much smaller than the instability 'predicted' by the steady-state theory. One can assume that the creation of protons is of the same order of magnitude as the creation of the matter and that this creation is only a function of space and that all protons which are created close to the Ni nucleus (10^{-13} cm) are captured by the nickel nucleus. This would give a half-life of the order of 10^{50} years for a reaction of type (1). Therefore, the experimental limit reported in this work is much too low to test the 'creation-of-particle' hypothesis of Bondi and Gold[1]. (However, it is not necessary to assume that in the steady-state theory the creation of matter is only a function of space. Hoyle[6] discussed the possibility that creation might depend on the mass density.)

I thank Drs. A. C. Pappas, G. Rudstam, K. Chackett, T. Alväger and G. Sørensen for their advice and Mr. H. Bersinger for his help.

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Olbers' Paradox

OLBERS[1] in 1826 was the first to show that the radiation density everywhere in an infinite static universe should equal the radiation density at the surface of the stars. Hence, Olbers' paradox is that the sky is dark at night.

Let us ignore the absorption of radiation by non-luminous matter. If E_s is the average radiation density at the surface of the stars then the background radiation density in a uniform static universe at time t is :

$$E = E_s\,(1 - e^{-t/\tau_0}) \qquad (1)$$

where $E = 0$ at $t = 0$. In this equation τ_0 is a 'mean collision time' of a photon between emission and absorption by the stars and is :

$$\tau_0 = 4\,V_0/cS \qquad (2)$$

where V_0 is a macroscopic element of volume sufficiently large to contain average conditions and S is the surface area of the stars contained in V_0. If $\rho_0 \sim 10^{-30}$ g cm^{-3} is the present mean density of luminous matter and ρ_s and r_s are the density and radius of a typical star, then $\tau_0 = 4\rho_s r_s/3c\rho_0$. For main sequence stars such as the Sun it follows $\tau_0 \sim 10^{23}$ years. Thus Olbers' conclusion that $E = E_s$ rests on the assumption that the stars have radiated continuously at their present rate for a time of $t > \sim 10^{23}$ years.

Bondi[2] has examined the assumptions underlying Olbers' argument. Of these assumptions the most important for my purpose are : (*a*) the average density of stars and their average luminosity do not vary throughout space and time ; (*b*) the known laws of physics apply. There is an obvious contradiction between these assump-

tions which was not necessarily apparent in pre-relativistic physics. According to the conservation of energy principle stars can radiate at their present rate only for a finite period of time. A static universe may therefore be of infinite age but the radiation life-time of the stars is necessarily finite. Thus if ε is the average luminosity – average mass ratio and t is the time during which the stars have been luminous, then necessarily $\varepsilon t < c^2$. For $\varepsilon \sim 1$ erg g^{-1} s^{-1} it follows $t < \sim 3 \times 10^{13}$ years. Therefore since $t/\tau_0 < \sim 3 \times 10^{-10}$ equation (1) becomes :

$$E = E_s \frac{t}{\tau_0} \tag{3}$$

where $E_s/\tau_0 = \rho_0 \varepsilon$. Hence radiation equilibrium of $E = E_s$ as deduced by Olbers is impossible. The night-sky is dark, or $E \ll E_s$, simply because the stars cannot radiate at their present rate for the immense period of time of 10^{23} years. Thus Olbers' paradox is meaningless; to insist that there is a paradox violates the conservation of energy principle.

The same conclusion follows by arguing in a slightly different way. Let us imagine that the stars are converted entirely into radiation. Then in a static universe the radiation density will be $\rho_0 c^2 \sim 10^{-9}$ erg cm^{-3}. This is roughly the radiation density of moonlight at the Earth's surface and is nothing like $E_s \simeq 10$ erg cm^{-3} at the surface of the Sun. If, more realistically, we imagine only that all hydrogen is converted to iron the radiation density will not affect profoundly the present brightness of the night-sky.

With $\varepsilon \sim 1$ erg g^{-1} s^{-1}, $\rho_0 \sim 10^{-30}$ g cm^{-3}, and using Bondi's[2] estimate of $E = 3\ 10^{-13}$ erg cm^{-3}, it follows from equation (3) that the stars have radiated for a time $t \sim 10^{10}$ years. Thus Olbers' assumptions, with the modification that the stars have radiated for a finite time of the order 10^{10} years, account for the present background radiation density in a reasonably satisfactory manner.

It is generally supposed that the existence of the extragalactic red-shift is a sufficient condition for the resolution of Olbers' paradox and for ensuring a low background radiation-level. I shall show that the existence of a red-shift, or indeed even a blue-shift, plays only a relatively minor part in determining the present radiation level in a variety of non-static models.

I suppose that the universe is divided into a large number of macroscopic elements of proper volume, each having perfectly reflecting walls. In each cell :

$$\mathrm{d}(EV) + p\,\mathrm{d}V = \delta Q \tag{4}$$

where $p = \frac{1}{3} E$ is the radiation pressure and δQ is the incremental gain in energy. In an isotropic and homogeneous universe the radiation conditions within each cell are identical at each instant of cosmic time t. It is therefore possible to dispense with the reflecting walls without in any way disturbing the radiation conditions. We now imagine that during the entire history of the universe a single cell of proper volume V is enclosed with walls which are perfectly reflecting from both inside and outside. At any instant an observer outside V finds that the radiation conditions are exactly the same as those found by an observer inside V. The observer outside V tends to evaluate the radiation density by making a time-retarded integration of the Doppler-shifted contributions from distant stars[3]. The observer inside V, however, tends to use the differential equations of classical thermodynamics for evaluating the density of the radiation emitted by the local stars in a cavity of varying volume. Since both methods must yield identical results I shall adopt the latter method because of its greater simplicity. From equation (4) it follows :

$$\frac{1}{V^{1/3}} \frac{\mathrm{d}}{\mathrm{d}t}(V^{4/3}E) = \frac{1}{4} cS(E_s - E) \tag{5}$$

where the right-hand side is the luminosity of the stars in V minus the rate of absorption of the background radiation by the stars (absorption by non-luminous matter is neglected). This equation can also be derived from the adiabatic relativistic equation using the Robertson line element. Since $V \propto R^3(t)$, where $R(t)$ is a proper unit of length, then :

$$\frac{\mathrm{d}}{\mathrm{d}t}(R^4 E) = \frac{R^4}{\tau}(E_s - E) \tag{6}$$

and $\tau R^{-3} = \tau_0 R_0^{-3}$.

Let us consider the range of models satisfying the relation $R \propto t^n$, where $n > -1$. On integrating equation (6) it is found for $t \gg t_1$, where t_1 is the time of the initial conditions, that

$$E = \frac{E_s}{n+1} \frac{t}{\tau} \tag{7}$$

and $t = nT$ where T_0^{-1} is Hubble's constant. Also $\tau t^{-3n} = \tau_0 t_0^{-3n}$, and the zero suffix denotes the present epoch. The static model is given by $n = 0$, and the steady-state model is given by $n = 1/3$ with $t = \frac{1}{4} T$. For $n > 1/3$ the radiation density diminishes with time and for $n < 1/3$ the radiation density increases. It would be absurd to suppose that the stars can exist when $E \geqslant E_s$ and therefore there is a critical epoch :

$$t_c = \left(\frac{1}{n+1} \frac{t_0^{3n}}{\tau_0}\right)^{\frac{1}{3n-1}} \tag{8}$$

such that for $n > 1/3$ the stars commence radiating at $t_1 > t_c$ and for $n < 1/3$ they cease radiating at $t_2 < t_c$. Some values of t_c are (using $t_0 \sim 10^{10}$ years) : Milne's model ($n = 1$), $t_c \sim 3 \times 10^3$ years ; Einstein–de Sitter model ($n = 2/3$), $t_c \sim 1$ day ; steady state and Dirac models ($n = 1/3$), $t_c = \infty$; static model ($n = 0$), $t_c \sim 10^{23}$ years.

It is seen from equation (7) that the background radiation density is less than the radiation density at the surface of the stars when $t < (n+1)\tau$. At present the background radiation level is low not so much because the universe is non-static but mainly because τ_0 is very large. This feature of our universe is of such overriding importance that the radiation-level is affected only in a comparatively slight way by whether the universe is expanding ($n > 0$) or contracting ($n < 0$). In general, for values of $n > 0$, the sufficient condition for a low radiation-level at present is $t \ll \tau_0$, which is the same as for a static model. This is also a necessary condition because of the conservation of energy principle. Thus the radiation-level is low in our universe at present and the night-sky is dark simply because the stars are so widely separated from each other, or, in other words, because the characteristic time τ_0 is so very large.

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RADIO ASTRONOMY

Absorption of Radio Waves in the Galaxy

Observations of the distribution of the galactic radio emission at 4·7 Mc/s for southern declinations[1] have shown evidence of pronounced absorption by interstellar ionized hydrogen within about 20° of the galactic disk. Between declinations −20° and −50° the disk is seen as a dark

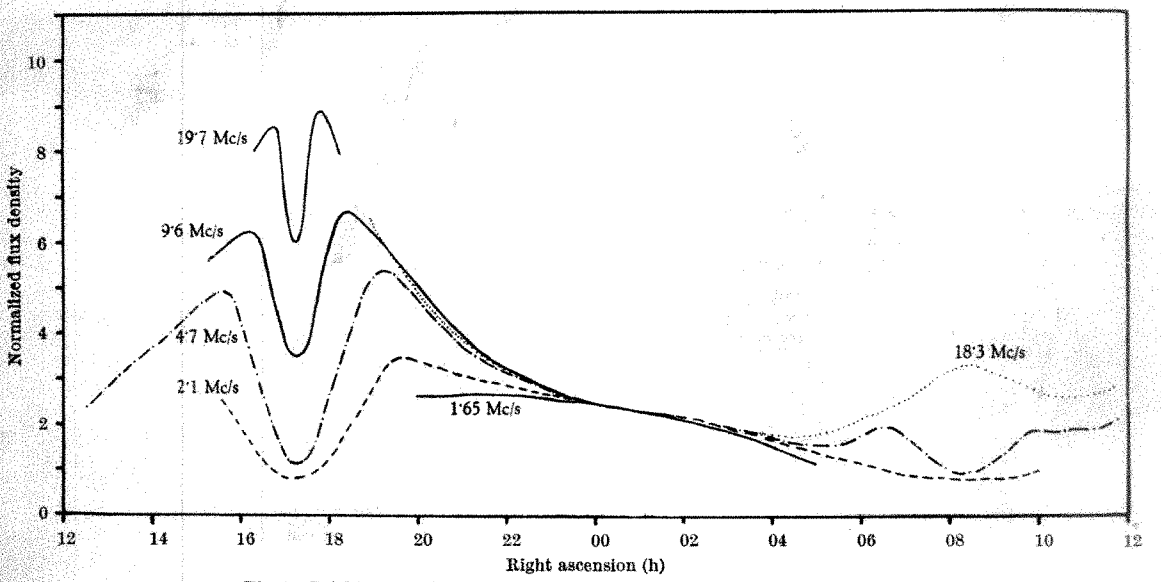


Fig. 1. Brightness profiles of the general background radiation at declination −37°

band approximately one-quarter as bright as the surroundings.

Since the absorption is proportional to e^{-KL}, where $K = 0{\cdot}4N^2T^{-3/2}f^{-2}$, it is a strong function of frequency, where K is of order unity. Observations at other frequencies would be expected to give substantially different brightness distributions and possibly permit the estimation of the absorption coefficient.

Fig. 1 shows a comparison between the 4·7 Mc/s brightness profile in right ascension at declination −37° with profiles obtained in a new series of observations at 1·65 Mc/s and 9·6 Mc/s. The antenna beamwidths were 2° × 5°, 4° × 10° and 32° × 40° at 9·6 Mc/s, 4·7 Mc/s and 1·65 Mc/s respectively. Also shown is a profile at 2·1 Mc/s supplied by G. Reber (private communication) and two from previous observations at 19·7 Mc/s (ref. 2) and 18·3 Mc/s (ref. 4), the beamwidths being 8° × 8°, 1·4° and 17° respectively. These profiles have all been normalized in the direction of greatest galactic latitude (−80°) at 0030 R.A.

The widening of the absorption trough at 1715 R.A. near the galactic centre with decreasing frequency is noteworthy and at the lowest frequency the effect of the absorption can be followed out almost to the galactic pole. The right ascension scan cut through the galactic disk a second time at 0820 R.A. in a direction remote from the centre and again pronounced absorption was observable at 4·7 Mc/s and 2·1 Mc/s, suggesting that the absorbing material exists at much greater distances than the Sun.

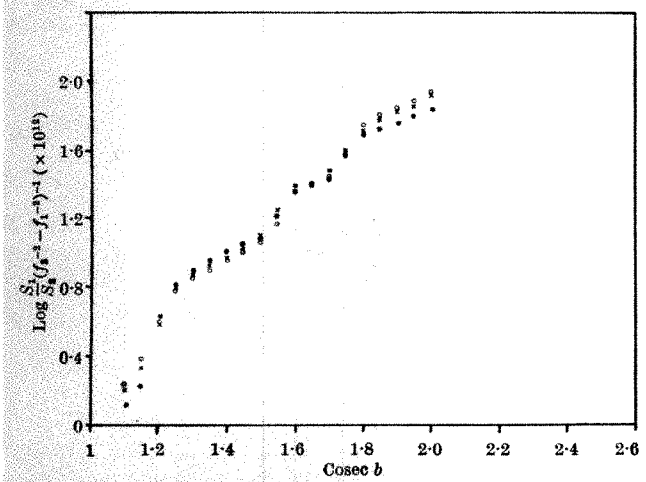


Fig. 2. Variation of normalized brightness ratios with galactic latitude from R.A. 2000 to R.A. 0030 (circles, 4·7/2·1; crosses, 4·7/1·65; dots, 2·1/1·65)

Now providing the ionized hydrogen is distributed uniformly in a parallel layer and substantially all the radiation originates outside the layer, the ratio of the normalized intensities S_1, S_2 at frequencies f_1, f_2 in a specified direction is given by:

$$A = \frac{\log S_1/S_2}{1/f_2^2 - 1/f_1^2} = \frac{0{\cdot}4N^2L_0}{T^3 f_2} (\mathrm{cosec}\, b - 1)$$

where b is the latitude angle, N the electron density, T the electron temperature and L_0 the semi-thickness of the layer. If the foregoing assumptions are correct, a plot of A against b will give a straight line which will be independent of the particular pair of frequencies used. Furthermore, its slope will be a measure of the absorption coefficient K.

In Fig. 2 the value of A is plotted using the profiles of Fig. 1 for the region from 2000 R.A. to 0030 R.A. Between cosec $b = 1$ and cosec $b = 2{\cdot}0$, A is independent of frequency. Deviation of the curves from a straight line indicates that the assumption of uniform density would need modification. However, the deviation is not great and we obtain a mean value for $N^2L/T^{3/2}$ of $6{\cdot}25 \times 10^{12}$ cm^{-5}(°K)$^{-3/2}$ ($b > 60°$). Taking $T = 10^{4\cdot0}K$ and $L_0 = 300$ pc gives $N = 0{\cdot}08$/c.c. This compares with $N = 0{\cdot}1$/c.c. previously obtained from low-resolution spectral intensity observations[3]. However, it should be emphasized that in the present derivation no absolute values of intensity have been used.

For galactic latitudes less than 20° and longitudes in the vicinity of the centre, the foregoing simple disk model does not hold, and A becomes a strong function of frequency in this region. However, the observed profiles may be obtained theoretically if it is assumed that the electron density increases towards the centre and that there is significant disk emission for these directions.

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RADIOPHYSICS

An Ion Gyrofrequency Phenomenon observed in Satellites

Barrington and Belrose[1], Gurnett and O'Brien[2] and Brice *et al.*[3] have reported that very-low-frequency emissions at frequencies of a few kc/s may be observed in satellites immediately following an atmospheric which has propagated upwards to the satellite. Brice and Smith[4] suggested that these triggered emissions were related to the lower hybrid resonance for propagation transverse to the Earth's magnetic field.

A related phenomenon has now been observed in the *Alouette I* and *Injun III* very-low-frequency data, examples being given in Figs. 1 and 2 respectively. These noises were first detected, aurally, by one of us (J. K.) on recordings of *Alouette I* made at Stanford. It was realized that the noises were genuine (and not interference from other equipment in the satellite) when similar noises were found at Stanford from tapes of very-low-frequency recordings of the *Injun III* satellite which were on loan from the State University of Iowa.

These noises are observed only after the reception of an atmospheric and are at frequencies much less than 1 kc/s. They show initially a rapid rise in frequency, following which the frequency is very nearly constant. This maximum frequency is 520 c/s in Fig. 1 and 400 c/s in Fig. 2. The satellites were at heights of about 1,000 km (for *Alouette I*) and 1,800 km (for *Injun III*) when the noises of Figs. 1 and 2 respectively were recorded.

It is suggested that these noises may be related to an ion gyrofrequency resonance, since the maximum frequency is approximately the gyrofrequency for protons in the plasma surrounding the satellite.

While the relationship of these noises to the preceding atmospherics suggests a 'triggering' process, it appears possible that the noises are simply dispersed forms of the original atmospheric impulse (with the dispersion arising from propagation from the ground to the satellite).

The work at Stanford was sponsored by the National Aeronautics and Space Administration under grant *NsG*–174–61.

The work at Iowa was supported in part by the Office of Naval Research under contract *N9onr*–93803 and by the

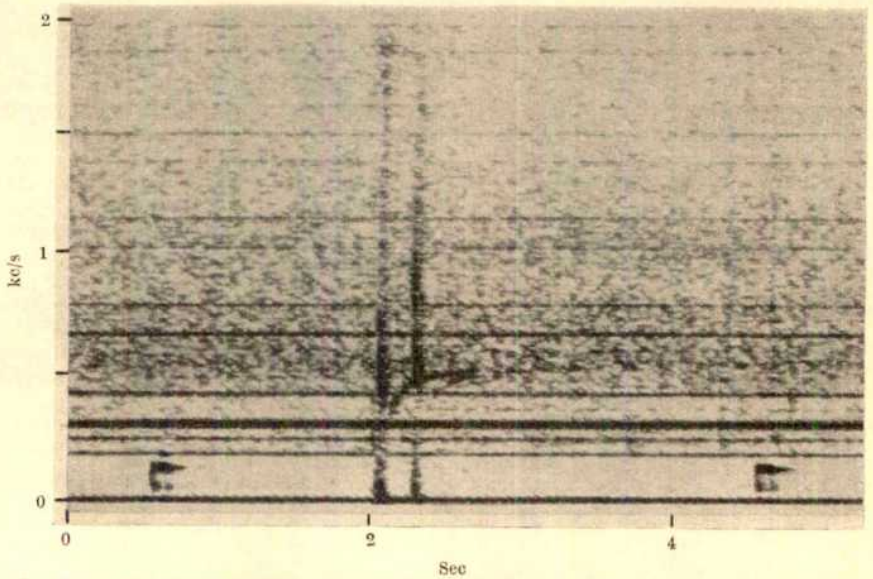


Fig. 1. Discrete noises which appear to be triggered by atmospherics at frequencies below 1 kc/s, recorded by *Alouette I* (March 18, 1963; 0807 : 05 U.T.)

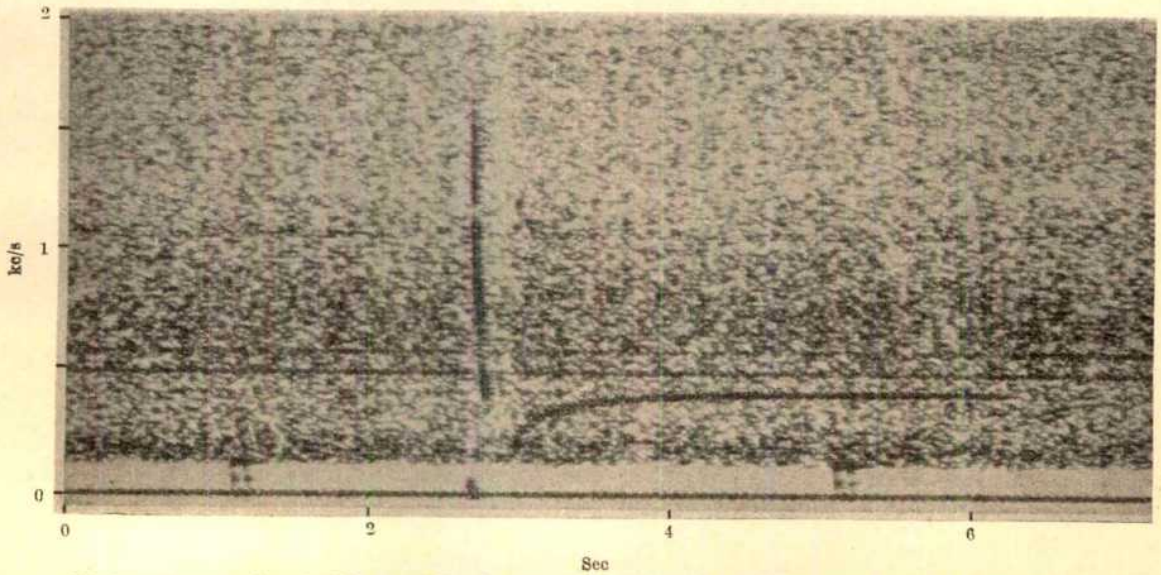


Fig. 2. A strong quasi-constant frequency discrete noise below 1 kc/s recorded by *Injun III* (February 17, 1963; 1231 : 12 U.T.)

National Aeronautics and Space Administration under grant *NsG*–233–62.

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PHYSICS

Extrathermal Electrical Conductivity Measurements in a Magnetoplasmadynamic Generator

A detailed description of the International Research and Development Co., Ltd., magnetoplasmadynamic (MPD) generator has been given previously[1,2]. On July 14, 1964, a series of power generation experiments with cæsium seeding of the helium were undertaken. This series differed from the previously reported runs[3] mainly in the generator and nozzle configuration and materials. To reduce end current leakage, the tantalum nozzle used previously was replaced by a boron nitride nozzle, the exit region of the generator lengthened by 2 in. and the interior of the diffuser inlet spray-coated with alumina. The generator channel was constructed from boron nitride in attempts to reduce the internal current leakage encountered previously with alumina[3]. Sixteen pairs of narrow (1/16 in. × 1/2 in.) tantalum electrodes replaced the five pairs of square (1/2 in. × 1/2 in.) electrodes used previously; the gap between the electrodes was large (3/16 in.), to reduce insulator and boundary layer electrical breakdown. Five pairs of tantalum pins (1/16 in. diam.) were used to monitor open circuit voltages, two pairs in the nozzle and three pairs in the generator exit region. Four static pressure tappings and three thermocouples were also located in the channel wall. During operation of the loop nozzle inlet gas temperatures in excess of 1,700° C were measured. The maximum channel temperature measured was 1,110° C with a corresponding flow velocity of 1,730 m/sec and a Mach number of 0·79.

Fifteen short-duration cæsium injections were carried out at different flow conditions during the loop operation; magnetic fields up to one tesla were used. The cæsium injection circuit gave a nominal seeding rate of 0·2 atomic per cent; this low rate was necessitated by the low capacity of the evaporator. In practice, while cæsium was injected for only a few sec (maximum 15 sec) observed open-circuit voltages lasted much longer and decayed only slowly to zero. In most cases voltages were measured several minutes after seeding had stopped. From these measurements it was concluded that the true seeding fraction was one-half to one-tenth the nominal value.

Open circuit voltages in the channel up to 36 V were generated near the channel inlet, falling to about half this value at exit; Fig. 1 shows values for three runs. The

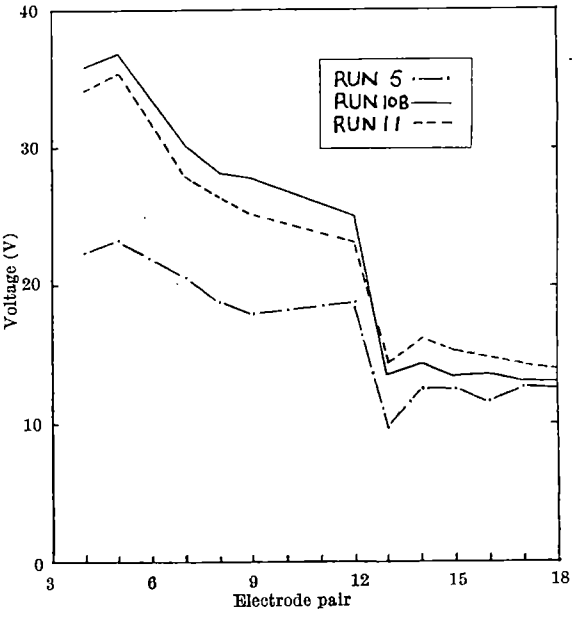


Fig. 1

fall in voltage occurs as gas velocity decreases in the channel; faulty electrical insulation may cause the rapid drop between electrodes 12 and 13.

The electrical conductivity of the plasma was obtained by switching each of the sixteen electrode pairs simultaneously on to five load resistors (18, 68, 280, 1,000 and 4,700 ohms) and open circuit in rapid sequence and displaying the resulting voltage on a 24-channel ultra-violet recorder trace. This sequence takes about a second and can be repeated continuously. From the ultra-violet traces the voltage–current characteristics for the plasma can be constructed. The slope of these characteristics yields the internal resistance (R) of the plasma, which is related to the electrical conductivity (s) by $s = L/RA$, where L is the distance between electrodes (m), A is the electrode area (m^2), S is in mho/m and R in ohms. If the

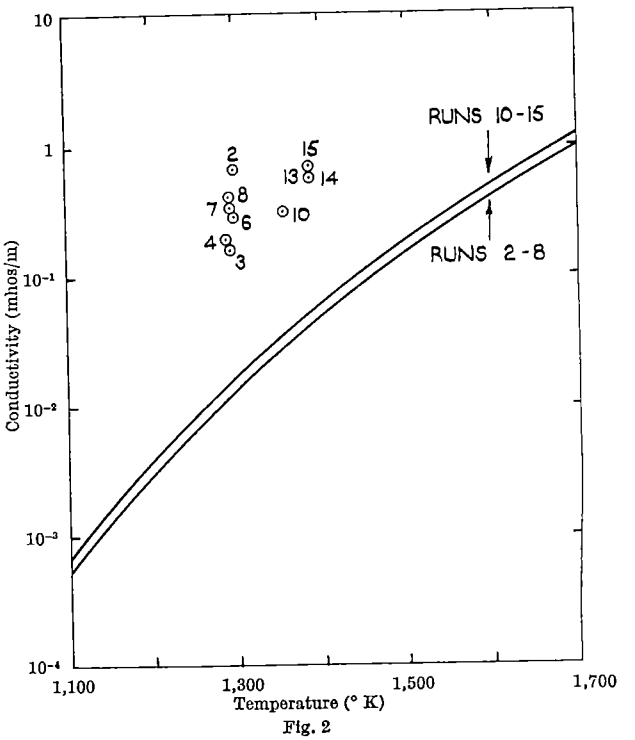


Fig. 2

whole volume of the channel carries current, A is the product of the channel width and the electrode pitch rather than the real electrode area.

Deviations from linearity on the voltage–current characteristics were observed in many cases at high current values; however, the voltage measurements are least precise in these regions: a linear relationship has been used to derive the conductivities quoted here. Fig. 2 shows the average electrical conductivity along the generator channel compared with thermal equilibrium values in the same temperature-range for several runs; a seeding fraction of 0·1 atomic per cent was used in obtaining the equilibrium values and the two curves refer to the appropriate static pressures at channel inlet (the lowest observed in the channel). The observed conductivities are up to fifty times greater than the thermal equilibrium values. Accounting for these values merely by incorrect temperature measurement requires, in the highest cases, errors of more than 300° C temperature measurement. All other factors (seeding fraction, channel pressure, current-carrying area, voltage sheaths near the electrode and internal current leakage) are likely to increase the difference between the observed and equilibrium conductivities. The measurement of such electrical conductivities indicates that some mode of extra-thermal ionization is occurring unless corrections to the observed temperatures are very large. Further experiments using more sophisticated measurement techniques are planned.

During the experiments generated current densities at the electrodes of 750 amp/m² were observed at less than 1,400° K. This is greater than the thermionic current density of tantalum but not that of cæsium; thermionic emission cannot therefore be neglected as an electron-producing mechanism at the electrodes if cæsium deposition occurs.

These experiments were planned and performed by Dr. B. C. Lindley, Mr. R. Brown and members of the International Research and Development Co., Magnetoplasmadynamics team. The work was supported in part by C. A. Parsons and Co., Ltd., and by the U.S. Office of Naval Research under U.S. Navy contract N 62558–4180, and published by permission of the directors of the International Research and Development Co., Ltd.

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Generation of Giant Pulses from a Neodymium Laser by a Reversibly Bleachable Absorber

The generation of high-peak power pulses of short duration from a ruby laser by the incorporation of a reversible bleachable absorber into the optical cavity has recently been reported[1]. This communication reports the observation of giant pulses at 1·06μ from a neodymium glass laser using the polymethine dye 3,3′-diethyl-9,11; 15,17-dineopentylene-thia-pentacarbocyanine iodide[2] as the bleachable absorber.

Initially, the dye molecules absorb light due to an allowed singlet–singlet transition at the laser wave-length and laser operation is inhibited. When the gain becomes high enough to overcome this absorption, laser action begins and the dye molecules are rapidly bleached. The cavity Q is suddenly increased and the stored energy is released in a giant pulse.

A 0·25 in. × 2 in. rod of neodymium-doped glass with external dielectric mirrors was used. The threshold for ordinary laser action was approximately 850 joules. A solution of the dye in a rectangular cell was placed in the cavity. The optical density of the dye cell was increased by steps from 0 to 0·3 in a series of experiments. The pulses were measured with a fast rise S-1 planar photodiode calibrated calorimetrically and observed on an oscilloscope having a 4 nsec rise time. The ordinary laser pulses (optical density zero) had a minimum width of about 1 μsec and a maximum amplitude of 12 kW with an input pumping energy of 1,800 joules. As the optical density was increased, the half-width of each pulse, as well as the number of pulses, gradually decreased until a limiting value of 25 nsec (full-width at half maximum) was reached when five pulses occurred. Further increase in optical density, although decreasing the number of pulses obtained, did not lead to an increase in the peak power of each pulse. Giant pulses of 1-MW peak power were obtained. The total energy output of such a giant laser pulse was typically 5 per cent of the total ordinary laser output. No deterioration of the dye due to exposure to the laser light was noted in approximately 100 flashes.

We thank See-Yin Wong for the preparation of the polymethine dye, Dr. W. Buchman for the use of the laser rod and photodiode, and Dr. J. Boyden for the design and construction of the calorimeter.

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Absorption Spectra of Silver Vapour

The line spectrum of silver vapour in absorption was first examined by R. G. Loyarte and A. T. Williams[1]. In their work the metal was vaporized in an electric oven of King's type at temperatures between 1,200° and 2,200° C, and the continuous background was furnished by a hydrogen discharge tube. They found altogether 11 lines lying between 3383 Å and 2320 Å, of which two strong ones situated on the long wave-length side of the region, namely, λλ3382·86 and 3280·66, were ascribed to the first doublet of the principal series of silver atom, and nine other weaker lines to ionized atom of the same metal. More recently, J. Ruamps[2], using the same type of oven, observed two band systems of silver in emission, one designated as system A extending from 4900 Å to 5050 Å, and the other as system B from 4100 Å to 4600 Å. Vibrational analysis of the second system led Ruamps to attribute it to silver. As to the more complicated system A, because of the experimental conditions, he was unable to ascertain whether it was due to silver or to other kinds of molecules such as AgN, AgO or AgC. Similar analysis of the bands of system B was afterwards repeated by B. Kleman and S. Lindkvist[3] both in emission and in absorption.

In view of the fact that silver commences to vaporize appreciably at a temperature somewhat lower than its melting-point (960·8° C), and that fused silica tubing can endure a temperature as high as 1,200° C for some time, it was felt to be of interest to investigate the spectral absorption of silver vapour within the workable temperature range of fused silica, so that better controllable experimental conditions could be achieved. The fused silica absorption tube with flat windows at both ends, used in our work, was about 80 cm in length. This tube was connected to a pumping system through which pure inert gas, argon or neon, could be introduced into the tube, in case of necessity. The central part of the absorption tube where a few grams of spectrally pure silver grains were placed could be conveniently heated to any

desired temperature below 1,200° C by means of a temperature-controllable electric furnace. The effective heating length of this furnace was about 40 cm. Spectrograms were taken by a Hilger medium-size quartz spectrograph. The indispensable continuous backgrounds for absorption in the ultra-violet and visible regions were furnished by a powerful hydrogen discharged tube and a tungsten ribbon lamp respectively. The time of exposure for a spectrogram varied from a few seconds to about half an hour, as required by each case.

Attempts to obtain an absorption spectrum of silver vapour with an evacuated absorption tube were unsuccessful. This was because of rapid diffusion resulting in an insufficient accumulation of the vapour in the heated part of the tube. To attain this aim, it was found necessary to introduce a suitable quantity of inert gas, say 30 cm mercury pressure of argon or neon, into the tube. Because of the presence of a foreign gas the effect of diffusion became greatly reduced, and an experiment could be carried on for several hours without the need of replenishing the absorbing material.

Under our experimental conditions, five absorption lines in the ultra-violet region and a system of bands in the visible region have been found. The wave-lengths of the five absorption lines are as follows:

$$\lambda\,\lambda:\ \left.\begin{matrix}3{,}382{\cdot}9\\3{,}280{\cdot}7\end{matrix}\right\}\ \text{doublet},\ 2{,}537{\cdot}0,\ \left.\begin{matrix}2{,}069{\cdot}4\\2{,}061{\cdot}6\end{matrix}\right\}\ \text{doublet}$$

The first two lines and the last two appeared to be doublets, and they have their counterparts in emission as are lines of silver. The first doublet began to turn up at about 700° C, and its intensity increased with rise in temperature. The second doublet, weak in intensity, appeared only at temperatures above 1,000° C. The doublet separations, $\Delta\bar{\nu}$, of the first and the second doublets are 921 cm^{-1} and 183 cm^{-1} respectively. From this it is inferred that these doublets belong to the principal series of lines, $5^2S_{1/2} - 5^2P_{1/2,3/2}$ and $5^2S_{1/2} - 6^2P_{1/2,3/2}$, of the silver atom. The singlet λ2537·0, which was also weak in intensity, showed its presence at about 650° C, but since then its intensity remained practically constant. In consideration of the facts that there is a persistent mercury line of wave-length very close to this line, and that the mercury vapour evolving from a manometer which was attached to the absorption tube might contaminate the silver sample therein by forming a trace of silver amalgam, it is believed that the line in question was due to mercury. Finally, the band system observed was composed of a number of very faint bands extending from about 4100 Å to 4600 Å. Because of their faintness no determination of the wave-lengths of these bands has been made. What can be said about this system is that its locality coincides with that of system *B* of silver. As to the band system *A*, mentioned in the paper of Ruamps, not a trace of it was observable.

It is remarkable that none of the nine absorption lines, identified by Loyarte and Williams as lines belonging to the ionized atom of silver, was observed. From this and the results of our work, and also of others, some conclusions about the nature of silver vapour may be drawn as follows: (1) Monatomic molecules form the predominant part of silver vapour at all temperatures from about 700° C up. (2) At temperatures below 1,200° C no spectrally perceptible amounts of ionic atoms exist in the vapour. (3) Perceptible amounts of diatomic molecules appear at temperatures above 1,000° C.

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METEOROLOGY

Polarization and Albedo

Schwerdtfeger[1] has recently directed attention to the mechanism underlying the dependence of short-wave or optical albedo on solar elevation and the presence or absence of cloud cover. He cites as one of the major causes of this dependence the polarization of incident radiation (solar) on reflexion at terrestrial surfaces. The few comments made here are intended to bring out the rather involved interrelation between polarization and albedo, which plays a very prominent part in the solution of radiant-energy transfer problems in the Earth's atmosphere.

A complete description of the ambient radiation field, including the state of polarization, in a plane parallel molecular atmosphere was obtained by Chandrasekhar[2], who assumed the ground to be a Lambertian reflector. The diffusely reflected light from a Lambertian surface is isotropic and natural and is independent of the state of polarization and angle of incidence of incident light. Sekera *et al.*[3] have given quantitative estimates of the radiation emerging from a planetary atmosphere on the basis of the foregoing theory. However, it is quite obvious that naturally occurring terrestrial surfaces exhibit reflective properties which are intermediate between those of a Lambertian reflector and a Fresnel (specular) reflector. The tendency of the reflecting terrestrial surface to behave either like a Lambertian or Fresnel reflector is in turn governed by the roughness parameter as defined by the Rayleigh criterion, which would imply that this tendency is highly dispersive. It also explains the dependence of albedo on solar elevation (angle of illumination).

The foregoing considerations point towards the measurement of spectral reflectance properties (that is, reflecting properties over fairly narrow, well-defined wave-length intervals) as a means of studying the variation of roughness of a given surface over the spectral region of interest. Such measurements would render possible the determination of when a given surface can be considered smooth over a given wave-length region as per the Rayleigh criterion. It would then be possible to determine over what spectral region the polarization features of the incident radiation are changed on reflexion at the given terrestrial surface.

Terrestrial surfaces can be generally considered rough over the visible region with certain notable exceptions—the notable exceptions being vast expanses of water (like calm sea surfaces, lakes) and smooth snow surfaces. Systematic investigations[4] of the dispersion of the angular distances of the neutral points of skylight polarization (the Arago, Babinet and Brewster points) from the Sun or the anti-Sun indicate that this is indeed the case. Theoretical investigations[5] of the ambient radiation field in an atmosphere the lower boundary of which reflects in accordance with the Fresnel formulæ have correctly predicted the disappearance of the neutral points from the Sun's vertical (the meridian through the Sun) for moderate values of elevation of Sun and smaller optical thicknesses (that is, towards the red end of the spectrum) and their reappearance in different meridional planes. Soret[6] has recorded such observations.

The transition of a terrestrial surface from smoothness to roughness (or the other way around) is also governed by the spatial resolution and the geometry of the experimental set-up. When observations of the reflected energy or intensity are made with an instrument with a small field of view very close to the reflecting surface, measured values will be influenced only by the small-scale structural irregularities characteristic of the surface, whereas when observations are made at a great distance from the reflecting surface—especially for grazing angles of illum-

ination—the reflexion process is predominantly governed by the large-scale surface features.

Coulson[7] has made extensive measurements of characteristics of solar radiation reflected by desert soil in the Nevada region. A photoelectric skylight polarimeter[8] was used in these measurements. Relative spectral reflectance values were determined. These measurements indicate that both the polarization and intensity of the reflected solar radiation are strongly dependent on the elevation of the Sun, the altitude and azimuth of the point of observation and the wave-length.

The azimuthal dependence of the intensity of the reflected radiation—especially the marked differences in the intensities of beams reflected in the solar and anti-solar directions—has been correctly attributed by Coulson to the differential amounts of shadow which are seen by the instrument. The spectral reflectance is observed to increase smoothly with increasing wave-length.

Yu Mullama[9] has investigated the problem of light diffusely reflected by a statistically structured surface taking into account the polarization of the incident radiation. He has also presented numerical computations for the specific case of reflexion and refraction at a water surface for two different wind velocities. It is felt that his computations may be extended to the investigation of the polarization and intensity of light reflected by wind-blown sand.

A proper understanding of the interrelation between albedo and polarization, in addition to being of immediate use in the solution of energy exchange problems in our own atmosphere, will play a prominent part in the study of the feasibility of polarimetric studies of surface characteristics of planets with thin atmospheres.

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26-Month Oscillation in Atmospheric Properties and the Apparent Solar Diameter

A biennial oscillation in tropical stratospheric winds[1-3] and total amounts of ozone[4-6] has been well documented during the past few years and its discovery has led to a re-examination of much of the earlier work on biennial oscillations in surface temperature, rainfall, tree rings, lake levels and other parameters which depend on conditions in the troposphere[7]. I have argued elsewhere[8] that the oscillation in the stratosphere is caused by changes in the troposphere, and this argument is in line with much of the present thinking about tropospheric–stratospheric interactions, which holds that the lower stratosphere receives its driving energy from below[9-11]. If this reasoning is correct then there must be sought a source for the 26-month oscillation in the tropospheric heat engine. One possibility is some type of internal oscillation; another is that the incoming radiation which drives the tropospheric heat engine (approximately 3000 Å to 2μ) is modulated with a 26-month oscillation.

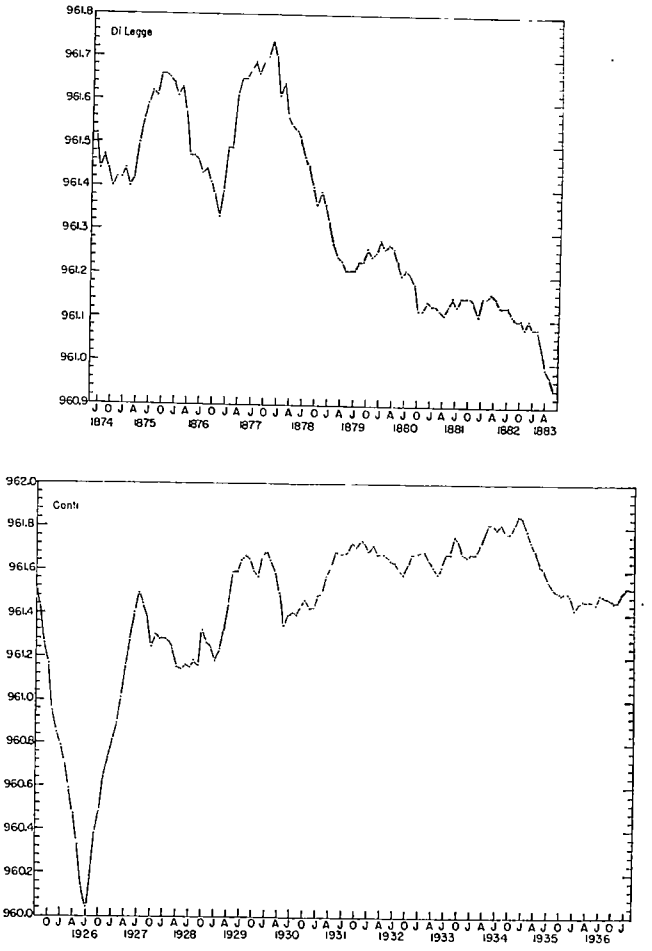


Fig. 1. Twelve-month running means values of solar radius. Units are seconds of arc at the mean Earth–Sun distance

Landsberg, Mitchell, Crutcher and Quinlan[12] have shown that the biennial temperature oscillation in general reverses phase between tropical latitudes and middle latitudes and also sometimes has a phase variation with longitude in middle latitudes. Such complex behaviour is not inconsistent with an extra-terrestrial modulation. An increase in the heat energy to be transported polewards in the troposphere may lead to an increase in the amplitude of the large-scale standing eddy flow patterns in the troposphere; this in turn would mean increased upward velocities at some middle latitude stations with concomitant cloudiness and lower temperatures. It has been shown elsewhere that the amplitude of these standing eddies in the troposphere has a direct effect on the ozone amounts in the stratosphere[13].

One possible source of such extra-terrestrial modulation of the incoming radiation is a periodic change in the solar diameter. It seems to me that this possibility has not been eliminated in spite of an enormous amount of observational work. Airy[14], Cullen[15], Lane Poor[16], Schur and Ambronn[17], and Di Legge[18], to name a few, have examined this question with a variety of observational techniques, and the present-day reader of these early papers cannot help concluding that the question of variability is unresolved. It is noteworthy that Abetti in his treatise on the Sun[19] is also of this opinion. Much of the material reported has been compounded into annual mean values which are not useful for the present purpose. The Italian astronomers have made a comprehensive investigation of the problem from observations collected between 1870 and 1940 and many of these have been published either directly or as monthly means and corrected to a mean Earth–Sun distance.

From two such series of these observations[18,20], each taken by a single observer, twelve-month running means have been calculated and are plotted in Fig. 1. The observations by Di Legge suggest the presence of a biennial oscillation in the apparent solar diameter. It is difficult to establish whether this is a real variation or whether the periodicity was introduced through the refractive correction based on tropospheric temperatures; in fact, there is some phase correspondence between the oscillation in Fig. 1 and the biennial temperature oscillation at Uppsala reported by Landsberg *et al.*[12]. The diameter data taken by Conti also show some evidence of the fluctuation. Undoubtedly a personal element is involved in these observations and values by different observers do not agree exactly. Such features as the large minimum in 1926 in Conti's curve are shown by other observers; these may be related to atmospheric opacity and aerosol content.

If radius changes of about 0·3 sec of arc, as evident in Fig. 1, did actually occur they would change the resulting radiation at the Earth by about 5 parts in 10,000, which is well below the sensitivity limit of present radiation instrumentation. An order of magnitude estimate shows that a similar change in input radiation could be produced if sunspots with a temperature of 4,800° K covered about 1/1,000 of the solar disk. A considerable amount of effort has been spent on the possible relationships between sunspot effects and the atmospheric circulation, and if the solar diameter observations represent a real variation it would seem desirable to examine the effects of diameter changes in more detail.

It may be argued that the Sun is not a cool star of the type normally associated with long-period variables. May not this be simply a reflexion of the fact that variables with such small amplitude cannot normally be detected? One purpose of this communication is to raise again the question of Armellini[21]: "Il sole è uua stella pulsante?", and to suggest that a further investigation with modern instrumentation might be appropriate. I shall be grateful for any daily or monthly values of diameter that might be available from regions outside Europe since it seems that data from a series of locations would allow one to unravel the effect of atmospheric refraction and temperature changes in the results.

This communication comments on one possible source of the oscillation which seems to warrant further examination. It is intended to discuss others in a separate communication.

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GEOLOGY

Pleochroism of Orthopyroxenes

DR. M. S. MURTY has recently concluded[1] on the basis of two analysed minerals that the strength of the pleochroism of orthopyroxenes of the charnockite series can be correlated with their titanium content, it having been noted earlier[2] that the pleochroism is independent of the iron content of these minerals. More recently, however, it was suggested[3] that the pleochroism of orthopyroxenes can be correlated with their alumina content and with the resultant contraction of their cell parameters, that is, it may be largely a physical effect.

Inspection of a semi-quantitative record of the intensity of pleochroism in 12 analysed orthopyroxenes of the charnockite series[2] does not support Murty's conclusion that the intensity of pleochroism can be correlated with the titanium content: indeed, the most pleochroic minerals have the lowest titanium contents but do contain more than 4 per cent alumina. Further examples can be taken from a compilation of 47 orthopyroxene analyses[3]; for example, specimens 186b/54 and 177/54, both from British Guiana, have roughly equal amounts of titania at 0·17 and 0·19 per cent respectively; their alumina contents, however, are 1·37 and 9·48 per cent and the latter mineral is very much more pleochroic.

In an examination of Scottish orthopyroxenes[4], two bronzites are shown to have roughly equal amounts of alumina, with 4·53 and 4·45 per cent, and are equally strongly pleochroic, but the first has twice the titania content, with 0·19 and 0·09 per cent respectively. Thus, on further inspection, Murty's data for two pyroxenes with titania 0·45 and 1·05 per cent, and alumina 2·83 and 4·06 per cent, the second pyroxene being more strongly pleochroic, could be taken to support my contention that the strength of pleochroism of orthopyroxenes can be correlated with their alumina content.

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IT has been noted by several investigators that the magnesium-rich orthopyroxenes are more strongly pleochrotic than the iron-rich members, indicating that the strength of the pleochroism is not dependent on the iron content. Kuno[1] observed no relationship between the strength of the pleochroism and the manganese content of the mineral, he having noted no pleochroism in the manganiferous pyroxene, but suggested a possible relationship with the titanium content. In a recent investigation[2], it was suggested that the strong pleochroism may be due to the high titanium content. But Howie (preceding communication) considers that the strength of the pleochroism is independent of the titanium and correlates with the alumina content.

Kuno[1] related the pleochroism in the pyroxenes of the Bushveld type to the exsolution of titanium together with calcium and iron. Howie[3] suggested that it is related to the presence of oriented schiller inclusions. Parras, as quoted by Deer *et al.*[4], considered that the intensity of the pleochroism is dependent on the amount of exsolution of diopsidic pyroxene and ferric iron.

Though oriented schiller inclusions and lamellæ were noted in the orthopyroxenes of the charnockite series, they apparently seem to have no relationship with the strength of the pleochroism as the inclusion free grains are also found to be pleochroic. In the light of the foregoing observations, I am inclined to believe that the strength of

the pleochroism is dependent on the titanium content. The observations of Kuno[1], that the strongest pleochroism occurs in the highest titanium mineral, moderate pleochroism in the low-titanium mineral and no pleochroism in the titanium-free mineral, could be taken to support my contention. Kuno's results do not support the contention of Dr. Howie that the strength of the pleochroism can be correlated to the alumina content, since non-pleochroic pyroxenes 1, 2 and 19 are found to contain 0·93–1·47 per cent alumina. It can be further seen that the strongest pleochroism is in the pyroxene 13 containing 1·70 per cent alumina and the less-intense pleochroism in pyroxene 7 having 2·42 per cent.

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Shatter-cone Orientation at Sudbury, Canada

Shatter-coning, a unique type of conical fracturing, appears to be a criterion for shock-induced over-pressures in rock. As such, they have been useful in identifying presumed astroblemes (ancient meteorite impact scars)[1]. So far, about eight shatter-coned structures have been identified around the world[1]. A good analysis of shatter-coning has recently been made by Short[2].

As well as indicating shock deformation at crypto-explosion structures the orientation of shatter cone apexes identifies the arrival direction of the shock waves. In other words, the shatter cones point toward the focus of origin of the shock force, that is, to 'ground zero' in the case of a meteorite impact source. Although in principle it should be possible to locate the direction of ground-zero, it has only rarely been possible to do so. Most shatter-coned structures are small so that shock-fracturing is confined to the central eye. Here the explosion event which immediately follows the shock-fracturing converts the eye of a shatter-coned structure into a nest of megabreccia. Hence the blocks of rock which contain shatter cones are disoriented, making it difficult to relate the orientation of shatter cones found in any particular block to the structure as a whole. The Kentland, Indiana, disturbance provides an exception. In the up-ended strata there, the shatter cones have been shown to be preferentially oriented stratigraphically upward. This suggests that the disturbance is an astrobleme rather than a cryptovolcano[3].

For astroblemes resulting from higher-energy impacts, the peripheral zone of shatter-coning may extend several miles into the country rock. At sites where this zone is spared great disruption and subsequent erosion, an optimum situation for cone-orientation examination should exist. Indeed, this was found to be the case at the Vredefort Ring structure where shatter-coning in the annular ring of upturned strata extends for at least seven miles from the margin of the granite core. Hargraves[4] first demonstrated a remarkable preferred orientation of the cones which has been confirmed by a detailed investigation by Manton[5]. Manton found the shatter cones to point inward toward the centre of the Vredefort Ring and to point upward about 45,000 ft. above the present ground surface. This provides highly suggestive new evidence that this ring structure is an astrobleme as proposed by Boon and Albritton[6], Daly[7] and Dietz[8].

In July 1964 we made a brief examination of shatter-cone orientation at another large shatter-coned structure, the Sudbury structure in Ontario, Canada, long famous for its nickel mines. One of us (R. S. D.) first discovered shatter cones at Sudbury in May 1962. In the summer of 1962 they were also independently found by Hargraves (personal communication) and recognized as similar to those which he had investigated at the Vredefort Ring.

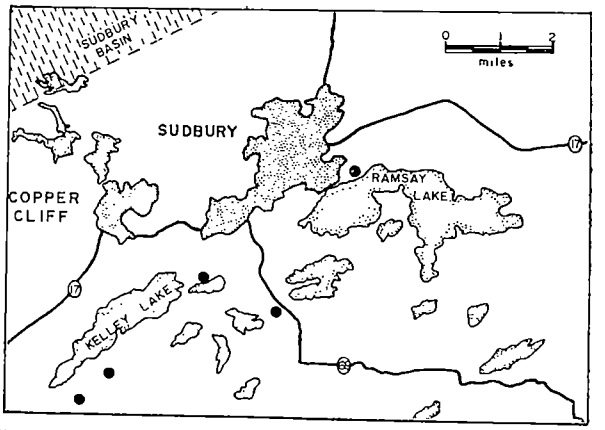


Fig. 1. Southern margin of Sudbury Basin. Solid circles show location of oriented, shatter-coned rock outcrops. Exposures near Kelley Lake are in Mississagi quartzite; outcrop at Ramsay Lake is in Ramsay Lake conglomerate



Fig. 2. Shatter cones in Mississagi quartzite along southern margin of Sudbury Basin structure. Their orientation is such that, when the rocks are returned to their pre-event position, the cones point inward toward the Sudbury Basin. This suggests that the structure is an astrobleme (meteorite impact scar)

In situ shatter-coning at five localities (four in the Mississagi quartzite and one in the Ramsay Lake conglomerate) was sufficiently well developed to allow a preliminary orientation investigation (Fig. 1). An especially fine display of oriented shatter cones is present in a road cut immediately south of the south-western end of Kelley Lake (Fig. 2).

Based on the reasonable assumption that the Mississagi quartzite was an essentially flat-lying formation at the time of the Sudbury event, 1·7 billion years ago[9], the preferential orientation of the shatter cones, on restoration of the beds to horizontal, is toward the centre of the Sudbury basin. The Mississagi quartzite now dips steeply and faces south-south-east. The axes of shatter cones in the Mississagi quartzite (and Ramsay Lake conglomerate) lie preferentially in planes that are essentially parallel to bedding planes. This essentially duplicates the situation at the Vredefort Ring.

Shatter-coning at the Sudbury structure seems to be largely, although not entirely, confined to the well-bedded Mississagi quartzite. The reason for this selectivity is not clear. However, it is empirically known from other

presumed astroblemes that initial compressibility of the rock, fine-grained texture and minimal porosity, and frequent discontinuities (bedding and joint surfaces) in the shock-wave path are conducive to extensive development of shatter cones.

We conclude that the shock force which shattered the collar rocks along the southern periphery of the Sudbury Basin came from the north, that is, from the direction of the Sudbury Basin. This is in accordance with expectations if the Sudbury structure is an astrobleme.

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CHEMISTRY

A Simplified Scintillation-counting Technique for assaying $^{14}CO_2$ in a Warburg Flask

AVAILABLE methods for assaying $^{14}CO_2$ in Warburg flasks are of two types. The first[1] involves trapping liberated $^{14}CO_2$ with an organic base, which makes it possible to count efficiently, but at the cost of a rather cumbersome procedure. Trapping with filter paper moistened with sodium hydroxide[2] or potassium hydroxide[3,4] provides a convenient method but with some loss of efficiency. The procedure described here is based on the use of a solvent system described by Belcher[5] that will accommodate up to 3 per cent of a 25 per cent aqueous potassium hydroxide solution with only 3 per cent quenching.

Portions of 0·01 M sodium carbonate containing standardized $Na_2{}^{14}CO_3$ (obtained from the Radiochemical Centre, Amersham) were pipetted into the main compartments of Warburg flasks. Water was then added to bring the total volume to 3 ml. Sulphuric acid (0·2 ml. 6 N) was added from the side arm and the evolved carbon dioxide was trapped in 0·1 ml. 20 per cent aqueous potassium hydroxide contained in the centre well. After shaking for 1 h to ensure complete absorption of the carbon dioxide, the contents of the centre well were transferred with a Pasteur pipette to 5-ml. solvent in a silica counting vial: maximum transfer was ensured by transferring some of the mixture to and fro three or four times. The solvent system consisted of 2,5-diphenyloxazole (PPO) 2 g, 1,4-*bis*-2-(5-phenyloxazolyl) benzene (POPOP) 25 mg, toluene (500 ml.) and methanol (500 ml.). Reagent-grade solvents were used. The solvent system differs from that described by Belcher[5] only in that methanol was used instead of ethanol. The solvent system is very sensitive to oxygen quenching, and oxygen-free nitrogen was therefore bubbled through each vial for 1 min before sealing with a well-fitting cap. Counting was performed in a Nuclear Enterprises liquid scintillation unit at 20° C. Table 1 shows that the recovery of $^{14}CO_2$ radioactivity was independent of the amount of carbonate in the flasks up to 10 μmole. After this, the contents of the centre well cease to be completely soluble in the scintillator solution. The method is thus applicable to volumes of carbon dioxide up to about 200 μl.

Table 1. LIQUID SCINTILLATION COUNTING OF $^{14}CO_2$ RADIOACTIVITY FROM WARBURG FLASKS CONTAINING STANDARD $Na_2{}^{14}CO_3$

Initial radioactivity (d.p.s.)	Carbonate present (μmole)	Count rate (c.p.s.)	Counting efficiency (per cent)
0	0	0·75	—
24·4	2	19·4	76·4
48·8	4	37·4	75·1
73·2	6	55·4	74·7
97·6	8	74·2	75·3
122·0	10	92·0	74·8
146·4	12	102·9	69·8
170·8	14	110·3	64·1

The counting efficiencies were calculated from the count rate obtained after subtraction of the blank value. It can be shown[6] that the radioactivity from ^{40}K in 0·1 ml. 20 per cent potassium hydroxide contributes 0·44 d.p.s. towards the blank.

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Kinetic Evidence for Nucleus Cluster Formation in Solid Decompositions

OVERALL topokinetic equations can be extended to take account of time-dependent nucleation functions other than the random law modified by ingestion[1,2]; but no analytic treatment of non-random spatial arrangements of nucleation sites has appeared. Apart from photo-micrographs of partially decomposed barium azide showing barium nuclei in clusters and arrays[3,4], and some single crystal investigations[5], there is little published evidence of the need for such a theoretical extension. However, recent kinetic evidence has suggested the existence of non-random arrangements of two kinds.

First, work on α-lead azide[6] and nickel oxalate[7], described elsewhere, in which the kinetic form of the accelerator period changes at $\alpha \sim 0{\cdot}25$ from a cubic towards a parabolic dependence, is consistent with linear arrays of three-dimensional nuclei which coalesce to form rod-like bodies. Secondly, in some preparations of silver oxide[8] and silver oxalate[9] a cubic dependence holds for $\alpha > 0{\cdot}05$ with negative deviations for $\alpha < 0{\cdot}05$, the intercept t_0 of the $\alpha^{1/3}$ versus t plot being negative.

This second kinetic form can arise from the growth of nuclei in isolated planar groups. The constituent nuclei first grow independently giving a linear $\alpha^{1/3}$ versus t plot, but later coalesce giving a nearly linear plot with a reduced slope, the number of nuclei in a group being given by the cube of the ratio of the initial and final slopes of the $\alpha^{1/3}$ versus t plot. For the data on a range of neutron-irradiated silver oxalate samples[9] plotted on a reduced time

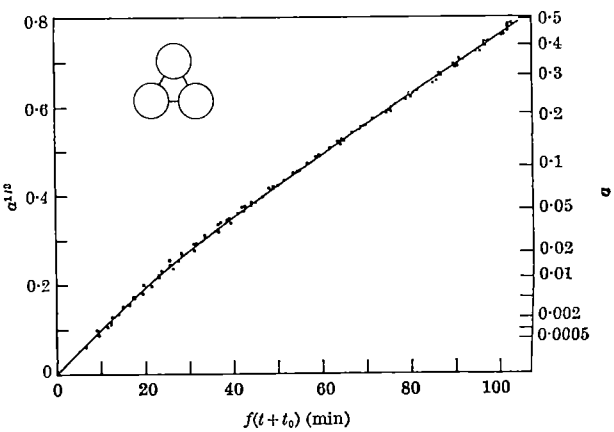


Fig. 1. The reduced plot $\alpha^{1/3}$ versus $f(t+t_0)$ for the thermal decomposition at 126° C of neutron-irradiated silver oxalate. The full line represents the volume development of an equilateral cluster. Data from Fig. 7 of ref. 9

axis in Fig. 1, this ratio is 1·4, thus the groups contain three nuclei. The full line represents the growth of three hemispherical nuclei at the apices of an equilateral triangle with proper allowance for single and multiple overlap. Calculations for three nuclei in a straight line do not accord with this.

A non-random arrangement of nuclei is thus adequate to explain these kinetic features. It is not necessary to introduce non-isothermal conditions or rapid growth of small nuclei.

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Brown (β) Titanium Trichloride

Recent papers by Clark[1] and Dijkgraaf[2] have shown that α-$TiCl_3$ may be distinguished from β-$TiCl_3$ by differences in the reflectance spectra of the two solids. In particular, the band at 19,000 cm^{-1} in the violet (α) form is replaced by a band at 24,000 cm^{-1} in the brown (β) form.

I have now found that the violet and brown aqueous acid solutions of the respective α and β forms can be distinguished from one another by absorption maxima at frequencies similar to those of the two solids (Table 1).

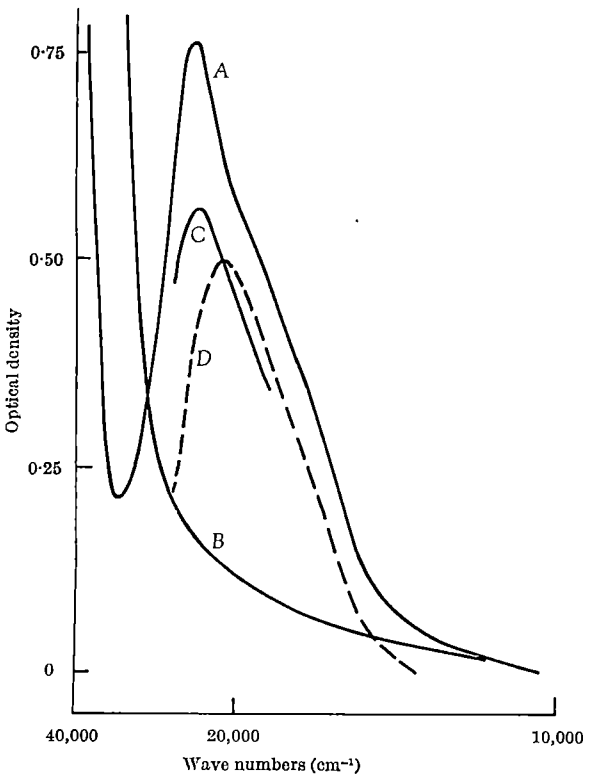


Fig. 1. Oxidation and reduction of a solution of brown (β) $TiCl_3$ (9·6 mg/ml. in 1·3 N sulphuric acid)

A, fresh solution; *B*, solution *A* after passage of oxygen for 30 min; *C*, solution *B* after cathodic reduction; *D*, solution *B* after prolonged oxidation and subsequent cathodic reduction

Table 1. Absorption Spectra of $TiCl_3$ in 1·3 N Sulphuric Acid

Form	Concentration (mg/ml.)	Wave numbers of absorption maxima ($cm^{-1} \times 10^{-2}$)		Colour
α	12·0	198 (Σ 3)	161*	Violet
β	9·6	233 (Σ 14)	178*	Brown

* Indicates an inflexion on a larger curve; Σ indicates extinction coefficient per 154 g of solute.

Distinctive spectra can also be obtained from solutions of the two forms in ethanol and acetonitrile.

These observations suggest that β-$TiCl_3$ preserves some of its distinctive structure in solution even when the medium is strongly solvating. A remarkable example of the stability of this relic structure is provided by a solution of β-$TiCl_3$ which has been subjected to an oxidation-reduction sequence (Fig. 1). It could well be assumed that such a succession of chemical reactions would convert any trivalent titanium into its most stable form; indeed, after anodic oxidation for several days the violet coloration of α-$TiCl_3$ is produced on cathodic reduction. If, however, the duration of the oxidized stage is limited to 1 h, it is surprising to find that the brown coloration is restored by cathodic reduction.

Further work on the structure of β-$TiCl_3$ is in progress.

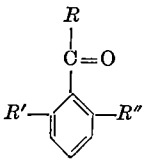
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Tetracyanoethylene Complexes as Indicators of the Carbonyl Inductive (−*I*) Effect in Sterically Hindered Aromatic Ketones

It is well established that the conjugation of the carbonyl group with the aromatic π-electron system in alkyl phenyl ketones (I, $R' = R'' = H$) is not hindered by the substitution of one methyl group in the *ortho*-position (I, $R' = CH_3$, $R'' = H$).



I

However, two such *ortho*-substituted methyl groups (I, $R' = R'' = CH_3$) force the oxygen of the carbonyl group out of the plane of the aromatic ring and so suppress carbonyl-ring conjugation. This is shown by the large diminution in the intensity of the ultra-violet absorption in the region 210–300 mμ, in the 2,6-dimethyl compounds, relative to the 2-methyl- and unsubstituted compounds[1].

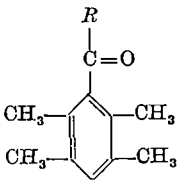
In principle, it should be possible to separate the inductive effect (−*I*) of the carbonyl group (independent of conjugation) from its mesomeric effect (−*M*) (dependent on conjugation) in several ways and so to observe the inductive effect independently in compounds of this type. Dipole moment investigations have achieved this[2]. Thus the moments of 2-methylacetophenone and 2,6-dimethylacetophenone are respectively 3·22 D (−*I* and −*M* operating) and 2·81 D (−*I* only operating).

Hammett σ-value investigations have also been directed to this general problem. Thus the value ($\sigma_p - \sigma_m$) generally gives a semi-quantitative measure of the separate effects, but this is only approximate[3].

It was shown in 1937 by dipole moment investigations[2c] that conjugation between the nitro-group and the aromatic ring in nitrodurene (3-nitro-1,2,4,5,-tetramethylbenzene) is suppressed because the oxygen atoms of the nitro-group are forced out of the plane of the ring by the steric hind-

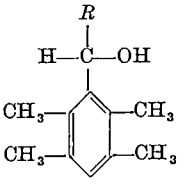
rance of the vicinal 2- and 4-methyl groups. The dipole moments of nitrobenzene and nitrodurene are, respectively, 3·95 D ($-I$ and $-M$ operating) and 3·39 D ($-I$ only operating).

Clearly, by analogy with 3-nitrodurene and with alkyl 2,6-dimethylphenyl ketones, alkyl duryl ketones (II) should exhibit suppression of conjugation between the carbonyl group and the aromatic ring. With this in mind, we have prepared a series of 3-acylated durenes (II; R = n-C_3H_7, n-C_4H_9, n-C_5H_{11}, $(CH_3)_2CH$) and examined them by a variety of spectroscopic means.



II

The ultra-violet absorption spectra of these compounds indicate the absence of carbonyl conjugation, since high-intensity absorption is not present in the region 210–300 mμ. The proton magnetic resonance spectrum of durene shows two singlet peaks (τ 3·4, τ 8·0), the former due to the two aromatic protons, the latter due to the twelve equivalent protons of the four methyl groups. The 3-acyldurenes, however, show two distinct aromatic methyl proton peaks, one unchanged at τ 8·0 (two methyl groups) and the other (two methyl groups) displaced about 0·2 p.p.m. downfield. This difference is ascribed partly to the inductive effect of the carbonyl dipole on the *ortho* methyl groups and partly to long-range deshielding by the diamagnetic anisotopic carbonyl field, there being virtually no effect on the *meta* methyl groups. The aromatic methyl protons in methyl *o*- and *m*-toluate have τ values 7·15 and 7·73 respectively, the downfield displacement in the former compound of 0·58 p.p.m. being presumably due to the *ortho* carbonyl group where the mesomeric effect may also operate[4]. In accordance with this interpretation, the secondary alcohols (III) corresponding to (II) show proton-magnetic resonance spectra in which the peaks due to *ortho* and *meta* methyl groups are separated by only 0·05 p.p.m.



III

It has been shown that tetracyanoethylene (TCNE) is a sensitive indicator of π-electron availability[5]. Charge-transfer complexes were therefore formed by mixing equivalent amounts of TCNE and the above acyldurenes in methylene chloride solution. Durene itself forms a deep red complex (λ_{max} 481 mμ) while the acylated durenes all show a paler colour (λ_{max} about 440 mμ). That this is not due to steric inhibition of complex formation is shown by the fact that the corresponding alcohols (III) show the same deep colour as durene itself (λ_{max} about 490 mμ).

Thus the acyldurene–TCNE complexes appear to indicate a π-electron deficiency in the aromatic ring with respect to durene[1b], and since the ultra-violet and proton-magnetic resonance spectra of the acyldurenes indicate very little if any mesomeric effect, this must be ascribed largely to the inductive effect of the carbonyl dipole.

3-Acyl durenes (II)[(R = n-C_3H_7, n-C_4H_9, n-C_5H_{11})] were prepared by Friedel–Crafts acylation of durene using the corresponding acid chlorides (1·2 mole) in carbon disulphide below 0°. The compound (II) (R = $(CH_3)_2CH$) was prepared by Friedel-Crafts acylation of durene with isobutyric anhydride (1·2 mole) in carbon disulphide at room temperature. All were colourless. 3-*Butanoyldurene* (II; R = n-C_3H_7) found: C, 82·55; H, 9·95; $C_{14}H_{20}O$ requires C, 82·35; H, 9·8 per cent: m.p. 37°. 3-*Pentanoyldurene* (II; R = n-C_4H_9) found: C, 82·85; H, 10·2; $C_{15}H_{22}O$ requires C, 82·55; H, 10·1 per cent: m.p. 40°. 3-*Hexanoyldurene* (II; R = n-C_5H_{11}) found: C, 82·65; H, 10·35; $C_{16}H_{24}O$ requires C, 82·75; H, 10·35 per cent: m.p. 62°. 3-*Isobutanoyldurene* (II; R = $(CH_3)_2CH$) found: C, 82·25; H, 9·95; $C_{14}H_{20}O$ requires C, 82·35; H, 9·8 per cent: m.p. 49°.

The ultra-violet absorption spectra were recorded on a Unicam *SP* 700 spectrophotometer; the proton-magnetic resonance spectra on a Perkin–Elmer 60 *Mcs* spectrometer.

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BIOCHEMISTRY

Isozymes of Salivary Amylase

It has previously been observed that crystalline salivary amylase gives two peaks in free electrophoresis[1]. The work recorded here shows that a sample of salivary amylase crystallized three times, which gave a single peak in the ultracentrifuge (Fig. 1), could be separated into four bands by disk electrophoresis on polyacrylamide gel[2] (Fig. 2).

The amylase was prepared according to a method previously described[1]. The crystals were dissolved at *p*H 10·1 by addition of ammonia, brought to *p*H 8·6 with acetic acid (final ionic strength 0·19) and centrifuged at 59,780 r.p.m. at 7·1° C in a Spinco model *E* ultracentrifuge. (We thank Dr. G. W. de Villafranca for the use of the ultracentrifuge.)

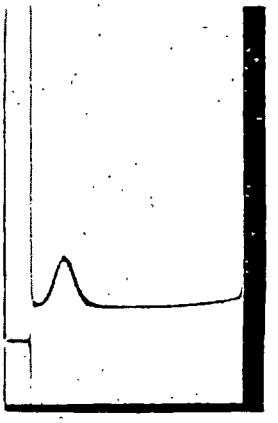


Fig. 1. Ultracentrifugation of salivary amylase, 4·9 mg/ml., *p*H 8·5, 0·19 ionic strength, 45 min at 59,780, 7·1° C in Spinco model *E* ultracentrifuge

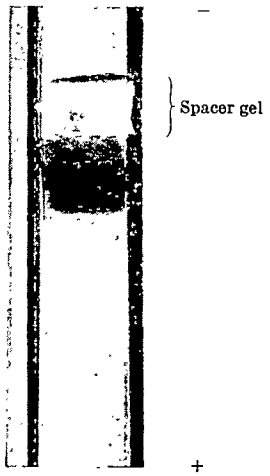


Fig. 2. Gel-electrophoresis of salivary amylase on polyacrylamide gel (Canalco) 1 h at 2·5 m. amp/tube. Buffer 0·05 M *tris* (hydroxymethyl) aminomethane, 0·384 M glycine, *p*H 8·3 (*p*H in the column during electrophoresis in this buffer is 9·5). 200 μg amylase (same preparation as in Fig. 1) on 0·5 cm × 6 cm column. Spacer gel on top, migration towards anode at bottom. Amido black staining

Disk electrophoresis was performed in a Canalco model 12 apparatus according to the method of Ornstein and Davis[2] as further elaborated in the Canalco instructions. The pattern shown in Fig. 2 was obtained on the same solution as that used for ultracentrifugation. Three bands show clearly in the photograph and a fourth faint band is discernible.

Amylase activity was determined in unstained columns run simultaneously with comparable columns which were stained with amido black at the end of the run. The unstained columns were frozen immediately after termination of the electrophoresis. They were cut in 1 mm slices and each slice was extracted with 0·5 ml. buffer of *p*H 6·8, 0·02 M phosphate, 0·045 M sodium chloride. The slices were crushed and shaken mechanically in the buffer for 3–4 h. Activity was determined on the basis of the starch iodine colour[1] and the amount of amylase calculated from the relation between amylase concentration and activity[3]. Further attempts to improve the method of extraction did not increase the recovery, which was 60–85 per cent of the activity of the amylase applied to the column.

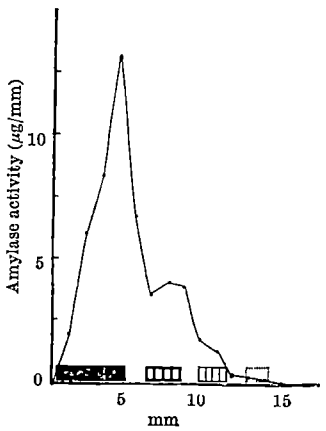


Fig. 3. Comparison of amylase activity and amido black staining of two polyacrylamide gel columns run simultaneously. Conditions as in Fig. 2 except 3 h run, 82 μg amylase. Migration towards anode at right. Position of the four protein bands and the intensity of staining represented by blocks at base of graph

In Fig. 3 the distribution of the amylase activity is compared with the position of the stained protein bands. The two columns were run simultaneously at *p*H 9·5 in the Canalco buffer, 0·384 M glycine, 0·05 M *tris* (hydroxymethyl) aminomethane, for 3 h at about 2·5 m.amp/column. The most slowly moving band is the broadest, the most strongly stained, and it gave the highest amylase activity. The bands became progressively narrower and less deeply stained toward the anode. The activity follows the same trend and even the faint, fastest moving band manifested amylase activity. The use of parallel columns for staining and activity introduces some uncertainty in the comparison. For a total spread of the four bands of 15 mm, duplicate stained columns may show 0·5 mm differences in the width of the bands and 1–2 mm difference in the total spread. The gel between the two slowest bands is more deeply stained than the weakest band, so that there is never complete separation of the bands. There is also some distortion during the cutting, and the staining procedure may alter the length of the column. However, the same general distribution of protein bands and amylase activity has been found with several amylase preparations. There may be some variation in the relative amounts found in each band; for example, the preparation shown in Fig. 2 had relatively less of the slowest-moving component than that of Fig. 3.

The use of methods such as electrophoresis in a supporting medium or chromatography has, in recent years, revealed the presence of several fractions in many enzymes which appeared homogeneous by other criteria such as ultracentrifugation. In the case of salivary amylase, Millin and Smith[4] obtained three fractions by chromatography on a calcium phosphate column. Fresh saliva was first separated into two fractions on 'Sephadex *G*25' and the amylase-containing fraction was rechromatographed on calcium phosphate using a discontinuous elution system of increasing concentrations of phosphate buffer. Five protein fractions were obtained and three of these had amylase activity, but none of the fractions seemed to represent single proteins. The three chromatographic fractions of amylase were obtained when saliva from a single individual was used. We have repeated and confirmed these observations, but have not yet had an opportunity to compare these fractions with the electrophoretic fractions. Our crystalline preparation originated from pooled saliva and the possibility remains that there are differences among the amylases secreted by different individuals. Millin and Smith observed that one of their three fractions was unstable. They, therefore, suggested that the two peaks found in our laboratory on free electrophoresis of the crystalline enzyme[1] was due to the loss of one fraction during preparation. Since four components have now been separated by gel-electrophoresis, loss of a particular fraction does not need to be presumed.

Patton and Pigman[5] found up to twelve components of parotid gland secretion by free electrophoresis and showed that one of these contained amylase activity. This might indicate that the different salivary glands each produce a slightly modified enzyme, though in their system it would be difficult to distinguish two or more components of amylase. In spite of the three chromatographic fractions found in fresh saliva, changes in the amylase during preparation of the crystalline enzyme, such as the partial deamination observed in insulin by Carpenter and Hayes, cannot be excluded[6]. Tsugita and Akabori's finding that 'Taka' amylase contains bound carbohydrate[7] prompted us to look for bound carbohydrate in salivary amylase. The anthrone test and the tests for hexosamine and sialic acid were performed[8,9]. All three gave results that corresponded to less than one-third of a group per mole of amylase, assuming 50,000 as the molecular weight[10]. This might well represent a small amount of mucin which had not been completely removed, but it might also mean that one fraction of the amylase contains carbohydrate, a situation similar to that observed by Plummer and Hirs for ribonuclease[11]. While the molecular weight of salivary amylase is usually assumed to be 50,000, the amino-acid composition[12] fits a minimum molecular weight of 18,000 to within ± 3 per cent of all the amino-acid but histidine (−5 per cent). This leaves the possibility

that the situation is similar to that of lactic dehydrogenase[13] and that the amylase might be a trimer of two very similar proteins. This could give rise to four fractions.

This work was supported in part by National Science Foundation grants *GE* 2591 and *G* 12385.

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Uncouplers of Oxidative Phosphorylation and Teratogenic Activity of Insulin

TREATMENT with insulin has well-established teratogenic effects in chick development. When administered during the earliest stages of incubation (first 2 days), it is responsible for the occurrence of rumplessness (chiefly absence of the tail vertebræ), whereas somewhat later (4th or 5th) day) it leads to a syndrome of micromelia and abnormalities of the beak (namely, a shortened upper beak or parrot beak). During these later stages, but not in the early ones, the teratogenic activity of insulin is strikingly exaggerated by the simultaneous administration of chlorpromazine[1]. The amounts of chlorpromazine which produce this effect were non-teratogenic and only moderately toxic if given alone.

When we reported these observations, we suggested that uncoupling of oxidative phosphorylation by chlorpromazine might well play an important part in bringing about the teratogenic synergism which follows combined administration of the two compounds. This seemed the more suggestive since Randle and Smith had advanced persuasive arguments for assigning to insulin also the role of an uncoupling agent in energy transfer[2,3]. It seemed important, therefore, to enquire if other compounds which serve as uncouplers of oxidative phosphorylation potentiate the teratogenic activity of insulin in a way similar to that observed for chlorpromazine.

The tests recorded here were conducted with four compounds for which uncoupling of oxidative phosphorylation is well known, namely, sodium salicylate, 2.4-dinitrophenol (DNP), *p*-hydroxymercuribenzoate (PHMB) and carbonyl cyanide *m*-chlorophenylhydrazone. Reasons for these choices, in addition to solubility in water and compatibility with adequate embryo survival, will be discussed later.

The results obtained with salicylate are given in Table 1. In the control group receiving 10 mg/egg salicylate at 96 h the immediate post-operative mortality was moderate, many of the hatched chicks (23·5 per cent) showed a marked opisthotonus, but the incidence of gross malformations among survivors of the 13th day was very low, the most conspicuous effect being a shortened upper beak (2·6 per cent). The same amount of salicylate, given in combination with 1·5 I.U. insulin, led to a great rise in embryo mortality and to a dramatic exaggeration in incidence of the malformations typically produced by insulin. Too few chicks hatched in this group to determine if the reduced incidence of opisthotonus (7·1 per cent) differed significantly from that observed among the salicylate controls. In regard to synergism with the teratogenic activity of insulin, salicylate was similar to chlorpromazine. It is of interest that according to Lane and Eshleman a high incidence of shortened upper beak occurs after treatment (at 96 h) with larger doses of salicylate than those used by us and that, except for curling of the toes, this was the only marked morphological defect[4].

Table 1. Results of experiments in which either insulin or sodium salicylate or both compounds were injected into the yolk sac of eggs of White Leghorn fowl after 96 h of incubation

Insulin (I.U.)	1·5	1·5	—
Sodium salicylate (mg)	—	10·0	10·0
Treated	292	273	295
Mortality per cent			
to end sixth day	25·7	54·6	9·5
7–13	2·7	10·3	12·9
14–22	27·4	30·0	48·8
Hatched per cent	44·2	5·1	28·8
Survivors of thirteenth day	209	96	229
Normal per cent	80·4	37·5	86·0
Micromelia per cent	14·8	44·8	1·3
Parrot or short lower beak per cent	5·7	21·9	0·4
Short upper beak per cent	3·3	19·8	2·6
Opisthotonus among hatched chicks per cent	—	7·1	23·5
Miscellaneous defects per cent	5·3	1·0	0·9

The data for experiments with 2,4-dinitrophenol are shown in Table 2. The injection of 100 γ/egg DNP at 96 h was non-toxic, the chicks hatching quite normally, and it was also non-teratogenic. When the same amount of DNP was combined with 1·5 I.U. insulin, embryo mortality during the first 48 h following treatment was three times that occurring after insulin alone. The incidence of micromelia and parrot beak was unaffected among survivors of the 13th day; but the frequency of shortened upper beak was raised more than thirteen-fold.

Table 2. Results of experiments in which either insulin or 2,4-dinitrophenol or both compounds were injected into the yolk sac of eggs of White Leghorn fowl after 96 h of incubation

Insulin (I.U.)	1·5	1·5	—
2,4-dinitrophenol (γ)	—	100	100
Treated	184	281	185
Mortality per cent			
to end sixth day	17·9	53·0	3·2
7–13	3·8	0·7	2·2
14–22	26·1	23·5	18·3
Hatched per cent	52·2	22·8	76·2
Survivors of thirteenth day	144	130	175
Normal per cent	84·0	70·8	97·7
Micromelia per cent	15·3	15·4	0·0
Parrot or short lower beak per cent	4·2	3·8	0·0
Short upper beak per cent	1·4	18·5	0·0
Miscellaneous defects per cent	2·1	3·8	1·7

In a third series of tests we used *p*-hydroxymercuribenzoate (PHMB) alone or in combination with insulin. Injection of 0·5 mg PHMB at 96 h was only slightly toxic and produced an incidence of 2·5 per cent micromelia and beak defects; with one-tenth of this dose, that is, 50 γ/egg, PHMB was non-toxic and non-teratogenic. When 50γ PHMB were administered in combination with 1·5 I.U. insulin, the incidence of micromelia was significantly increased over that found after insulin alone, but there was no significant change in the frequency of beak defects.

Finally, we experimented with carbonyl cyanide *m*-chlorophenylhydrazone[5,6]. Five and 15 γ/egg of this compound were tested alone and in combination with 1·5 I.U. insulin. Both dosages were well tolerated and non-teratogenic when given alone. Both amounts greatly raised embryo mortality when used together with insulin, but there was no effect whatever on incidence of malformations.

We have now tested the effects on the teratogenic activity of insulin exerted by five compounds which are known to interfere with coupled oxidative phosphorylation. Two of these compounds, namely, chlorpromazine and sodium salicylate, produce synergistic results over the whole spectrum of insulin teratogenicity (at the 96-h stage) as shown by a great exaggeration in the incidence of malformations of the appendicular as well as the facial

skeleton. In combination with 2,4-dinitrophenol the frequency of insulin-induced defects of the maxilla was greatly raised, but the incidence of other abnormalities remained unaffected. When the administration of insulin was combined with that of *p*-hydroxymercuribenzoate, the results were the opposite to those just discussed, that is, the teratogenic effect of insulin on long bone development (micromelia) showed potentiation, but the incidence of abnormalities of the facial skeleton was not influenced. There was, finally, no quantitative alteration in the teratogenic effects of insulin by the added administration of carbonyl *m*-chlorophenylhydrazone.

It has been clearly shown "that chlorpromazine and dinitrophenol act at different points in the sequence of phosphorylation reactions"[7], and it seems likely that multiple loci of such interference exist[8]. This is in good agreement with our findings. There is also much evidence for similarities between insulin and uncoupling agents in their effects on metabolic events, and especially on dehydrogenase activity[9,10]. It is true that the uncoupling agents are not altogether specific for the enzymatic events of oxidative phosphorylation[11], but since many of our earlier observations had already pointed to interference with dehydrogenase activity as the most likely primary step by which insulin interferes with normal development[12], the present results may be taken as strong supporting evidence. The recent conclusion by Eisenhardt and Rosenthal[13] that "uncoupling agents operate by interfering with the synthesis of high-energy intermediates" may well apply to our material.

We thank Dr. P. G. Heytler, Central Research Department, E.I. du Pont de Nemours and Co., Wilmington, Delaware, for providing the carbonyl cyanide compound used in our tests. This work was supported by a grant from the U.S. National Science Foundation.

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Metabolic Fate of some Chlorinated Phenoxyacetic Acids in the Stem Tissue of *Avena sativa*

AN extension of the previous work[1] on the hydroxylation of phenoxyacetic acid by mesocotyl tissue of *Avena sativa* has demonstrated a broad relationship, at least along one pathway of metabolism, between the fate of certain phenoxyacetic acids and their pattern of ring substitution.

The techniques used are the same as those already reported. All metabolites were isolated in mg quantities by solvent extraction followed by chromatography. Characterization of the metabolites or their derivatives was by comparison with authentic compounds.

Phenoxyacetic acids with an unsubstituted 4 position were hydroxylated at that position, and the resulting phenolic acid was accumulated as the 4-0-β-D glucoside. Thus 2-chlorophenoxyacetic acid was converted into a more polar compound which could be hydrolysed with acid or β-glucosidase to glucose and a phenolic acid (m.p. 146° C). The acid showed selective light absorption in the ultra-violet region, λ_{max} 288 mμ at *p*H 2; λ_{max} 306 mμ at *p*H 12. This phenolic acid corresponds exactly, both in terms of these characteristics and the infra-red spectrum, with 2-chloro-4-hydroxyphenoxyacetic acid[2].

Similarly 2,6-dichlorophenoxyacetic acid was converted into a glucoside which yielded glucose and a phenolic acid on acid or enzymatic hydrolysis. Elementary analysis of the ammonium salt of this glucoside supports the formulation as a mono glucoside of a phenolic acid derived from 2,6-dichlorophenoxyacetic acid (found: C, 39·2; H, 4·77; Cl, 16·65; calc. for $C_{14}H_{19}NO_9Cl_2$: C, 40·5; H, 4·6; Cl, 16·87). This phenolic acid had λ_{max} 283, 287 mμ at *p*H 2 and λ_{max} 302 mμ at *p*H 12 and was identical in these respects with authentic 2,6-dichloro-4-hydroxyphenoxyacetic acid.

In contrast, phenoxyacetic acids with a chlorine atom at position 4 were not hydroxylated to any appreciable extent, but neutral products were obtained which proved to be substituted phenoxyacetylglucoses. Compounds of this type were obtained from 4-chlorophenoxyacetic acid and 2,4-dichlorophenoxyacetic acid (2,4-D). The ultra-violet spectra of these metabolites were identical with their parent acids. The infra-red spectrum of the 2,4-D metabolite exhibited strong absorption at 3,300 cm^{-1} and 1,050–1,100 cm^{-1} characteristic of hydroxyl groups in the sugar moiety. Acid or enzymatic hydrolysis yielded equimolar quantities of glucose and the parent acid. The 2,4-D metabolite was obtained in the crystalline form, and was acetylated to give a material having an infra-red absorption spectrum identical with that of 1-0-2,4-dichlorophenoxyacetyl - 2,3,4,6 - tetraacetyl - β - D - glucose, which was obtained by condensing tetraacetylbromoglucose with the silver salt of 2,4-D.

The metabolic fate of 2,4,6-trichlorophenoxyacetic acid is exceptional: hydroxylation occurs at position 3 and the glucoside was isolated. Enzymatic hydrolysis yielded a phenolic acid having λ_{max} 291 mμ at *p*H 2 and λ_{max} 308 mμ at *p*H 12 identical with synthetic 3-hydroxy-2,4,6-trichlorophenoxyacetic acid.

In the case of 2,6-dichlorophenoxyacetic acid, in addition to the glucoside already described, a glucose ester of the unchanged acid was isolated.

Since the hydroxylation of auxins, benzoic acid, and cinnamic acids has been demonstrated by other workers[3–5], there can be little doubt that an enzyme system capable of effecting this transformation exists in plant tissues.

We thank Prof. G. E. Blackman for his advice.

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Detection of 'Activated' Compounds on Paper

THE presence of acylating compounds on paper is frequently deduced from reaction with hydroxylamine and subsequent treatment with ferric chloride to reveal the hydroxamates so formed. This method has been used for the detection of nucleotide peptides and similar 'activated' complexes[1–8].

During attempts to characterize the products of a yeast enzyme system reported to form 'activated' peptides[9,10] colours were observed after spraying chromatograms or electropherograms with ferric chloride irrespective of any previous treatment with hydroxylamine. It is well known that ferric ions form coloured complexes with a variety of functional groups; accordingly, a large number of compounds were tested with ferric chloride and hydroxylamine on paper under various conditions.

Typical results are listed in Table 1. It is clear that many substances react with ferric chloride alone and that the colours are usually intensified after treatment with hydroxylamine, particularly in the presence of borate, *p*H 7·7 (cf. ref. 4). Small amounts of genuine hydroxamates may perhaps be formed from amino-acids under these conditions[12]. All the common amino-acids were tested, and most gave some colour with ferric chloride alone. Only cysteine, leucine and phenylalanine failed to give colours under any of the conditions tried. A series of dipeptides of glycine were also tested and gave reactions similar to those of the C-terminal amino-acid. Representative amides, esters, uronic acids and lactones, substances known to form hydroxamates[13–18], were also tested and examples are included in Table 1. The purple colour produced by the genuine hydroxamates is fairly characteristic, but the test for hydroxamate formation on paper is of doubtful significance except when a duplicate paper gives little or no reaction with ferric chloride alone.

Table 1. Reactions on Paper of Various Compounds with Ferric Chloride and Hydroxylamine

Compound	*A*		*B*		*C*	
Aspartic acid		—		—	+	P
Glutamic acid	+ + +	R–B	+ + +	R–B	+ + +	R–B
Glycine	+ +	R–B	+ +	R–B	+ + +	R–B
Methionine	[+]	O–P	+	O–P	+	O–P
Cytosine		—	[+]	Blue	[+]	Blue
Guanine	[±]	Pink		—	+	P
Yeast RNA	+	G	+	G	+	G
Arabinose		—	±	Pink	±	Pink
Glucose		—	+	B	+ +	Pink
Glucosamine hydrochloride	+	Y	+	P	+	P
Ascorbic acid	+	P	+ +	P	+ +	P
Pyruvic acid	+	B	+ +	P	+ +	P
Imidazole	+ + +	O–B	+ +	P	+ + +	P
Glutamine		—	+ +	P	+ + +	P
Glucuronic acid		—	+	P	+	P
1,5-Gluconolactone		—	+ +	P	+ +	P
Triacetin		—	+ +	P	+ +	P

Treatment of paper: *A*, sprayed with ferric chloride reagent[1] only; *B*, sprayed lightly with 0·1 M acetate buffer, *p*H 4·0, then sprayed with salt-free hydroxylamine[11], heated at 60° for 5 min, then sprayed with ferric chloride; *C*, sprayed lightly with 0·05 M borate buffer, *p*H 7·7, followed by hydroxylamine and ferric chloride. [ ] Colour appears overnight. P, purple; G, Grey; R–B, red-brown; O–B, orange-brown; O–P, orange-pink; Y, yellow changing to brown; B, buff changing to pink.

With regard to the colours produced on electropherograms of yeast extracts[1,4] it has now been found that these can be readily reproduced by electrophoresis of mixtures of amino-acids especially after evaporation of the latter in the presence of acetate buffer. An aqueous ethanolic extract of yeast was prepared and subjected to electrophoresis as described by Harris and Wiseman[4]. For comparison a mixture of tryptophan, threonine, asparagine, aspartic acid, glutamic acid and lysine (10 mg of each) was taken up in 25 ml. of 0·1 M acetate buffer, *p*H 5·0, and the mixture concentrated to 2·0 ml. by rotary evaporation. After centrifugation a sample of the supernatant solution was electrophoresed under the same conditions. The two electropherograms were virtually indistinguishable under ultra-violet light, after spraying with ninhydrin and after the hydroxylamine–ferric chloride test. Both exhibited patterns very similar to those described for the yeast extract by Harris and Wiseman[4]. In addition, closely similar pink-brown colours were observed on duplicate papers treated with ferric chloride alone.

Substances reacting with hydroxylamine to form genuine hydroxamates are certainly present in the yeast extracts as judged by the reaction in solution, where it is possible to control conditions more precisely and to examine the spectral characteristics of the product. Their specific detection on paper, however, is virtually impossible in the presence of materials which react with ferric chloride in a non-specific manner. Extensive fractionation[2,3] is clearly necessary before any reliance can be placed on the hydroxylamine–ferric chloride test.

We thank Dr. A. H. Cook for his advice.

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Paper Electrophoresis of Heat-treated Serum Albumin in the Presence of Congo Red

It is known that if the specific spatial arrangement of the peptide chains of some proteins, such as egg albumin and serum albumin, is altered by heat, they coagulate and lose many of their specific properties. This phenomenon of denaturation is generally irreversible.

Van der Scheer and associates[1] demonstrated electrophoretically the formation of a colloidal component *C* in normal horse serum which had previously been heated to 65°. As Beilinsson[2] and Ball *et al.*[3] had shown that the coagulation of egg albumin by heat could be prevented by treating the albumin solution with sugars and sugar alcohols, it was thought that a similar treatment of blood serum and plasma might also prevent coagulation and even prevent the formation of the *C* component. Hardt *et al.*[4] demonstrated that heat coagulation and the formation of the *C* component were prevented when bovine plasma was saturated with D-glucose. Later the same workers[5] compared the effects of some monosaccharides and mannitol as well as D-glucose in plasma and serum on the formation of the *C* component. Haurowitz *et al.*[6] showed that heat-treated ovalbumin and serum albumin combine with congo red to form complexes which remain red at *p*H 2, while mixtures of congo red with the unheated proteins have a purplish colour at *p*H 2, and the denatured protein combines with more congo red than the native protein.

The work recorded here describes the effect of congo red on the coagulation and the paper electrophoretic behaviour of beef serum albumin.

Serum albumin coagulates when it is heat-treated in boiling water and then does not migrate on paper electro-

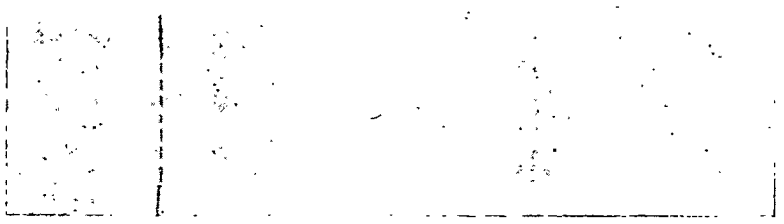


Fig. 1. The migration of serum albumin heated with congo red at (*p*H 6·4) The photograph was taken using a red filter to remove the red colour of congo on the strip

phoresis. But according to our unpublished experiments, it remains clear and migrates when it is heat-treated in alkaline solution. We now find that albumin also remains clear and migrates when it is heat-treated in the presence of congo red. The aqueous solution of congo red is alkaline (about *p*H 10). But albumin also remains clear and migrates when it is heat-treated with congo red acidified to *p*H 6·4, whereas it coagulates and does not migrate when it is heat-treated without congo red at the same *p*H.

In our experiments albumin was prepared by isolation from beef serum using ammonium sulphate[7]. The precipitate was dialysed to remove the salts, the albumin content (2·2 per cent) was determined refractometrically and the *p*H (5·8) electrometrically. To 100 mg congo red in aqueous solution was added 6·3 ml. of 0·1 N sulphuric acid and the volume made up to 50 ml. with water. In two test-tubes were put 1 ml. (22 mg) of serum albumin; to one was added 5 ml. of acidified congo red solution and to the other 4·9 ml. of water and 0·1 ml. of 0·01 N sodium hydroxide to adjust the *p*H to 6·4. The two tubes were then heated in a boiling-water bath for 15 min and cooled. The mixture containing congo red was clear while the other was coagulated. The *p*H value of the dye-containing mixture was 6·4 after heating. Paper electrophoresis of the dye-containing mixture, using an L.K.B. type *B* apparatus with barbitone–sodium barbitone buffer, *p*H 8·6, 225 V and ionic strength 0·1, was carried out for 7 h. The strips were then dyed with bromo-phenol blue, washed and dried. Fig. 1 shows the migration of albumin heated with congo red at *p*H 6·4. The experiments show that congo red prevents coagulation of serum albumin at *p*H 6·4 and permits paper-electrophoretic migration of the protein heat-denatured in this way.

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Biliary Phospholipids in Various Species

While carrying out investigations on the fatty acids of bile and plasma lecithins differences were noted in the overall biliary phospholipid pattern in man, dog, rat and ox.

Bile lipids were extracted, separated and analysed by previously described methods[1]. Purity after silicic acid column chromatography was checked by thin-layer[2] and paper chromatography[3]. Phospholipid standards were prepared from rat liver and red blood cells according to the methods of Glenn *et al.*[4] and Spitzer and Balint[5].

Lipid extraction with chloroform–methanol[6] or by refluxing with hot ethanol–ether yielded similar results.

The distribution of phospholipids obtained is shown in Table 1. Compounds noted as trace amounts could only be detected by thin-layer chromatography. Besides the phospholipids indicated, trace amounts of what appeared to be cardiolipin and phosphatidic acids were also detected in the four species examined. The final identification of all compounds was based on comparison with standards using thin layer or paper chromatography. The phosphorus-to-ester ratio determined on the lecithins of the species examined gave an average value of 1 : 1·88 (range 1 : 1·71–1 : 2·10).

The results obtained in this work confirm those of Turner[8], Isaksson[9] and Nakayama and Blomstrand[10] that the major phospholipid of bile is lecithin. The detection of phosphatidyl ethanolamine and lysolecithin in human bile also confirms the work of others[8–10].

Isaksson[9], in his work on bile from man, dog and ox, concluded that lecithin was the only phospholipid present. The results obtained in this work indicate a more complex phospholipid pattern. The detection of phosphatidyl inositol and sphingomyelin by Nakayama and Blomstrand[10] in human bile could not be confirmed.

Table 1. Average Percentage Distribution of Bile Phospholipids

Compound	Man (10*)	Rat (4)	Dog (3)	Ox (2)
Phosphatidyl ethanolamine	3·6	6·0	*N.D.*†	1·1
Phosphatidyl serine	*N.D.*	*N.D.*	1·8	1·2
Phosphatidyl inositol	*N.D.*	Trace	Trace	Trace
Lecithin	92·2	92·2	95·8	89·8
Sphingomyelin	*N.D.*	Trace	2·3	*N.D.*
Lysolecithin	3·9	Trace	*N.D.*	7·8

* No. of animals.
† *N.D.* not detected.

The phosphorus-to-ester ratio of less than 1 : 2 obtained for lecithin is consistent with the reported presence of choline plasmalogens[10]. Attempts at a similar elucidation of the phosphatidyl ethanolamine and phosphatidyl serine proved unsuccessful, due to bile pigments in these fractions interfering with the hydroxylamine reaction in determining ester linkages.

The results presented indicate that there are species differences in the phospholipid composition of bile. In all species examined, lecithin was the major phospholipid amounting to 90 per cent or more of the total. It is difficult to assign any significance to the differences noted at this time. This project was supported by grant *AM*-07563-02 from the National Institutes of Health, U.S. Public Health Service.

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PHYSIOLOGY

Simulation of a Neuronal Network operating Rhythmically through Recurrent Inhibition

A SYNCHRONOUS afferent volley initiates a series of rhythmic discharges in the ventrolateral nucleus in the thalamus[1], due to repetitive activity in a recurrent inhibitory pathway of the thalamic neurones[2]. A similar pattern of rhythmic activity occurs spontaneously in animals anæsthetized with barbiturates. An inhibitory phasing theory has been offered as an explanation for the spontaneous rhythm[3]. According to this theory, a period of rhythmic activity, usually lasting 1–2 sec, is triggered off by the discharge of one cell, which, through a recurrent inhibitory pathway utilizing one or several interneurones, is producing inhibitory postsynaptic potentials in a number of neighbouring cells. When the inhibitory potentials subside, the neurones will be in a state of post-anodal exaltation, often leading to depolarization of the cell with discharges. Similar depolarizing potentials have been seen in response to hyperpolarizing pulses[4–6] or inhibitory postsynaptic potentials[7,8] in many types of neurones. In the thalamus, the post-inhibitory firing will initiate another cycle, engaging an increased number of cells, and so on, until a large proportion of the neurones in a group beat in unison. At this point, a cell, discharging prematurely, may recruit other neurones to discharge at its own rhythm, and thereby start the disruption of the dominant rhythm.

The theory has been tested by simulation of a neuronal network on a digital computer, IBM 1620. Eighty neurones were made to discharge randomly, all at a given probability of firing (PF) (Fig. 1*A*). Following the discharge of any cell, up to five other neurones were subjected to a drop of their PF to zero for 10 units of time (UT), simulating the widely distributed postsynaptic inhibitory potentials. This was immediately followed by an increase of the PF to a higher level for 2 UT, simulating the post-anodal exaltation, before the cell resumed its random PF (Fig. 1*A*). The number of neurones undergoing the change in the PF, sequential to the discharge of another cell, was not constant, but varied between 0 and 5 as shown in Fig. 1*B*. The wide distribution of the inhibitory postsynaptic potentials is due to the extensive branching of the axon of the inhibitory interneurones.

The starting of the network results in the discharge of a large number of the simulated neurones. A corresponding pattern occurs in the animal thalamus in response to an afferent volley. In Fig. 1*C*, the initial waves in the diagram indicate the synchronous and rhythmic discharges

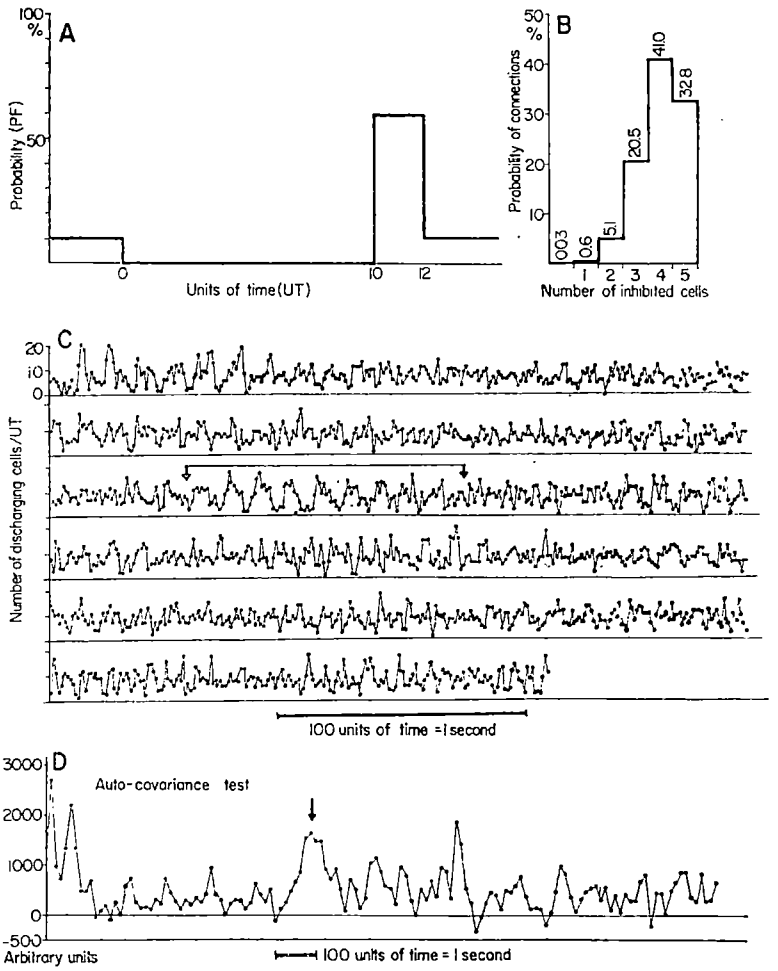


Fig. 1. *A*, The thick line indicates the change of the probability of firing of certain neurones in the model, triggered by the firing of another cell. *B*, The graph gives the probability, expressed as a percentage, of the number of neurones undergoing the probability change shown in *A*, following the discharge of a single other cell. *C*, The number of discharging neurones per unit time (UT, the scanning time of the computer) is plotted against time. The two arrows combined with a line indicate a section of marked rhythmic activity at a frequency corresponding to a period of 12 UT. The time bar below the graph indicates 100 UT, which corresponds to 1 sec in animal experiments. *D*, Auto-covariance plot of the curves in *C*, calculated as described in the text. Note the shorter time axis

of a number of cells, the appearance being similar to that observed in the cat[1,9].

Leaving the network to itself, there appear periods of rhythmic activity having a slow onset as well as offset (Fig. 1*C*, arrows). The occurrence of these periods and their shape are similar to the corresponding spontaneous activity patterns in the feline thalamus[3], and can be analysed by auto-covariance calculations. The auto-covariance is expressed as:

$$\sum_{i=j}^{j+T-1} (x_i - \bar{x}_i)\,(x_{i+\tau} - \bar{x}_{i+\tau})/n \quad \text{with} \quad j = 0, T, 2T, \ldots .$$

In Fig. 1*D*, the quantity is plotted for $T = 12$ units of time. The interval marked with arrows in Fig. 1*C* appears in Fig. 1*D* (single arrow) as a period of marked dominance of the frequency corresponding to 12 UT. The amplitude of the curve in Fig. 1*D* gives the degree of synchrony of the rhythmic waves in the network, shown in Fig. 1*C*.

Thus, the model is capable of producing activity with certain characteristics in common with the two known types of thalamic rhythmic activity. The results lend some support to the theories based on a widespread inhibition and the process of post-anodal exaltation, advanced as explanations for such activities.

Two parameters of the network turned out to be of particular importance for the production of rhythmic activity. First, the degree of the post-inhibitory increase of the firing probability, secondly, the number of neurones being inhibited subsequent to the firing of another cell. These results suggest that future animal experiments should be focused on the mechanism underlying the post-anodal exaltation, and the connexions and properties of the inhibitory interneurones in the thalamus.

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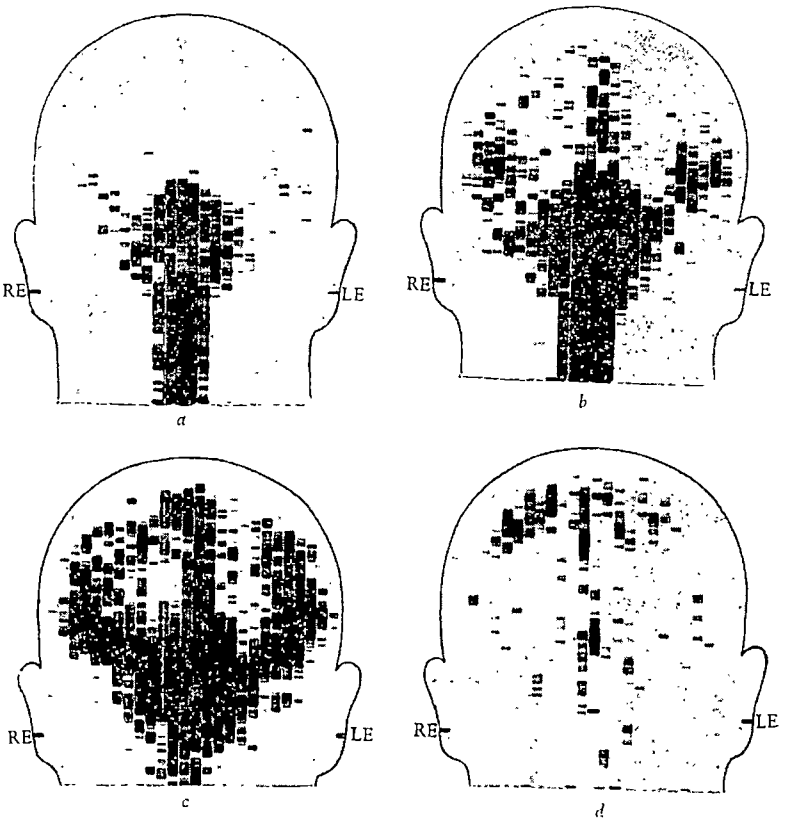


Fig. 1. One (*a*), three (*b*), six (*c*) and twenty-four (*d*) hours radio-iodinated serum albumin cisternograms

Movement of the Cerebrospinal Fluid in Human Beings

Considerable controversy and doubt exist regarding the movement of the cerebrospinal fluid, and a convincing demonstration of bulk flow of this fluid is still lacking. Since obstruction of the ventricular paths invariably results in hydrocephalus, obviously a net transfer of fluid must occur from the ventricular system out into the subarachnoidal spaces. A caudad or cephalad movement of the cerebrospinal fluid in the subarachnoidal spaces, however, has been questioned[1,2].

Iodine-131 serum albumin or iodine-131 autologous cerebrospinal fluid has been injected intraventricularly and intrathecally in more than fifty patients for various diagnostic reasons[3–5]. External scintillation scans of the head are performed at various time-intervals. In this way the γ-emitter is followed in its movement through the cerebrospinal fluid pathways (Fig. 1). The labelled compound, injected intraventricularly, begins to flow into the basal cisterns within a few minutes. From the basal cisterns the activity proceeds, mainly through the anterior subarachnoid paths and the Sylvian fissures, to the convexity of the brain. At 12–24 h, most, and sometimes all, of the activity is detectable along the superior longitudinal sinus. The tracer, when injected in the lumbar area, is detected in the basal cisterns in about 1 h and from here it follows the same pattern as above. It has been calculated that 20–33 per cent of the activity injected intrathecally reaches the endocranium within 12 h. Considerable activity remains in the lumbosacral cul-de-sac even at 24 h. Positioning and the physical activities of the patient between scans do not affect the pattern of spreading. Where pathological localized blocks of the free movement of the cerebrospinal fluid occur, the radioactive material follows remaining open routes to reach the hemispheric convexities. In such instances, the radioactivity in the open pathways, and in the obstructed route proximal to the block, is in higher concentration. These data suggest a 'transport' or 'sweeping' phenomenon. Although an ebb-and-flow component may be present, there is definitely a net forward progression of the cerebrospinal fluid. Technetium 99*m* pertechnate (mol. wt. 163) injected intraventricularly in one, and intrathecally in three patients, failed to be transported with the cerebrospinal fluid but was obviously resorbed locally at the site of injection.

It is probably true that the cerebrospinal fluid is formed everywhere and resorbed everywhere in the cerebrospinal fluid cavities. It is also true, however, that at least part of this fluid moves in a flow-like fashion towards the hemispheric convexities, possibly due to the vascular[6–8]

and choroid plexus pulsations[9], plus the *vis a tergo* of the newly produced fluid.

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HÆMATOLOGY

Abnormal Hæmoglobin and Glucose-6-phosphate Dehydrogenase Deficiency in Malayan Aborigines

THE aborigines in Malaya form a distinct group of the Malayan population. They live more or less separated from the other groups and are genetically as well as socially entirely different from the group at present known as Malays. They are divided racially, according to William-Hunt[1], into the Negritos, the Senoi and the aboriginal Malays. The Senoi group is the largest and consists of many different sub-groups, of which the Temiar (or Temer), Semai and Temuan are the most important. Of the aboriginal Malays, the Jakun and the Semelai are the largest sub-groups. This classification is not universally accepted, but it forms a convenient basis for further discussions. Most of the aborigines live in the jungle, where the frequency of parasitic and tropical diseases is high and where infant mortality from malaria and dysentery is high.

Recently much interest has been aroused for the investigation of the distributions of abnormal hæmoglobins and G-6-P dehydrogenase deficiency especially in association with malaria. Because of the distinct characteristics of the aborigines from the genetic point of view as well as from their way of living and their environmental challenges, it was thought to be of interest to examine the frequencies of abnormal hæmoglobins and G-6-P dehydrogenase in these aboriginal groups. Vella[2] found a high frequency of hæmoglobin E (29 per cent) in 41 Semelai examined. G-6-P dehydrogenase deficiency has not previously been reported in Malayan aborigines.

Healthy aborigines, predominantly Senoi, were obtained from the Senoi military camp in Kuala Lumpur and staff personnel of the Ulu Gombak Aborigines Hospital. The soldiers were recruited from the jungle in Central and Northern Malaya. A group of patients with miscellaneous diseases, mostly tuberculosis, were also obtained from the Ulu Gombak Aborigines Hospital, which serves aborigines from all parts of Malaya.

The dye method of Motulsky and Campbell[3], taking a clearance time of more than 120 min as abnormal, was used as a screening test. Intravenous blood was obtained from the soldiers, while blood was obtained from the finger from patients. Each positive test was repeated on an intravenous blood sample and the methæmoglobin reduction test of Brewer *et al.*[4] or the glutathione stability test of Beutler[5] or both were also performed.

Hæmoglobin analysis was carried out by the paper electrophoretic method described by Smith and Conley[6].

A total of 278 male and female aborigines were examined for abnormal hæmoglobin and 607 males examined for G-6-P dehydrogenase. The results are presented in Tables 1 and 2. Part of the result of the investigation on abnormal hæmoglobin in Malayan aborigines has been reported by Lie-Injo Luan Eng[7].

Of 154 healthy aborigines, 29·2 per cent had abnormal hæmoglobin E, while of 124 patients 25·8 per cent carried abnormal hæmoglobin E in the blood. The frequency for the total 278 examined was 27·7 per cent. The frequency for hæmoglobin E in the Semai (26·5 per cent) seems to be about as high as had been found by Vella[2] for 41 Semelai aboriginal Malays examined (29 per cent). The Temiar group, however, had a very high frequency, above 45 per cent in healthy as well as in hospital patients. It would be interesting to examine the abnormal hæmoglobins in the Negritos, the third main group of Malayan aborigines.

Of 607 persons examined for G-6-P dehydrogenase, 12·2 per cent of 197 healthy persons and 19·3 per cent of 410 patients were deficient, giving a frequency of 17·0 per cent for the total group. The frequency is, in general,

Table 1. ABNORMAL HÆMOGLOBIN IN MALAYAN ABORIGINES

Main ethnic group	Sub-group	Healthy persons				Patients				Healthy persons + patients			
		No.	Hb A+E	Hb E	% Abnormal	No.	Hb A+E	Hb E	% Abnormal	No.	Hb A+E	Hb E	% Abnormal
Senoi	Semai	68	14	4	26·5	43	12	0	27·9	111	26	4	27·0
	Temiar	44	11	9	45·5	18	7	2	50·0	62	18	11	46·8
	Temuan	18	0	0	—	19	3	0	—	37	3	0	8·1
Aboriginal Malay	Semelai	4	2	0	—	25	4	0	—	29	6	0	20·7
	Jakun	4	0	0	—	5	0	0	—	9	0	0	—
	Lanoh	—	—	—	—	1	0	0	—	1	—	—	—
Unknown		16	4	1	—	13	4	0	—	29	8	1	31·0
All ethnic groups		154	31	14	29·2	124	30	2	25·8	278	61	16	27·7

Table 2. G-6-P-DEHYDROGENASE DEFICIENCY IN MALAYAN ABORIGINES

Main ethnic group	Sub-group	Healthy persons			Patients			Healthy persons + patients		
		No. examined	Deficient	%	No. examined	Deficient	%	No. examined	Deficient	%
Aboriginal Malay	Semelai	4	1	—	52	4	7·7	56	5	8·9
Senoi	Semai	76	14	18·4	105	26	24·8	181	40	22·1
	Temiar	44	3	6·8	93	22	23·7	137	25	18·2
	Temuan	21	3	14·3	93	15	16·1	114	18	15·8
	Jahut	1	0	—	12	3	25·0	13	3	23·1
	Jakun	4	0	—	—	—	—	4	0	—
Unknown	—	47	3	6·4	55	9	16·4	102	12	11·8
All ethnic groups		197	24	12·2	410	79	19·3	607	103	17·0

high in all aboriginal groups, but it seems to be much higher in the Senoi than in the aboriginal Malays. However, the number of each group examined was not large enough to warrant a definite conclusion. The frequency in patients is definitely higher than that in healthy persons. Whether this is due to the inclusion of patients admitted to the hospital in actual hæmolytic crisis due to G-6-P dehydrogenase deficiency or whether G-6-P dehydrogenase-deficient persons are more susceptible to certain diseases cannot be said. As we also mentioned in other papers[8], there seems to be a definite correlation between the frequencies of hæmoglobin E and those of G-6-P dehydrogenase deficiency in different areas in South-east Asia. It has been suggested from observations in other areas of the world that malaria plays a part in the causation of this correlation, due to the simultaneous protection it gives to carriers of certain abnormal hæmoglobin genes and the G-6-P dehydrogenase gene[3,9]. That malaria plays a part in the high frequency of abnormal hæmoglobin and G-6-P dehydrogenase deficiency in the aborigines in Malaya is a possibility which certainly has to be kept in mind, since indeed the infant mortality due to malaria has always been high in the aborigines. However, more detailed investigations have to be carried out before conclusions can be made, since some other factor or factors may have played a part as well. Inbreeding may be an important factor to account for the high frequencies of the aforementioned hereditary abnormalities and should certainly be investigated further.

We are grateful to the Senoi Praaq Regiment and the commanding officer, Enche Mohamed Ruslan bin Abdullah, for permission to obtain blood samples from his men, and also to Enche bin Abdullah bin Sainy, of the same unit, for his assistance. We also thank Dr. J. M. Bolton, director of the Ulu Gombak Hospital, for permission to obtain blood from the personnel and patients of the hospital.

This work was supported by the U.S. Public Health Service research grant *GM*–11329–03 from the ICMRT Program, Office of International Research, National Institutes of Health, U.S. Public Health Service.

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Coenzyme Q_0 Derivative from Fibrin Clots

During my investigation of clotting and lytic factors from fibrin clots, I observed that on boiling in 1 N hydrochloric acid the clots disintegrated and consistently turned a faintly purple colour. Attempts to extract the pigment from the fibrin residue were unsuccessful. To learn more about this compound, 1 l. of normal citrated human plasma was recalcified, the fibrin digested with 1,000,000 units of crystalline bovine trypsin in distilled water at 37° C overnight, and the undigested sediment, making up about 0·5 ml., washed 3 times in saline and once in distilled water. 2 ml. of 1 N hydrochloric acid were then added, and the mixture was placed in a boiling water bath for 10 min. The change in colour of the sediment was again noted, yet following centrifugation some pigment was also seen in the supernatant. It was slightly reddish, but on neutralization with sodium hydroxide became yellow. After drying in the presence of the salt, the pigment could not be taken up in any organic solvent except methanol, and in it only partially. Since chromatographic separation proved possible, strong adsorption to solids appeared to be one of its characteristics. The partially purified pigment in distilled water gave a distinct ultra-violet spectrum, with a minimum at 250 mμ and a maximum at 265 mμ. Some pigment was liberated from digested fibrin with β-glucuronidase, but the amount found in the supernatant was much smaller than when boiling with acid was carried out.

In a previous communication[1], a menadione derivative had been reported to be present in the supernatants of digested fibrin clots, partly uncombined and partly conjugated with glucuronic acid. It thus seemed reasonable to speculate that the pigment here encountered might be derived from a cell constituent of similar chemical constitution. All cells contain ubiquinones or coenzymes Q with isoprenoid chains of various lengths in the 6-position. To prepare from them a compound analogous to the vitamin K derivative[1], only coenzyme Q_0, 2,3-methoxy-5-methyl-1,4-benzoquinone, could be used. It was dissolved in benzene and sprayed on Whatman No. 1 filter paper, where it produced a deeply yellow colour after evaporation of the solvent. The colour disappeared at a rate proportional to the saturation of the atmosphere with water vapour. Oxygen or light was not necessary for the conversion. Filter papers were extracted with hot, distilled water. Desiccation left a yellowish powder, which displayed the same solubility characteristics as the pigment from fibrin clots. Their ultra-violet spectra were identical, and also their diffusion rates on Whatman No. 1 filter paper with isobutanol, acetone and distilled water in equal proportion as solvent.

The type of transformation undergone by menadione and coenzyme Q_0 appears to be dimerization. The properties of a colourless photodimer of menadione have been described in some detail[2]. It was prepared according to instructions for comparison with the orange pigment derived from the same source[1]. The latter differed from the photodimer in being soluble in alkali and ether. However, their ultra-violet spectra appeared to be the same, and their infra-red spectra differed only in one stretch at 3·4μ, given by the orange, but not the colourless, compound—a deviation still requiring chemical interpretation. Dimerization of quinones, which consists of the formation of *cyclo*butane rings between two molecules, may be the function of an enzyme secreted by blood platelets. This could explain their dual role in blood clotting and activation of fibrinolysin[3], and perhaps also the anticoagulant effect of such compounds as dicoumarol and diphthiocol, in which two molecules of 3-methyl-4-hydroxycoumarin or phthiocol, both known to have some vitamin K activity, are bound together by a methylene bridge instead of being dimerized.

The derivative prepared from coenzyme Q_0, like the analogous menadione compound, did not affect fibrin formation or decomposition *in vitro*. Following injection into an animal, however, some of it might have been expected to be conjugated with glucuronic acid in the liver, thus inducing distinct physiological effects. White mice, weighing 20–25 g, were injected subcutaneously with 0·5 mg of pigment in 0·1 ml. of distilled water and controls with equal amounts of normal saline. They proved not to be suitable for differential fibrinolysin tests since they normally have a fairly high serum titre of this substance.

Observations made in some, but not all, animals under deep ether anæsthesia 2 h after injection of the pigment included congestion of the skin blood vessels and occasional spontaneous hæmorrhages. The only consistent finding differentiating test animals from controls was a rise in the neutrophil/lymphocyte ratio. Blood was obtained at intervals from the tail vein by cutting off a portion of the tail, which prevented healing for several hours; or blood from the tail was used only for the first sample, while the second sample was taken from the severed jugular vein of the sacrificed animal. With the first method, there was a considerable increase in neutrophils even in controls in response to the injury inflicted. Greatest changes were generally recorded 2 h after the original injection. Before injection the average neutrophil/lymphocyte ratio was 1/4. In control animals it rose to 2/3–4/5 when the first method was used and remained unchanged by the second. The respective figures in test animals were 3/1–4/1 and 2/3. Experiments with larger animals will be carried out as soon as greater amounts of coenzyme Q_9 derivative can be prepared.

This work was supported in part by a grant from the Kansas Heart Association. I thank Dr. K. Conrow, Department of Chemistry, Kansas State University, Manhattan, for his advice on the chemical aspects of this work, and Dr. M. Tishler, Merck Sharp and Dohme Research Laboratories, Rahway, N.J., for a gift of coenzyme Q_9.

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Reticulo-endothelial Cells as 'Nurses' for Plasma Cells and Erythroblasts in Baboons

BABOON (*Papio anubis*) marrows were taken from either the sternum or iliac crest stained with Leishman and Giemsa and examined as thin smears. In the majority of these smears nests of plasma cells as well as early erythroblasts were seen surrounding a central reticulo-endothelial cell (Fig. 1).

The arrangements of these plasma cells around the reticulo-endothelial cell resembled the erythroblast islands around the central reticulo-endothelial cell described by Bessis[1], Bessis and Breton-Gorius[2], and Policard and Bessis[3], in their electron microscope investigations of hypersideræmic anæmias (Fig. 2).

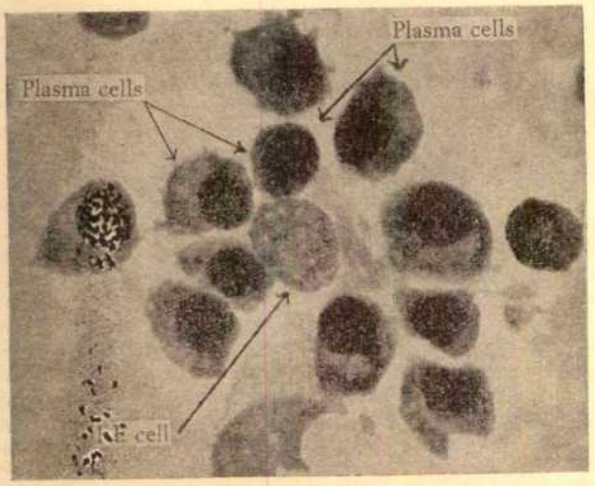


Fig. 1

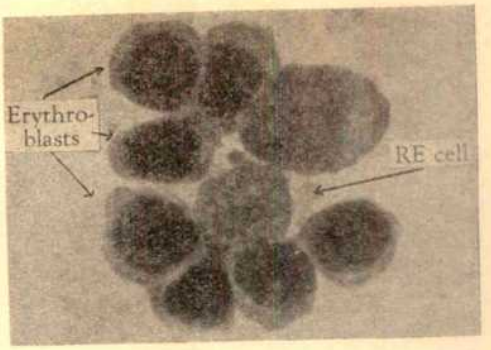


Fig. 2

Bessis *et al.* have suggested that a transfer of ferritin molecules occurs by pinocytosis across the adjacent boundary between the reticulo-endothelial cell and the erythroblasts.

If such a transfer of ferritin does occur we wonder whether any exchanges are going on between the reticulo-endothelial cells and the surrounding plasma cells described here. Plasma cells are thought to be concerned with various immunological processes, and the possibility exists that a transfer of 'immunological bodies' or their precursors may be occurring.

In some of the baboons infections such as diarrhœa, pneumonia or *Plasmodium kochi* were present. Other animals were, however, normal.

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HISTOCHEMISTRY

Electron-microscopical Localization of Products from Histochemical Reactions used to detect Cholinesterase in Muscle

IN 1949 Koelle and Friedenwald[1] described a procedure to determine the sites of cholinesterase activity in tissues. Essentially, the method depends on the hydrolysis of acetylthiocholine, or similar esters, by the tissue enzyme and the formation at or near the site of enzymatic activity of a precipitate due to the combination of copper ions and the split thiocholine. Recently this and other methods have been used to determine the electron-microscopical localization of the enzyme in mammalian muscle[2–6].

In the experiments recorded here, the frog sartorius muscle, fixed in glutaraldehyde, was incubated for 20 min–7 h in a medium containing acetylthiocholine. After incubation the muscle was washed briefly and 'developed' in ammonium sulphide (cf. Couteaux and Taxi[7] for further details). Small specimens from the muscle were then further fixed in osmic acid, dehydrated in ethanol and embedded in 'Araldite'. Sections of this material were examined unstained or stained with lead citrate.

It was found that this treatment produced fine electron-dense particles (10–100 Å) which were scattered throughout the muscle fibre. A marked concentration of these particles occurred in certain parts of the endoplasmic reticulum, namely in the centre element of the triads and in some longitudinal tubules (150–200 Å wide) which run parallel to the myofibrils and seem to be connected to the central elements. Occasionally, these tubules, densely filled with

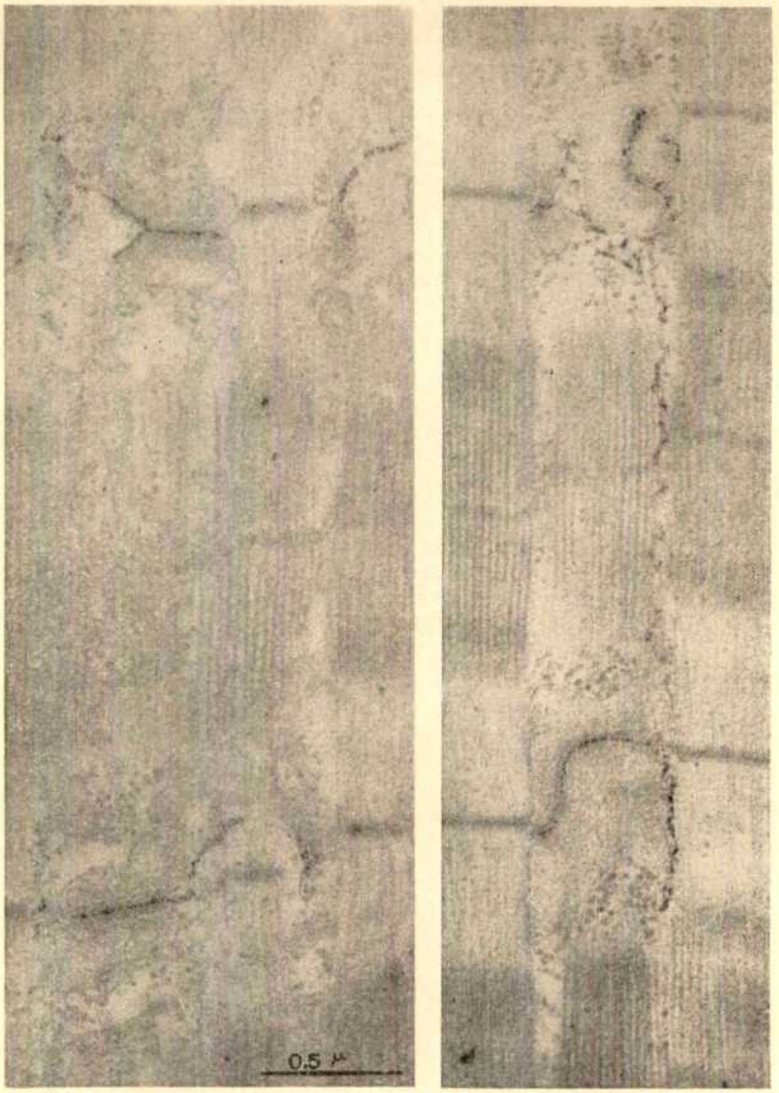


Fig. 1. Particles in the endoplasmic reticulum of two muscle fibres

the particles, can be traced throughout the whole length of a sarcomere (about 2·5μ). Dense particles can also be seen in myelin like figures and tubules which occur frequently near muscle nuclei, and occasionally in mitochondria, resembling the accumulation of metal ions described by Peachey[3].

With incubations of more than 1 h, the synaptic cleft at the nerve muscle junction was completely filled with the precipitate. Vesicles lying immediately under the muscle membrane in the synaptic and juxta-synaptic regions were also densely filled with the material. Fine particles were distributed generally inside the nerve terminal, but were specially concentrated in the synaptic vesicles. The distribution resembles that previously reported by Barrnett[5] (see also refs. 2–6). Particles were also concentrated in the basement membrane of the muscle fibre in the region surrounding the neuromuscular junction, and in the basement membrane of neighbouring muscle fibres, even though they did not contain myoneural junctions. This almost certainly indicates that there can be appreciable diffusion of the products, since no similar accumulation was seen in the basement membrane of a muscle fibre a few hundred μ away from the synaptic region. At the myotendinous junction, where cholinesterase is also concentrated[9,10], the electron-microscopical distribution of the precipitate resembles closely that at the nerve-muscle junction.

If the treatment with ammonium sulphide is omitted the Cu-thiocholine complex can be washed away i phosphate buffer (*p*H 7–7·4) and during the subsequen dehydration with ethanol. With this procedure particle which previously were seen to be diffusively distribute throughout the muscle are almost completely removed But the special accumulation in the central element o the triad and its associated longitudinal tubules is sti clearly visible (Fig. 1). This pattern resembles th distribution of ferritin particles recently described b H. E. Huxley[11].

At the neuromuscular junction, the very dense precip tate in the synaptic cleft is also removed, leaving in th gap only a few scattered particles. In the nerve ending essentially only the particles associated with synapti vesicles are left (Fig. 2). On close examination it appear as if some of the particles were stuck to the membrane, an not inside the synaptic vesicle.

One question that is raised by these findings concern the significance of the accumulation of particles i the transverse and longitudinal tubules of the triad in the muscle. Obviously, this could mean that these ar actual sites of cholinesterase activity, which would accoun at least partly for the cholinesterase activity found i muscle fibres away from the myoneural junction[12,1] But, in view of the demonstrable diffusibility of th precipitate, it remains still possible that the acetylthio

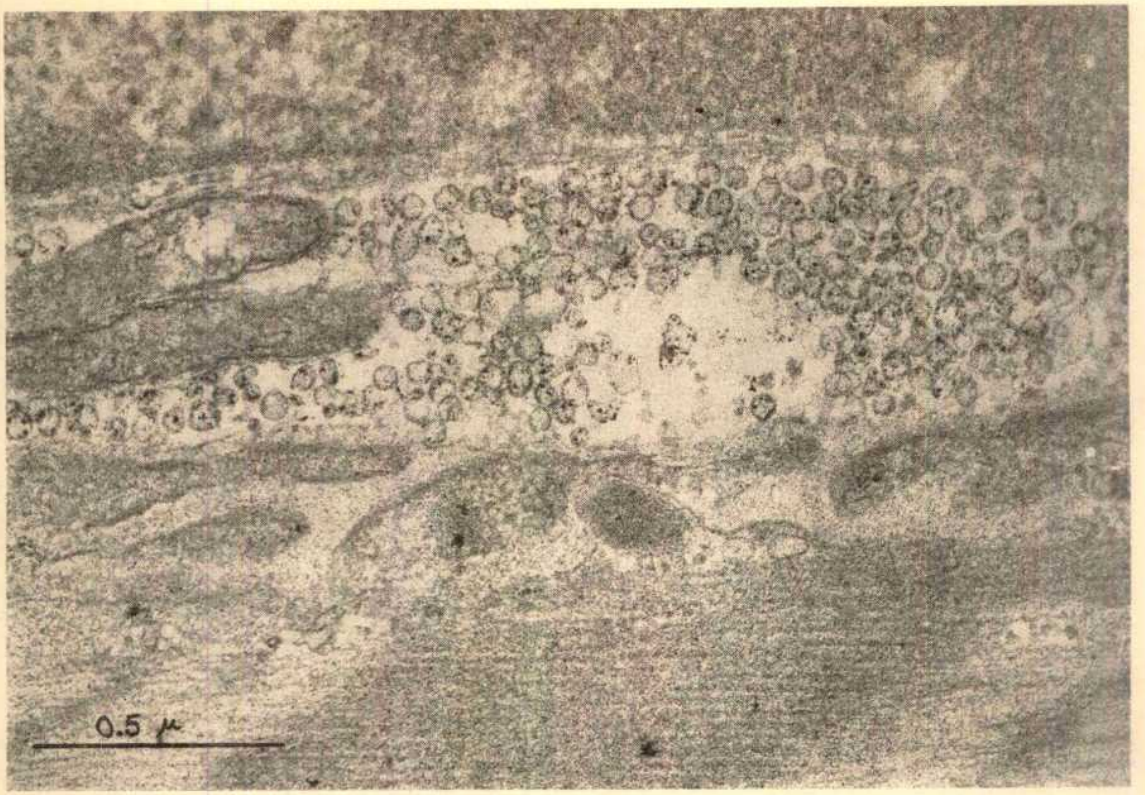


Fig. 2. Neuromuscular junction. Muscle fibre below, axon at centre and Schwann cell nucleus at top of picture. Notice the particles in the synaptic vesicles inside the axon

choline is split somewhere else within the muscle, for example by myosincholinesterase[14], and that the products are secondarily retained by the endoplasmic reticulum. Similar alternatives could explain the presence of particles in the synaptic vesicles.

I thank Miss J. Bott for her assistance.

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Histochemical Evidence on Localization of Succinic Dehydrogenase in the Synapse of the Central Nervous System of Rabbit, Rat, and Guinea-pig

Succinic dehydrogenase activity of the normal spinal motor neurone was demonstrated as spotted in type at the cell membrane of the motor neurone in the spinal cord[1] of the guinea-pig.

This communication describes the localization of succinic dehydrogenase activity on the surface of various types of neurones in the medulla oblongata, pons, mesencephalon, cerebellum and cerebral cortex of the adult rabbit, guinea-pig and rat. The same staining procedure as that of a previous investigation was used[1].

The spotted succinic dehydrogenase reaction, which appears at the outer surface of the cell bodies, was demonstrated in the multipolar cells of the reticular formation (Figs. 1 and 2), the nerve cells of the motor nucleus of the trigeminal nerve and the cells of origin of the motor fibres of the oculomotor nerves. In addition, the cytoplasmic succinic dehydrogenase activity in these cells showed a homogeneous, moderately positive reaction.

All animals showed less spotted enzyme activity at the surface of cell bodies of the superior olivary nucleus (Fig. 3), the inferior colliculus (Fig. 4) and the mesencephalic nucleus of the trigeminal nerve (Fig. 5).

This was especially true in the mesencephalic nucleus of the trigeminal nerve and the cerebral cortex in which it was not possible to visualize the spotted succinic dehydrogenase activity at the outer surface of the cell bodies at all.

According to Smythies *et al.*[2], the boutons in the cerebral cortex do not resemble those in the spinal cord and are not closely applied to the surface of the cell body.

Succinic dehydrogenase activity was strong to moderately positive in the neurophil of the cerebral cortex and in the intracellular space which includes the neuroglia. Histochemical demonstration of spotted succinic dehydrogenase activity at the walls of the cell bodies in the cerebral cortex was indistinct.

The localization of succinic dehydrogenase activity on the walls of the nerve cell body was accompanied by a similar localization at the walls of blood capillaries in the brain. As shown in Figs. 6 and 7, the spotted enzyme activity was arranged along the walls of blood vessels and at axon surfaces. These findings also occurred in the medulla oblongata, pons and mesencephalon in all animals. The localization of succinic dehydrogenase in the Purkinje layer of the cerebellum was variable (Fig. 8). The spotted succinic dehydrogenase activity of the Purkinje layer in the cerebellar cortex differs from that of the medulla

oblongata, pons, etc. The spotted positive reaction for succinic dehydrogenase showed a variable pattern as well as aggregates in the intracellular space in the Purkinje layer, surrounding the cell body, and in the nearby granular layer. From a histochemical point of view, the foregoing shows spotted localization of succinic dehydrogenase. This is associated with the synapses of Purkinje cells as seen with silver impregnation, as well as the network or basket formation from the basket cells (cf. ref. 3).

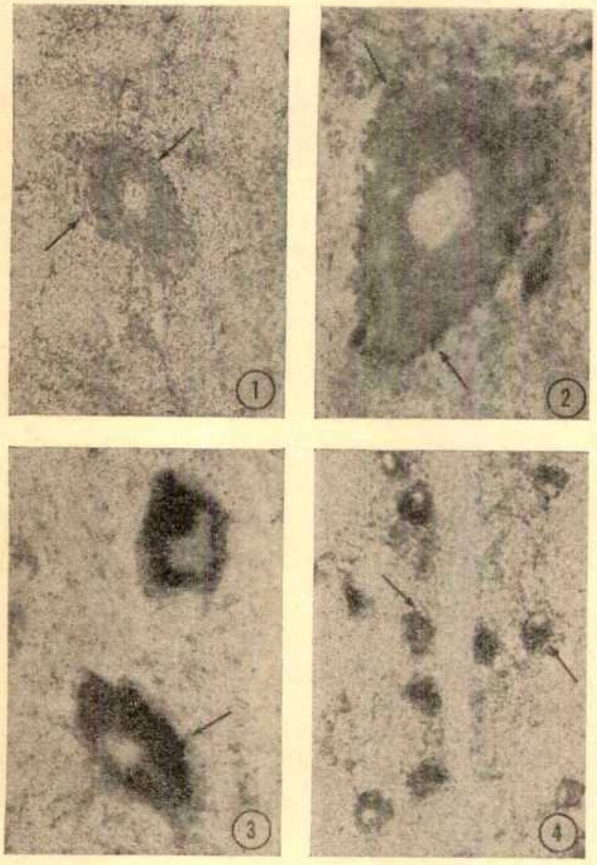
In electron-microscope observations on the synapse, the bouton terminaux or end-feet were shown to contain numerous mitochondria[4–8].

The spotted succinic dehydrogenase activity at the walls of the nerve cell bodies probably demonstrates the enzyme activity of synaptic mitochondria.

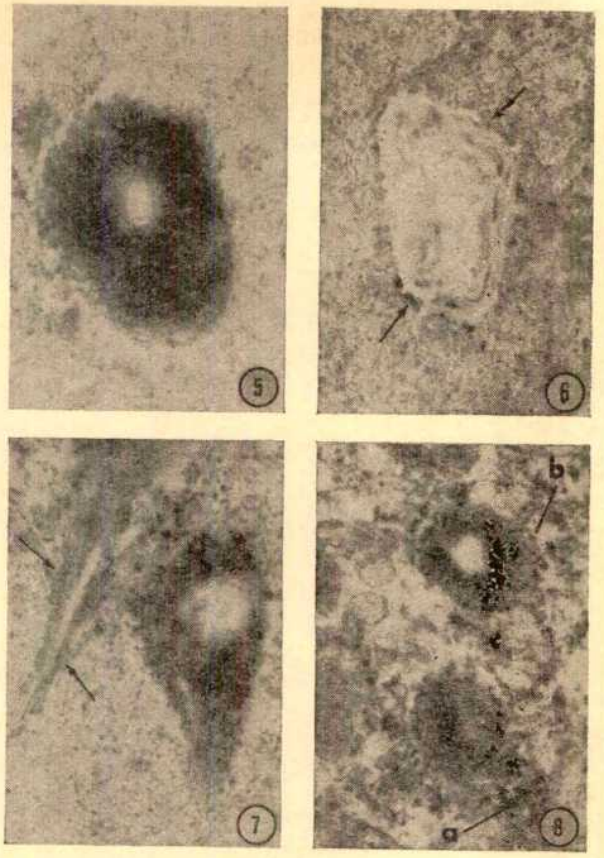
Friede[9] demonstrated spotted succinic dehydrogenase activity of the cell membrane in chromatolytic cells in the nuclei of origin of the vestibulospinal and reticulospinal fibres. Our observations were interesting in showing that succinic dehydrogenase activity in neurones was located at the membranes of the cell bodies and their axons and in the walls of blood capillaries in the normal brain.

Glycogen and other polysaccharides can also be found at the membranes of the nerve cell[10–12].

The higher levels of spotty synaptic succinic dehydrogenase activity seem obviously to be correlated with glycolysis of synaptic glycogen and thus play a part in the energy changes concerned with impulse transmission.



Figs. 1–4. 1, Succinic dehydrogenase activity in a multipolar cell of the reticular formation of rabbit. Note spotty reaction (arrows) on outer surface of cell. Cytoplasmic reaction is moderately positive (× *c.* 170). 2, Succinic dehydrogenase activity in multipolar cell of reticular formation of guinea-pig. Note numerous spotty positive areas (arrows) on outer surface of cell body (× *c.* 660). 3, Succinic dehydrogenase activity of the cells in the superior olivary nucleus of guinea-pig. Note strong positive reaction in the cytoplasm and spotty succinic dehydrogenase activity on outer surface (arrow) (× *c.* 660). 4, Succinic dehydrogenase activity of the small cells of the nucleus of the oculomotor nerve of rabbit. Note strong positive reaction in cytoplasm and spotty activity on outer surface of cells (arrows) (× *c.* 100)



Figs. 5–8. 5, Succinic dehydrogenase activity in the cell of the mesencephalic nucleus of the trigeminal nerve of rat. Note strong positive reaction in the cytoplasm and much fainter reaction on the outer surface of the cell body (× *c.* 660). 6, Succinic dehydrogenase activity on the walls of blood vessel in the medulla oblongata of rat. Note spotty positive areas on wall (arrows) (× *c.* 660). 7, Succinic dehydrogenase activity at the surface of axon and cell body in pons of guinea-pig. Note spotted positive reaction along the axon and surface of cell body (arrows) (× *c.* 660). 8, Succinic dehydrogenase activity in the Purkinje layer in cerebellum of guinea-pig. Note spotty reaction for the enzyme. The spots are aggregated in the intracellular space (*a*) and surround the cell body (*b*) (× *c.* 660)

This work was supported by grant *NB*–01914 from the U.S. National Institute of Neurological Diseases and Blindness.

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BIOLOGY

Structural and Enzymatic Changes in Muscle due to Ageing

So far, there has been no systematic and detailed investigation on the enzymes and their changes with ageing. The activity of various enzymes was found to change with ageing in different ways[1]. No change occurs

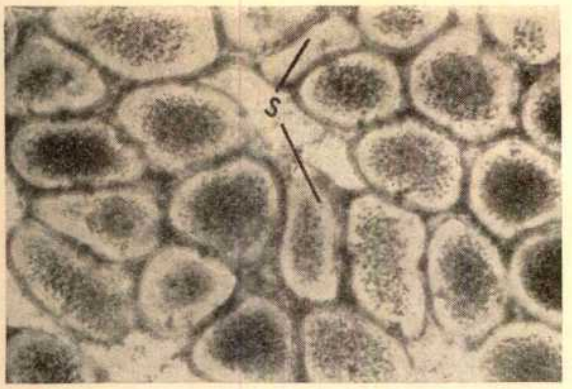


Fig. 1. Photomicrograph of the cross-section of the fibres of the human spinal muscle, showing the localization of lipase activity. Note the high degree of variation in the shape of the fibres. The compressed fibres with negligible enzymatic activity are also seen (*S*)

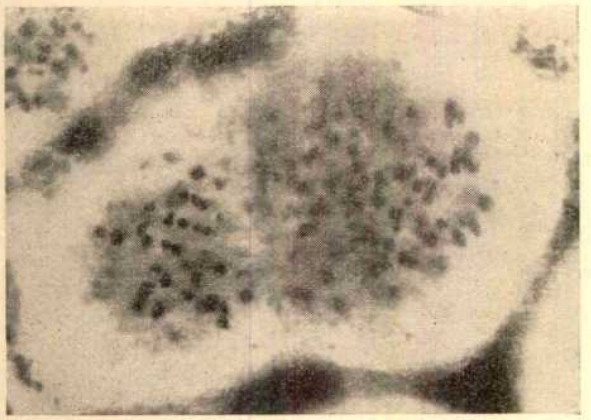


Fig. 2. A single fibre enlarged. Note the outer margin encircling the central active zone

in certain tissues, while in others the activity increases or decreases with ageing. A detailed investigation was therefore designed on the cellular and enzymatic changes in the human muscle.

The human spinal muscle of an individual 47 years old was the material for the investigation recorded here. The lipid-hydrolysing enzyme was localized histochemically[2] in the fibres.

The lipase activity is localized as brownish black precipitates of lead sulphide in the fibres (Fig. 1). The enzymatic activity is localized more or less uniformly except for a few fibres where the activity is negligible. A high degree of variation in the shape of the fibres has also been observed. The enzymatic activity is mainly restricted towards the centre of each of the component fibres, leaving an outer margin (Fig. 2). The outer margin with negligible enzymatic activity is nothing but the thickening or the condensation of the endomysium. The endomysial connective tissue sheath encroaches more and more, thus diminishing the active sarcoplasm—a feature which becomes prominent during ageing.

The fibres with negligible enzymatic activity are few in number, much compressed and shrunken in nature. These fibres are not a common appearance in the normal developing muscle or in fully grown and mature muscle. However, the changes in the fibre nature and the enzymatic activity in no circumstance can be due to defects in fixation. If it were due to any neuromuscular disorder, fibre degeneration would occur; but the degenerating fibres are never found singly. A cluster of fibres or the fibres of a fasciculi become affected. Since the pattern of degeneration is altogether different in the present investigation, the possibility of a neuromuscular disorder is also ruled out.

The negligible enzymatic activity in these compressed fibres indicates the inefficiency of esterifying and utilizing fat for energy. The compressed fibres with decreasing and depleting enzymatic activity are the degenerating fibres due to advancing age. Disturbances in energy metabolism as an initial phenomenon in ageing have been reported[3]. The ability of the muscle fibre to divide during old age for the replacement of the degenerated and perished cells was also observed in the present investigation[4]. The structural and enzymatic changes that have been observed in the present attempt are, therefore, considered to be the effect of ageing on muscle fibres.

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Identification of a Kinetin-like Substance from Pea Seedlings, *Pisum sativum*

The importance of kinetin and related compounds in controlling different physiological processes in higher plants has been widely recognized, although attempts to isolate and identify such substances in plant material have so far failed[1]. Chromatographic separation of pea seedling extract yielded some pertinent information which is reported here.

English peas, variety 'Laxton's Progress', were grown in a flat of sterilized soil for 17 days before they were harvested. Four thousand plants were included in each extraction and the whole experiment was repeated twice. The plants were gathered and divided into three parts (top, middle and bottom). The plant parts were cut into small pieces and were then homogenized in a Virtis apparatus for 10 min with 10 per cent hydrochloric acid. The homogenate was filtered under a high vacuum pump and concentrated in a Rinco evaporator. The solid evaporated samples thus obtained were dissolved with 10 ml. of distilled water. The crude extracts were then separated by means of descending chromatography using a solvent system consisting of iso-propanol, distilled water, and concentrated hydrochloric acid (130 : 37 : 33). Known concentrations of kinetin (obtained from California Biochemical Research) and adenine (100 p.p.m.) were used as controls, along with the unknowns for identification. The R_F values were determined by developing the paper with location reagent consisting of 2 per cent silver nitrate and 0·4 per cent bromophenol blue (v/v). The blue-coloured spots thus obtained were eluted with 10 ml. of absolute ethyl alcohol and the concentrations of the eluted samples were determined by colorimetric tests using a Coleman Junior Spectrophotometer at a wave-length of 650 mμ, with known concentration of kinetin as the standard. A bioassay test was attempted with *Raphanus sativus* leaf disk[2], but it was not successful.

The plant extract from the top parts gave a comparable spot on the chromatogram which had the same R_F value as kinetin (0·88). Two more spots were developed which occupied the same region as adenine in the control. However, the kinetin spot was not clear in extracts of middle and bottom parts, although in these there was a faint suggestion of its presence in the extract from the middle portions. All three were eluted in alcohol and the concentration was determined as described earlier. The results obtained are presented in Table 1.

The top portion gave a 3–5 p.p.m. concentration of kinetin-like substances, whereas the middle portion gave less than 1·0 p.p.m. and the bottom none. This suggests that the presence of kinetin-like substances may be restricted to growing regions only.

Table 1. Concentrations of Kinetin-like Substances (p.p.m.) in Pea Seedlings as Determined by Colorimetric Tests*

	Kinetin-like substances in Top portions	Middle portions	Bottom portions
English pea, variety 'Laxton's Progress'	3·0–5·0	Less than 1·0	None

* Results include both replications.

This work gives some indication as to the presence of kinetin-like substances in higher plants. It is found that these substances are mainly present in the top portions of the plants. However, the chemical identification requires an elaborate investigation.

I thank Prof. B. C. Pal of this Institute for his advice.

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Effect of the Implantation Site on the Development of Grafted Limb Bud Mesoderm in Chick Embryos

A small rectangular block of the mesoderm immediately subjacent to the superficial venous plexus was excised from the wing bud apex in stages 25–26 of chick embryos and replaced with an isolate of mesoderm from the prospective thigh or proximal foreleg region. The graft extended from the dorsal to the ventral surface of the wing bud apex and did not lie in contact with the apical ridge of the ectoderm; it was separated from the latter structure by the mesoderm intervening between the apical ectoderm and the superficial venous plexus, namely, by about 15–20 layers of mesodermal cells of the wing bud (histological control).

Embryos at stages 25–26 only were used for the two following reasons: (*a*) according to the supporters of the thesis of a return inductor activity exerted by the apical ridge, the distal territories of the limb would be "capable of differentiation independently of the influence of the apical ectoderm" by stage 24 (ref. 2); (*b*) the graft inserted at the limb bud apex at stages 25–26 maintains its distal position and is not displaced to more proximal regions in consequence of the distal growth of the bud as occurs when younger stages are used.

In the majority of the operated embryos the more or less complete skeleton of one or two toes and 1–3 fingers of the hand formed in the terminal part of the wing. The toes are covered distally with scales associated with feathers and end with a claw (Fig. 1). The mentioned result is not related to any special orientation of the axes of the grafted material in respect to the axes of the host wing bud. The terminal foot structures in the wing seem to develop from the grafted proximal material of the hind-limb bud; however, the possibility that mesenchymal cells from the wing area surrounding the graft may invade the latter and take part in the development of the foot rays cannot be ruled out at present.

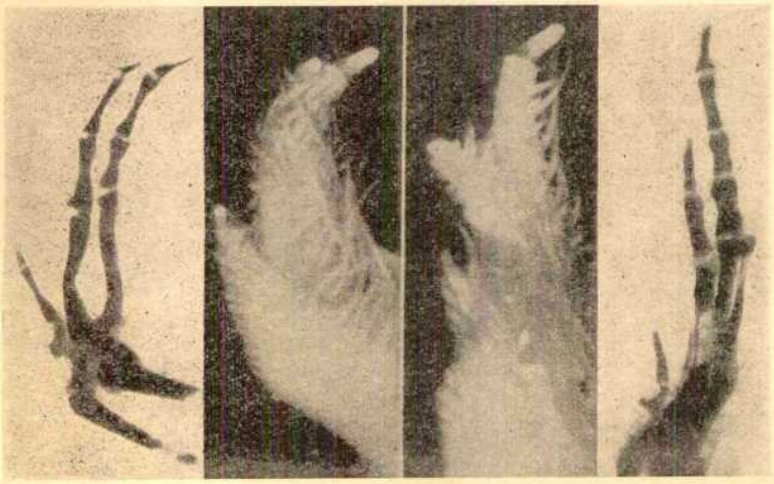


Fig. 1. Embryos of 13 days operated at stage 25–25·5. Notice the phalanges and the claw of the toes developed on the host wing

These experiments and their result bear a close resemblance to those of Saunders *et al.*[1–3], who grafted a small block of prospective thigh mesoderm subjacent to the apical ectodermal ridge of the wing bud in stages 18–23 of chick embryos. Grafts which remained in contact with the apical ectoderm frequently formed typical foot parts terminally on the wing; grafts separated from the apical ridge through interposition of wing mesoderm developed at wing levels proximal to the wrist and did not form toes. According to the authors mentioned, the return inductor activity of the apical ridge of the wing bud affected the regional character of morphological differentiation of the grafted thigh mesoderm and not its specific leg quality.

Our experiment indicates that changes of the regional character of morphological differentiation of prospective thigh or proximal foreleg material grafted to the wing bud apex may take place in stages 25–26 embryos independent of a contact of the grafted mesoderm with the apical ridge of ectoderm. The change in the developmental fate of the grafted hind-limb mesoderm could be related to organogenetic influences exerted by the mesenchymal territories of the wing apex directly on the material of the graft.

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ENTOMOLOGY

Occurrence of Microvilli and Micropinocytosis in Trophocyte of *Bombus*

Recent investigations have suggested that micropinocytosis plays an important part in the formation of egg yolk in insects. Roth and Porter[1] have recently described the occurrence of this process at the periphery of developing oocytes in the mosquito. Anderson[2] has observed a similar process in the oocyte of *Periplaneta*. It has been described in the small milkweed bug *Lygaeus kalmii* by Kessel and Beams[3]. The last-named workers suggest that micropinocytosis plays an important part in the formation of some yolk constituent, but it is questionable whether it is responsible for all yolk production.

Individual follicles of *Bombus* trophocytes were fixed in 1 per cent solution of osmium tetroxide at 0° C buffered with phosphate to *p*H 7–8 for 1·5 h. Following rapid dehydration in a series of cold ethanols the tissue was embedded in 'Araldite'. Sections were obtained with grey interference colours, using the Huxley microtome, and mounted on untreated grids. The sections were stained for 5 min with lead citrate[4] before being examined in an *EM6* electron microscope. Micropinocytosis occurs at the periphery of the developing oocytes of *Bombus*, where it appears to fulfil a similar role to that suggested above. At the stage of development when microvilli appear at the periphery

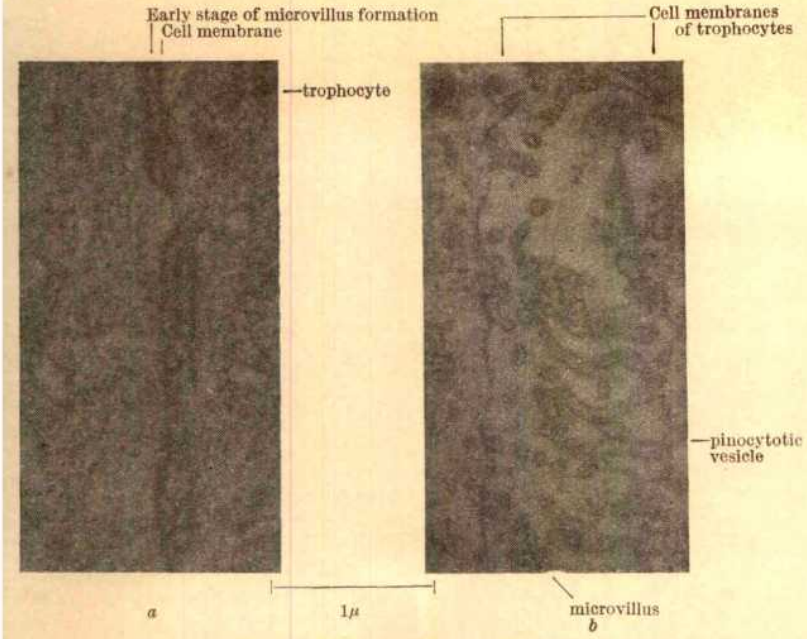


Fig. 1. Electron-micrographs of trophocyte membranes in *Bombus*

of follicle cells and micropinocytosis occurs at the periphery of the oocyte areas of the cell, membranes of the trophocytes (Fig. 1*a*) also form microvilli where they are in contact with other trophocytes or trophocyte follicular epithelium. These microvilli grow as development continues and project into spaces between the trophocytes which are formed by the cell membranes of adjacent trophocyte no longer being in contact (Fig. 1*b*). This may give a network of spaces between the trophocytes, allowing substances to be passed to them or removed from them. In the later stages of yolk synthesis micropinocytosis occurs in the pits between the microvilli. It is possible that processes occurring in these regions bear some similarity to those in the oocyte.

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Effect of Two Chemosterilants on *Glossina morsitans*

The use of sterile males offers a theoretical advantage over the use of insecticides in insect control[1]. This advantage is greater in the control of low-density pest species such as the screw-worm[2]. The sterile male technique has therefore been suggested for the control of tsetse flies, and Potts[3] examined the effects of γ-radiation on tsetse fly pupæ. Some exploratory work has now been done on the effects of two chemosterilants, 'Apholate' (2,2,4,4,6,6-hexahydro-2,2,4,4,6,6-hexa-*cis* (1-aziridinyl)-1,3,5,2,4,6-triazatriphosphorine) and 'Metepa' (*tris*(1-(2-methyl)aziridinyl) phosphine oxide) on *Glossina morsitans*.

Wild pupæ were collected in the bush at Singida and taken to Arusha, where the adult flies were allowed to emerge in a room heated to 27° by day and allowed to cool to 19° overnight. On emergence the flies were dosed topically with 0·5 μg of chemosterilant dissolved in 0·5 μl. of methyl-ethyl-ketone. After treatment the flies were kept in a room at 27° and fed on sheep every three days. Female flies were mated three days after emergence and male flies after eight days[4]. Treated flies were mated once with treated or untreated flies. Any pupæ resulting were kept to breed out a second generation.

In Table 1 are given the mean numbers of pupæ produced in each treatment by those female flies which lived more than 20 days and therefore had the opportunity of producing pupæ. It is noteworthy that treating the males caused a reduction of only some 40 per cent in the mean number of pupæ produced by the untreated females. Mating treated females with treated or untreated males produced no pupæ with 'Apholate' and very few with 'Metepa'.

Treating only the female flies with 'Apholate' shortened their mean life to 16·6 days and with 'Metepa' to 15·8 days. Untreated control females had a mean life of 34 days. The shortening effect was more marked when the treated females were mated with treated males, the mean female life using 'Apholate' being 8·8 days and with 'Metepa' 5·3 days. However, it is surprising to find that untreated females mated with treated males lived longer than the 34 days of the control group, 52 days using 'Apholate' and 46 using 'Metepa'. The mean life of males treated with 'Apholate' was 12·5 days, with 'Metepa' 13·1 days, and the life of the control-group males was 17·2 days.

Figures are given in Table 2 relating to the pupæ and adult flies produced by parents treated with chemosterilant. The numbers involved are small and the figures correspondingly unreliable. It would seem, however, that the percentage emergence was low when the male parent was treated with 'Metepa', the effect being less marked with 'Apholate'.

The action of the two chemosterilants on the pupæ was also examined. Wild pupæ were dosed on arrival with 0·1 μg of chemosterilant, placed in boxes and the flies allowed to emerge in the normal way. Of 95 pupæ dosed with 'Apholate', only 42 per cent emerged and all the flies were dead within a week. 79 per cent of a batch of 100 pupæ dosed with 'Metepa' emerged compared with 72 per cent among the controls. The male flies in the 'Metepa' group had a mean life of 7·7 days and the females 12·9 days. The corresponding life-times for control groups were 17 and 34 days.

Table 1. Pupæ produced from matings of flies treated with 0·5 μg doses of chemosterilant applied topically

Treatment	Mating	No. of females	% of females living over 20 days	No. of pupæ	Mean pupæ per female over 20 days	No. of visible abortions per female over 20 days
'Metepa'	♂ × S♀	85	19	8	0·5	0·37
'Metepa'	S♂ × ♀	12	67	14	1·75	0
'Metepa'	S♂ × S♀	78	6	3	0·6	0
'Apholate'	♂ × S♀	27	22	0	0	0
'Apholate'	S♂ × ♀	52	73	56	1·47	0·03
'Apholate'	S♂ × S♀	46	0	0	0	0
Control (undosed)	♂ × ♀	245	38	254	2·76	0·26

(S♂ or S♀ = treated fly)

Table 2. Pupæ and adult flies from parents treated with chemosterilants

Treatment	Mating of parents	No. of pupæ	Mean pupal weight (mg)	% emergence	Mean life of flies (days)
'Metepa'	S♂ × ♀	14	19·7	27	2·6
'Apholate'	S♂ × ♀	56	21·0	48	4·5
'Metepa'	S♂ × S♀	3	19·5	100	0·7
'Metepa'	♂ × S♀	8	19·9	63	4·2
Control	♂ × ♀	150	20·6	72	5·6

Two of the females from the 'Metepa'-treated pupæ lived long enough to produce pupæ of their own after mating with untreated males. One of these produced two pupæ and the second none. The surviving males were mated with untreated females, but only two lived long enough to produce pupæ, although neither did so in fact.

These exploratory experiments strongly suggest that these two chemosterilants applied topically are not likely to be useful in the practical control of tsetse flies. The treatment of pupæ, produced by a breeding colony or collected in the field, is unlikely to be effective because of the low percentage emergence when 'Apholate' is used, while with either chemosterilant the flies have a shorter life than normal. The consequences of shorter life among treated flies, even if perfectly sterilized, have been examined by Simpson[5].

Treatment of the adult males followed by their release in the bush is equivalent to the matings $\delta\delta \times \varphi$, which have been found to give only some 40 per cent reduction in fertility coupled with a shortening of the life of the flies. Furthermore, multiple mating of the females is possible in the field[6]. In consequence, these two compounds are unlikely to be suitable for field use in this way. Treatment of the resting sites of the flies in the bush would result in the sterilization of the females as well, so giving a greater effect. However, this presupposes an adequate residual life for the film of chemosterilant and disregards their possible toxicity to mammals or the ecological side-effects of their widespread use.

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Metabolism of Nicotine by Tobacco-feeding Insects

Recent experiments in this laboratory have indicated that the green peach aphid, *Myzus persicae* (Sulz.), avoids the nicotine in tobacco plants by selective feeding in the phloem[1], whereas the tobacco hornworm, *Protoparce sexta* (Johan.), excretes nicotine and other ingested alkaloids before a toxic dose can accumulate[2]. The present communication presents evidence that in addition to the foregoing mechanisms, insects feeding on tobacco may metabolize nicotine to other alkaloids. Such pathways have been previously reported from insects[3] that do not feed on tobacco and from mammals[4,5]. Comparative experiments on an insect which does not feed on tobacco, the house fly (*Musca domestica* L.), are also included.

Actively feeding, immature stages of the tobacco budworm, *Heliothis virescens* (F.), the cabbage looper, *Trichoplusia ni* (Hubner), the tobacco wireworm, *Conoderus vespertinus* (F.), and nymphs of the differential grasshopper, *Melanoplus differentialis* (Thom.), were collected on flue-cured tobacco, *Nicotiana tobacum* L., and brought into the laboratory. The insects were fed on alternate host plants for two days to prevent interference of ingested alkaloids in later chemical analysis. In each of five replicated experiments, ten larvæ were treated on the dorsum of the thorax with 200 μg of free base nicotine in 1 μl. of acetone. The larvæ were held for ten hours, killed, and placed in acidified methanol. Similar experiments were conducted with 100 cigarette beetle larvæ, *Lasioderma serricorne* (F.), which were treated with 10 μg of nicotine, and 500 2-day-old adult house flies which were treated with 5 μg of nicotine and held for 18 h.

The insects and their fæces were separated and homogenized in 25–100 ml. of acidified methanol. Nicotine and related metabolites were extracted using methods previously reported[2]. Aliquots of the final chloroform extracts were chromatographed on paper using a *tert.*-amyl alcohol solvent system[6]. The alkaloids were located by the cyanogen bromide method[6] and corresponding areas of untreated chromatograms were eluted with hydrochloric acid, filtered, and subjected to ultra-violet spectroscopy on a Beckman *DK*-2 recording spectrophotometer. No alkaloids were apparent in untreated insects subjected to identical conditions of extraction, chromatography, and spectroscopy.

The results in the experiment with budworms and cabbage loopers showed no apparent metabolism of nicotine. The only alkaloid detected had an R_F (0·19) and ultra-violet maximum (259 mμ) corresponding to nicotine. As previously reported with tobacco hornworms[3], larvæ exhibited no symptoms of nicotine poisoning. In addition, when budworms and loopers were fed on tobacco, the four alkaloids apparent in the fæces corresponded chromatographically and spectroscopically to the alkaloids in the tobacco plant.

The tobacco wireworm, cigarette beetle, differential grasshopper and house fly metabolized nicotine to 1, 2, 4, and 3 other alkaloids respectively (Table 1). In all cases at least 70 per cent of the alkaloids recovered were metabolites, the principal one corresponding chromatographically to cotinine. When grasshopper nymphs were fed on tobacco the fæces contained three alkaloids not detected in the tobacco plant. The principal metabolite again corresponded to cotinine.

Table 1. Metabolism of Nicotine by Certain Insects

These data are reported from experiments using the *t*-amyl alcohol system. Results with the butanol–ethanol–ammonium solvent system showed no significant difference. The quantities of alkaloids were estimated from the ultra-violet absorption maxima. The loss of topically applied free base nicotine from the surface of insects is frequently as high as 50 per cent due to volatilization. Recoveries of injected doses normally exceed 90 per cent

Insect	Alkaloids recovered R_F	Alkaloids recovered μg	% recovered alkaloids	% recovery
Tobacco wireworm	0·18	201	29·3	
	0·82	485	70·7	69
Cigarette beetle	0·18	90	22·5	
	0·82	290	72·5	
	0·89	20	5·0	80
Differential grasshopper	0·18	86	17·8	
	0·24	10	2·0	
	0·29	10	2·0	
	0·82	340	70·0	
	0·89	40	8·2	49
House fly	0·13	26	4·5	
	0·18	69	12·1	
	0·24	24	4·2	
	0·82	453	79·2	46
Nicotine	0·18	—	—	—
Cotinine	0·82	—	—	—

In contrast to the tobacco-feeding insects, nicotine and its metabolites were not readily excreted in the house fly, more than 90 per cent of the administered dose remaining in the insect after 18 h.

The principal metabolite from the grasshopper and the house fly corresponded to and co-chromatographed with cotinine in two solvent systems, *tert.*-amyl alcohol and butanol–ethanol–ammonium. This metabolite was further characterized by ultra-violet and infra-red spectroscopy. The ultra-violet maxima and minima corresponded closely to a known cotinine sample and agreed with data from a recent study on the metabolism of nicotine in the dog[6]. Fig. 1 shows that the infra-red spectrum of the isolated grasshopper metabolite corresponds to the known cotinine sample. The spectrum of the house fly metabolite also corresponds to cotinine, although the 3μ region is not distinct. As cotinine is hygroscopic it seems probable that interference due to water occurred.

The results of this investigation show that, in addition to the tobacco hornworm, two other lepidopterous insects

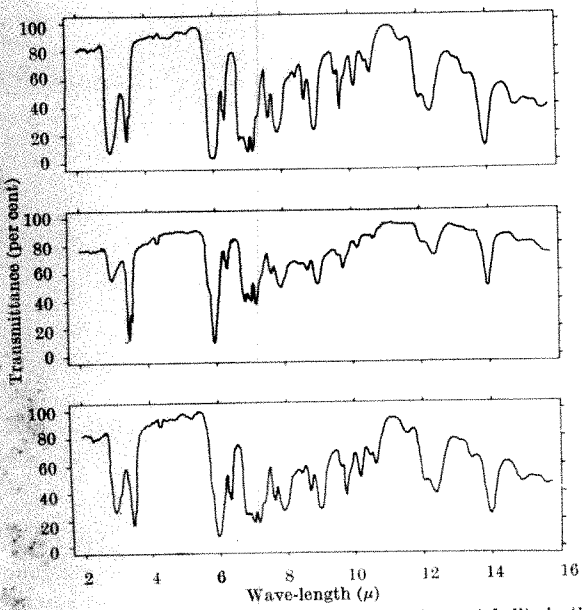


Fig. 1. The infra-red spectra of the major nicotine metabolite in the differential grasshopper and house fly. Top, cotinine; centre, *Musca* metabolite; bottom, *Melanoplus* metabolite

which have become adapted to tobacco as a host plant do not metabolize nicotine but have an efficient excretory mechanism. The coleopterous and orthopterous pests of tobacco examined were shown to metabolize nicotine to cotinine and other alkaloids. As cotinine has previously been shown to be non-toxic to insects[2], it is suggested that metabolism to cotinine represents an additional detoxification mechanism in tobacco-feeding insects. The metabolism of nicotine to cotinine may be widespread, since it occurs among mammals[4,5] and insects, although some insects, including some tobacco-feeding insects, cannot carry out this reaction. If this mechanism is important in the adaptation of insects to plants containing nicotine, its importance lies in the extent to which it occurs rather than the presence or absence of the necessary enzymes.

This work was supported by U.S. Public Health Service research grant *ES* 00044 from the Division of Environmental Engineering and Food Protection.

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PSYCHOLOGY

Retention as a Function of Previous Learning

It has been argued by Underwood[1] that proactive inhibition is a major factor determining retention. On the basis of data from a large number of earlier experiments he suggests that the recall of verbal tasks is a function of the number of previously learned similar tasks: the greater their number, the lower is the recall score after 24 h. This loss of retention is assumed to reflect interference from the earlier tasks (proactive inhibition).

There is, however, an alternative interpretation of the results discussed by Underwood. When a subject learns several similar tasks in succession, the number of trials required to reach a given learning criterion decreases gradually (positive transfer), and the average number of correct (reinforced) repetitions per item decreases correspondingly. If we assume that recall of verbal list items is a function of the number of (reinforced) repetitions, we would thus expect results similar to those discussed by Underwood even if there were no interference from previously learned items. This interpretation receives support from the following type of results. When subjects learn one list a day for several successive days, there is a high positive correlation between trials to criterion and percentage recall after 24 h (for example, Warr[2]). Warr has further shown that probability of recall of verbal items is directly dependent on the number of correct anticipations of those items during learning.

In the present experiment an attempt was made to devise a situation in which trials to criterion and average number of correct anticipations of the items were constant for a given task T independently of the number of previously learned similar tasks. Evidence for proactive inhibition in such a situation could not be given the alternative interpretation discussed here, but would support Underwood's conclusions. The design of the experiment was based on the following consideration: the cumulative transfer usually found when several similar tasks are learned in succession is mainly due to learning of efficient techniques and methods of learning, to emotional adjustment, etc. ('general transfer'). Proactive interference effects, on the other hand, should be primarily related to the contents of the tasks. Consequently, sufficient pre-training in the use of a specific method, with one type of content, should secure that little or no additional transfer occurs when tasks with a different type of content are learned afterwards by the same method.

Thirty-six subjects—mainly firemen, who had ample time for the task—were given 4·5 h pre-training in the use of the paired associate method of learning. Ten lists were used: seven lists with numbers, two word lists, and one list with figures. In the experiment proper, nine lists of paired nonsense syllables of equal and low association value were used, one of these being the critical list T. The subjects were divided into four groups. Group I learned only T, Group II learned two other lists and T, Group III learned five other lists and T (three lists a day), and Group IV learned eight other lists and T. All lists were learned to the criterion of a single correct trial. The last-learned list, T, was recalled after 24 h.

Table 1. Loss of Retention due to Proactive Inhibition

Group	No. previous similar tasks	Mean trials to criterion, list T	Percentage recall of T after 24 h
I	0	13·7	61
II	2	9·6	55
III	5	13·6	48
IV	8	12·1	39

The main results are presented in Table 1. The average number of trials required to learn T was approximately equal in all groups ($F = 1{\cdot}83$). No statistically significant transfer effects occurred in the learning of syllable lists in any group. In the recall scores, on the other hand, there were clear-cut differences between the groups: the score was lower the greater the number of previously learned syllable lists. An analysis of variance of the recall scores yields an $F = 13{\cdot}2$ ($P < 0{\cdot}001$). It may be pointed out that to the extent that pre-training experiences affect the recall scores (by giving rise to additional proactive interference) they should tend to decrease the differential effects of the learning of syllable lists on the recall of T. A control group, learning list T without any preceding pre-training, required on the average 16·9 trials to reach the criterion, and recalled 69 per cent of

the items after 24 h. The differences between the recall scores of Groups I–IV thus appear to be primarily due to proactive interference.

The correlation between speed of learning and recall of T was negative but low. However, there were indications in the results of a positive correlation between the number of reinforced repetitions and the probability of recall of individual items, supporting Warr's findings.

The results yield clear-cut evidence for the assumption that proactive inhibition is a factor determining retention, although the effects were smaller than might be expected from Underwood's curve. At the same time the results are consistent with the argument that part of the differences in retention interpreted by Underwood in terms of proactive inhibition may simply reflect differences in degree of learning of the items.

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Illusory Perception as a Constancy Phenomenon

Gregory[1] offers a general account of visual illusions, relating the apparent distortions in them to the perceptual process of size constancy. He observes that Tausch is the only previous writer to have considered constancy in relation to illusions. However, Gibson[2] has incorporated illusory perceptions within the context of size constancy, and suggests that perception of size is a by-product of a constant scale, which Gregory calls 'constancy scaling', at different distances. Arguing that illusory figures are "flat projections of typical views of objects lying in three-dimensional space", Gregory notes that "the parts of the figure corresponding to distant objects are expanded and the parts corresponding to nearer objects reduced". He states the same principle more simply when he writes: "Those parts of the figures which would normally be further away in 3-D space appear too large in the illusion figures"[3]. These principles are applied to a series of illusions, including those of Müller-Lyer, Ponzo and Hering. Gregory therefore postulates a common process modifying retinal images in constancy scaling and in the perception of illusory figures.

If this general principle operates, it is difficult to understand why the distorting process does not occur under all the conditions in which distance is perceived in two-dimensional figures. In Ponzo's figure the same one of the two central lines appears longer, no matter whether it is seen as nearer or farther after rotating the figure through 180°. Similarly when only the top or bottom half of either forms of Hering's illusion is inspected, the parallel lines appear to be distorted, no matter which way the perceived depth or distance appears to be in the background field. Further, Gregory's contention that "the scaling can be set directly by depth features of flat figures"[4] does not seem to be supported experimentally in all illusions. Green and Hoyle[5], for example, found that the Poggendorff illusion did not give rise to 3-D perception under reduced cue conditions. Their finding also is substantiated by Gibson[6], who reports that when the texture of a pattern becomes indeterminate the observer no longer sees the dimensions of a surface but a depthless shape perceived in the frontal plane.

Gregory's predictions, when applied to different orientations of visual illusions, are therefore not supported. In fact the illusions are more stable than Gregory's predictions would suggest, since changing the constancy relationships does not necessarily change the illusory effects. However, if the constancy interpretation of illusions is to be accepted it must be applicable to all conditions of inspection, and not only to a single orientation of the figure.

Although we do not have an alternative general explanation of the cause of visual illusions, we would offer some suggestions concerning specific factors operating in the illusory patterns being discussed. First, the relative position of the two horizontal lines in Ponzo's figure seems more important than the distance at which they appear to be located. Then, the distortion in Hering's illusion, or in Gibson's texture gradient, occurs as a function of the density of background lines: that is, the closer together these lines are, the more distorted the illusory lines appear, irrespective of which part of the pattern looks nearer or farther away. Further, predictions can only be made about which sections of the illusory lines will be more distorted, and not about the direction of distortion, that is whether the illusory lines will appear closer together or farther apart, as Gregory's account would require. Finally, some of the illusions considered by Gregory (that is, Hering's, Gibson's texture gradient, Orbison's field of concentric circles and the Necker cube) disappear entirely when the viewing slant is altered or when they are viewed from a distance, although the cues for depth, which set the constancy scaling, still operate[7]. We conclude, therefore, that constancy scaling operates only under a set of very limited conditions of inspection of illusory figures.

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There are several hints in the literature of perception of a possible tie-up between constancy and the illusions, but Tausch[1] seems to have produced the first reasonably solid treatment, described clearly by Teuber[2], though he has not developed a fully consistent theory. Brown and Houssiadas's reference to J. J. Gibson's *The Perception of the Visual World*[3] in this connexion is surprising, for Gibson holds a view of constancy which precludes this kind of theory. Gibson starts off (p. 163) somewhat disconcertingly: "The aim of this chapter is ultimately to show that the question of why things retain their sizes and shapes under different circumstances is a false question". (The rest of the chapter is, however, devoted to this question.) He develops a theory of depth perception which he attributes to Koffka[4]—the size-at-a-distance theory—which is that all three spatial dimensions are equally available to the perceptual system. But in denying that depth has to be specially computed, Gibson rejects the notion of constancy scaling essential to this theory of the illusions. When Gibson uses the word 'scale' he is evidently not referring to a process of size adjustment normally giving constancy, for several times he explicitly denies such processes in depth perception.

Gibson says of an illusion figure (p. 181): "All three cylinders are the same size on the page; it is not an illusion at all but a demonstration that apparent size depends on apparent distance". But the fact is that illusion figures are not seen as lying in depth when presented on normal textured paper: hence the problem. It was for this reason I removed background texture by using luminous illusion figures for experimental purposes.

I think Brown and Houssiadas are incorrect in attributing this kind of theory to Gibson, while his account of the illusions seems the least satisfactory part of his treatment of perception.

The main question raised by Brown and Houssiadas is whether the distortions occur under all conditions in which distance is perceived in a two-dimensional figure.

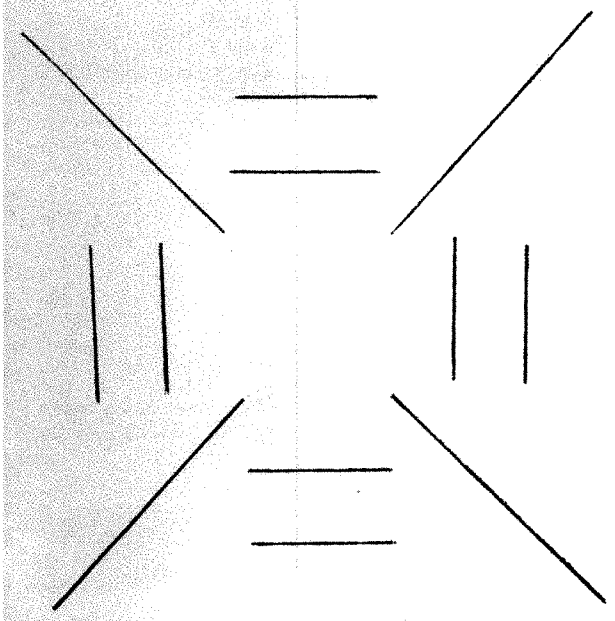


Fig. 1. This shows four differently orientated Ponzo figures. Brown and Houssiadas argue that the illusion should change with the orientation: but the perspective is unchanged, and so this is incorrect

(Actually, as I point out[5], it need not be perceived; for we must suppose that constancy scaling can be set directly by perspective and other depth cues, even when these are countermanded by other features such as the texture of the background. Perspective depth is 'paradoxical'—suggested but not seen as in the real world of objects lying in space.) Now, they are mistaken in regarding their test cases as exceptions; for when a perspective figure is rotated, the perspective does not change. What changes is the effective viewing position, and this is a very different matter. This should be clear from the example given of four differently orientated Ponzo figures—their perspective is the same and so is the illusion. They lie respectively on the 'ground', to the sides and in the 'sky'. The same consideration holds for the case of the partly obscured Hering figure, and the rest.

Brown and Houssiadas give insufficient data on their final point for a useful comment, but it should be pointed out that there is only one distance from which a given perspective is strictly correct. Their findings might be very important in this connexion.

So far no valid objections seem to have been raised to my form of the theory[5]; but the evidence for it is rather indirect. I have, therefore, devised a technique for obtaining objective measures of apparent depth. This makes it possible to relate directly apparent depth with illusory distortions. An established relation between apparent depth in the luminous illusion figures, where depth is not countermanded by texture, and the extent of the illusion in the same figures but viewed on a normal texture background, would be very strong evidence for the theory. Working with Miss Linda Townes at the Massachusetts Institute of Technology, and in a preliminary experiment with Mr. I. Morrell at Cambridge, England, I have found just such a relation.

How to measure apparent depth. The basic scheme is to use binocular depth vision (either the range-finder effect of convergence, or disparity between the retinal images) as a standard against which to measure depth given by perspective. This is done, using convergence, by keeping both eyes open but allowing only one to see the (generally luminous) display figure, by cross-polarizing the light from the display to one eye. A small dim reference light is introduced into the display by means of a half-silvered mirror, this light being seen by both eyes. Its distance is adjusted until it appears the same distance as any selected part of the display figure, viewed with a single eye. Since the reference light is seen by binocular vision, we thus obtain a measure of depth as determined by perspective features of the figure. The positions of the reference light are automatically recorded on graph paper, and so we obtain a complete plot of visual space.

So far I have used this technique on the Müller-Lyer illusion, and on ambiguous figures such as the Necker cube. I am now working on the Poggendorff illusion, and find fluctuating depth with related changes in the illusion. The results on the Müller-Lyer are remarkable: there is a correlation of about 0·9 between the extent of the illusion (as determined by the fin angle; measuring the illusion by setting an adjustable comparison line to equality) with the same figures but luminous, their depth being measured with this technique.

My suggestion[5] that figural after-effects are due to constancy scaling being upset by lengthy fixation of depth clues can now be tested.

A further fact, which seems to hold for all the illusions where background produces a distortion, is that the illusion evidently disappears when the affected lines are displaced in depth from the disturbing background, by introducing disparity with a stereoscope.

This work was supported by the National Aeronautics and Space Administration. I thank Prof. H-L. Teuber for his advice.

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[1] Tausch, R., *Psychol. Forschung*, **24**, 299 (1954).
[2] Teuber, H-L., in *Handbook of Physiology*, Sect. 1, *Neurophysiology*, edit. by Feild *et al.* (Washington, 1960).
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Alpha-frequency of Electroencephalogram and a Stabilized Retinal Image

IT is known that the alpha rhythm is suppressed in most subjects when patterned targets are viewed in normal vision[1]. It is now also well known[2–4] that when a pattern is viewed as a stabilized retinal image, perception of it in whole or in part may be lost intermittently. It is evidently of interest to discover what modification, if any, of the alpha component of the human electroencephalogram occurs when a stabilized pattern has actually disappeared.

Until quite recently, methods of achieving a stabilized image have involved the wearing of contact lenses. Despite the fact that compensation for eye movements by this particular method is sufficiently good to promote fragmentation and occasional total disappearance of the image, some mechanical destabilization due to minute translations of the lens relative to the scleral surface must occur[5]. Though this cannot account for all reappearance of the image[6], it undoubtedly induces periodic regenerations of the order of three or four seconds, and since alpha develops relatively slowly in normal situations[7], a clear-cut correlation with such brief events might be hard to achieve. (Since this communication was written, we have heard that comprehensive investigations of the relationship of alpha to image disappearance using the contact lens method of stabilization have been performed by Fender *et al.* at the California Institute of Technology, with results in general agreement with those reported here.)

The after-image method of investigating stabilized patterns[8] removes destabilization as an interfering variable, and, since in any sequence of viewing a prolonged after-image, total disappearances of the pattern tend to become increasingly more common with the passage of

time, any correlation that exists between disappearance phenomena and the electroencephalogram should manifest itself more clearly. In fact, if suppression of alpha is, as has been proposed, associated with the perception of patterns, then one would expect more frequent occurrence of alpha towards the end of experimental sessions.

In this experiment the electroencephalogram was recorded from a single pair of chlorided silver scalp leads placed over the occipital and parietal areas about 2 in. from the midline. The amplifier sensitivity was 50 μV/cm over the 5–20 c/s band and the recording paper moved at a speed of 1 cm/sec.

Each trial was of 4-min duration from the time of the initial flash. The subject lay relaxed on a couch in a dimly lit room and was instructed to close his eyes directly the flash had been given, and report, by pressing a key, the occasions on which the target had totally disappeared. The image was a simple patterned target, consisting of a square with an inscribed cross. The figure in outline was cut from thick black paper and illuminated from behind by a 1,000-joule flash of 25 msec maximum duration. The flash was at a distance of 6 ft. from the subject's head, the image subtending at this distance a retinal angle of 5°. The target was dimly illuminated from behind by a low-power bulb for a few seconds before the flash to afford the subject a proper fixation point.

After the experiment, records were divided into 1-min sections and periods of clear alpha activity were marked off visually. Both frequency and amplitude were used as criteria and measurements were made independently by two observers. Disagreement between the observers was found to be small.

The subject's record indicated that total disappearance rarely, if ever, occurred in the first quarter (1 min) of the run, and began to occur towards the end of the second quarter. The pattern had apparently totally disappeared for the final 1·5 min of the trial. Fig. 1*a* plots the total time for which alpha was present in each minute following the flash. The bars are means for four trials on one subject, the standard error of the means being indicated by the vertical lines. Note that alpha was almost entirely absent in the first 2 min, and increased rapidly during the final two, reflecting the incidence of recorded disappearances.

Two independent sets of control observations were considered to be desirable. In the first set, the subject's 'resting' alpha was recorded. The subject relaxed with open eyes in the dimly lit room for 30 sec, this being followed by a 4-min period with eyes closed when the electroencephalogram record was taken. Distribution of alpha for the 4-min sessions is plotted in Fig. 1*b*. It will be noted that alpha began to occur about 15 sec after the eyes had been closed, and in the second quarter had reached a level only achieved in the fourth quarter after the patterned flash.

It was recognized that the observed alpha suppression might be due to the after-effects of the bright flash used to plant the after-image on the retina, and not necessarily to the perception of a patterned target. To take account of this possibility, a complete series of measurements was made following a flash in which no pattern or contours were present. For this series of trials the black paper mask and pattern 'cut-out' were moved from the front of the flash apparatus, and the whole device was covered with diffused tracing paper and fired within 12 in. of the subject's eye. This produced a brilliant explosion of light which completely filled the subject's monocular field. With the flash intensity remaining at 1,000 joules, in this control the actual intensity expressed in terms of input or excitation of the retina must have been many times greater than when the pattern 'mask' was used. The flash was described by subjects as a startling and 'somewhat painful' experience. Fig. 1*c* indicates that while alpha was certainly suppressed for a period, as might be expected owing to the strength of the flash in such proximity to the eye, it began to return in the first minute,

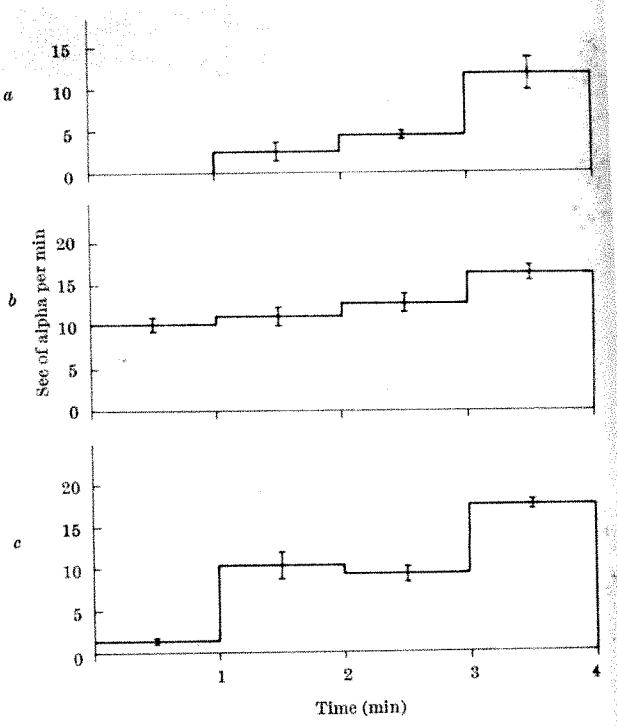


Fig. 1

and by the end of the second minute had risen to the 'resting' level. It is clear that the general suppression of alpha noted in Fig. 1*a* is not simply a function of visual shock or general disturbance of the subject. The complete experiment was repeated on a second subject with almost identical findings.

It therefore seems evident that the suppression of alpha is related to the presence of a pattern in the visual field. How closely this is linked to 'attentional' phenomena is not clear, though it should be mentioned that the key-pressing task, which might conceivably be expected to inhibit alpha somewhat was deliberately confined to the basic experimental condition (that is, the subject pressed the key when the target was absent). (In fact, pilot investigations had indicated that the muscular act of random key-pressing did not seem to affect alpha in any obvious way. Typically in the experimental sessions, the key was depressed only four or five times in a 4-min run. The role of 'attention' in relation to pattern perception cannot, of course, be established from a preliminary investigation of this kind. Nevertheless, since the subjects eyes remained closed in all three conditions of the experiment, and since in all conditions the visual input did not change after the single initial flash, it now seems to be certainly established, perhaps for the first time, that neither of these two variables plays a vital part in the promotion or suppression of alpha. Rather, the independent importance of signals that define contours, boundaries and possibly 'shape' seems to be demonstrated.

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[1] Adrian, E. D., and Matthews, B. H. C., *Brain*, **57**, 355 (1934).
[2] Ditchburn, R. W., and Fender, D. H., *Optica Acta*, **2**, 128 (1955).
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[4] Pritchard, R. M., *Sci. Amer.*, **204**, 72 (1961).
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[7] Lindsley, D. B., in *Intern. Symp. on Feelings and Emotions*, edit. by Reymert, M. L. (McGraw-Hill, New York, 1950).
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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, October 19

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Dr. J. L. Garraway: "Studies on certain Organo-Sulphur Compounds as Fungicides".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. G. Klein (Stockholm): "The Immunology of Experimental Tumours".*

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (at Enfield Technical College, Queensway, Ponders End, Enfield, Middlesex), at 7 p.m.—Dr. T. R. E. Kressman: "Ion Exchange".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.15 p.m.—Mr. Eric E. Shipton, C.B.E.: "Over the North Patagonian Icecap".

Tuesday, October 20

SOCIETY OF CHEMICAL INDUSTRY, Agriculture Group (at 14 Belgrave Square, London, S.W.1), at 2 p.m.—Short Paper Meeting on "Microanalysis in Agriculture".

PHOTOBIOLOGY GROUP (at the Linnean Society of London, Burlington House, Piccadilly, London, W.1), at 2.15 p.m.—Scientific Meeting and Annual General Meeting.

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, London, S.W.1), at 5.30 p.m.—Mr. O. A. Kerensky and Mr. Gordon Little: "Medway Bridge—Design"; Mr. M. Kier, Mr. Frode Hansen and Mr. J. A. Dunster: "Medway Bridge—Construction".

INSTITUTION OF ELECTRICAL ENGINEERS, SCIENCE AND GENERAL DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Prof. F. Brailsford and Mr. R. Fogg: "Anomalous Iron Losses in Cold-Reduced Grain-Orientated Transformer Steel".

UNIVERSITY OF LONDON (at King's College, Strand, London, W.C.2), at 5.30 p.m.—Prof. F. K. Hare: "New Wings for Climatology—a Science Reborn" (Inaugural Lecture).*

UNIVERSITY OF LONDON (in conjunction with the Society for the Study of Addiction, at King's College, Strand, London, W.C.2), at 5.30 p.m.—Prof. Leonard Goldberg: "Dependence on Alcohol" (J. Y. Dent Memorial Lecture in Pharmacology).*

UNIVERSITY OF LONDON (in the Beveridge Hall, Senate House, London, W.C.1), at 5.30 p.m.—Prof. Sir Robert Platt: "The Ethical Basis of Medical Science". (First of fourteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)*

INSTITUTION OF MECHANICAL ENGINEERS, RAILWAY ENGINEERING GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Traction-Motor Problems".

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (joint meeting with the Brighton College of Technology Chemical Society, at the Brighton College of Technology, Lewes Road, Brighton, Sussex), at 7 p.m.—Prof. Keith Simpson: "Some Applications of Science to Crime Investigation".

Wednesday, October 21

ROYAL METEOROLOGICAL SOCIETY (at 49 Cromwell Road, London, S.W.7), at 5 p.m.—Mr. P. M. Saunders: "Sea Smoke and Steam Fog"; Mr. A. A. Townsend: "Natural Convection in Water Over an Ice Surface".

BRITISH COAL UTILISATION RESEARCH ASSOCIATION (at the Federation of British Industries, 21 Tothill Street, London, S.W.1), at 5.30 p.m.—Prof. A. R. J. P. Ubbelohde, C.B.E., F.R.S.: "The Chemistry of Carbon and Graphite" (Thirteenth Coal Science Lecture).

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. G. G. Gouriet: "Through Communications to Electronics" (Chairman's Address).

UNIVERSITY OF LONDON (at King's College, Strand, London, W.C.2), at 5.30 p.m.—Prof. J. L. Synge, F.R.S.: "Hamiltonian Theory of Rays and Waves". (Further lectures on October 22 and 23.)*

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, RADAR GROUP (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Mr. M. G. Henley: "The Effect of Tolerances on Automatic Landing".

INSTITUTION OF MECHANICAL ENGINEERS, HYDRAULIC PLANT GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "What Don't We Know About Non-Return Valves?"

SOCIETY FOR ANALYTICAL CHEMISTRY, MICROCHEMICAL METHODS GROUP (at "The Feathers", Tudor Street, London, E.C.4), at 6.30 p.m.—Discussion Meeting on "The Determination of Phosphorus Applied to Organic, Inorganic and Biological Materials", introduced by Mr. E. Q. Laws.

UNIVERSITY OF LONDON (at the Institute of Neurology, National Hospital, Queen Square, London, W.C.1), at 7 p.m.—Prof. J. Z. Young, F.R.S.: "An Experimental Approach to Neurology".*

INSTITUTION OF ENGINEERING INSPECTION (at the Royal Society of Arts, John Adam Street, Adelphi, London, W.C.2), at 7.15 p.m.—Mr. R. S. Sharpe: "The Detection and Assessment of Defects".

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION, LEA VALLEY SUB-SECTION (at the College of Further Education, Hagsdell Road, Hertford), at 7.30 p.m.—Dr. R. S. Cahn: "The Future of Chemical Publications".

Wednesday, October 21—Thursday, October 22

INSTITUTION OF MECHANICAL ENGINEERS, INTERNAL COMBUSTION ENGINES GROUP (at the College of Advanced Technology, Gosta Green, Birmingham, 4)—Symposium on "Thermal Loading of Diesel Engines".

Thursday, October 22

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p.m.—Dr. Colin Smith: "Words, Words, Words—Satire, Thinker or Humbug?"*

UNIVERSITY OF LONDON (in the Recital Room, Goldsmiths' College, New Cross, London, S.E.14), at 4.45 p.m.—Dr. Michael Kaye: "Philosophy Today".*

INSTITUTION OF MINING AND METALLURGY (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Mr. D. G. Armstrong: "Variations in Stroke Waveform in a Laboratory Jig"; Mr. A. G. Moncrieff: "Theory of Thickener Design Based on Batch Sedimentation Tests".

LINNEAN SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. K. A. Kermack: "*Aegialodon dawsoni*, the Earliest Known Trituberculosectorial Tooth"; Dr. J. R. E. Mills: "Form and Function in the Teeth of Tenrecoidea".

INSTITUTION OF CIVIL ENGINEERS, ENGINEERING MANAGEMENT GROUP (at Great George Street, London, S.W.1), at 5.30 p.m.—Informal Discussion on "Selection for Management in the Army", introduced by Col. R. L. Clutterbuck.

INSTITUTE OF REFRIGERATION (at the National College for Heating, Ventilating, Refrigeration and Fan Engineering, Southwark Bridge Road, London, S.E.1), at 6 p.m.—Colonel H. Randal Steward: "Twenty-five Years of Make and Mend" (Presidential Address).

ROYAL AERONAUTICAL SOCIETY (at 4 Hamilton Place, London, W.1), at 6 p.m.—Mr. J. Zbrozek: "Atmospheric Gusts".

OIL AND COLOUR CHEMISTS' ASSOCIATION (at Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Mr. H. Oosterhof: "Inherent Properties of Paint Latex Films in Relation to Their Performance".

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (at the South-East Essex Technical College and School of Art, Longbridge Road, Dagenham, Essex), at 7 p.m.—Mr. R. A. Saunders: "The Uses of Mass Spectrometry in Organic Chemistry".

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION, KENT SUB-SECTION (at the Medway College of Technology, Horsted, Maidstone Road, Chatham, Kent), at 7 p.m.—Prof. C. C. Addison: "Liquid Sodium".

Thursday October 22—Friday, October 23

POWDER METALLURGY JOINT GROUP OF THE IRON AND STEEL INSTITUTE AND THE INSTITUTE OF METALS (in the Hoare Memorial Hall, Church House, Great Smith Street, London, S.W.1), at 10 a.m. daily—Annual Meeting.

Friday, October 23

INSTITUTION OF MECHANICAL ENGINEERS, APPLIED MECHANICS GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "The Mechanics of Sugar-Cane Crushing".

ROYAL AERONAUTICAL SOCIETY, ROTOCRAFT SECTION (at 4 Hamilton Place, London, W.1), at 6 p.m.—Prof. H. Focke: "German Thinking on Rotary Wing Development" (Fifth Cierva Memorial Lecture).

OCCAM SOCIETY (at the Northampton College of Advanced Technology, St. John Street, London, E.C.1), at 6.15 p.m.—Mr. A. Healey: "The Philosophy of Finance".

Saturday, October 24

JOINT BIOLOGY COMMITTEE (in the William Beveridge Hall, University of London, Senate House, Malet Street, London, W.C.1), at 10 a.m.—One-Day Course on "The Educational Value of Biology".

LONDON COUNTY COUNCIL (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Miss Mildred Archer: "Birds and Flowers in Indian Painting".*

Monday, October 26

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Du Cane Road, London, W.12), at 4 p.m.—Dr. B. Ackner: "Stress and Psychosomatic Disorders".*

MEDICAL RESEARCH COUNCIL (at the National Institute for Medical Research, Mill Hill, London, N.W.7), at 4.30 p.m.—Prof. Sven Gard (Karolinska Institutet, Stockholm): "An Approach to the Study of Virus Synthesis".

INSTITUTE OF NAVIGATION (at the Royal Geographical Society, 1 Kensington Gore, London, S.W.7), at 5 p.m.—Dr. C. Koeman: "The Life and Work of Lucas Janszoon Waghenaer" (The Eva G. R. Taylor Lecture).

UNIVERSITY OF LONDON (in the Botany Theatre, University College, Gower Street, London, W.C.1), at 5 p.m.—Dr. K. L. Manchester: "Some Aspects of the Action of Insulin" (Further lecture on November 2).*

BRITISH SOCIETY FOR THE HISTORY OF SCIENCE (in the Council Room of the Science Museum, Exhibition Road, London, S.W.7), at 5.30 p.m.—Prof. A. Rupert Hall: "Galileo and the Science of Motion".

INSTITUTION OF ELECTRICAL ENGINEERS, SCIENCE AND GENERAL DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Prof. A. L. Cullen: "Millimetre Wave Examination of Gas Discharges".

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, JOINT I.E.R.E.–I.E.E. COMPUTER GROUPS (at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 5.30 p.m.—Colloquium on "Design Automation".

ROYAL INSTITUTION, LIBRARY CIRCLE (at 21 Albemarle Street, London, W.1), at 5.30 p.m.—Mr. Julian Royle: "A Modern Approach to Colour Printing".

ROYAL AERONAUTICAL SOCIETY, HISTORICAL GROUP (at 4 Hamilton Place, London, W.1), at 7 p.m.—Mr. S. B. Jackson: "The Parachute—an Historical Review".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURER or ASSISTANT LECTURER (with a good honours degree and preferably some teaching, research or industrial experience) IN PHYSIOLOGY IN THE DEPARTMENT OF BIOLOGY—The Academic Registrar, Brunel College, Woodlands Avenue, Acton, London, W.3 (October 23).

READER IN MECHANICAL ENGINEERING; and a LECTURER or ASSISTANT LECTURER IN MECHANICAL ENGINEERING—The Academic Registrar, Brunel College, Woodlands Avenue, Acton, London, W.3 (October 23).

CHAIR OF NUMERICAL ANALYSIS WITHIN THE DEPARTMENT OF APPLIED MATHEMATICS—The Registrar, The University, Liverpool (October 30).

POST-DOCTORAL FELLOW IN THE DEPARTMENT OF CHEMISTRY, for research on the structure and adsorptive properties of active charcoals—The Professor of Chemistry, The University, Hull (October 31).

RESEARCH ASSISTANT or SENIOR RESEARCH ASSISTANT (with a qualification in electrical engineering or physics and industrial or research laboratory experience) IN THE DEPARTMENT OF ELECTRICAL ENGINEERING, to assist in an experimental investigation, supported by D.S.I.R., into the high voltage breakdown of gases—The Secretary, Welsh College of Advanced Technology, Cathays Park, Cardiff (October 31).

TUTORIAL STUDENT (with a good honours degree or an equivalent qualification) IN THE DEPARTMENT OF CHEMISTRY, to carry out research on polyelectrolytes—Prof. G. M. Burnett, Department of Chemistry, University of Aberdeen, Aberdeen (October 31).

X-RAY CRYSTALLOGRAPHER (with previous research experience in X-ray crystallography) IN THE PHYSICS DEPARTMENT, for crystallographic studies of materials (including single crystals) at high pressures—The Secretary, Northampton College of Advanced Technology, St. John Street, London, E.C.1 (October 31).

DEMONSTRATOR IN PATHOLOGY—The Registrar and Secretary, The University, Bristol (November 2).

SECOND CHAIR OF PHYSICS—The Secretary and Registrar, The University College of North Wales, Bangor, North Wales (November 2).

LECTURER (with at least an honours degree of high standing in mathematics or equivalent qualifications, and able to produce evidence of strong research interests and experience) IN THE SCHOOL OF MATHEMATICS, The University of New South Wales—The Agent General for New South Wales, 56–57 Strand, London, W.C.2; and The Appointments Section, The University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia (November 6).

RESEARCH ASSISTANT (with an honours degree in botany or agricultural botany) to investigate the effects of storage conditions on the life span of crop seeds—The Secretary, National Institute of Agricultural Botany, Huntingdon Road, Cambridge (November 6).

SENIOR LECTURERS, LECTURERS or ASSISTANT LECTURERS IN PHILOSOPHY—The Secretary, University of Lancaster, Bailrigg House, Lancaster (November 7).

CHAIR OF PHYSIOLOGY at Makerere University College, Uganda—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (November 8).

CHAIR OF STATISTICS at Queen Mary College in association with the Institute of Computer Science—The Academic Registrar, University of London, Senate House, London, W.C.1 (November 9).

LECTURER (with special qualifications and interests in economic geography) IN GEOGRAPHY—The Secretary, The Queen's University of Belfast, Belfast, Northern Ireland (November 10).

LECTURER IN PURE MATHEMATICS—The Secretary, The Queen's University, Belfast, Northern Ireland (November 13).

LECTURER/SENIOR LECTURER IN PHILOSOPHY at the University of Sydney, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 13).

LECTURER (with a good honours or postgraduate degree in philosophy) IN PHILOSOPHY at the University of New South Wales—The Agent General for New South Wales, 56–57 Strand, London, W.C.2; and The Appointments Section, The University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia (November 14).

LECTURER IN GEOGRAPHY at the University of Otago, Dunedin, New Zealand—The Secretary-General, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, November 15).

LECTURER (suitably qualified graduate with specialism in educational sociology) IN EDUCATION at the University of Otago, Dunedin, New Zealand—The Secretary-General, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, November 15).

READERS, SENIOR LECTURERS and LECTURERS IN PHYSICS—The Registrar, University of Warwick, Coventry (November 16).

SENIOR LECTURER or LECTURER (with a special interest in vertebrate zoology) IN ZOOLOGY at the University of Khartoum—The Registrar, University of Khartoum, c/o The Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (November 30).

MYCOLOGIST (with a good honours degree) to work on soil-borne diseases of cereals—The Secretary, Rothamsted Experimental Station, Harpenden, Herts (December 31).

AGRICULTURAL OFFICERS (with a degree in agriculture and, preferably, with several years experience of agriculture in the tropics) in Fiji, for extension work, mainly with peasant farmers (crops include cocoa, coconuts, groundnuts, pulses, rice and livestock)—The Appointments Officer, Room 301, Department of Technical Co-operation, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 213/62/01.

CHIEF PHARMACIST, Category V (male or female) at the Central Middlesex Hospital, Park Royal, London, N.W.10—The Group Secretary, Central Middlesex Group H.M.C., Acton Lane, London, N.W.10.

JUNIOR RESEARCH ASSISTANT for fundamental research on the chemistry and physical chemistry of soil minerals—Prof. R. M. Barrer, F.R.S., Chemistry Department, Imperial College, London, S.W.7.

RESEARCH ASSISTANT (with qualifications in chemical engineering, chemistry or physics or another relevant discipline) IN THE DEPARTMENT OF CHEMICAL ENGINEERING, to work on a new project in the field of liquid–liquid extraction of metal ions, supported by a D.S.I.R. research grant—The Academic Registrar, Loughborough College of Technology, Loughborough, Leicestershire, quoting Ref. 23/G.

RESEARCH FELLOWS (with a good honours degree in engineering, physics, or mathematics or their equivalent, interested in acoustics and vibrations, and preferably experience in research or in industry) at the Institute of Sound and Vibration Research—The Secretary and Registrar (Room 9), The University, Southampton.

REPORTS and other PUBLICATION

(not included in the monthly Books Supplement)

Great Britain and Ireland

Catalogue of Publications of the Cement and Concrete Association cu available for distribution in the United Kingdom. Pp. 40. (Londo Cement and Concrete Association, 1964.)

Bulletin of the British Museum (Natural History). Entomology. V No. 6: Diptera from Nepal. The Fruit Flies (Diptera: Tephritidæ). Elmo Hardy. The Blow Flies (Diptera: Calliphoridæ). By Maurice T. Pp. 145–179. (London: British Museum (Natural History), 1964.) 14

The Institution of Electrical Engineers. *Current Papers*, No. 1 (. 1964). Pp. 4. Published monthly. Annual subscription 20*s*. (L The Institution of Electrical Engineers, 1964.)

Transactions of the Royal Society of Edinburgh. Vol. 66, No. 1: Structure of Some Petioles Associated with *Rhetinangium* Gordon. By G. Long. Pp. 1–8 + 2 plates. 6*s*. Vol. 66, No. 2: Observations on gotid Eurypterids. By Charles D. Waterston. Pp. 9–34 + 4 plates. 1 Vol. 66, No. 3: A Petrified Lower Carboniferous Lepidodendron Sl Rooting Organs Identified with *Calamopsis* Solms-Laubach. By Alt Long. Pp. 35–48 + 4 plates. 10*s*. 6*d*. (Edinburgh: Royal Soci Edinburgh, 1964.)

Department of Scientific and Industrial Research. Summary of P of the Geological Survey of Great Britain and the Museum of Pr Geology for the year 1963. Pp. iii + 99. (London: H.M. Stationery 1964.) 6*s*. 6*d*. net.

Department of Scientific and Industrial Research. Ergonomics for try, No. 6: Noise in Industry. By D. E. Broadbent. Pp. 16. (L H.M. Stationery Office, 1964. Obtainable (free) from DSIR Warren Laboratory, Stevenage, Herts.)

National Union of Teachers. First Things First: A Memorandum dence Submitted to the Central Advisory Committee for Education (En under the Chairmanship of Lady Plowden. (An inquiry into primary tion in all its aspects and the transition to secondary education.) (London: National Union of Teachers, 1964.) 2*s*. 6*d*.

Ministry of Housing and Local Government; Scottish Develo Department; Ministry of Health and Local Government, Northern I Radioactivity in Drinking Water in the United Kingdom—1963 R Pp. 23. (London: H.M. Stationery Office, 1964.) 1*s*. 9*d*. net.

University of Bristol. Annual Report of the Long Ashton Agricultu Horticultural Research Station (The National Fruit and Cider Institu 1963. Pp. x + 193 + 4 plates. (Bristol: The University, 1964.) 15*s*.

London and Home Counties Regional Advisory Council for Techno Education. Bulletin of Special Courses in Higher Technology, Manag Studies and Commerce, 1964–65. Part 1: Autumn Term. Pp. 126. (L London and Home Counties Regional Advisory Council for Techno Education, 1964.) 5*s*.

Other Countries

Annals of the New York Academy of Sciences, Vol. 115, Article 1: Bl in the Surgical Patient. By Seymour Gollub, Alex W. Ulin and 77 authors. Pp. 1–542. (New York: New York Academy of Sciences, 7 dollars.

Style Manual for Biological Journals. Prepared by the Committee or and Style of the Conference of Biological Editors. Second edition x + 117. (Washington, D.C.: American Institute of Biological Sciences Published for the Conference of Biological Editors.)

Smithsonian Institution: United States National Museum. Bullet 239: The Recent Mollusca of Augustus Addison Gould—Illustrations Types Described by Gould, with a Bibliography and Catalog of his S By Richard I. Johnson. Pp. v + 182 + 45 plates. (Washington, D.C.: G ment Printing Office, 1964.) 1.50 dollars.

United Nations: Department of Economic and Social Affairs. The Nations Development Decade: Proposals for Action—Report of the tary-General. Pp. xii + 125. (New York: United Nations; London: Stationery Office, 1962.) 1.25 dollars; 9*s*. 6*d*.

Collision Efficiencies of Two Spheres Falling in a Viscous Mediu Reynolds Numbers up to 19·2. By Uris Shafrir and Morris Neil (University of California Occasional Papers, No. 1.) Pp. ix + 140. (Be and Los Angeles: University of California Press; London: Cam University Press, 1964.) 3 dollars.

Tenside, 1 Jahrgang, Heft 1 (September 1964). Redaktion: Dr.-I Huttenlocher und Dr. rer. nat. M. Reinhardt. (Zeitschrift für F Chemie und Anwendung grenzflachenaktiver Stoffe.) Pp. 1–40. (Mü Carl Hanser Zeitschriftenverlag, G.m.b.H., 1964.)

World Health Organization. Public Health Papers, No. 24: Care o dren in Day Centres. Contributors: F. Davidson, B. Górnicki, Intern Labour Office, S. Lebovici, Irene Lezine, Julius B. Richmond, Eva Sc Kolmer and Stig Sjölin. Pp. 189. (Geneva: World Health Organiz London: H.M. Stationery Office, 1964.) 7 Sw. francs; 12*s*.; 2.25 dollar

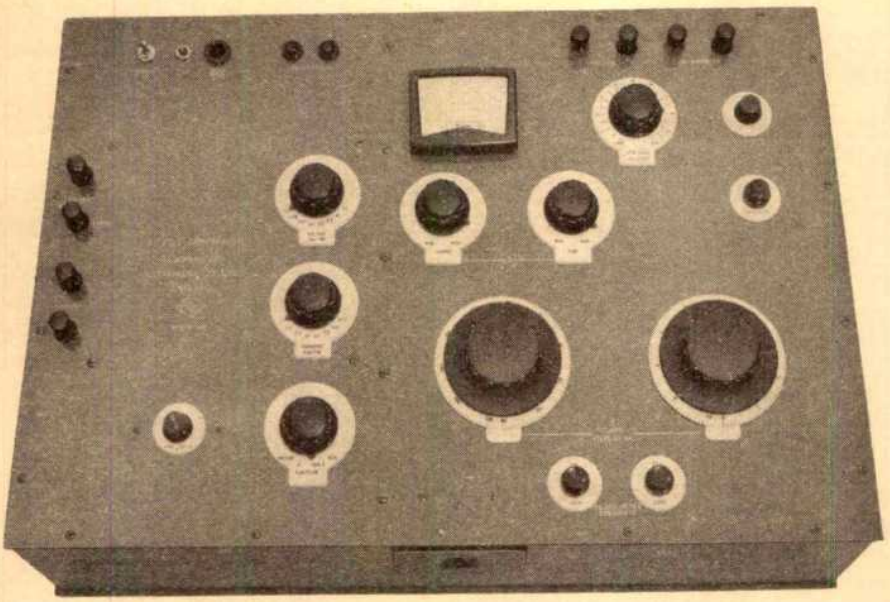
World Health Organization. Technical Report Series, No. 286: Re in Immunology—Report of Five Scientific Groups convened by the Di General of the World Health Organization. Pp. 97. (Geneva: World Organization; London: H.M. Stationery Office, 1964.) 5 Sw. francs; 1.75 dollars.

Editorial and Publishing Offices of "NATURE"
MACMILLAN (JOURNALS) LIMITED
ST. MARTIN'S STREET, LONDON, W.C.2
Telephone Number: Whitehall 0081. Telegrams: Phusis London W.C

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REGIUS CHAIR OF ZOOLOGY

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Further particulars can be obtained from the Registrar, Senate House, Bristol, 2, to whom applications should be sent by October 30.

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PROMOTING INDUSTRIAL RESEARCH IN BRITAIN

*RESEARCH FOR INDUSTRY, 1963** follows the usual pattern of recent years, comprising the report of the Industrial Grants Committee and the directory of the research associations, together with brief progress reports on the work of ten associations, the grants of which came up for renewal during the year. There is also a short article by Sir Charles Goodeve on the research associations as a collaborative network. After noting the establishment of the Standing Consultative Conference on Textile Research and the Consultative Committee of Directors of Food Research Organizations (which has been meeting for some 10 years), Sir Charles directs attention to the technical role of the Committee of Directors of Research Associations, formed in 1945, and to the objectives of the Industrial Automation Group set up by that Committee in 1963. The contracting process which marked the 1962 report continues, and the report is now no more than 54 pages (15 of which are occupied by the directory) compared with 75 in 1962, 140 in 1961, and 148 in 1960.

In the aggregate, therefore, *Research for Industry* now supplies no more information than was supplied about the research associations before the Department of Scientific and Industrial Research began to report on them separately from its annual report—in fact the 1963 report supplies less. The annual reports of the individual research associations increasingly provide the principal sources of information, and, although the report of the Industrial Grants Committee deals with some major questions of policy, it scarcely provides the wide conspectus that is desirable. Certainly it does not meet many of the major points of criticism which have been put forward in many recent discussions on the general functions of the research associations. There is, in fact, nothing in this report to dispose of the criticism of triviality in the amounts of grants to some associations, which has been made in influential quarters, or to indicate that the Council itself or its Industrial Grants Committee is aware that even the most carefully devised scheme of grants can be ineffective if the income of a research association or laboratory does not reach at least a certain minimum level. The decision of the British Internal Combustion Engine Research Association to transform itself, for lack of industrial support, into a sponsored research institute is a quiet reminder.

The total income of the grant-aided research organizations rose from £8·8 million in 1962 to £9·5 million in 1963: the grant from the Department of Scientific and Industrial Research increased from £1·96 million to £2·1 million. During the past two years the grant policy has radically changed; a flat rate is now being offered in order to stimulate growth or attract support from new sources—except when incentives are necessary. In 1963, 14 out of 16 associations were given a flat rate over a range of industrial income, and the grants represented 30–100 per cent of the industrial income. There is also a new approach to minimum and maximum limits for grants: the minimum income is no longer set so high as to imply a sudden fall in income if the association fails to raise additional industrial income in the first year of a new quinquennium, while the maximum grant represents a reasonable target for growth if the industry is disposed to achieve it, and if this target is exceeded the maximum grant may be extended.

The Industrial Grants Committee notes two further important points. The new policy is designed to avoid directors of research becoming too preoccupied with securing additional industrial support in order to qualify for a grant, and it is also intended to enable research associations to plan their future programmes without fear of discontinuities in income. Both these tendencies could help to eliminate some of the trivialities or waste which have been the subject of criticism, and the scheme of 'ear-marked' grants in support of selected research and development projects, which came into operation in 1963, could also help. Although the Industrial Grants Committee supported in principle 9 out of 13 applications for such grants, only one such grant was offered and accepted during the year—that to the British Welding Research Association for work on metal fatigue—though five more have been offered since the end of the year.

Two of these are also to the British Welding Research Association. One, of up to £84,000 over five years, is in support of research in serious welding problems, while the other, of up to £15,300 over five years, is for research into the brittle fracture of metals, and is part of a broad programme involving university departments as well as the Research Association. The other three ear-marked grants are: half the cost, estimated at £114,700 over three years, of an investigation by the Cotton Silk and Man-made Fibres Research Association of new methods of spinning; up to half the cost of a project (£14,000 over two and a half years) of the British Launderers' Research Association to speed the introduction of automation in its industry, and one of up to £39,400 over four years to the eight textile research associations to support research on water conservation and treatment of effluents throughout the textile industry.

The Industrial Grants Committee recognizes that the introduction of the new ear-marked grants raises issues of policy, including the broad question of how Government grants should eventually be related to the support secured by research associations from their industries. Clearly, the new policy could involve to some extent the Council of Scientific and Industrial Research determining the direction which the research pursued by particular associations or groups of associations should follow, and this has far-reaching implications which call for careful consideration. The Industrial Grants Committee itself suggests that the new terms awarded in 1963 to the Association of Special Libraries and Information Bureaux (Aslib) may provide a pointer to future developments, although Aslib is not typical of research associations. According to the new terms, separate grants were negotiated for research and for other activities; under the research part of the grant, virtually the full cost, up to an annual limit of £20,000, comes from the Government,

* Department of Scientific and Industrial Research. *Research for Industry, 1963*: a Report on Work done by Industrial Research Associations in the Government Scheme. Pp. v+54+19 plates. (London: H.M. Stationery Office, 1964.) 5*s*. 6*d*. net. See also p. 334 of this issue of *Nature*.

while for the general activities the grant is on the usual 50 per cent basis of grant-earning income up to a maximum of £17,500.

Some such arrangements might perhaps permit the ear-marked grant system to work smoothly, though where the amount of such grants is high or of the other activities and research carried out by the research association is relatively small, it would be worth while considering whether the research association model is appropriate or should, in these cases, be replaced by a structure of the research station type. Obviously there are other considerations, but the question of support from a reluctant or backward industry is far from being solved, in Britain or elsewhere, and deserves more penetrating and constructive thought than it has yet received. In this connexion it is to be noted that the Industrial Grants Committee has itself called for a thorough review of the research role, control and sources of support of the British Coal Utilization Research Association, but on different grounds—that of probable dominance by one or two large member organizations. Meanwhile, renewal of grant has been recommended for a further five years ending December 31, 1968, and it is hoped that the enquiry in progress will result in a formula which will either strengthen the laboratory in its work for the coal industry or define its functions more widely in relation to fuels in general.

Two other matters noted in the Industrial Grants Committee's report deserve comment. The most satisfactory feature of the scheme for special assistance to industry has been the variety of projects undertaken. This scheme, started in 1959, was planned to run for five years to help research associations in setting up or in improving liaison and information services, and generally in aiding the application of research results in industry. Almost half the research associations have participated. The Production Engineering Research Association, for example, employed a specially equipped mobile laboratory to give direct assistance to member firms in adopting new techniques to production operations. The British Non-Ferrous Metals Research Association demonstrated and developed prototype instruments for control of production processes and inspection of production. The British Welding Research Association runs special advisory services on the design of welded structures, the British Iron and Steel Research Association organizes similar services on ergonomics and safety, while the British Leather Research Association runs a special liaison service on effluents.

While the results of these projects are not easily assessed in economic terms, there have been some important results, notably in the baking industry through the introduction of the Chorleywood Bread Process, in the wool industry through the wider use of the Wool Industries Research Association 'Cop-Grader' and in production engineering through the introduction of improved metal-cutting and metal-forming techniques. Apart from this, the schemes have substantially influenced the industries served and they may well have encouraged research-mindedness generally. Most associations are continuing their projects as part of their normal programme of work.

The other matter is the extension to England and Wales of the scheme for the appointment of industrial liaison officers in technical colleges pioneered in Scotland by the Department in association with the Scottish Education Department. The scheme is welcomed by the Industrial Grants Committee, which considers that, particularly in relation to small firms, these liaison officers should provide a useful complement to the more specialized technical information and advisory services of individual research associations, and it is hoped that in time the officers will materially increase existing contact between industry and research associations. The Committee recognizes that it is important that the research associations should be linked with the activities of the liaison from the outside, and it proposes to consider further how such a full partnership can be fostered. This recognition of the importance of such information work is timely, but it would be even more encouraging to see a keener appreciation of the vital importance in all this of a really adequate system of library and information services for science and technology in all their aspects pervading the Council itself as well as the Advisory Council for Scientific Policy or its successor and the Ministries responsible for science, technology and education.

SOURCE OF CELLULAR ENERGY

The Mitochondrion

Molecular Basis of Structure and Function. By Prof. Albert L. Lehninger. Pp. xx+263. (New York and Amsterdam: W. A. Benjamin, Inc., 1964.) 9.90 dollars.

ALTHOUGH the structures now called mitochondria —a name proposed by Benda in 1898—have been known for more than a century, relatively little progress was made in the investigations of their physiological properties until about 20 years ago. During the 1940's two decisive advances were made. It became possible, by differential centrifugation in special media, to isolate mitochondria from other cell components, thanks to the pioneer work of Bensley, Claude, Hogeboom, Schneider and Palade, without major damage, and in particular without destroying their functional activities. These became thus amenable to detailed investigation on isolation. At about the same time, electron microscopy and its allied techniques such as thin-sectioning made striking advances in the elucidation of the fine-structure of mitochondria. The explosive developments initiated by these new techniques are described, sifted and analysed in *The Mitochondrion: Molecular Basis of Structure and Function*, by Prof. A. L. Lehninger, who himself has been in the forefront of mitochondrial research since 1948.

It soon became established that the mitochondria (and their counterpart in bacteria and certain other unicellular organisms—the 'membrane') are the chief 'power plant' for all cells depending on oxidation as a source of energy. It is the site where the energy of oxidation is released and converted into the utilizable form of high-energy-rich phosphate bonds. In addition, the mitochondria perform several functions, especially transport of ions and water and some chemical syntheses, which involve an expenditure of energy. The design of this power plant, in spite of its tiny dimensions (in rat liver $0{\cdot}1 \times 3\mu$), is of extraordinary complexity. One of the difficulties of analysing this complexity lies in the fact that many of the intramitochondrial chemical processes involve molecules which are embedded in an elaborate fine-structure, not readily accessible to present-day tools of investigations.

Prof. Lehninger's presentation is exceptionally competent. An enormous amount of material has been skilfully sifted and condensed to the essentials. The style is terse, clear and forceful, and the illustrations and diagrams are well chosen. The discussion is lively, directing attention to the gaps and hinting at likely future developments. The aim "to provide a comprehensive guide to current literature" has been splendidly achieved. The book is as up-to-date as any book can be expected to be in these

days of rapid progress: it has been produced in less than seven months.

Prof. Lehninger begins with a succinct account of the historical development of the subject. He then deals with the structural aspects, and this is followed, in several chapters, by a discussion of the oxidative processes (citric acid cycle, fatty acid oxidation and ketone body oxidation and formation, the properties of the electron transport chain and the mechanism of oxidative phosphorylation, including the energetics of mitochondrial metabolism and the control and integration of the various metabolic processes). Other chapters deal with the active transport of ions by mitochondria and with energy-coupled changes of volume and structure. One chapter is devoted to the ultra-structure in which the author discusses the information relevant to bridging the gap between electron microscope and chemical investigations of the basic molecules, or molecule assemblies, which have to be fitted into the structure.

Particularly stimulating is the final chapter on the bio-genesis of mitochondria, a subject on which information is still very scanty. Various hypotheses are discussed and include the *de novo* synthesis of mitochondria from sub-microscopic precursors present in the cytoplasm, the formation from other membranous structures in the cell, and the growth and division of pre-existing mitochondria. Mitochondria certainly have the capacity to synthesize some of their protein from amino-acids. Other mitochondrial components are synthesized extramitochondrially and taken up 'ready made' by the growing (or adapting) mitochondria. But the source of the mitochondrial matrix—the outer cell membrane, the endoplasmatic reticulum, the nuclear membrane, the nucleolus and Golgi apparatus have all been suspected—is still unknown.

The book integrates in a happy way the different kinds of mitochondrial investigations—biochemical, physiological and structural. No doubt it will find many friends among students at all levels. HANS KREBS

TRAINING THE INDUSTRIAL WORKER

Training within the Organization

A Study of Company Policy and Procedures for the Systematic Training of Operators and Supervisors. By David King. Pp. xxii + 274. (London: Tavistock Publications (1959), Ltd., 1964.) 38*s*.

NOW that the State has taken over responsibility for the development of industrial training and has empowered Industrial Training Boards to extract a levy from companies for training purposes, the attention being directed by industry and commerce to training has shown a noticeable increase. Attention, however, is not enough and many industrial managements are finding that the training required of them is much more than the sit-by-him variety. The help of specialists is being increasingly sought and, where they can show a practical approach to methods of training which lead to demonstrable improvements in workers' efficiency, managements are more than ready to accept their recommendations

Training within the Organization aims to provide a systematic approach to the training of operators and supervisors which, so far as it goes, could be most useful to those concerned with the training of operators. King first presents a case-study of a company in Norway which was faced with the need to improve the performance of women employed in garment making. He describes how the elements of jobs were differentiated by analytical methods, how a training sequence and programme were built up, how an instructor was selected and trained, and how the trained novitiates were introduced to the more austere conditions on the factory floor. In a neighbouring factory a similar approach to the training of supervisors led to the setting-up of self-governing working groups.

In the second part of the book the author examines the process of learning and shows how it should be used in training. The third section establishes the place of operator training in the company organization and, in the fourth section, detailed procedures in the training of operators are explained. The author also includes a chapter indicating how the effectiveness of training can be reviewed.

All in all, the case for the systematic training of operators in manual skills is well presented and the book will meet only with the criticism that it contains little that is new and has not been more succinctly presented by others elsewhere. If Mr. King had ventured outside the now familiar training schemes for sewing machinists, garment folders or stocking linkers, his book would have gained wider support, but the narrowness of his approach detracts from the appeal of the book. The inference throughout the whole work is that all that is needed in training operators is improvement in dexterity and that the systematic approach to the inculcation of skills is the universal panacea. The cognitive aspects of an operator's job are almost entirely ignored, and in those jobs where knowledge is at least as important as systematic training to develop manual skills, dexterity could be but a small part of the overall training required. In a chemical plant, for example, or in oil refineries, the operators need not only to be highly skilled manually but also to be well aware of what is taking place in pots and vessels and when and how to read instruments so that manual skills can be properly used. Nor will an approach which is not difficult to introduce on inexpensive equipment like sewing machines offer much comfort to managers faced with the problem of training operators for capital equipment like rolling mills, or for jobs that can only be done by operators working in teams, particularly where complete new teams are seldom required.

Mr. King's exhaustive approach to the development and administration of company training schemes also leaves much to be desired. Rather than detailed procedures, managers and supervisors would welcome guidance about what are, for them, complicated differences between City and Guilds and National Certificate Courses; when and where to recommend operative, craftsman, Intermediate Certificate and full technological courses; differentiation between Higher National Certificates and Higher National Diplomas and what endorsements mean to Section 'C' examinations of the professional institutions. To suggest that operator training schemes can be developed without reference to accompanying educational courses is to take an unrealistic specialist view which implies that we have not progressed far from the concept of the employment of 'hands' in industry.

The attention directed to the training of supervisors is so scanty that it is difficult to understand why it was included. T. H. HAWKINS

ECONOMICS OF EDUCATION

The Control of Education

By John Vaizey. Pp. 263. (London: Faber and Faber, 1963.) 30*s*.

THE Control of Education, the publishers claim, tells us "about education—its value, its cost, its control". In fact, it tells us little about these things but a great deal about Mr. Vaizey's opinions concerning them.

Mr. Vaizey has collected together a number of talks and papers which he has given at various times covering a variety of topics such as "Education and Economic Development"; "Manpower"; "A Policy for Higher Education"; "The Status of Teachers". There has been

very little editing. Since each essay is a unit in itself, it would have been helpful to be told when, and for whom, each had been written. As it is, we are left to grope along a series of dark corridors which turn out to be unconnected.

Some of the content is clearly aimed at developing countries and has little relevance to Great Britain. Much of it concerns education in Britain, but is oddly out of date. Thus, although published in 1963, his chapter on the block grant was presumably written before the introduction of the block-grant system in 1959 and is printed without revision or apology. It repeats the fears expressed by those who then opposed the system and contains no reference to the fact that by 1963 Britain had access to experience of the system as it had been worked out over the first three years of operation. An examination of these years would have shown that Mr. Vaizey's fears prior to 1959 have not been realized; there is, therefore, little point in repeating them as economic laws in 1963.

Mr. Vaizey has earned a deserved reputation as a pioneer of the investigation of the economics of education. Perhaps he is writing too much too quickly. Certainly some of his expressed opinions—including those to which he seems to attach most importance—merit more examination and research than he gives them. It is a good debating point to say that it is the shortage of teachers that keeps the financial structure of education alive. But is it anything more than a debating point ? It is true that to supply the service with the 100,000 additional teachers which it badly needs will cost an extra £300,000,000 a year. But does it follow, as Mr. Vaizey asserts, that had these teachers been available "the local taxation system and the general grant system would have come under intolerable strain some time ago" ? The system is flexible. We already spend £800,000,000 more a year on education than we spent 10 years ago, with no visible signs of a breakdown. Even if there is a ceiling to the rate share of this increase (and we have no idea what it might be), nevertheless the ratio of grants to rates can be adjusted to take the strain.

This is a point which Mr. Vaizey ignores; he seems to cling to the idea that a higher proportion of government grant is inadvisable because it leads inevitably to diminution of local control. This belief that local independence can only be assured by a high degree of local fiscal autonomy used to be widespread, but local authorities themselves are now beginning to question its validity.

Mr. Vaizey, however, not only still holds to this view, he also believes that only a very large authority can pay its way and that the present structure of local government must, therefore, give way to a system of regionalization. This is another *non sequitur*. There is, no doubt, a minimum size of administrative unit necessary for economy and efficiency. But what is this size ? And beyond the minimum, how true is it to say 'the larger the better' ? These are some of the vital questions which the professional educational economists should be considering, but they must be answered on the basis of careful costing, not of pontifical pronouncement.

N. Morris

TOWARDS THE EDUCATION OF THE WHOLE MAN

South Kensington to Robbins

An Account of English Technical and Scientific Education since 1851. By Michael Argles. Pp. xiii+178. (London: Longmans, Green and Co., Ltd., 1964.) 21*s*. net.

South Kensington to Robbins presents the history of scientific and technical education, against its social and economic background, since the Great Exhibition of 1851. In the light of recent developments, and because of the imminent massive expansion in the whole field of technology and technical education, it treats a subject which is interesting, important and timely. The author, the assistant librarian of the University of Lancaster, has produced a first-rate book, which compresses a considerable amount of information into a small space. The documentation is exceptionally well done. He pays tribute to some important figures in the history of English scientific education whose value has never been properly assessed or who have been overlooked. Such men were Albert Prince Consort, Lionel Playfair, Joseph Whitworth, Quintin Hogg (Senior), Roscoe, Lord Percy, A. W. Hofmann and his students. The story of English technical and scientific education, until the Second World War, is both surprising and sad. England did not lack men with knowledge and vision, including some trained in Germany, knowing the position of science and technology there, who were eloquent in their plea for a rational position for the 'pure' and 'applied' sciences in education. In 1851, after the successful conclusion of the Great Exhibition, Playfair said, "Until our schools accept as a living faith that a study of God's works is more fitted to increase the resources of the nation than a study of the Amours of Jupiter or of Venus, our industrial colleagues will make no material headway against those of the Continent". Even the obviously better technical equipment of the Germans at the beginning of the First World War was no lesson to England, so far as education was concerned, and the Spens report of 1938, just before another great war, has to confess that there was "little development of Secondary Schools of quasi-vocational type for those who desired to enter industry and commerce at the age of sixteen".

We have been waiting for Robbins for a number of years and, now that it is with us, we are still waiting, to a large extent, for it in terms of a new Government. But a report, even when followed by the expenditure of large sums of money, does not necessarily secure either success or efficiency. *The Times* considered that "by concentrating on the demand for places, as they interpret it, the members of the Committee have paid too little attention to the difficulties in which the universities would find themselves. The change of scale, the influx of students of questionable academic bent, the thinner spread of first-rate teachers are bound to influence profoundly the nature and quality of British universities . . . the Robbins rate of expansion . . . is not proof against the criticism that it would work to dilute academic standards". The conscientious provincial university teacher struggling with the least apt quartile of his students—"the already tapped sources of untalent"—may well wonder where are the "great untapped sources of talent". It is fair to say that cogent rebuttals of the "more means worse" type of argument have been made.

The foreword to the book is by Charles F. Carter, the Vice-Chancellor of the University of Lancaster. It is a masterpiece in one paragraph. It should be read by every responsible citizen. "Education remains a mysterious process, we are sure neither of how to achieve results, nor of what results we want to achieve. Technical education, though it may seem to have the simple function of providing the developed skills needed by society, must now in reality prepare people to develop skills which have not yet been imagined, required by technical or commercial processes not yet conceived; and it must do this in a way which has regard to the universal need of general education. . . . What we have done has usually been too little and too late; we still have to learn to educate for the needs of the future and not for the needs already passing which we have just belatedly come to understand. There is still a great lack of research and experiment".

Many more teachers who understand and practise modern techniques of teaching, examining and the use of libraries will be required. The university lecture which is a mere transcription of the lecturer's notes into those of the students, without the matter going through the minds of any of them, is not good enough: it never was !

Technology is not enough. It is a means to an end. A better technology does not ease the problems of the starving millions unless it is applied with freedom and goodwill on all sides. It does not even ensure a greater

supply of convenient and cheaper apparatus for better living unless restrictive practices on the part of both employer and employee are removed. If it is largely a matter of John keeping up with Ivan, Jo and Johann in the production of better rockets and aircraft, it has not served its purpose, and may be positively harmful. The development of scientific and technical education cannot be considered *in vacuo*: it must become a harmonious part of the education of the whole man. W. L. SUMNER

SPECIALIZED CONTRIBUTIONS TO PLANT SYSTEMATICS

Recent Researches in Plant Taxonomy

Edited by Dr. W. B. Turrill. (Vistas in Botany, Vol. 4.) (International Series of Monographs on Pure and Applied Biology. Division: Botany, Vol. 7.) Pp. xiii+314. (London and New York: Pergamon Press, 1964.) 105*s*. net.

THE original *Vistas in Botany* was a volume in honour of the bicentenary of the Royal Botanic Gardens, Kew. It appeared in 1959, and was at the same time Volume 2 in an international series of monographs on pure and applied biology. Volume 2 of *Vistas in Botany* was Volume 5 in this same series, and it contained reviews of topics in the general field of economic botany, thus acting as a useful extension of the original volume. Volume 3 was wholly physiological. Volume 4 bears the sub-title *Recent Researches in Plant Taxonomy*.

In view of the contents of the volume, the sub-title is rather misleading, for here are seven articles, essays or comprehensive reviews in which reference to recent research is made in very varying degree. Again, the seven topics, though all in some way connected with the practice of plant taxonomy, are treated so very differently by the different authors that the volume as a whole lacks unity and it is hard to see at exactly what class of reader it is aimed. The present position in flowering plant taxonomy is better stated, and the literature as a whole better covered, in the recent work by Davis and Heywood (*Principles of Angiosperm Taxonomy*), so that the value of the present volume lies rather in the weight of authority with which particular authors of international renown are able to write on their own chosen topics.

The first 22 pages are occupied by an article on philosophy and classification by J. S. L. Gilmour and S. M. Walters. In this perspicacious and scholarly article there is plenty of food for thought, but its content does not range far beyond what may be found in earlier contributions by one or other of these authors. The second chapter, of similar length, is that of Prof. G. Erdtman on palynology. Its effect is to leave one conscious of the almost excessively detailed examination to which pollen grains have been exposed in recent years. While such items as the tables on pp. 28 and 29 afford a useful summary of information, the figure on p. 31 will incline most botanists to leave pollen grains strictly to the specialists. For the few, the minute detail will provide a fascinating challenge, and they will be attracted both by Prof. Erdtman's clear exposition and by the technical brilliance of his illustrations.

Embryology in relation to taxonomy, by Prof. P. Maheshwari, occupies 43 pages and is an excellent summary of a subject which does not always receive the attention it deserves from taxonomists. This chapter is superbly illustrated and concludes with a bibliography of 167 references. These features, together with the clear presentation of the selected facts, make it a most valuable part of the book.

The next 87 pages are occupied by what is not only by far the longest chapter in the book but also the one that is, both from the technical and linguistic points of view, the most difficult. One feels that "Cytologie, Taxonomie, und Evolution bei Samenpflanzen", by Dr. F. Ehrendorfer, will appeal only to comparative specialists already well acquainted with this subject and anxious to read Dr. Ehrendorfer's authoritative views on it. The 21 figures are not uniformly easy for a student to grasp, but the bibliography of 346 titles should be useful.

There follow two chapters by the late Dr. W. B. Turrill, one of 37 pages on "Plant Taxonomy, Phytogeography and Plant Ecology", the other, of 13 pages only, on "Floras". Both suffer from excessive use of generalized statements. To trace the delicate interconnexions between taxonomy, phytogeography and ecology is no easy task; and somehow the article fails to achieve this aim. The arbitrary fragmentation of the subject into 'bits' does not help, and it is hard to see how 'types and typology' or 'herbarium problems' bear directly on the topic under review. At times a combative attitude obtrudes and there is some needless repetition. Much of the very sketchy chapter on "Floras" is self-evident and seems to be aimed at an entirely different class of reader from those visualized in the earlier contributions.

The final chapter on fossil plant taxonomy, by Dr. K. I. M. Chesters, is a most readable summary of a wide range of palæobotanical work. It will be useful to many non-specialist readers, but would have benefited greatly by illustrations on the liberal scale provided by Prof. Maheshwari.

The book is pleasantly produced, but better proof-reading would have eliminated numerous imperfections. Some are misprints, such as "micropore mother cells", "dabatable", "suported" and "phyllum" (phylum); others are examples of unfortunate phrasing such as "in the degree that" on p. 188, or confused construction where a sentence is left with no main verb, as on pp. 271 and 277. One finds the use of "globulets" for globules, "millenni" for millennia (p. 199) and "petrification" for petrifaction (p. 261) annoying, but "climate vegetation" in place of climax vegetation (p. 198) and "rhizomorph" for rhizophore (p. 265) are more serious. "Subdividable" for divisible and "analogical" for analogous are further verbal atrocities which ought not to have been allowed to mar a book of this class. With well under 300 pages of reading matter the book is a little expensive at five guineas.

E. V. WATSON

A WREATH FOR THE ENGLISH BOTANISTS

Sertum Anglicum, 1788

By Charles-Louis L'Héritier de Brutelle. Facsimile with Critical Studies and a Translation. (The Hunt Facsimile Series, No. 1.) Pp. xcviii+36+34 plates+12. (Pittsburgh: The Hunt Botanical Library, Carnegie Institute of Technology, 1963.) n.p.

NO one, however charitable, can honestly maintain that Charles-Louis L'Héritier, Seigneur de Brutelle, has any particular claim to fame as a botanist. He was at most a competent disciple of Linnaeus, content to elaborate and augment the works of his master at a time when many of his more enlightened compatriots were following the banner of de Jussieu away from the rather sterile formalities of the sexual system.

L'Héritier's few, and generally incomplete, publications are valued as rarities; they are, for the most part, of grand dimensions and amply illustrated by exceptionally gifted artists; they also happen to contain the first published identifications, descriptions and figures of a number of plants, some of them now well known to others besides taxonomists. For these reasons a facsimile edition of the rare (and incomplete) *Sertum Anglicum* is to be welcomed, indeed doubly welcomed, because the Advisory

Committee of the Hunt Botanical Library (sponsoring the publication) has had the good sense to make room for several valuable introductory essays, including a masterly biographical and bibliographical account of L'Héritier's life and works by Dr. F. A. Stafleu. Anyone who has ever had the misfortune to be involved in the nightmarish task of trying to date L'Héritier's publications will be eternally grateful to Dr. Stafleu for his researches; and those who have pondered on the character of the man who could perpetrate such bibliographical horrors will find illumination in the biography.

L'Héritier may have been a praiseworthy magistrate, but as a personality he was the type specimen of an exasperating and not uncommon species—devious, secretive and, as de Candolle remarked, "avec une sorte de complaisance aux petites intrigues". Sir Joseph Banks was caught up in the network of "petites intrigues" almost as soon as the misguided L'Héritier landed at Dover, and at once erupted in full fury: "Of all the impudent Frenchmen in the whole world he is the most impertinent and dangerous". But L'Héritier, with the pertinacity characteristic of his breed, survived the eruption, and more than eight months later, in May 1787, was still visiting Soho Square, and Banks was writing, somewhat wearily, "L'Héritier is still here to the amazement of everyone and has given no signs of an intention of returning. He has left off working at Dombey's Herbarium some time and now runs most diligently from garden to garden, buying plants in profusion and books without end".

Banks was to suffer his persistent visitor for a further six months, and, to crown his discomfiture, was to receive, in January 1789, the first fascicle of the *Sertum Anglicum*, dedicated to the English botanists, and recalling "how courteously everyone received me; how the scholars showed themselves accommodating and ready to reveal their treasures; how especially the men so learned in botany assisted me obligingly". Poor, poor Sir Joseph!

And poor, poor L'Héritier! His fate was tragic in excess of his deserts. But this lurid and mysterious story lies far beyond the scope of a review.

R. D. Meikle

AUSTRALIAN GRASSLAND RESEARCH

Grasses and Grasslands

Edited by C. Barnard. Pp. viii+269. (London: Macmillan and Co., Ltd.; New York: St. Martin's Press, Inc., 1964.) 50*s*.

THE subject of this book has received attention by research teams in many countries, and Australian scientists have made major contributions in this field. It is pleasing therefore to have the Australian findings relating to grasses and their utilization brought together under one cover. This is done within the context of much basic knowledge concerning the biology of the grasses, a necessary preliminary to their proper use as agricultural plants. There are fourteen chapters, written by present and past members of the staff of the Division of Plant Industry in Canberra.

The volume is therefore a series of specialist essays giving a mass of detailed information on a number of subjects. Each is more or less complete in itself, but there is perhaps insufficient cross-reference. The chapters are well documented, each with its own extensive list of references; many are annotated by diagrams and photographs, some depicting plant parts and others a range of grassland types belonging to the Australian environment.

The first chapter deals with historical aspects of grass and grassland development. It indicates also the relationship between grass and the grazing animal. This affords a useful introduction in which reference is made to agronomic sequences and also to the importance of grasslands in the agricultural economy. The next nine chapters are concerned with the biology of grasses, their taxonomy, distribution, morphology, histology, physiology of growth and reproduction, and with cytogenetics and plant breeding. Whereas the range limits of grass species may be determined by climate, distribution is greatly affected by agricultural influences. The distribution of grasses offers an index of the whole environment, but this probably holds more widely and in other families.

Grasslands as plant communities are considered in four separate chapters dealing respectively with distribution, nutrition, animal influence and soil conservation. The parts played by climate, topography, the biotic factor and fire are described as factors in grassland ecology. The nature of climax is noted and it is suggested that the mono-climax theory is untenable because climate is not the sole factor which determines the distribution and type of grassland.

The supply of soil nutrients has an important influence on the composition and production of pastures. Grassland improvement in Australia is much concerned with the legume, and emphasis is placed on the legume/grass relationship. Grazing management is discussed in relation to different grassland groups in Australia, where the trend on mesophytic grasslands has been towards winter-growing species and introduced annuals, a trend much influenced by the use of subterranean clover. There is, however, much to commend the perennial as the dominant constituent of pasture land. In arid situations some of the changes which follow grazing are harmful to pastoral production, and modified patterns of grazing are required. This matter needs emphasis, for in many countries grassland management is a most important factor in determining pasture type. Grazing may be too lenient, but more often harm is done because of over-pressure by animals.

Management has the dual purpose of providing animal feed and maintaining a protective cover on the soil. Soil conservation is basically the correct use of land, but we need to know how different swards behave as soil covers, especially at periods of stress when the sward may be open and under much grazing pressure. Stocking rates may have to be adjusted to meet this situation to minimize loss of soil.

The book is well produced, and the text reasonably free from minor error (the name of one author is omitted from the list of contributors, however). The subject is important, and the volume should prove of lasting value to a wide audience with an interest in grasses and grassland in general.

W. Davies

FORMOSAN FOREST FLORA

Woody Flora of Taiwan

By Hui-Lin Li. (A Morris Arboretum Monograph.) Pp. x+974. (Philadelphia, Pa.: The Morris Arboretum; Narberth, Pa.: Livingston Publishing Company, 1963.) 18.75 dollars.

THIS well-presented and attractive volume is divided into two unequal parts, a short introduction and a much bulkier systematic treatment of the woody plants of Taiwan. The introduction gives short accounts of the physical features of Taiwan and of its vegetation, including a brief history of botanical collecting and a very short review of its forestry. An analysis of the geographical affinities of the Taiwan woody flora is included. These turn out to be strongest with Eastern Asia, such elements (on the species level) comprising some 40 per cent of it, as might be expected for this Island province only 140 km offshore of the Asian land-mass. Of the rest, 25 per cent consists of various tropical elements and some 35 per cent is considered, in this account, to be endemic. This seems a remarkably high figure, and one wonders

how it would stand up to a more thorough comparison of the flora with that of mainland China, and to thorough monographic revision of the genera, especially the tropical ones.

The floristic part proper consists of a systematic descriptive account of the woody plants of Taiwan, arranged under families on the Englerian system but with the few monocotyledons at the end. Unfortunately, no indication of the author's criteria on which a plant is admitted, as woody, to this Flora is given; that there are many borderline species which are often variable in their habit is well known. The treatment claims to be a critical one, as indeed it should be, for time and money spent on those that are not so are, for tropical and sub-tropical areas at least, time and money wasted. Within the limitations of its size and format it does give quite full keys, descriptions, and citations of places of original publication of names and synonyms, and of herbarium specimens. These latter are of particular value in illustrating the author's taxonomic conclusions. Nevertheless, the work shows some disquieting features. It includes a number of incorrect names and erroneous citations, and at least one invalidly published name; while some names recorded for the Taiwan flora have not been accounted for. Furthermore, some of the more recent monographic work that has concerned, in part, the Taiwan flora (especially its tropical elements) seems to have been overlooked, and some of the literature cited appears not to have been personally checked by the author. Thus the book does not inspire confidence as a source of basic taxonomic data for the working taxonomist. To botanists in general, however, it will be welcome as a source-book of information on the woody plants of Taiwan.

As it is a woody flora its interest to the forester must also be considered. The main purpose of a Flora of any region must surely be to enable anyone who is not a taxonomist to determine as reliably and as easily as possible the correct name of any plant he finds growing in that region, and to give such references to synonymy and literature, as to enable him to pursue further information on it should he so require. Judged on these considerations, this Flora does quite well on the second count but not so well on the first. It omits virtually all introduced species; foresters familiar with the wanderings of many conifers and eucalypts will realize the limitations this imposes. There are numerous more or less satisfactory line-drawings, but these seem to have been made mostly from herbarium specimens—there are no photographs to show such field characters as bark type or shape of crown, nor is there any mention of them, or of vernacular names, in the text.

But in the final analysis a Flora stands or falls by the quality of its keys. Those in this book may or may not be reliable, use and time alone will tell, but they are not easy to use. The characters used are often quite needlessly difficult technical ones, and there is no glossary, nor any diagrams, to explain terms that are used. The Chinese forester may well find the difficulties of this entirely English text insurmountable. There are also some errors in the keys, for example, a whole line that should read "3. Ovary of a single carpel", has been omitted below the second lead of couplet 2 on p. 27, while on p. 159 the key characters of *Schisandra* and *Kadsura* have been transposed.

One is left with the impression that this book falls between two stools, being insufficiently rigorous, detailed and thorough in its taxonomic approach to serve as an authoritative regional Flora, nor, on the other hand, is it sufficiently suited to the needs of the local forester to be considered as a working forest Flora. It does, however, bring to the notice of botanists in general, and students of the Taiwan flora in particular, the richness of the Formosan woody flora, and, to the latter, their good fortune in having at hand such a wealth and diversity of the raw materials of botany.

C. JEFFREY

PLANT AND ANIMAL COMMUNITIES OF BRITAIN'S ROCKY SHORES

The Ecology of Rocky Shores

By Dr. J. R. Lewis. Pp. xi+323 (40 plates). (London: The English Universities Press, Ltd., 1964.) 42*s*. net.

THERE is now an impressive literature on intertidal life. Beginning in the eighteenth century, flourishing exceedingly in the mid-Victorian days of Philip Henry Gosse, during this century interest has spread to all the shores of the World. The great surveys of Australian, South African and North American rocky shores by T. A. Stephenson represent major enquiries into the distribution of animals and plants. They also revealed the universal presence of three horizontal zones he termed supralittoral fringe, midlittoral zone and infralittoral (or sublittoral) fringe, each delimited not by any fixed position in relation to tidal levels but by distribution of certain indicator species.

On the basis of his own investigations, initiated under T. A. Stephenson, which have taken him from the shores of western Ireland to those of Caithness, together with those of others concerned with specific aspects of the many problems involved, Dr. Lewis has produced what is reasonably described on the cover as "the first comprehensive account to be published of the dominant plant and animal communities of the rocky shores of the British Isles". The book is admirably produced with excellent photographs and diagrams, stylized representations of zonation, and a most useful systematic index.

Dr. Lewis prefers to divide shores into a littoral fringe with an upper limit of *Littorina* and *Verrucaria* (lichen), a eulittoral zone separated from it by the upper limit of barnacles (the two constituting the littoral zone) and a sublittoral zone bounded by the upper limit of *Laminaria*. The height to which each extends and the width it attains become greater with increased exposure. This is immediately demonstrated in an introductory comparison between a fully exposed shore at Parkmore Point, Co. Kerry, and an extremely sheltered one within Clachan Sound, Argyll.

Consideration is then given to factors influencing zonation—tides, exposure (all-important and yet measurable only by subjective criteria), topography and substratum, climate, time of low water of spring tides and presence of other organisms—and then to those influencing the geographical and local distribution of shore species. The next two parts of the book are devoted to descriptions of zonation, first on open coasts, with separate consideration of the eulittoral areas dominated by barnacles, by mussels and by Fucaceae, and then in lochs and very sheltered bays. The concluding chapters are devoted to the consideration of the many problems that present themselves.

Precisely why should a species disappear as a headland is rounded? Is this due to inability to settle or, having done so, to maintain or reproduce itself? Why should mussels occur on some eulittoral shores and barnacles on others, apparently similar? Why is it impossible to predict what exact combination of an invariable assemblage of sponges, hydroids, polyzoans and ascidians will occur in any region of tidal rapids? Anomalies abound, and the clearer the general picture the more obscure become its details. But part of the answer, as Dr. Lewis notes, is that shore populations are always changing, young individuals replacing old ones, new populations settling from the plankton and with all manner of intermittent, seasonal and perhaps purely chance factors exerting their separate or interlocking effects. There is on the shore no inevitable succession leading to a stable climax.

The most specialized and successful shore animals are the acorn barnacles, which are of exceptional interest on British shores. There is the overlap in the eulittoral of the northern *Balanus balanoides* and the southern *Chthamalus*

stellatus, each with its own ecological requirements, the latter generally demanding greater exposure and extending higher. Where they overlap the two species in some places compete, in others occupy distinct zones indicating other controlling factors. Nor is distribution static; the two follow the present general northward trend of marine populations. Added to these is the Australasian *Elminius modestus* the spread of which since 1945 has been so carefully followed. Here other ecological needs, structural limitations and more frequent reproduction, have all to be considered.

All is admirably dealt with and, if Dr. Lewis does in places labour somewhat too desperately for exact description and explanation, he does in text, photograph and diagram convey an impression of the richness and variety of intertidal life on Britain's rocky shores which will come as a revelation to many and be of the greatest value to all who study these shores. This book is a major addition to knowledge concerning the fauna and flora of these islands.

C. M. Yonge

BIRDS OF THE AMERICAS

Birds of the Labrador Peninsula and Adjacent Areas

A Distributional List. By W. E. Clyde Todd. Pp. xii + 819 + 8 plates. (Toronto: University of Toronto Press; London: Oxford University Press, 1963. Published in association with Carnegie Museum.) 18 dollars; 144*s*. net.

The Birds of Colombia and Adjacent Areas of South and Central America

By R. Meyer de Schauensee. Pp. xvi + 427 (20 plates). (Narberth, Pennsylvania: Livingston Publishing Company, 1964.) 10.00 dollars.

EACH of these books deals with the avifauna of a particular area in the New World, one sub-arctic, the other tropical; both are products of the expeditionary enterprises of museums in, as it happens, Pennsylvania. There the resemblance ends. The work on Labrador is purely a research report, with the limited scope indicated by its sub-title. The other is a dual-purpose book, presenting the results of research in summary form and at the same time serving as a guide to the identification of the birds to be found in Colombia and adjacent areas.

Clyde Todd is indeed a veteran worker, having entered on his career as a professional ornithologist in 1891. This has been mainly associated with the Carnegie Museum in Pittsburgh, where he now has the status of curator emeritus of birds. From there, in the period 1901–58, he organized 25 ornithological expeditions to Labrador; most of these he led in person, and occasionally he was single-handed. The object was to study the distribution of the bird species found in that inhospitable area, and the existence of such 'life-zones' as climatic and physiographic factors could be shown to determine.

The result of these prolonged and arduous investigations has been a massive accumulation of detail, all this time deliberately withheld from publication. The information is now presented in its entirety, and fully documented with references to other sources; it makes a volume of outsize dimensions and more than 6 lb. in weight. Inevitably, it is strictly a work of reference.

Meyer de Schauensee is curator of ornithology at the Academy of Natural Sciences of Philadelphia, where he and various colleagues have made special investigations of the immensely rich bird-life of Colombia. As a work of reference his book usefully summarizes what is known about the distribution of species and sub-species in Colombia, with indications of the wider range. It is, however, designed primarily as a handbook, and is accordingly provided with descriptions to aid identification and with numerous illustrations both in colour and in line; habits are mentioned only briefly, in the general introduction to the systematic treatment of each family. It is an attractive volume, admirably adapted to its purpose.

The work has much more than a local relevance, and its appearance is particularly to be welcomed because, as the author puts it, "no modern book exists in English on tropical birds of continental South America". In these circumstances the book will be very useful beyond the confines of Colombia itself; it covers three-fourths of the birds found in a much larger adjacent area, and more than half of those found in the tropics of Central and South America from Yucatan to Paraguay.

It is interesting to contrast the two areas. In Labrador there are only 49 species or sub-species that are found throughout the year, with 4 others that are winter visitors from further north; 200 are breeding summer visitors, and 34 others (excluding 'accidentals') are migratory transients or non-breeding summer visitors. The total compares with 1,556 species (2,640 sub-species) recorded from Colombia—a figure without known parallel. This is a sign not only of the richness of the tropics, and of the neotropical region in particular, but also of the great diversity of the country; Colombia has coasts to the Caribbean and the Pacific, parts of the Orinoco and Amazon systems, grassy plains, continuous forests, and mountains rising above the snow-line to 19,000 ft.

Landsborough Thomson

CARPENTRY TOOLS

The History of Woodworking Tools

By W. L. Goodman. Pp. 208. (London: G. Bell and Sons, Ltd., 1964.) 45*s*. net.

THE History of Woodworking Tools provides the first comprehensive account of such tools in the Western hemisphere. It seems surprising that objects which play such a vital part in everyday life have not been more often investigated but, as Mr. Goodman points out, there seems to be a general impression that through the ages there has been very little change in the form of the more common tools. He goes on to suggest that this is not the case, but that in almost every detail of their design and construction, changes and improvements have occurred.

Woodworking tools are described in this book under eight headings, starting with the axe and adze. The earliest of these were the antler axes, used by small groups of people living in Denmark in the Mesolithic period. Axes and adzes of flint and stone followed, hafted in various ways, and then a variety of tools in bronze, iron and steel, some of them very specialized forms.

The origin and development of the plane are not so clear. The Greeks had a word for it, but the earliest examples are Roman, and numerous fragments survive from this period as well as representations carved on tombstones. The old belief that this tool was forgotten in the Dark Ages and re-invented in the twelfth and thirteenth centuries is disproved by new evidence from Frisia and Anglo-Saxon England. Few tools of any kind of the period from 800 until 1600 survive, but there is much pictorial evidence, sometimes puzzling or obviously inaccurate, according to the interest taken by the artist in practical matters. In this context, the building of Noah's Ark is often a fruitful subject for contemplation. From 1600 until the present day there is abundant material. Indeed, the two centuries after 1600 are described as the 'Golden Age' of woodwork, because the rising standard of living and the appearance of new types of wood from the New World and the Far East demanded increasing skill from joiners and cabinet-makers, and a wide range of special tools.

The first true saws seem to date back to the early Bronze Age, although flint knives with a serrated edge go back to earlier times. The saws were made of copper, and the arrangement of the teeth shows that they must have been used with a pulling action. The far more effective saw with

a pushing stroke must have been developed by the Romans, who understood the importance of setting the teeth. They also experimented with an extension of the handle along the top of the blade, thus anticipating modern backed saws, and developed several types of framed saw in various sizes which continued in use through the Middle Ages until the present day in some backward areas.

Boring tools include the bow-drill depicted on early Egyptian reliefs, the auger used by the Romans, and the brace, known as a fully developed tool in northern Europe in the fifteenth century and probably originating farther east. Chisels and gouges, which date back to prehistoric times, have a section to themselves. Other essential items of the carpenter's equipment are also discussed and include his bench, also probably a Roman innovation connected with the invention of the plane, devices for measuring and setting out, such as rulers and dividers, squares and levels, and even his bag.

The book is attractively produced and well illustrated, with 200 photographs or line drawings of both actual tools, and tools shown in pictures, illustrated books, sculpture or wood-carvings from all over Europe. Many of the objects depicted, such as the seventeenth- and eighteenth-century planes, show much decoration, so that Mr. Goodman has not only produced a useful work of reference for craftsmen, but also an important book for all who are interested in the ordinary every-day life of people at all periods.

JOAN LIVERSIDGE

INTERNATIONAL SPACE RESEARCH

Space Research IV

Proceedings of the International Space Science Symposium, Warsaw, June 4–10, 1963. Edited by P. Muller. (Organized by the Committee on Space Research—Cospar —and the Polish Academy of Sciences.) Pp. xvi + 997. (Amsterdam: North-Holland Publishing Company, 1964.) 220*s*.

THE fourth International Space Science Symposium arranged by the Committee on Space Research (COSPAR) was held at Warsaw in June 1963, and, as in previous years, the proceedings have been published in a handsome 1,000-page volume by the North-Holland Publishing Company. The papers are mainly concerned with geophysics and interplanetary physics—with Earth, Air, Sun and Space. There is little on the tracking or dynamics of satellites, while the biological papers are being published in a separate volume.

Since the subject-matter of space physics extends without interruption from cloud-level to the outermost limits of the universe, any attempt to classify the papers is liable to be as fallacious as librarians' attempts to classify books. But in both spheres the attempt must be made, and in the present volume the division into, basically, six sections is surprisingly successful. There are 90 specialized papers in the six sections, and it is worth mentioning a few individually, even though others equally worthy must be omitted.

The first section deals with the upper atmosphere, which in practice means the study of the main neutral constituents of the atmosphere from the stratosphere to about 2,000 km height. L. G. Jacchia and J. Slowey report the most detailed analysis yet made of the variations in upper-atmosphere density and temperature at heights near 600 km, from the orbit of the *Explorer* 9 satellite. K. Y. Kondratyev discusses how to interpret the measurements of the Earth's outgoing radiation made by meteorological satellites.

The next section is on the ionosphere, which is to be interpreted as the charged particles with ordinary thermal energies at heights from 100 to 2,000 km, and sometimes higher. R. C. Sagalyn and M. Smiddy report on the electrical microstructure of the *D* and *E* ionospheric regions. The measurements of electron density made (by different methods) from the *Alouette* and *Ariel* 1 satellites are described by G. L. Nelms and P. Rothwell respectively.

The third section, entitled "Magnetosphere", is primarily concerned with the high-energy trapped particles of the radiation zones at heights ranging from 400 km (near the Brazilian magnetic anomaly) up to the outer limits of the Earth's atmosphere, 50,000 km or more. There are three papers, by Yu. I. Galperin, V. I. Krassovsky and others, on the results from the *Cosmos* 3 and 5 satellites, while L. A. Frank, J. W. Freeman, jun., and J. A. Van Allen describe their mapping of the outer boundary of the magnetosphere with the aid of *Explorers* 12 and 14.

The fourth section describes investigations of solar radiation, chiefly ultra-violet and X-ray, and includes many new measurements from the *Oso* 1 and *Ariel* 1 satellites, and from high-altitude rockets. The fifth section is devoted to the interplanetary medium (the plasma streams and meteoric material). There are useful papers by T. Obayashi and E. I. Mogilevsky on the interaction of the solar wind with the Earth's magnetosphere, and by T. R. Kaiser and C. T. D'Aiutolo on meteoric particles. Finally, there is a group of papers on cosmic rays, X-rays and nuclear reactions in space.

The specialized papers are preceded by a valuable series of review papers. The first, a frankly critical review of the ionospheric *F*-region by J. A. Ratcliffe, offers a new method of presenting a scientific paper, as the bare bones without the all-too-solid flesh of verbiage which normally hides them. Though some readers may yearn for more complete sentences, this vivid staccato style may well be more conducive to progress than the stodgy tone of the average scientific paper, because it brings out the main points at issue so clearly. The dozens of unanswered quickfire questions inevitably give the impression, however, that little has been discovered in thirty years of ionospheric investigations.

There are two important review papers on the aurora and airglow by B. J. O'Brien and V. I. Krassovsky. O'Brien notes that the energy required to run the world-wide aurora is on average 4×10^{17} ergs/sec, while the tube of particles streaming out from the Sun, as intercepted by the outermost atmosphere, brings about 3×10^{19} ergs/sec. So 1 per cent of the solar-wind energy could sustain the aurora. But the detailed process—the life-history of the particles—is still unknown.

P. V. Vakulov and others contribute a thorough review of cosmic rays, their energy spectra and their time variations, utilizing measurements both in space probes and on the ground. W. N. Hess discusses the life-times and life-histories of high-energy particles trapped in the radiation zones, and there is a useful review by E. R. Mustel of the Sun and the interplanetary plasma. C. W. Snyder and M. Neugebauer contribute an interesting report on the measurements of the solar wind made by *Mariner* 2, which showed that the velocity of the plasma varied between 300 and 800 km/sec, with a 27-day recurrence tendency and a mean of about 500 km/sec.

The book, like its predecessors, is well printed and produced, with good type and paper, though misprints are more frequent than in previous volumes. Each of the papers is intended to have abstracts in English and Russian, but many of the Russian ones are unfortunately missing. Several of the Russian papers are rather difficult to read because the English is far from fluent: most specialists will be prepared to accept this slight inconvenience as a small price to pay for the advantage of having so many Russian papers available in English within a year of the conference. Recording as it does the main advances in space research, reported by the leading space scientists of all countries, this volume should be in every library covering the subject, and many space scientists will wish to obtain a personal copy for reference.

D. G. KING-HELE

Georges Cuvier

Zoologist—a Study in the History of Evolution Theory. By William Coleman. Pp. x+212. (Cambridge, Mass.: Harvard University Press; London: Oxford University Press, 1964.) 40*s*. net.

THE life of Cuvier is a 'success' story, a rise from provincial obscurity to eminence as an imperial inspector of public instruction under Napoleon and peer of the realm under the restored Bourbons. It is also a record of sustained, highly organized and immensely productive enquiry into the structure of animals. "Dissection and description," in the words of Mr. Coleman, "could produce the clear and indisputable facts, and comparison could generalize them and reveal their real significance. The principal generalizations thus formed were the famous anatomical rules: the correlation of parts, which established the interrelated physiological operations and morphological features which guaranteed the life and integrity of the organism, and the subordination of characters, which determined the taxonomic value of the structures making up the organism."

After Aristotle, whom he held in profound respect and whose teleological attitude he endorsed, Cuvier is the supreme comparative anatomist. His contributions to classification (as distinct from taxonomy) are profound; the principles underlying the modern subdivisions of the animal kingdom spring directly from the criteria on which he based establishment of his four *embranchements*. Belief in special creation, based on scientific rather than religious belief, did not prevent investigation of fossils, to the reconstruction of which he applied the pregnant conception of the correlation of parts. The first true palæontologist, he nowhere contemplated a succession of species with extinct forms providing evidence of progressive advance. He thought only of a series of populations successively eliminated by catastrophes.

Opposing with equal vigour the evolutionary ideas of Lamarck and the concept of the archetype propounded by the German *Naturphilosophen* and so ardently championed by his other great contemporary, Etienne Geoffroy St. Hilaire, Cuvier has more in common with Linnæus. Both sought, the latter in the *Systema Naturae* and the former in the *Régne animal*, to lay bare the plan of creation. Failing inevitably in this, they nevertheless, by establishment of the species concept and by clear demonstration of structural relationships, laid firm foundations for later establishment of the fact of evolution.

In this concisely written book, Mr. Coleman has not attempted a life of Cuvier with its public triumphs and private tragedies. He confines himself to an analysis of ideas contained in Cuvier's unpublished as well as his published work, and in so doing provides an important contribution to the history of zoological thought.

C. M. Yonge

Iconographia Mycologica, VIII

By O. Verona and T. Benedek. (Mycopathologia et Mycologia Applicata, Vol. XVIII, Supplementum.) Pp. iv+plates *A*166–181, *B*52–65, *C*106–115, *D*30–39. (Den Haag: Uitgeverij Dr. W. Junk, 1962.) n.p.

Iconographia Mycologica, IX

By O. Verona and T. Benedek. (Mycopathologia et Mycologia Applicata, Vol. XIX, Supplementum.) Pp. iv+plates *A*182–203, *B*66–73, *C*116–135. (Den Haag: Uitgeverij Dr. W. Junk, 1963.) n.p.

Iconographia Mycologica, X

By O. Verona and T. Benedek. (Mycopathologia et Mycologia Applicata, Vol. XX, Supplementum.) (Den Haag: Uitgeverij Dr. W. Junk, 1963.) n.p.

THIS series of unbound plates, 50 to a volume, offers clear line drawings to illustrate the genera of microfungi. Usually only one species is figured, occasionally a small genus is treated in greater detail; for *Fusoma* Corda, Plate *A*169, no species is named, though conidia and conidiophores are drawn.

The scope of the work is very wide, covering every group of fungi from the lower Phycomycetes to the Uredinales, a field too vast for any modern specialist. Hence, in the chaotic state of fungus systematics, the authors must expect serious problems in the interpretation and typification of some generic names. They are not well-known taxonomic mycologists, and their work would have greater authority if the source of each plate were clearly stated. At present one is left to guess how much is original, how much copied and from which source. When, as with *Englerulaster macowanianus* (Plate *C*114), for example, a figure is attributed to a standard authority, it may be found to have been somewhat drastically redrawn. This plate is taken from Arnaud, but that next to it, *Morenoella mollinediae* (*C*113), is not clearly so attributed, though he is cited as authority for the genus. Comparison with Arnaud's Pl. XXI shows the lower part of *C*113 to be copied closely from his work and the remaining portions to be apparently inspired by him, but they are so modified that direct borrowing cannot be demonstrated.

A brief description accompanies each plate. The book is in English, sometimes not happily worded, and the proof-reading leaves something to be desired. For example, on p. *D*38 we read: "*Schizonella* parasitizes Ciferaceae" and, under *Cintractia*, "living on Ciperaceae"; in each case 'Cyperaceae' is intended. This would appear to be a work which could fill a useful role for mycologists who lack access to an adequate library, but which would profit by more critical taxonomic supervision.

R. W. G. Dennis

The Annual Species of *Medicago*

By Chaia Clara Heyn. (Scripta Hierosolymitana, Vol. 12.) Pp. 154. (Jerusalem: At the Magnes Press, The Hebrew University; London: Oxford University Press, 1963.) 36*s*. net.

SOME genera seem to have been designed by Nature to defeat the plans of botanists; some re-planned by botanists to obscure the designs of Nature. *Medicago* has had the worst of both worlds, the natural intricacies of its taxonomy being further confused by the complexities of its nomenclature. Fortunately Dr. Heyn has appeared, like a botanical Ariadne, offering guidance through the labyrinth, and showing us that the way, though tortuous enough, is not insuperably perplexing.

The revision is restricted to the annual members of the genus, and includes full descriptions and synonymy of 28 species and 35 varieties, supplemented by keys and a generous number of really excellent illustrations, photographs and text diagrams. Notes on habitat and geographical distribution, and lists of specimens examined, accompany the description of each species and variety, and where the author feels the need for further elucidation there are explanatory notes on taxonomy and nomenclature. All this information, together with a readable introduction and a good bibliography, will come as a boon, not only to professional taxonomists, but to all those agriculturists and plant-breeders who have hitherto struggled with this intractable genus.

Dr. Heyn has had the good luck to live in a country where it is possible to examine living specimens of most of the species she describes; it is gratifying to add that she has taken full advantage of her situation, and provided us with a revision which will be of lasting value. Only very occasionally, as in the treatment of *Medicago aculeata* and its varieties, or in the comments on the typification of the genus *Medicago*, does one venture to question her conclusions, and, in such a labyrinth, it would surely be unreasonable to expect that our guide should never once falter or stumble.

R. D. Meikle

RADIO ECHO SOUNDING THROUGH AN ICE SHELF

By M. E. R. WALFORD

British Antarctic Survey and Scott Polar Research Institute, Cambridge

IN December 1963 a tractor journey of 200 miles was made on the Brunt Ice Shelf from the British Antarctic Survey base at Halley Bay (Base *Z*, 75° 31′ S., 26° 40′ W.) and some unexpected results were obtained with a radar instrument developed for the penetration of polar ice masses. They seem to be different from the observations made by Waite (private communication) with a higher frequency apparatus on the Ross Ice Shelf. The techniques have been described by Evans[1] and the important parameters of the present apparatus are given in Table 1.

The range of a radar target is indicated by the echo delay-time, with an accuracy determined mainly by the rise time of the receiver. The shortest measurable range is limited by the recovery time of the receiver after the transmitter pulse and in practice, by echoes from nearby surfaces, probably stratifications in the snow. The aerials can be seen in Fig. 1: they transmit and receive most power in their equatorial plane, which includes the vertical and the direction of travel. Due to refraction, the sensitivity in the ice is greatly reduced beyond 35° to the vertical. There is no other means provided

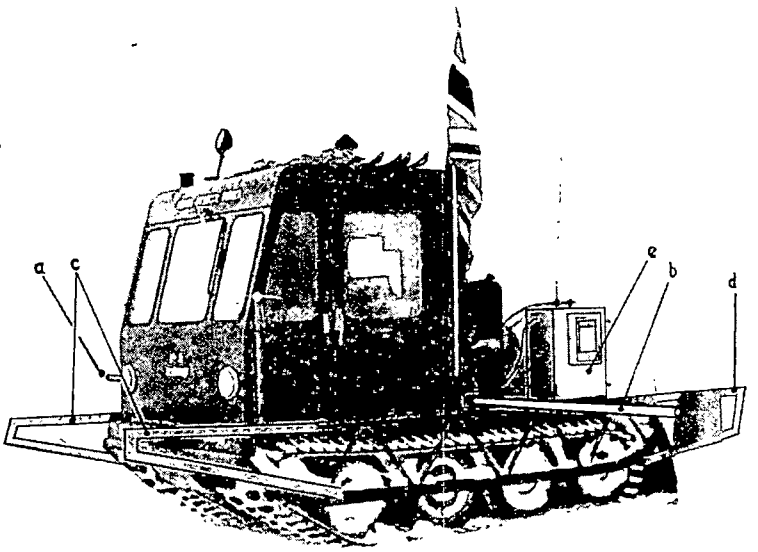


Fig. 1. Muskeg vehicle with separate unipole aerials (*a*, *b*) for transmission and reception. The 'Dexion' extensions (*c*, *d*), which increased the effective electrical length of the vehicle, were found necessary to reduce the direct coupling between transmitting and receiving aerials and to provide correct electrical loading. The box (*e*) contains the electronic equipment except for a display unit mounted in the vehicle cab

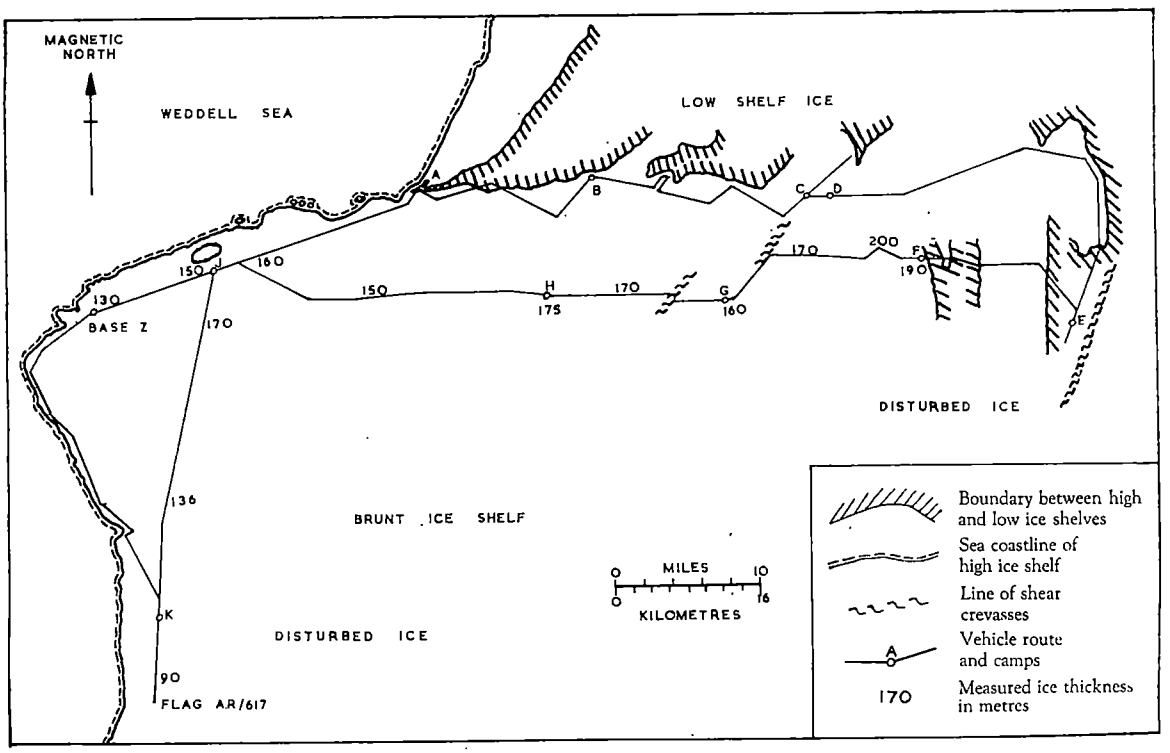


Fig. 2. Sketch map (after Finlayson, British Antarctic Survey Sledging Report, 1963) showing the vehicle route on the Brunt Ice Shelf. Base *Z*, Halley Bay, has Geographic co-ordinates 75° 31′ S, 26° 40′ W.; the magnetic deviation is 1·7° west of true north. Results obtained between Base *Z* and Camp *F* and between Camp *J* and Flag *AR*617 are plotted in Fig. 3. Most of the ice shelf is covered by troughs and ridges lying roughly north-east to south-west

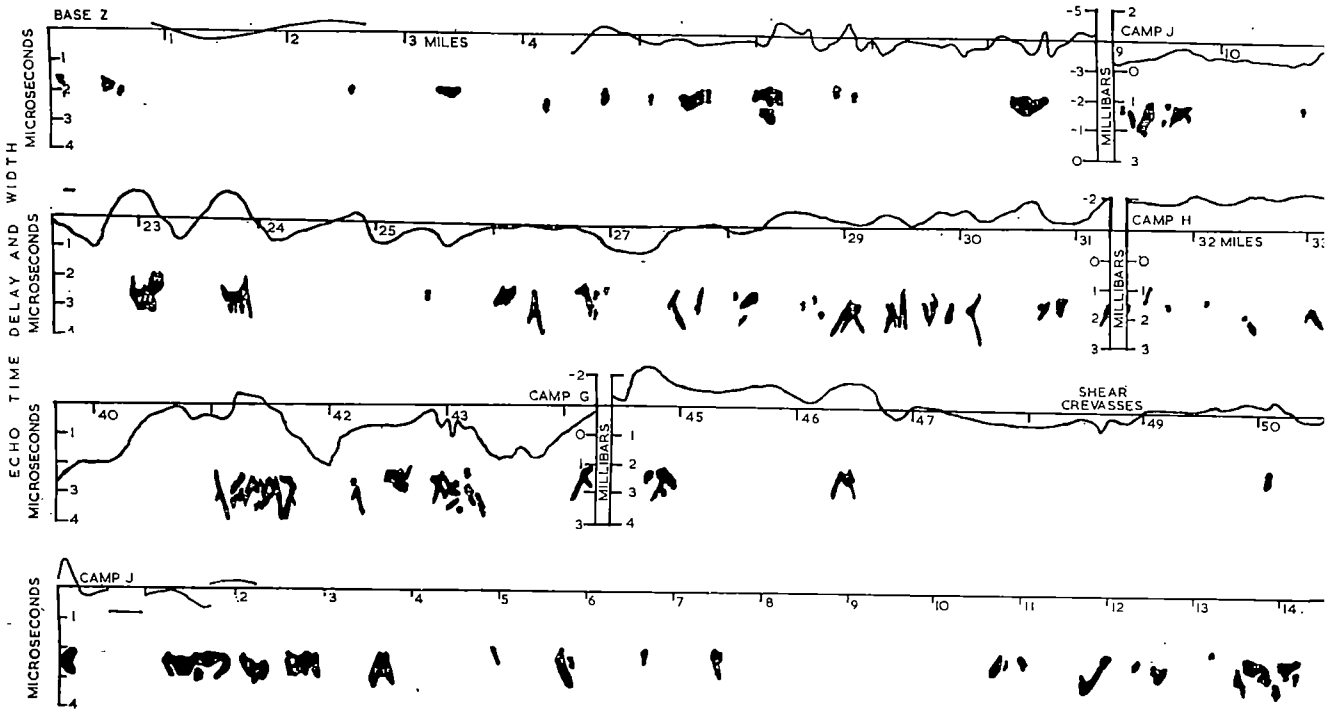


Fig. 3. Barometric pressure and radio echoes plotted against sledge wheel mileages. Pressure, the continuous curve, is plotted in millibars deviation from the daily mean pressure at Base *Z* corrected to mean sea-level. The blocked-in areas represent the time-delay and duration of radio echoes in microseconds. If pressures and time-delays are converted into heights and depths the respective vertical exaggerations are 50 times and 5 times. Note that on the lowest plot (Camp *J* to Flag *AR*617) no barometric pressures are available and that the mileage scale is different from the upper three plots. The inset shows the loci of echoes from discrete targets at various minimum radar ranges from the vehicle track. They are strictly appropriate only to the upper three plots and are calculated assuming 160 m/μsec for the velocity of radio waves in ice

for determining the direction from which an echo is received.

Range information is obtained in the form of a time-interval displayed on a cathode-ray tube as a fraction of the period of a crystal oscillator. The velocity of propagation has been estimated from an experiment in which the transmitting and receiving aerials were separated a distance s apart in a region where the ice depth appeared to be constant. The echo time-delay, t, for the slant path through the ice was measured for a series of spacings and the velocity deduced from the gradient of s^2 against t^2. The method is unfortunately rather sensitive to the slope of the bottom surface of the ice shelf, so a large uncertainty remains in the present result, which is 160 ± 20 m/μsec. Laboratory measurements of the permittivity of natural ice cores (Evans, 1964, in the press) and unpublished field measurements made in Greenland suggest that the most probable velocity is in the upper half of this range and that it depends on the ice density.

The sketch map in Fig. 2 shows that the Brunt Ice Shelf is bounded to the north and east by low shelf, perhaps 50 m thick, while to the south a broken surface slopes down to the junction with the inland ice sheet. Waves of a few metres height (greater in the east) have troughs lying north-east to south-west. Other surface disturbances indicate that there is probably local grounding on the sea bottom.

Depth soundings were made on the route from Camp *J* to flag *AR*617, and from Base *Z* to Camp *F*. The section from Base to Camp *J* was examined about a month earlier. Navigation by compass and sledge wheel proved simple and adequate. The error of closure for the loop of 150 miles was 4 miles which, if significant, is probably due to the difficulty of maintaining a straight track in undulating country. Information was recorded in a notebook using a concise notation to deal with the flow of data. An echo was characterized by its time-delay and code letters designated width, rise time, amplitude, and variation with vehicle movement. In addition, the barometric pressure, sledge wheel mileage and time, were read frequently.

In Fig. 3 the blocked-in areas show the time-delay and duration of radio echoes in microseconds, and the upper curve represents the barometric pressure deviation in millibars from the daily mean pressure at Base *Z* corrected to mean sea-level (Table 2). One millibar is equivalent to about minus 8 m change in altitude in air and 1 μsec is equivalent to 80 ± 10 m depth of ice. Thus, estimated random errors of one-tenth of a millibar and one-tenth of a microsecond in reading the instruments correspond to errors of 0·8 m surface elevation and 8 m depth.

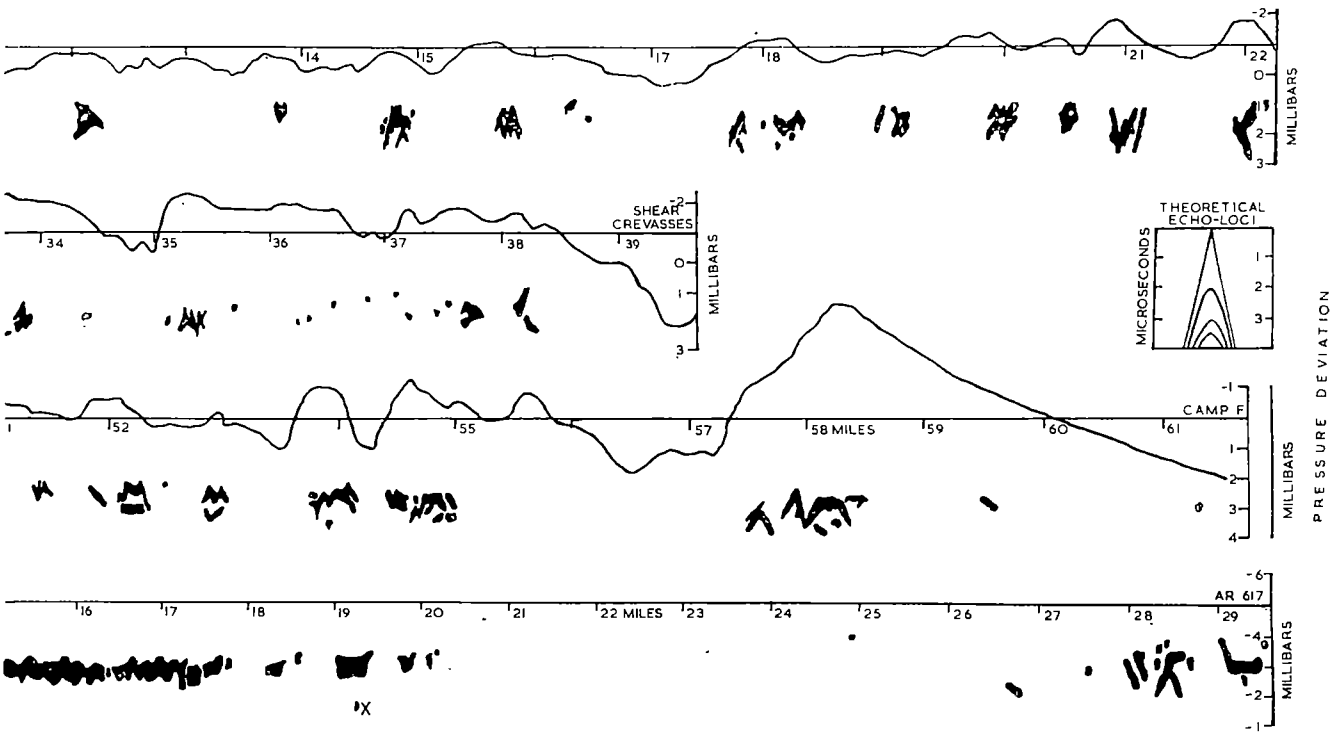
Table 1. Equipment Parameters

Radio frequency band	32–39 Mc/s
Receiver sensitivity	127 db below peak transmitter power
Transmitter pulse width	0·3 μsec
Pulse rise time (10–90 per cent at receiver output)	0·1 μsec
Pulse repetition frequency	10^4/sec
Aerials	Horizontal unipoles

Table 2. Daily Mean Pressures at Base *Z* corrected to Mean Sea-Level

Date, 1963	Journey	Pressure in mbar
Oct. 21	Base *Z* to Camp *J*	975
Dec. 7	Camp *F* to Camp *G*	977
Dec. 8	Camp *G* to Camp *H*	983
Dec. 9	Camp *H* to Camp *J*	989
Dec. 11	Camp *J* to Flag *AR* 617	992

We note first that echoes occur in groups; within groups the echo power averages 30 dB above receiver noise and peak powers of 55 dB are observed in places. Thus we have to explain a huge variation in mean echo strength



from place to place on an almost uniform ice shelf. Secondly, it is clear that the occurrence of the groups is correlated with the barometric readings showing that echoes occur only under the higher portions of the top surface. Finally, within the groups, the locus of an individual echo usually decreases in range to a minimum and then increases again as the vehicle continues along its track. If two distinct echoes converge in range and coalesce the resulting echo amplitude varies with vehicle position in a roughly sinusoidal manner with wave-length 3 to 50 m and fading range of 10 to 20 dB. Otherwise the strength of a single echo changes slowly in a distance less than the shelf thickness.

Echoes at much longer range than the minimum for the group are examined by turning the vehicle and aerials about a vertical axis. The resulting variation in echo strength shows that the targets are at a large angle to the vertical. The only clear exception, marked x in Fig. 3, appears to be due to a pulse which, partially reflected on first reaching the top surface, traverses the ice shelf four times before reception.

Since echoes at range longer than the minimum for the group are found to be oblique, we explain the locus of an individual echo by supposing that it is due to a small, isolated reflecting region which scatters the incident power into a wide solid angle. Theoretical loci for different minimum ranges are shown in the inset to Fig. 3, and can be seen to fit the observed echo shapes adequately. The curvature depends on the velocity of propagation used in the calculation; a target in air would produce a locus extended by a factor 1·8 along the mileage axis. On this explanation the true vertical extent of the ice shelf must be obtained from the minimum time-delay found in a group of many echoes, not from an isolated sounding. Such depths are marked at a number of places in Fig. 2.

Variations in height of the top surface of the ice shelf are often greater than 10 m in amplitude and are separated from one another by distances of about a kilometre; approximately five times the measured ice thickness. If these variations are nearly in hydrostatic equilibrium and the snow density does not increase more rapidly with depth below troughs than below ridges[2], then the thickness of the shelf must vary by about ten times the variation in surface elevation. This is compatible with the hypothesis that ridges occur where the shelf contains icebergs of inland origin, the troughs in between consisting of sea ice and drift snow[3]. If the shelf is not in hydrostatic equilibrium it is difficult to explain how the necessary vertical components of shear stress could arise[4,5].

It is surprising that the radio-echo technique has failed to indicate the ice thickness except below surface ridges. We can only suppose that an absorbing layer at least 10 metres thick at the bottom of the shelf presents no sharp discontinuity in electromagnetic impedance and so reflects no energy. Ragle, Blair and Persson[6] have measured the dielectric loss factor of ice samples taken from the bottom on the Ward Hunt Ice Shelf in the Arctic and found them about 20 times greater than for ice samples taken from near the top surface. The transitional layer could be caused by liquid sea-water permeating into pure ice or by the accumulation of new ice frozen from sea-water. The observed echoes, under ridges, would then arise only where neither process occurs or, following Barclay, where the bottom of the ice shelf consists of inland ice, not sea ice.

This work is part of a programme supported by the British Antarctic Survey. We thank the Committee of the R. W. Paul Instrument Fund for providing a grant to cover the entire cost of developing the radio echo-sounding apparatus.

[1] Evans, S., *Polar Record*, 11, No. 73, 406, and No. 74, 495 (1963).
[2] Zumberge, J. H., *et al.*, *I.G.Y. Glaciological Rep. Ser.*, No. 3, May 1960 (I.G.Y. World Data Centre *A*; Glaciology. American Geographical Society, New York, 1960).
[3] Barclay, L. W., *Royal Society I.G.Y. Antarctic Expedition, Halley Bay, 1955–59*, 4, 329 (London, Royal Society, 1964).
[4] Robin, G. de Q., *Norwegian-British-Swedish Antarctic Expedition, 1949–52, Sci. Rep.*, 5 (Oslo, Norsk Polarinstitutt, 1958).
[5] Swithinbank C. W. M., *Norwegian-British-Swedish Antarctic Expedition, 1949–52, Sci. Res.*, 3 (Oslo, Norsk Polarinstitutt, 1958).
[6] Ragle, R. H., Blair, R. G., and Persson, L. E., *J. Glaciology*, 5, No. 37, 39 (1964).

BIOLOGICAL ASPECTS OF THE POPULATION EXPLOSION*

By Prof. A. S. PARKES, C.B.E., F.R.S.
Physiological Laboratory, University of Cambridge

The Explosion

My first task must be to justify the use of such a word as 'explosion' in connexion with an increase of population, however rapid and unprecedented. This perhaps is not as difficult as might be thought. The explosive growth of world population has taken place over about a century, especially over the past fifty years, during which the number of human beings has doubled. Such an increase in numbers in so small a fraction of human history is indeed remarkable.

It may be that animate beings, recognizable as human, have been in existence on this Earth for 200,000 years. If for the sake of argument we accept this estimate at its face value, it means that it took 199,000 years, that is up to A.D. 1000, for numbers to reach about 300 million. This implies a very slow rate of increase, an average of a mere 1,500 a year, and there is no doubt that during the vastly greater part of human history a high birth-rate was almost neutralized in the ordinary course of events by a high death-rate, and further that from time to time numbers were sharply reduced by epidemics.

By 1650 world population may have been of the order of 500 million. Things had evidently begun to move, but even so the 1,000 million mark was probably not reached until about 1830. Another 1,000 million, to make 2,000 million in all, were added by 1930 and since then more than another 1,000 million have joined the flock. The total in 1962 was thought to be about 3,180 million. In the past 30 years, therefore, as many people have been added to the human race as were to be found after the first 2,000 centuries or so of man's existence. Thirty years in 2,000 centuries corresponds to about 0·5 sec in 1 h, and anything that happens relatively in 0·5 sec to the time-scale of 1 h can reasonably be called an explosion. We are indeed in the early stages of a population explosion of which no one can foresee the end or the full consequences. Any biologist knows what a population growth curve of this kind means; whether the curve relates to bats or bacteria it means that something is going to happen, and in Nature that something is a crash. It remains for the human subject to decide whether in his case that something will be pleasant or unpleasant.

I want now to discuss three matters in connexion with the population explosion.

Cause of the Explosion

First, taking the world as a whole, the enormous increase in population is not due to increased fertility—it has been caused very largely by advances in the medical and related sciences, which have resulted in a comparatively sudden and dramatic fall in the death-rate in areas where the birth-rate remains high. In such circumstances there are more young people than old people to receive the benefits of medical science; as a result the immediate consequence is a tremendous excess of young people. In some parts of the world nearly half the population is less than 14 years of age. In many parts, half is less than 18 years of age. The reproductive potential arising from an age distribution of this kind needs no emphasis. Moreover, the impact of medical science on world population is accelerating.

The tempo of this increase in the expectation of life will no doubt slacken, but evidently, taking the world as a whole, it has a long way to go before everyone dies essentially of senescence; and what about senescence? In spite of the very large increase in the expectation of life at birth, the expectation of life at 65 or 70 years of age has increased comparatively little (though, of course, many more people live to expect it). In other words, medical science, fully engaged in the conquest of disease, has done little towards defeating senescence. It is a moot point whether or not medical science should add this problem to its programme. Whatever the demographic effects might be, and there seems to be some doubt about this, the social effects would be disturbing, to say the least, if a large majority, instead of a tiny minority, of people lived to be 100 years old.

Differential Rates of Increase

The second point to which I wish to direct attention is that the increase in population is very different in different parts of the world.

There is thus a continuous shift in the balance of world population, and figures of this kind hold a very pointed lesson for Great Britain. So long as present trends continue the inhabitants of these islands will constitute a progressively smaller proportion of the human race. How then are we going to hold even our present somewhat attenuated position in the world? Evidently, if we cannot rely on quantity we must rely on quality, and for this reason, if no other, serious consideration of the genetic improvement of the race, if only by negative means, should not be delayed indefinitely. In the meantime, most people would agree that we should try to make the most of everyone we have, and that presumably means education and more education and training and more training. But education and training in what? Great Britain is reaching a position in a competitive, populous and technological world where it can ill afford to fritter away its talent. We hear much about the brain drain to the United States. Is it not time we heard more about the brain drain into unproductive forms of scholarship? In asking this I do not wish to imply that all forms of science are obviously productive. In fact there is no clear distinction between 'pure' and applied science: either may, and often does, merge into and stimulate the other.

Nature's Limiting Factors

My third point about the population explosion is that it provides a good illustration of a very important biological principle. In Nature the first job of a species is to survive, and to this end reproductive capacity is such that any living thing able to multiply without hindrance to the limit of its reproductive capacity would soon swamp the world. The fact that this does not happen shows very clearly that all populations are held in check by limiting factors, of which the main ones are food supply, disease and enemies. This applies even to the slow-breeding mammal and to man himself. In human terms these limiting factors are famine, pestilence and war—the traditional scourges of mankind. In Nature, the relaxation of one limiting factor allows a population to increase until it bumps up against the same factor again, perhaps in a modified form, or against one of the other two. In the case of man, medical science has greatly relaxed the limiting factor in infective disease, and under natural conditions the present population explosion would be halted by the reappearance of control by disease, using

* Substance of an Evening Discourse delivered before the British Association (Southampton) on August 31.

the word in its widest sense, by famine or by war. It remains for man to break the grip of these harsh limiting factors of Nature. How is this to be done?

Explosive Power of Geometric Progression

It has often been said that it should be possible to increase food supplies and other resources as fast as the population increases. This, however, would not be enough. At the present time it is admitted that one-quarter of the world's population is malnourished and another quarter undernourished. If the general standard of living throughout the world is to be raised to an acceptable level it will be necessary for resources to increase faster than population, and at present this is certainly not happening adequately. No doubt resources will be increased very largely, but there is no possibility of the increase being commensurate with the reproductive potential of the human race. It is deplorably easy to overlook the force of geometric progression. The present rate of increase of the world population is about 2 per cent per annum, and here I want to quote from the report published last year by the U.S. National Academy of Sciences on the *Growth of World Population*.

"Although the current 2% per year does not sound like an extraordinary rate of increase a few simple calculations demonstrate that such a rate of increase in human population could not possibly continue for more than a few hundred years. Had this rate existed from the time of Christ to now the world population would have increased in this period by a factor of about 7×10^{16}. In other words there would be about 20 million individuals in place of each person now alive or 100 people to each square foot."

The Academy report goes on to extrapolate into the future:

"If the present world population should continue to increase at its present rate of 2% per annum then within two centuries there will be more than 150 billion* people."

That is fifty times as many people as we have to-day, to be fed, housed, educated, exercised, transported and given the amenities of life, and all this in a habitat which in many places is already being destroyed by man's activities. The American report concludes:

"Calculations of this sort demonstrate without question not only that the current continued increase in the rate of population growth must cease, but also that this rate must decline again. There can be no doubt concerning this long-term prognosis. *Either the birth-rate of the world must come down or the death-rate must go back up.*"

This quotation shows the futility of arguments based on the indefinite increase of resources. Even if technically possible to produce, adequate resources would be little consolation to people for whom there was not even standing room.

Pathological Togetherness

Nor, it seems, would adequate resources stave off the biological effects of overcrowding. I have already said that I was using the word 'disease' in its widest sense: and here I want to quote from a recent paper by Hudson Hoagland on "The Cybernetics of Population Control". In speaking about the rabbits of Minnesota, Hoagland says:

"These populations rise and fall through cycles of several years' duration. There is a build-up followed by a dying off. Why do these marked oscillations in numbers of rabbits occur? It was observed that when the animals died off there was usually plenty of food—they didn't starve. There were no evidences of an excessive number of predators. Furthermore, the bodies showed no sign of any specific epidemic that killed them."

* 'Billion' in the United States and elsewhere equals a thousand millions; in Great Britain and elsewhere it equals a million millions.

Hoagland cites many examples of the rise and fall of natural populations where the fall could not be attributed to lack of food, enemies or infective disease. He also refers to experiments by Calhoun in which rats kept at merely double the density compatible with a healthy population showed low fertility associated with a great variety of physiological and behavioural abnormalities.

Here then is a new aspect of population control by disease—the pathology of overpopulation, of which the symptoms are highly suggestive of the stress syndrome characterized by hyperactivity of the pituitary–adrenal axis.

For those who dislike the expression pathology of overpopulation, I would recommend that coined by Calhoun, 'pathological togetherness'.

The 'Demographic Cycle'

I should emphasize that the idea of the crash of populations caused by overcrowding must be clearly distinguished from the idea of the so-called demographic cycle in man, which postulates that with an increased standard of living the birth-rate falls naturally without conscious fertility control and will do so in the East as it has already done in the West. This idea seems to have little biological foundation, because: (*a*) according to present indications a fall in the birth-rate in the East will have to precede, not follow, an increase in the standard of living; (*b*) in the absence of any conclusive evidence of a decline in the biological fertility of the people concerned, the fall in the birth-rate in the West has presumably been caused by the conscious control of fertility by some means or other; (*c*) present evidence is that the demographic cycle can go into reverse, and that with a very high standard of living, the birth-rate tends to rise again, as it has done in the United States and to a lesser extent in Great Britain since the Second World War.

The Human Limiting Factor

The indefinite increase of resources, therefore, even if it could be achieved, might lead only to pathological togetherness, and the idea of a natural demographic cycle is largely illusory. Some more positive human limiting factor is evidently required if we are to avoid the harsh limiting factors of Nature. This fourth, human and humane factor, can only be the possession of the will and the means for the conscious control of human fertility. Stated simple and bluntly, the fact is that medical science has put the human race in a position where man's urge to mate can be reconciled with the bearing capacity of our planet only by conscious limitation of the results of mating. Surely few would wish to achieve this aim by means other than the control of conception.

This raises the important question of what constitutes conception in man—the fertilization of the egg or its implantation in the uterus some days later. My own view is that implantation, not fertilization, constitutes conception, and I base this view on the biological analogy that no one maintains that a hen conceives when her egg is fertilized or that she aborts when she lays it. On this view contraception can properly be exercised up to the time of implantation. Whatever the precise definition, if the idea of contraception is to be accepted for both population control and family planning, then surely methods should be as effective, simple and acceptable as possible. Yet up to recently, available methods of contraception were archaic in principle and a disgrace to science in an age of spectacular technical progress. This unhappy situation arose from the taboo which for long surrounded the subject and which not only drove it underground but also inhibited scientific work in the field.

Fortunately, the situation has now changed, and for this we owe much to the biological breakthrough, pioneered by Pincus and Rock, which has resulted in the

development of oral contraception for the female by means of 'the pill'. It is not my purpose to discuss the biological background or the very high effectiveness of this remarkable form of medication. Both are most interesting and important, but it may be that in years to come the Pincus–Rock pill will be thought of as having made its main contribution in the psycho-social field. I say this because the pill, by reason of having dissociated fertility control from the act of coitus, has promoted discussion of the matter in a remarkable way.

The advent of the pill—that dream of family planners—has naturally raised serious questions about the possible long-term effects of such medication. Here again, this is no place to discuss technical pros and cons, but I would make some comments of a general nature. The first is that the possible hazards of using contraception must be balanced against the possible or even certain hazards of not doing so—apprehension, oppressive pregnancies, abortion, unwanted children, overpopulation. The second is that the pill is the most effective and acceptable form of fertility control so far available. The third is that the intensive research now in progress will almost certainly produce equally or even more effective and acceptable methods in the not-distant future, so that alternation of methods will make it possible to avoid possible hazards arising from the long-term use of any particular one.

Size of Family and Population Growth

There is now available, therefore, at least one very efficient biological method of preventing conception. Undoubtedly other and better ones will follow. We shall thus have the means to control the growth of population. Will this solve the problem?

Obviously the first requirement is the spread of information and the avoidance of unwanted children: we must hope that this will be only a matter of time. The real long-term question is motivation. Will people want to limit their families to a level which, in the light of present medical trends, is compatible with no more than an acceptable rate of population increase? We have seen that an annual increase of 2 per cent cannot be maintained, and it is arguable that the population should be stabilized at its present level so that increase of resources can be used to increase the standard of living in parts of the world where it is low, rather than to provide for more people. There are, however, disadvantages in a static population and probably some slight increase, possibly 0·5 per cent per annum, would be acceptable. This raises the very interesting question of what average size of family an increase of this small amount would represent. Allowing for mortality rates as found in the West and for a generation interval of 25 years, the answer seems to be something less than 2·5 (Table 1).

Table 1

Average size of completed family per married couple	Rate of growth of population (% p.a.)
2	—
2¼	3/4
3	1¼
3½	2¼
4	3

Motivation towards small families, that is families of 2 or 3, for either personal or demographic reasons, is going to be very difficult in areas of traditionally high birth-rate. This is especially true where a large family is a status symbol or a source of cheap labour. In the East, therefore, the chance of getting the average size of family down to 2¼ in the near future is anything but good. What about the West? Large families of the size often seen in Victorian times are now rare; but they are not unheard of even among educated and knowledgeable people. This raises in acute form the question posed by A. V. Hill in his presidential address to the British Association in 1952, the question of whether the rights of man, about which much is heard, can under modern conditions be held to include the right to unlimited reproduction. It would seem that now or in the visible future the answer must be: no. Even a family size of four would be catastrophic if it became general.

Here then is an acute social problem of motivation. Evolution has endowed man not only with an urge to mate, but also with a parental urge at a level appropriate to a low survival rate. Medical science has altered one, but not the other. Our present dilemma is indeed a striking example of the effects of unilaterally interfering with Nature. What must be done to redress the balance? We cannot put back the clock of medical progress and we cannot cope on the planet Earth with unlimited numbers of people. Emigration to other planets is not likely to be a practical solution within the visible future. Mass reduction of fertility, as, for example, by the medication of a staple food, would be possible only under a rigid dictatorship. Possibly economic pressures and inducements will have to be used. Certainly, by some means, the parental urge will have to be further diverted from quantity to quality, with all that quality implies. I do not pretend to know the answers; but as a biologist I would like to conclude with this thought—that the development of both the means and the will to match his fertility to his environment and so to avoid the harsh limiting factors of Nature will be a fundamentally significant stage in the continued evolution of the human race.

A NEW THEORY TO EXPLAIN CROSSING-OVER BETWEEN GENES ON CHROMOSOMES

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Belling's Theory, 1931

Belling's[1] cytological investigation of synapsis and chiasmata in liliaceous plants with large chromomeres led to his proposal in 1931 of a theory of crossing-over based on his direct cytological observations. He stated that the 'new' chromatids, formed after synapsis, were produced: (1) by the duplication of the 'old' chromioles to produce 'new' chromioles; (2) by the outgrowth, extension and union of interchromiolar (non-genic) filaments on the 'new' chromioles. He inferred that crossing-over occurred by cross union of the two 'new' strands formed after synapsis. Belling proposed that when a twist occurred in the two 'old' synapsed strands, followed by duplication of the 'old' chromioles to produce 'new' chromioles, a cross union, producing a 'cross-over', might occur at the point of the twist between two of the 'new' non-sister strands (Fig. 1). The two 'old' strands would remain 'non-crossed-over'. Belling's 1931 theory proposed that crossing-over never occurred between the two 'old' strands but only between the two 'new' strands. Therefore, each tetrad of chromatids always produced two

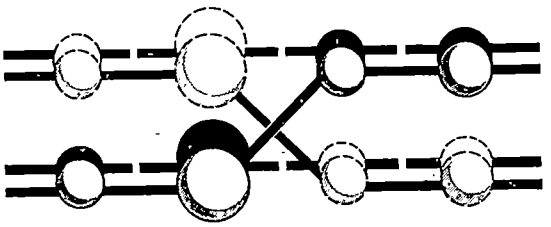


Fig. 1

'non-crossed-over', 'original', 'old' strands along the entire length of the chromosome; the cross-overs (or crossed-over strands) of the tetrad would involve only the two 'new' strands. Belling rejected the concept of breakage of any of the strands; crossing-over, according to him, was only due to the cross union of the newly formed 'interchromiolar' connexions.

I shall propose: (1) that the interchromiolar connexions are probably histone rather than DNA; (2) that the DNA in the chromosome is discontinuous; (3) that the gene is a particulate structure containing DNA. The kind of crossing-over discussed herein occurs between and not within genes and probably involves histones rather than DNA; the new theory does not concern the kind of genetic recombination which occurs in viruses nor between the DNA components of chromosomes.

Contradiction of Belling's 1931 Theory

Belling's 1931 theory was rejected by Beadle and Emerson[2], who argued that the *Drosophila* data proved it to be incorrect. Their analysis of chromatid interference in *Drosophila* contradicted Belling's early theory, because, according to Belling, only the two 'new' strands would be involved in exchanges. Bridges[3] was the first to demonstrate by genetical analysis that crossing-over occurred at the four-strand stage. Beadle and Emerson, using attached-*X* flies prepared by Bridges, demonstrated genetically that multiple exchanges could involve more than only the two 'new' strands; three or four strands were involved with high frequencies in multiple exchanges. The involvement of more than two strands proved that the 'old' strands also could be involved in crossing-over. Beadle and Emerson named the three different kinds of multiple exchanges '2-strand', '3-strand' and '4-strand' multiple exchanges. Multiple exchanges were usually studied over two adjacent regions and designated as 'double' exchanges. The ratios of the three different kinds of possible double exchanges were the basic data of the analysis. Beadle and Emerson concluded that the ratio of 2- : 3- : 4-strand 'doubles' in *Drosophila* was 1 : 2 : 1. Their results did, indeed, suggest that 'double' exchanges occur at random, but this was probably because the genes which they studied were too far apart to escape ambiguities due to undetected multiple exchanges which 'randomized' the ratios. Bonnier and Nordenskiold[4], using the same material, failed to confirm their findings. Beadle and Emerson's conclusions were contradicted by Lindegren[5], who showed that in *Neurospora* the relative frequencies of the three kinds of doubles was 2- > 3- > 4-strand double exchanges. These frequencies have been corroborated in recent unpublished analyses of heterozygous yeast hybrids marked for 10 different regions in one chromosome.

Belling's Revised 1933 Theory

Belling[6], after the criticism of Beadle and Emerson, revised his theory to fit what he considered to be the facts on the basis of their *Drosophila* data. In his revised theory, Belling proposed that the chromioles were indeterminate with regard to 'newness' or 'oldness' and that this indeterminancy could produce sister-strand crossing-over. But he continued to reject breakage as a possible cause of crossing-over.

Cleavage Planes of the Chromioles

In Belling's revision of his theory he assumed that the axis of cleavage of the chromioles was at random, leading to a high frequency of what would be equivalent to sister-strand crossing-over. But the axis of cleavage of the chromioles is probably predetermined and parallel to the long axis of the chromosome. In the analysis of heterozygous attached-*X* females, Bridges found only occasional females homozygous for recessive genes, proving that crossing-over had occurred when the 4-strands were present. But most of the daughters of attached-*X* females are heterozygous. The high frequency of heterozygous daughters compels acceptance of the view that the line of cleavage of the two attached-*X* centromeres in the mother must be predetermined to pass through the dotted line shown in Fig. 2. If cleavage occurred in other planes, recessives, trisomics and other irregular kinds of daughters would occur with high frequency. These considerations prove: (1) that the attachment of the centromeres in attached-*X*'s must occur at specific and corresponding points on the surface of the two respective centromeres; (2) that the insertions of the interchromiolar connexions into the centromeres must be precisely located; (3) that the cleavage plane must pass through both the attached centromeres between the insertions of the interchromiolar connexions; (4) through the points of attachment; (5) at right angles to the attachment points. Extension of these considerations to the cleavage of the 'old' chromioles suggests: (6) that the cleavage planes of chromioles are likewise orderly and predetermined; (7) that the 'old' and 'new' chromioles are distinguishable from each other.

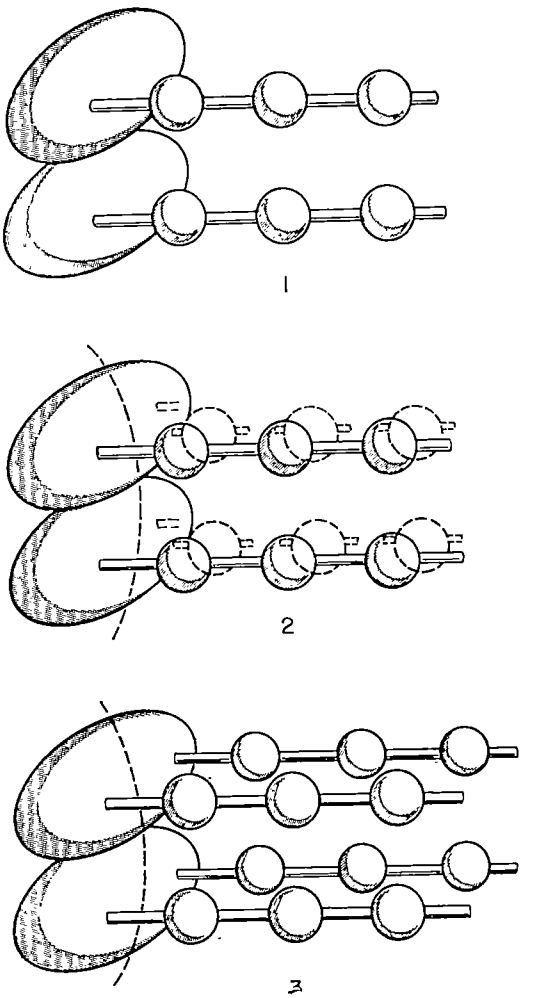


Fig. 2

Darlington's Theory

Darlington's[7] theory, like Belling's, proposed: (1) that crossing-over occurred at the 4-strand stage before the chiasmata appeared; (2) that the chiasma was produced by opening up the synapsed strands after the cross-over had occurred. Darlington disagreed completely with Belling on the cause of crossing-over, because Darlington contended that all cross-overs were due to breakage and reunion. He asserted that twisting of the chromosomes caused breakages at the 4-strand stage which then led to cross reunions. I have tested many different kinds of strands by twisting four strands together until breakage occurred; under these circumstances the strain of twisting is always relieved at first by the breakage of only one strand, never by the breakage of two or more strands. For this reason, I have always been more inclined toward Belling's theory.

The New Theory

Neither Belling's nor Darlington's theory explains the observed frequencies of double exchanges. The analysis of yeast hybrids has proved beyond question that in yeast, as in *Neurospora*, the frequency of 2-strand doubles exceeds the frequency of 3-strand doubles and that 4-strand doubles are the least frequent of the three categories. I shall propose a new explanation of crossing-over consistent with this distribution. This theory proposes: (1) that the primary cause of crossing-over is a twist in the synapsed strands with cross unions between non-homologues, as proposed by Belling in 1931; (2) that some twists may lead to the breakage of one of the two twisted strands before the 4-strand stage; (3) that duplication of the chromioles after such a break will produce three pairs of free 'interchromiolar' connexions; (4) that in such circumstances a union of a 'new' and an 'old' strand is possible; (5) that, since there will be six free 'interchromiolar' connexions after the chromioles have divided and have produced their outgrowths, the three pairs of free ends may either grow together to produce no new relationships or the strands may reunite to produce cross-overs. The theory holds that breakage of one strand, occurring at a twist point before the chromosome has become quadripartite, is the event which makes union of an 'old' and a 'new' strand (as well as sister-strand crossing-over) possible. If breakage at the twist points is relatively rare, the frequency of 2-strand double exchanges would greatly exceed the frequency of 3-strand doubles, as has been demonstrated in both *Neurospora* and yeasts. If breaks occurred at two adjacent twist points, an occasional 4-strand double could occur.

This explanation is consistent with the observed frequencies of the three different kinds of double exchanges. If twists and breaks occur with high frequency the distribution of double exchanges might be random—1 : 2 : 1. (Belling's revision predicted a 1 : 2 : 1 frequency distribution of 2- : 3-: 4-strand doubles.) The new theory proposes: (1) that all cross-overs occur at the 4-strand stage by cross union between chromioles of the interchromiolar histone filaments; (2) that most cross-overs are due to twists in the synapsed strands with cross unions occurring according to Belling's 1931 hypothesis to produce cross-overs only between the two 'new' strands; (3) that, occasionally, twists are so tight that one of the two 'old' strands at the twist point breaks leaving six free ends; (4) that in this circumstance a cross union may occur between two 'new' strands or between an 'old' and a 'new' strand, or may not produce a detectable cross-over, depending on how the unions occur; (5) that, if a non-sister cross-over between an old and new strand occurs, then a Belling-type cross-over at an adjacent twist could produce a 3-strand double (if a sister-strand cross-over occurred between two Belling-type exchanges, a 3-strand double could also occur); (6) that, if a detectable cross-over has occurred at the first twist due to the breakage of a single strand, and if, at an adjacent twist, a different strand breaks, a 4-strand double might be produced.

This work was supported by a research grant from the American Cancer Society.

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ALARM SUBSTANCES AND ALARM BEHAVIOUR IN SOCIAL HYMENOPTERA

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HIGHLY developed social communities of Hymenoptera make use of alarm communication. An alarm can be given by optical, mechanical or chemical means. The possibility of chemical alarm communication has been known for a long time. Huber[1] found in *Apis mellifera* that the sting odour is an attack releaser, while Staeger[2], Weyrauch[3] and Goetsch[4] supposed alarm could be released by optical or mechanical means. The last-named, however, also indicated alarm substances. The problem has recently been examined by several investigators. In all communities of Hymenoptera examined chemical alarm communication could be demonstrated; this was shown in the honey-bee by Free[5], Lecomte[6], Ghent *et al.*[7] and Boch *et al.*[8] and in some ants by Wilson[9,10], Sudd[11], Brown[12] and Hunt[13]. Some alarm substances have already been identified, such as citral in *Atta sexdens*[14], different ketones in *Tapinoma nigerrimum*[15] and *Iridomyrmex pruinosus*[16] and isoamylacetate in the honey-bee[8].

These fragmentary and somewhat contradictory results led to a comparative investigation of alarm and alarm substances[17].

In the honey-bee colony an alarm can be demonstrated best when only single bees are sitting in front of the entrance to the hive, as they do at dawn or on a cool day. Then, when some bees are excited by pressing, they run into the interior of the hive and after a short time a great number of workers come out of the hive, run about excitedly, and are ready to sting. The same behaviour can be simulated when a squashed sting is put at the hive entrance. The workers come out of the hive interior, approach the sting, and smell it. Then they run excitedly about on the hive board, ready to attack. The attraction effect has been used by many investigators[7,8,17] as a test reaction. Excited bees without a sting are not able to alarm other workers. This shows that only chemical stimulants can release an alarm. The excited worker bee exhibits a characteristic behaviour (Fig. 1) which resembles the fanning reaction. The bee erects its abdomen, opens the sting chamber and its sting is extruded. Sometimes a droplet appears on the tip of the sting. In this posture the bee runs buzzing among the others, which are excited and ready to attack. If more bees are excited the pleasant odour of the alarm substance

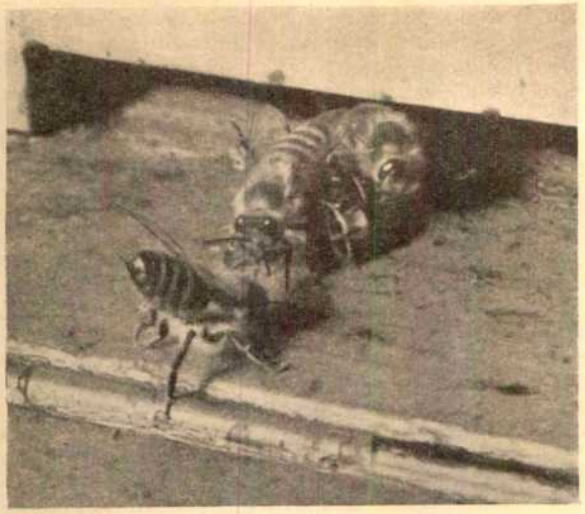


Fig. 1. An alarmed honey-bee which has been excited by pressing. In the opened sting chamber the sting and a bristled membrane can be seen. In the latter the alarm substance is exposed stored

can be recognized, which is released by the alarm fanning. The alarm secretion is exuded in the region between the bristles of a setose membrane which connects the oblong plates of the sting apparatus. It can evaporate when the sting is protruded into the open air.

At present we do not know in which gland the substance is produced (Fig. 2). Four different glands are situated in the sting of the honey-bee[18]. Only one of them, the big poison gland, runs directly into the sting shaft. The three other glands open into the sting chamber; the alcaline or Dufour gland, the paired Koshewnikov gland lying on the quadrate plates, and the newly discovered paired sting sheath gland. Ghent and Gary[7] supposed that the origin of the alarm substance is the Koshewnikov gland. But the bees showed no reaction to a large number of fresh glands simultaneously squashed. Experiments with poison glands, alcaline glands, and sting sheath glands produced the same result. The sting sheath gland can be eliminated in a second way. Freshly emerged bees have no alarm substance. The sting sheaths were removed from such bees. Nevertheless, after a week when the alarm secretion normally was fully developed, these bees also had a scented, alarm-releasing sting.

Comparison with the queen honey-bee is also not significant. Although the female has no alarm substance in her sting apparatus the same glands can be found. They are the same size or are even larger than the glands in the worker bee.

In the worker honey-bee another weak alarm substance has been found—the contents of the mandibular gland. Boch[19] also discovered that it attracts other bees. Its smell is quite different from that of the sting odour.

The alarm substance is of great importance not only for alerting the workers but also for a subsequent attack by the bees. It lowers the threshold of sensitivity for the attack reaction and enables the bees to locate the spot which has already been stung. This spot, however, has to be optically attractive at the same time, that is, it has to be dark or it has to be moving[7,17].

Alarm substances have also been demonstrated in the wasps *Vespa germanica* and *Vespa vulgaris*. If a wasp is seized or pressed in the nest entrance it gives the alarm and a number of workers rush out from the interior of the nest. They fly off and attack objects in the surrounding area. The same effect can be obtained with a squashed sting. The alarm substance, with an odour resembling fermenting fruit, is in the venom. It is an easily evaporating ether-soluble substance which is mixed with the venomous substances[20].

The alarm reaction corresponds to the wasp's morphological characteristics. The excited wasp turns the tip of its abdomen into the direction of the disturbance and sprays a few jets of venom for several centimetres. Like the honey-bee, the wasp alarm secretions act specifically. A number of other substances were tested (plant and animal odours, chemicals and repellents). The typical alarm could only be released by the alarm substance itself. In *Vespa*, however, an interspecific effect of the alarm secretions has been demonstrated: in *Vespa vulgaris* the venom of *V. germanica* released an alarm and vice versa. Whether the alarm substances are identical, only chemical analysis can prove. The close relationship of the wasps and the same odour for the human nose make this probable. *Polistes* venom and *Apis* alarm secretion are not effective. In the same way as in the honey-bee, the stings of *Bombus* and *Vespa* release no alarm. Wasp queens, too, possess alarm substances which are sprayed off in the same way as in worker wasps, when being pressed. The queens, however, do not join in attack. In wasps the alarm substance has the same importance for attack as in the honey-bee. Here, too, scent-marked subjects were more often and more intensely attacked than were odourless wasps.

Ants, with their bad sight, communicate principally by chemical means. Odour trails laid by the foragers lead the workers to the feeding places[21,22]. Nest mates, offspring, and dead ants are recognized by a specific odour and treated accordingly[4,23–25]. Alarm, too, is mostly given chemically. Most of the myrmicines possess mandibular gland substances[4] (*Pogonomyrmex*[9], *Solenopsis*[24], and *Atta*[14]). The heads of four species of *Myrmica*, *Tetramorium caespitum*, *Pheidole pallidula*, *Messor in-*

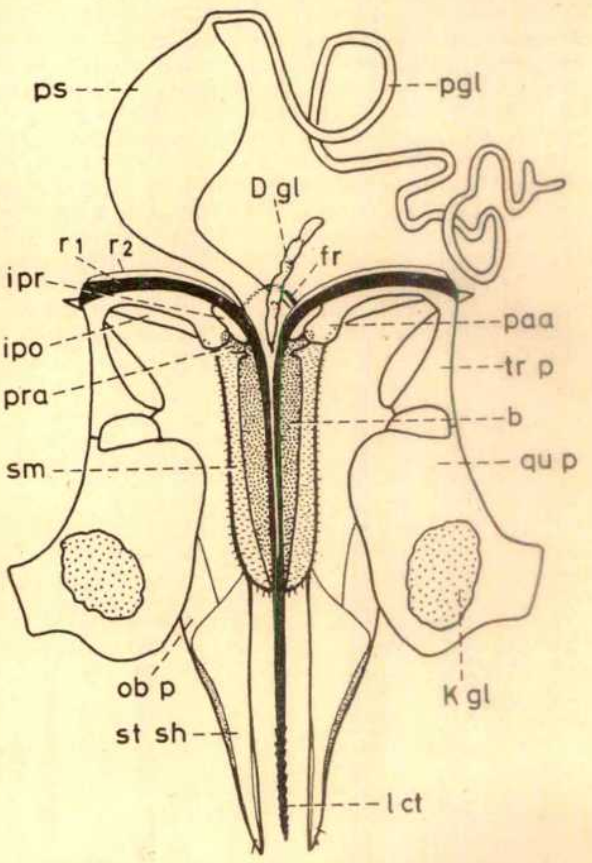


Fig. 2. Sting of a worker bee (modified after Zander, Snodgrass, Oeser and Maschwitz). *b*, Bulb of the stylet; *D gl*, Dufour gland; *fr*, furcula; *ipo*, incisura postarticularis; *ipr*, incisura prearticularis; *K gl*, Koshewnikov gland; *let*, lancet of the sting; *ob p*, oblong plate; *paa*, pars articularis; *pgl*, poison gland; *pra*, processus articularis; *ps*, poison sac; *qu p*, quadrate plate; r_1, r_2, first and second basal rami; *sm*, hairy setose membrane; *st sh*, sheath of the sting (sheath gland); *tr p*, triangular plate

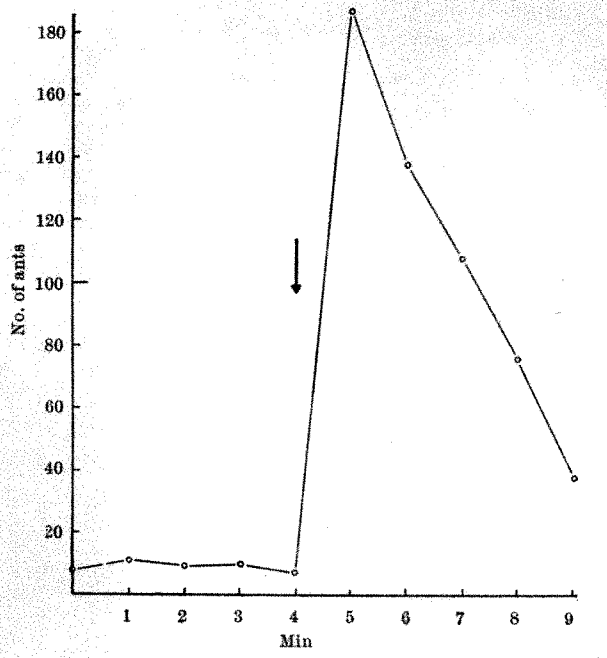


Fig. 3. *Formica polyctena*, reaction on alarm substance (4 trials summarized). The number of ants were counted which were attracted by the alarm substance (one squashed ant)

stabilis, *M. barbarus*, *Cremastogaster scutellaris*, *C. auberti* and *Aphaenogaster testacea-pilosa* release an alarm reaction. The alarm substance originates, so far as we know, from the mandibular gland (*Myrmica*, *Tetramorium*). At the same time *Tetramorium*, *Myrmica*, *Messor*, and *Pheidole* possess abdominal alarm substances, which are produced, so far as we know, in the poison gland (*Myrmica*, *Tetramorium*). In field experiments, *Aphaenogaster* and *Cremastogaster* showed no reaction to gasters of squashed nest mates near the trail or at the feeding place. In the first four genera alarm is therefore doubly secured. Perhaps the abdominal substances were used for trail marking, also. Venom, as well as the mandibular substance, is given off in situations of danger. When *Myrmica* is biting other ants or when it is pinched one can smell the characteristic ketone-like scent of the mandibular substance. When the ant is seized with tweezers one can observe that the venom, too, emerges from the extruded sting without the ant having stung.

A multiple securing of the alarm can also be found in *Formica* (*Formica polyctena*, *F. cinerea* and *F. fusca*). Mandibular gland secretions, as well as Dufour gland and venom gland contents, alarm the workers of *Formica*. The last two substances are released together. This could be demonstrated by neutralizing the formic acid of the secretion. *Lasius* (*L. niger*, *fuliginosus*, *flavus* and *brunneus*) and *Plagiolepis pygmaea*, too, release Dufour gland secretion together with formic acid. In these two genera, however, only the former substance causes alarm.

Therefore, the typical acid-spraying behaviour of *Formica* is not only a defence reaction but also an alarm behaviour. *Serviformica* and *Lasius* only release a droplet of the alarming venom when disturbed. In doing so *Lasius niger* and *flavus* often erect the gaster like the honey-bee and run between the nest mates, which become excited in this way.

The sting apparatus of the dolichoderines is reduced. For their defence these ants use anal gland secretions, which in *Tapinoma nigerrimum* and *Iridomyrmex pruinosus* mainly consist of strong-smelling, poisonous ketones[15,16]. These ketones function as venoms as well as alarm substances[15,10,16]. We found an alarm function for the anal glands in *Tapinoma erraticum* and *Bothriomyrmex meridionalis*. The alarm substance was released in droplets when pressure was exerted on the ants.

So far, only the location of alarm production and the method of its release have been reported. The behaviour of the alarmed ants will now be described.

An attack can be met in two ways. The workers either fly away or counter-attack. Both reactions occur among ants. In the nest, where brood and queen have to be defended, ants attack their enemies. They approach the alarm spot, circle around it with partly opened mandibles and smell it (Fig. 3). Some of them bite the smelling object and try to pull it about. At the feeding place, the defence of which is not important, different reactions can be observed. Some ants show typical flight reactions (*Tapinoma erraticum*, *Aphaenogaster testacea-pilosa* and *Lasius niger*). The honey-water feeding ants become restless after some time when they have smelt the alarm substance and fly into their nest. Newcomers will return. Between attack and flight reactions transitions can be found. In good fighting ants, for example, *Formica polyctena* or *Tapinoma erraticum* ssp. *nigerrima*, alarm can serve for recruiting other ants in order to overwhelm a strong prey.

As in wasps, species-specificity has also been investigated in ants. Wilson and Pavan[10] have shown that the alarm-

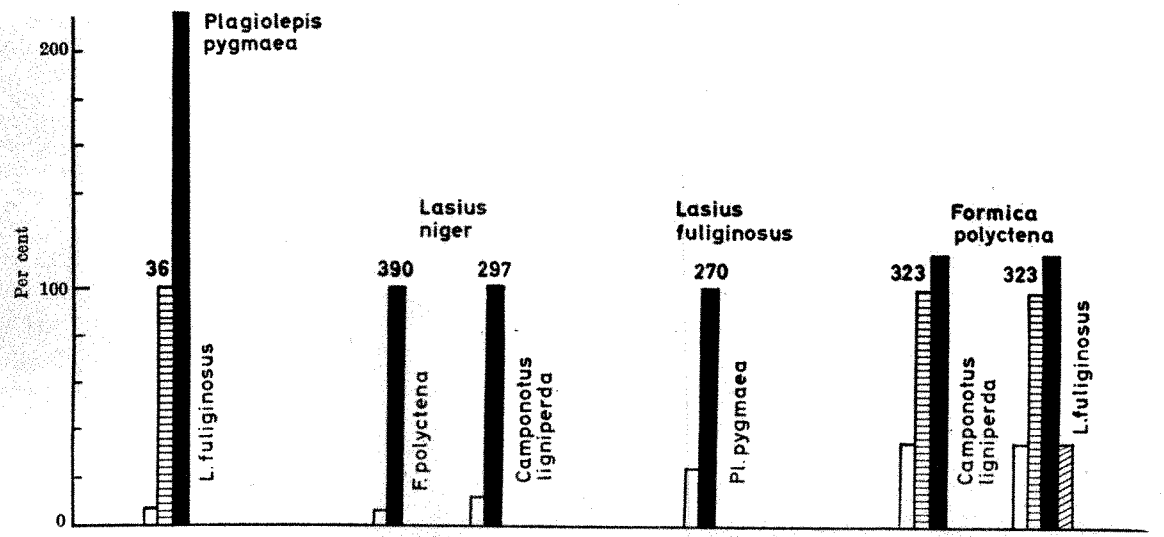


Fig. 4. Specificity of alarm substances within the sub-family of camponotines. Test with Dufour gland secretion. Left column (white), control test without odorous substances; right column (black), number of ants which reacted to the alarm substance of a strange species (percentage of workers which reacted to the alarm substance of their own species (horizontally hatched column)). Absolute values of the median columns (100 per cent) are approximate. Obliquely hatched, mandibular substance of *Lasius fuliginosus*

releasing ketones of *Tapinoma*, *Liometopym* and *Monacis* act intergenerically.

In some genera species-specificity was examined. In *Myrmica* it has been shown that the venom of *M. laevinodis*, *sulcinodis* and *rubida* stimulates alarm in *M. ruginodis*, and vice versa. The mandibular gland substances, too, are not species-specific in *ruginodis*, *sulcinodis* and *laevinodis*. Mandible gland substance from *M. rubinda*, however, is not so effective as the secretions of the other species, although its gland is much bigger than that of *M. ruginodis*. This is understood because it belongs to a different sub-species (*Neomyrma*) from the three others. For the human nose its odour is also different from that of the three others. The mandibular gland substances and the Dufour gland substances of the three species of *Formica* (*polyctena*, *cinerea*, *fusca*) are reciprocally effective, likewise the Dufour gland substances of four species of *Lasius* (*niger*, *fuliginosus*, *brunneus* and *flavus*). For the human nose the Dufour gland substances of *Lasius*, *Formica* and *Plagiolepis* have the same odour, therefore their intergenerical effectiveness was investigated. The results were positive (Fig. 4).

For *Myrmica ruginodis*, however, with the exception of the abdominal substance of *Pheidole pallidula*, the alarm substances of other myrmicines, such as *Messor*, *Tetramorium*, *Leptothorax*, are ineffective.

In summary, it may be said that the alarm substances of the social Hymenoptera are always connected with organs and glands of defence (with the mandibles, the sting, or anal glands). They originate either separated from the venom and are mixed with it when released (honey-bee and Camponotinae) or they originate directly from the venom gland. In the latter case they can be identical with the venom (formic acid in *Formica*, ketones in Dolichoderinae) or not (*Vespa*, Myrmicinae). The specifically acting substances are released when the workers are excited. The alarmed insects usually attack but sometimes they fly away. The alarm substances are not species-specific as a rule; in Camponotinae, even intergenerically acting substances were found.

So far only highly organized Hymenoptera colonies with great numbers of workers have been treated. They all possess alarm substances. Some Hymenoptera colonies, however, have been found which cannot give a chemical alarm. In bees three species of bumble bees were found (*Bombus lucorum*, *hortorum* and *hypnorum*), in wasps *Polistes dubia*, and in ants *Ponera coarctata* (Ponerinae) and *Myrmecina graminicola* (Myrmicinae). All these colonies are very small and have only a few workers, usually less than 100. A disturbance can be directly noted by all nest inhabitants. Therefore development of a chemical alarm system seems to be biologically unnecessary for them.

I thank Springer-Verlag (Heidelberg) for permission to reproduce the illustrations, which appeared first in *Zeitschrift für vergleichende Physiologie*, **47**, 596 (1964).

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OBITUARIES

Dr. L. E. Bayliss

LEONARD BAYLISS, who died on August 20 in his sixty-fourth year, was widely known for three achievements: his work on the rheology of blood, his notable two-volume *Principles of General Physiology* in 1959 and 1960 and, particularly to medical students, as co-author of *Human Physiology* in five editions (1930–62).

Blood rheology investigations began about 1930 after he had spent a year in Philadelphia with A. N. Richards collecting glomerular fluid from frogs' kidneys with micropipettes and comparing its oncotic pressure with that of the plasma. Earlier, he had worked with his uncle, E. H. Starling, professor of physiology in University College London, on heart–lung–kidney preparations of dogs, investigating oxygenation and *p*H of the blood and metabolism of the preparation. About then, Fåhraeus discovered the decrease in the apparent viscosity of blood in small tubes, but failed to induce blood to flow through tubes as small as arterioles because they became blocked with agglutinated red corpuscles. Bayliss, with micropipette technique and with defibrinated blood from heart–lung preparations which agglutinates less, was able to measure the apparent viscosity in blood flowing in 20μ glass tubes, that is, arteriole size, and found it about one-half that when the same blood passed through tubes of 500μ diameter or more. The paper describing this was refused for publication, presumably because the conclusion was so unlikely or perhaps because physiologists would not understand it. It remained in his drawer for a decade or more. Bayliss was always reluctant to publish. The conclusion leaked out as a 'personal communication' in a paper on the apparent viscosity of blood in hind limbs (1933) in which arterioles are about the same size, 20μ, and form the main resistance to the flow of blood. Happily, the apparent viscosity in hind limbs was the same as that in Bayliss's small glass tubes.

The anomalous viscosity of blood was attributed by Fåhraeus to axial concentration of the cells which slipped, as an almost solid rod, through nearly cell-free serum which separated the rod from the glass of the tube. Bayliss's later investigations attempted to test this hypothesis by comparing the viscosity obtained by direct measurement with that calculated from the distribution of optical density of different layers of blood flowing in microtubes when distortion due to refraction has been overcome or mathematically allowed for. His latest review on the rheology of blood is in the great *American Handbook of Physiology*, Section 2 on "Circulation" (1; 1962).

Fifty years ago, physiology had established itself as an independent science, pursued for the most part by medically qualified physiologists in the hope of increasing our understanding of normal, and to some extent disease, processes in man. Hence, many of the text-books included 'Human Physiology' in their titles, though they described, almost exclusively, experiments on animals other than man. Into this setting, Sir William, father of Leonard

Bayliss, launched his *Principles of General Physiology*, which aimed at application of physical and physico-chemical principles, rather outside the knowledge or interests of most medically qualified physiologists at that time, to the functions of cells, tissues and animals. Moreover, he ranged widely over the animal kingdom beyond the frog, cat and dog, which were the conventional objects of physiological investigation. This stirred almost explosive interest among physiologists, many of whom, from home and abroad, were welcome guests at Sir William and Lady Bayliss's hospitable house in Hampstead. Into this setting, Leonard grew up to hear much discussion and to gain practical experience in his father's private laboratory and workshop, in addition to formal education at University College School and later in Trinity College, from which he took his degree in physiology at Cambridge and then his Ph.D.

After a year as physiologist at the Marine Biological Laboratory at Plymouth, he became lecturer in physiology in the University of Edinburgh. By 1939, however, physical chemistry, physiology and experimental zoology had advanced mightily in the twenty-five years since the original publication of the *Principles*, and Bayliss resigned from his salaried post to devote himself to study so as to produce a new synthesis of the cognate parts of these subjects; this he did at his father's professional home, University College, London. The War intervened, and during these years he must have been immensely active in the Army Operational Research Group, about which he spoke with real enthusiasm (see following note).

With the shortage of university staff after the War, he became reader in physiology at University College and managed, practically single-handed, in 1946 to restart the Hons. B.Sc. course which had perforce lapsed during the war years. Having set that on its feet, he resigned in 1950 in order to have leisure to pursue his ambition to create a new synthesis in the form of the *Principles of General Physiology*. This he achieved in two volumes published in 1959 and 1960, although he had continued teaching and research in an honorary capacity much as before.

He and his wife, Dr. M. Grace Eggleton, who survives him, were a devoted couple, both working in the same department and nearly always together, either in London or at their cottage in Sussex. He was a modest, frugal and exceedingly kind person, always ready to interrupt what he was doing to discuss someone else's problem; unambitious and reluctant to publish papers, though he published quite a few about widely diverse branches of physiology from water-diuresis to action-potentials. A glimpse of his generous character appears in a quotation from his preface to the *Principles of General Physiology*, 1, in the acknowledgments: "But the most valuable of all my assistance has come, whether they knew it or not, from my colleagues and students in the course of discussion and instruction". F. R. Winton

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One morning in the autumn of 1940 I was gazing ruefully at the smoking ruins at University College, when Leonard Bayliss came and stood beside me. I had been looking for scientific people for Blackett's party at Anti-Aircraft Command and said, "Leonard, do you want a job?" The job seemed particularly pertinent then, and he said "Yes" at once. For nearly five years he worked with the Army Operational Research Group under its various titles, and was a leading member of its establishment throughout the War, contributing greatly to its success.

The efforts of this Group were of tremendous value to the A.A. Command. As General Pile wrote in his despatch (*London Gazette*, December 16, 1947), "While full credit must be given to the troops of all kinds, and indeed their conduct . . . was beyond all praise, the foundation of success was laid by the scientist, both civilian and in uniform". In his book *Ack-Ack* is a photograph of Pile talking to Bayliss, with other members of the team around.

The original party was split early into two sections: (*a*) those engaged in radar problems; (*b*) those dealing more generally with fire control and analysis. Bayliss was put in charge of the latter. A colleague of those days writes that he was "always delightfully helpful and stimulating"; and he was himself the author of many reports on A.A. gunnery, A.A. predictors, accuracy of fire against aircraft at various heights, and later against flying bombs. These were enlivened occasionally by touches of the gentle irony that his friends will recall.

It may seem remarkable that one who had no previous experience of war and its problems should have been able, immediately, to take so active and successful a part. But this does not seem so remarkable to those who knew Bayliss's breadth of knowledge and ingenuity, and his engaging readiness to help. A. V. Hill

Dr. Arvid Hjalmar Uggla

By the recent death of Dr. Arvid Uggla in his eighty-second year, the learned world has lost a great authority on everything connected with Linnæus. When Dr. J. M. Hulth of the University Library, Uppsala, published his excellent *Bibliographia Linnaeana* in 1907, he stated his intention of undertaking a work on Linnæus's manuscripts, but his promotion to the head librarianship of the University Library, Uppsala, and his early death prevented him from carrying out this project. After his service in the Swedish Army during the First World War, Arvid Uggla returned to the University Library, Uppsala, becoming one of its principal librarians. From about the year 1928, when Uggla published his first paper on Linnæus, he began a series of visits to England with the purpose of a close study of the Linnæan manuscripts. His profound study of these numerous manuscripts solved some problems long overdue. Some of these solutions were published in the year-book (*Årsskrift*) of the Swedish Linnean Society (Svenska Linné-Sällskapet) but many remain unpublished. Unlike previous research workers, he also thoroughly investigated the many manuscripts of the younger Linnæus, and was able to show that, although lacking the elder Linnæus's genius, that somewhat denigrated young man was quite a considerable naturalist. Although he prepared a valuable hand-list in manuscript and deposited a copy at the Linnean Society of London, his projected catalogue of the Linnæan manuscripts remains unpublished. It is hoped that Svenska Linné-Sällskapet will publish this catalogue as its tribute to his memory.

Only those who have made some study of the Linnæan manuscripts can form any idea of the difficulties connected with them. Apart from the fact that many of the manuscripts still consist of loose leaves (at times in doubtful order) there are the ever-present difficulties connected with reading the sometimes almost illegible handwritings. Uggla had the quick mind and keen eye necessary to do such work to perfection. In addition, he had wonderful powers of memory, which enabled him to associate immediately with other manuscripts the one he was investigating.

Apart from the numerous papers published in the *Årsskrift* and elsewhere, Uggla published several separate works. Among them, with E. Malmeström (now Bishop of Uppsala), he collaborated in a revised edition of Linnæus's several autobiographies in *Vita Caroli Linnaei* (1957).

Svenska Linné-Sällskapet devoted its *Årsskrift* for 1962, published in 1963, as a *festschrift* to Dr. Uggla on his eightieth birthday, February 13, 1963. In it will be found the reproduction of an excellent bas-relief portrait of Dr. Uggla, and a bibliography of his writings. From 1927 he was secretary of the Swedish Linnean Society and editor of its *Årsskrift*, as well as superintendent of the Linné Museum, Uppsala.

Uggla was one of the kindest and friendliest of men, and much liked by all who knew him. Although a bachelor he had great sympathy with young people, and even when very busy himself would willingly give time and effort to help others. Nevertheless, at times he could be very reserved, as if, like Browning's grammarian, he feared 'to draw a circle premature'. There is but little doubt that with him his research work came first. About two years ago he suffered a stroke, but made a good recovery. He died from heart failure on July 30, 1964.

Among the many honours conferred on Arvid Uggla were two Swedish knighthoods and the foreign membership of the Linnean Society of London. S. SAVAGE

Dr. M. Doporto

DR. MARIANO DOPORTO, director of the Irish Meteorological Service, died suddenly in Dublin on September 8.

Dr. Doporto was born in Cáceres, Spain, in 1902, and studied physics at the Universities of Madrid and Barcelona. He joined the Spanish Meteorological Office in 1921, and was in charge of the weather forecast centre at Barcelona at the outbreak of civil war in Spain. Some time afterwards he left Spain, and in 1939 joined the newly formed Irish Meteorological Service, serving first at the flying-boat base in Foynes. Two years later he moved to Dublin, and in 1948 succeeded Mr. A. H. Nagle, becoming the second director of the Service. At about the same time he became an Irish citizen.

Dr. Doporto was the author of a large number of scientific papers. His most important contributions concerned the extended solutions of the general equations of motion and the cellular structure of the atmosphere. In 1943 he deduced theoretically that an upper isopycnic layer must occur at a height of about 25 km, a prediction which was later verified. Shortly before his death, he was working on a possible association between noctilucent clouds and the isopycnic layer at 80 km.

Dr. Doporto's skill as an organizer and administrator was much in evidence in the development of the Irish Meteorological Service during the past sixteen years. He was responsible for the completion of the network of observing stations, and for the installation of laboratories dealing with atmospheric radioactivity and the chemical analysis of air and rain. He served on the Board of the Dublin Institute of Advanced Studies and took a leading part in international meteorological affairs through the World Meteorological Organization and the International Union of Geodesy and Geophysics.

Dr. Doporto leaves a widow and three sons.

P. M. AUSTIN BOURKE

NEWS and VIEWS

Automatic Data Processing in the Manchester College of Science and Technology : Prof. G. Black

DR. GORDON BLACK has been appointed to the (part-time) chair in automatic data processing in the Faculty of Technology in the Manchester College of Science and Technology. Dr. Black, who is forty-one years of age, was educated at the Warrington County Grammar School and the University of Durham. After gaining a B.Sc. degree at Durham in 1945, he worked for a short time at the Royal Aircraft Establishment, Farnborough, in the Research and Development Department, before moving in 1946 to the British Scientific Instrument Research Association. He remained with the Association until 1956 and during this ten-year period he qualified for a Ph.D (London) degree and a diploma of the Imperial College of Science and Technology, following extra-mural research extending over two years in the field of technical optics. He also spent three further years on research in association with the Computing Laboratory at the University of Manchester, working on automatic numerical optimization. He was elected a Fellow of the Institute of Physics in 1951. In 1956 he accepted an appointment as principal scientific officer with the United Kingdom Atomic Energy Authority at Risley, Warrington, working mainly on computer applications. He was appointed senior principal scientific officer in the Computing Section at Risley in 1958 and became deputy chief scientific officer (technical manager) of the Computing Section in 1959. His staff totals 60 (including 30 graduates) and he has been concerned with the operations of three computers, the *Mercury*, the IBM 704/7090, and the *PACE* analogue computer.

Professional Civil Servants

State Service for August 1964, the journal of the Institution of Professional Civil Servants, reports the debate on motions presented at the annual delegate conference, briefly summarizes the conflict between the Institution and the Ministry of Aviation over the conditions of service of Air Traffic Control Officers (into which a committee is now enquiring) and notes the protests which are reaching the Institution from professional and technical staff in the Ministry of Public Building and Works. These protests are aimed at the excessively autocratic nature of the new organization, which appears to embody the worst features of the old Ministry of Works organization. Professional officers in the new Ministry complain that administrative control now limits the freedom they formerly possessed in exercise of technical judgment in discharging their duties, while the overhead expenses of the Department are significantly greater than those of the Works Department in the old Service Ministries. The Institution also directs attention to the Treasury's resistance to the proposal that professionally qualified officers should be known simply by the title of their profession—architect, engineer, etc., instead of by the terms, 'basic' and 'main', which are now meaningless inside as well as outside the Civil Service; for these terms the Institution suggests the substitution of the terms 'senior' and 'principal', respectively.

Commonwealth Scholarship Plan

THE fourth annual report of the Commonwealth Scholarship and Fellowship Plan closely follows the pattern of previous reports and again includes a special section on Commonwealth visiting fellowships and other senior awards under the Plan (Pp. 76. London: H.M.S.O., 1964. 5*s*.). Five senior Fellows from Britain took up awards in New Zealand, Australia, India (2) and Canada. Four from Australia visited Britain (2), Canada and India; two from New Zealand took up prestige fellowships in Britain, one from India in Canada, and one visitor's award from Southern Rhodesia enabled an adviser to visit Australia. Awards held in the first terms of the academic year increased from 820 in 1962–63 to 924 in 1963–64. Britain has now reached her target figure and both Canada and Australia have nearly achieved theirs. Jamaica offered her first scholarship, tenable at the University of the West Indies, and, with one exception, all the countries which promised to institute awards have now done so. Of the 390 scholarships taken up during the year, 219 were awarded by Britain, 84 by Canada, 26 by Australia, 22 by India and 13 by the West Indies. Although the Plan appears to be working effectively and making a real contribution to the educational needs of the Commonwealth, there are some difficulties. Perhaps

the most important is that of attracting strong fields of applicants for awards offered by the less-developed Commonwealth countries. This is primarily a question of publicity, and the report stresses the need for accurate, detailed and up-to-date information regarding facilities in the universities of these countries. Reference is again made to the shortage in some countries of graduates eligible to apply, and some of these countries are urging more awards at undergraduate level. There is also the recurrent difficulty of inducing some Scholars to return home on completion of their tenure, and although an appreciable number of Scholars have now returned to their home countries, of 324 whose awards had terminated by March 31, 108 sought deferment for periods of up to two years.

University Extra-mural Work in Britain

The report of the Universities Council for Adult Education, 1962–63, entitled *Change and Tradition in University Extramural Work*, notes new developments announced in broadcasting and other encouragement received from different quarters for adult education (Pp. 24. Liverpool: Universities Council for Adult Education, The University, 1964). Reports from the various extra-mural departments indicate the variety of the demand as well as the opportunities, and the intensity of the demand is illustrated by the persistence with which the courses were pursued during the severe winter that year. The increased possibility of planning classes on a regional basis is emphasized in some of these reports, as well as the possibility of a new local pattern of extra-mural classes, with a choice of subjects offered in neighbouring towns. The report emphasizes also the need for adult education centres, with a varied programme, and spaced so that each can act as a centre for the region. Besides the growing momentum of day-release courses for trade unionists there is a marked spread and development of extra-mural courses for social workers. There is also a general development of extra-mural courses of groups which have a common vocational interest, illustrated by a particularly encouraging response to some courses for magistrates and the interest aroused among local authorities by a week-end conference on "Coastal Erosion" arranged by the University of Southampton. Residential courses arranged by university extra-mural departments seem likely to increase, and there is also more general adoption of linked week-end courses. The increased need for extra-mural departments to provide facilities for training and research in adult education is again stressed, but the report concludes by deploring the Ministry of Education's announcement in February 1964 that no additional grant-aid would be available in 1964–65 to finance further full-time appointments or expansion of full-time work. This reversal of policy came when the plans of extra-mural departments were already advanced. The Council firmly protests against such short-sightedness, which achieves trifling economy but frustrates and stultifies the efforts of extra-mural departments to respond to the growing public awareness that education is a life-long process in which the universities have a recognized and continuing part to play.

Teaching and Research in Public Health Engineering

A two-day conference of "Teachers of Public Health Engineering in Universities and Colleges of Technology" was held at the Imperial College of Science and Technology, London, during September 17–18. This followed a successful first meeting held in the University of Newcastle a year ago. It was attended by participants from the Universities of Birmingham, Leeds, London (Imperial College and University College), Newcastle, Sheffield and Strathclyde, and from Kingston College of Technology, Manchester College of Science and Technology, and the National University of Ireland. Discussions on the first day were devoted to the teaching of public health engineering at both undergraduate and postgraduate levels, the recruitment and selection of students, the provision of short courses on specific topics and assistance to universities overseas. On the second day the conference discussed a number of subjects of current research or of possible future research. Among these were various aspects of water conservation, including investigations on the economic optimization of water and sewage treatment processes, tertiary treatment of sewage, some possible new methods of waste treatment and the application of chemical engineering processes in the field of public health engineering. There was also discussion on the presentation of the results of research and on co-operation with other research bodies. It was agreed that a further meeting should be held in the University of Strathclyde, Glasgow, in September 1965.

Fourth World Congress on Industrial Safety

This year the Royal Society for the Prevention of Accidents, in co-operation with the International Labour Office and the International Social Security Association, has organized, and is host to, the Fourth World Congress on Industrial Safety. To mark the occasion, the *British Journal of Industrial Safety* (**6**, No. 68; Summer 1964) devotes a whole issue to contributions made by some sections of British industry towards the greater safety and health of their employees. It is gratifying to know that "The United Kingdom is proud of its contribution to industrial safety. Its statutory provisions have been used by other countries as a blueprint for their own laws, and many of its industries have established safety programmes which have achieved creditable results over the years". After a foreword by R. K. Christy (H.M. Chief Inspector of Factories), the titles of contributions are: "There is no Profit without Safety", by G. H. Beeby (chairman, British Titan Products Co., Ltd.); "Research for Health: Work on Pneumoconiosis", by Dr. R. L. Gordon (Safety in Mines Research Establishment, Ministry of Power); "Accidents in the Construction Industry", by D. Short (H.M. Senior Inspector of Factories, Building and Civil Engineering); "Safety through Standards in British Industry", by R. Berry (divisional chief technical officer, British Standards Institution); "The Practice of Medicine in Industry", by Dr. R. Murray (medical adviser to the Trades Union Congress); "Safety in Steel", by R. Barry (head of Accident Prevention, British Iron and Steel Federation, and editor of *Safety*); "Safety in Chemicals", by N. F. Patterson (chairman, British Chemical Industry Safety Council); and "Accident Prevention in the Rubber Manufacturing Industry", by H. Jackson and S. F. Smith (respectively deputy chairman of the Leyland and Birmingham Rubber Co., Ltd., and chief officer for safety, health and welfare, the Dunlop Rubber Co., Ltd.). Each year, in conjunction with the National Industrial Safety Conference, the Royal Society for the Prevention of Accidents organizes a trade exhibition of industrial safety equipment. It was appropriate that this year the 'Intersaf' Exhibition was staged in London (July 13–17) to coincide with the fourth World Congress on the Prevention of Occupational Accidents and Diseases.

Mobile Foundry Laboratory

Among many matters of interest mentioned in the annual report of the British Non-Ferrous Metals Research Association for 1963 is the description of a travelling laboratory designed to assist the non-ferrous foundry industries (Pp. 47. London: The British Non-Ferrous Metals Research Association, 1964). An 18-ft. caravan is fully equipped to demonstrate techniques, and is staffed by a metallurgist especially experienced in foundry matters and a highly qualified technician. Established especially to assist those producing copper alloy castings, it is already making a considerable impact on this branch of industry (see also p. 308 of this issue of *Nature*).

Future of Chemical Publications

A DISCUSSION meeting on "The Future of Chemical Publications", organized by the Chemical Society, was held at the University of Nottingham during September 22–24. It was attended by about 190 people drawn equally from those concerned with practical chemistry and those engaged in administration or information work. About 18 months ago the Chemical Society appointed Dr. R. S. Cahn, who was then its editor, to conduct a survey of chemical publications that should be embodied in a report containing recommendations for action by the Chemical Society in the immediate and the not so immediate future; the report is to be submitted not later than Easter 1965 and it is the Chemical Society's hope to publish it. The objects of the discussion meeting were to place before interested British chemists some details of the present situation and the lines along which Dr. Cahn as director of publications research for the Chemical Society was thinking, as well as to solicit opinions and suggestions from the audience. Principal papers were read by Prof. J. S. Anderson (Oxford) on "The Scientific Urgency", Dr. R. S. Cahn on "The Future of Primary Publications", and Dr. F. A. Tate (assistant director and acting editor of the Chemical Abstracts Service, Columbus, Ohio) on "The Chemical Abstracts Service: Present and Future". Shorter papers were contributed by Dr. L. C. Cross (editor, the Chemical Society), Prof. W. G. Overend (honorary publications secretary, Society of Chemical Industry), Prof. J. D. Bernal (Birkbeck College, London), Mr. C. M. Hutt (Academic Press, Inc. (London), Ltd.), Dr. H. J. Rogers (chairman of the editorial board of the *Biochemical Journal*), Mr. J. H. Black (Library and Information Department, Shirley Institute, Manchester), Mr. Allen J. Sprow (National Science Foundation, Washington, D.C.), Dr. H. T. Hookway (Department of Scientific and Industrial Research), Dr. R. E. Fairbairn (Imperial Chemical Industries, Ltd.), Dr. M. P. Barnett (Institute of Computer Science, London) and Dr. H. Coblans (Association of Special Libraries and Information Bureaux, London). A great deal of time was left for discussion from the floor, and many views were freely expressed about the needs of chemists and about the value of the suggestions put forward by the speakers. No firm conclusions were reached or sought at this meeting, but, as intended, most of those present were able to gain a deeper insight into chemical publications, while the officials of the Chemical Society gained much, ascertaining the views of chemists in regard to their varying activities.

International Support of Cancer Research

A CONFERENCE on "The International Support of Cancer Research" was held in Stockholm during September 7–9 under the auspices of the Union Internationale Contre le Cancer. The objectives of the conference were to survey present proposals for increased international co-operation in cancer research and related medicine and biology, to estimate research needs in the various regions of the world, and to make recommendations. Reports were received of the present and future requirements of cancer research in the various countries of the world. While present support of cancer research varies widely from country to country, it was concluded at the conference that an urgent need exists, additional to national support, for international support on a considerable scale, probably of the order of many millions of dollars annually. Special emphasis was placed on basic research, the extension of international investigations in epidemiology and demography, and programmes of scientific and technical training and exchange. The conference favoured the establishment of an International Cancer Research Agency, sponsored and financed by Governments, to which the Union Internationale Contre le Cancer would make its medical and scientific contribution in concert with the World Health Organization and other organs where appropriate. The establishment was envisaged of an agency to act as recipient and manager of funds, with a Scientific Advisory Council appointed from a panel nominated by the Union. This agency would be responsible for the allocation of support on the basis of specific applications, and also from its own investigations of research requirements throughout the world. The conference considered it of special importance that such support would be accorded not only to projects transcending national boundaries, but also to intra-national projects in the case of countries not at present able to sustain these from their own resources.

International Commission on Zoological Nomenclature

NOTICE is hereby given of the possible use by the International Commission on Zoological Nomenclature of its plenary powers in connexion with the following cases, full details of which will be found in the *Bulletin of Zoological Nomenclature* (**21**, Part 3; August 7, 1964): (1) Designation of a type-species for *Lingula* Bruguière [1797] (Brachiopoda). *Z.N.(S.)* 1598. (2) Validation of the generic name *Axopora* Milne Edwards and Haime, 1850 (Hydrozoa). *Z.N.(S.)* 1610. (3) Validation of a neotype for *Cancer setiferus* Linnaeus, 1767 (Crustacea, Decapoda). *Z.N.(S.)* 1617. (4) Designation of type-species for *Purpura* Bruguière, 1789, and *Muricanthus* Swainson, 1840; validation of *Ocenebra* Gray, 1847; validation of Thaisidae Suter, 1913 (Gastropoda). *Z.N.(S.)* 1621. (5) Suppression of *Certhia chrysotis* Latham, 1801 (Aves). *Z.N.(S.)* 1653. Any zoologist who wishes to comment on any of the foregoing cases should do so in writing to the Secretary, International Commission on Zoological Nomenclature, c/o British Museum (Natural History), Cromwell Road, London, S.W.7, before February 7, 1965.

Isidore of Seville

FROM the time when the Roman Empire was converted to Christianity, that is, from A.D. 400 until A.D. 1200, the surviving remains of classical learning and science were preserved in monasteries, where manuscripts were treasured, valued and written. Of the many writers of the period the most learned was Isidore of Seville or Isidoreus Hispalensis (570 ?–636). He was born either at Seville or Carthagena and became Bishop of Seville, the premier Catholic See in Spain, in 599. The canons of the Councils at Seville in 618–19 and at Toledo in 633, during his episcopate, formed the basis of the constitutional law of Spain. Isidore was a voluminous writer and widely read in classical authors. He wrote an introduction to the Old and New Testaments, a defence of Christianity against the Jews, three books of *Sentences*, books on ecclesiastical offices and monastic rules, and a history of the Goths, Vandals and Suevi. The works of most interest to scientific historians are *Etymologiae* (edited by W. M. Lindsay, 1912) and *De Natura Rerum*. The former was an encyclopædia of the then known sciences. In a recent monograph (*Isidore of Seville: The Medical Writings*. An English translation with an Introduction and Commentary, by William D. Sharpe. *Trans. Amer. Philos. Soc.*, Philadelphia (pp. 75), N.S., **54**, Pt. 2, 1964). Dr. W. D. Sharpe has translated the medical writings in the *Etymologiae* into English, and has made a careful examination of Isidore's sources for his compilation (including late Latin medical literature) and of the work itself. He points out that this part of the encyclopædia was written for amateurs of medicine rather than for professional physicians. Use was made of it by Bede, Alcuin, Rhabanus, Maurus and Vincent of Beauvais. In manuscript copies the *Etymologiae* enjoyed wide popularity during the Middle Ages, and was often reprinted during the first century after the invention of printing.

U.K. Honours Courses in Mathematics

A PAMPHLET on honours courses in mathematics in the universities of Great Britain, prepared by A. P. Rollett, was published in 1963 by the Association of Headmasters and the Association of Head Mistresses (*Honours Courses in Mathematics in the Universities of Great Britain*. Pp. ii+61. London: Incorporated Association of Headmasters, 1963. 3*s*. 6*d*.). In this, those honours degree courses which required mathematics as at least one-half of the course were described, with notes on entrance qualifications and an informative general introduction. This guide has already proved its worth in helping the prospective undergraduate to discover those universities which are offering courses most suited to his or her particular aptitudes and tastes, while the introduction does something to help him to bridge the gap, always tending to widen, between school mathematics and university mathematics. A first supplement, compiled by W. J. Langford, has now been issued, in which details of courses available in the ten colleges of advanced technology are given, and alterations and additions to the 1963 university information are noted (*Honours Courses in Mathematics in the Universities and Colleges of Advanced Technology in Great Britain, Supplement No. 1*. Pp. 28. London: Incorporated Association of Headmasters, 1964. 1*s*. 6*d*.). The usefulness of these signposts to a somewhat tangled domain is beyond question. It is good to know that new editions may be expected in 1965, and that information about courses in regional technical colleges and in training colleges may then be included.

New Labelled Compounds from the Radiochemical Centre, Amersham

OF the radioisotope preparations available for blood cell labelling, di-isopropylphosphorofluoridate-^{32}P is considered by many workers to provide most nearly the qualities of an 'ideal' label; it appears not to elute, does not produce measurable cell damage and is not re-utilized. The Radiochemical Centre, Amersham, has now made available a sterile solution in propylene glycol. The solution cannot be heat-sterilized because of thermal instability and reaction with the solvent, but γ-irradiation has been successfully adopted as the sterilization process. It is supplied in the following forms:

Packaging	Price
Ampoules containing 200 μc. in 1-ml. propylene glycol	5 ampoules (1 mc.) £15; 10 ampoules (2 mc.) £25; then £10 per 5 ampoules
Penicillin-type bottles containing 1 mc. in 5-ml. propylene glycol	1 bottle (1 mc.) £12; then £8 per bottle
'Clinbritic' vaccine bottles containing bulk solution at 200 μc. per ml.	1 mc. £12; then £6 per mc.

The following compounds have been introduced since the issue of the Radiochemical Centre *Supplementary List* No. 1: DL-adrenaline-7-*T*; allyl isothiocyanate-^{35}S; carbaminoylcholine(methyl-^{14}C) chloride; dimethyl sulphoxide-^{35}S; dodecane-1-thiol-^{35}S (dodecyl mercaptan-^{35}S, lauryl mercaptan-^{35}S); iodinated polyvinyl pyrrolidone-1125, sterile solution; œstradiol-4-^{14}C (benzene/2 per cent methanol solution); œstrone-4-^{14}C (benzene solution). Further information can be obtained from the Radiochemical Centre, Amersham, Bucks.

Codes of Practice on Prestressed Concrete

THE ever-increasing use of prestressed concrete in building and civil engineering in the United Kingdom and in most European countries stems largely from interchange of ideas and the results of international research. Codes of practice and specifications may, and often do, vary from one country to another, and contractors and engineers concerned with overseas contracts must necessarily have prior knowledge of such variations. As a practical means of collating information on this subject from different sources, not only for builders and civil engineers, but for all those concerned with design and construction in prestressed concrete, translations of the present-day specifications and codes of practice operative in different European countries are available (*Codes of Practice on Prestressed Concrete*. Nos. 1–7. London: Cement and Concrete Association. 20*s*. each). Of this valuable series, seven are now available: No. 1 (Germany), *Prestressed Concrete: Code of Practice for Design and Execution* (Version of October 1953, *DIN* 4227); No. 2 (Belgium), *Code of Practice for the Use of Steel in Prestressed Concrete Structures* (December 1960, document 215/4); No. 3 (Holland), *Directives for Prestressed Concrete* (Amsterdam, Stuvo, 1962); No. 4 (Austria), *Directives for the Design and Construction of Prestressed Concrete Structures* (Wien, Bondi and Sohn, 1960); No. 5 (Switzerland), *Standard Specifications relating to Concrete, Reinforced Concrete and Prestressed Concrete Structures* (Zürich, Société Suisse des Ingénieurs et des Architectes, 1956); No. 6 (Poland), *The Design of Prestressed Concrete Structures* (Konstrukcje z betonu Sprezonego, Warsaw, 1957); No. 7 (Finland), *Code of Practice on Prestressed Concrete* (Helsinki, Rakennusinsinööriyhdistys r.y., 1958).

Coal Research in Australia

THE Division of Coal Research, Commonwealth Scientific and Industrial Research Organization (Chatswood, New South Wales), publishes periodically a useful bulletin, *Coal Research in C.S.I.R.O.*, to provide ". . . an outline of the investigations in progress at the laboratories at North Ryde". A recent issue (No. 23; July 1964. Melbourne) emphasizes the importance of research into processes offering new potential methods for utilization of certain by-products of coal carbonization in gasworks and steelworks. It is noted that profitable disposal of by-products of coal carbonization has hitherto played an important part in the economics of this process, including coke in the case of gasworks, but due to the rapid growth and competition of the petrochemical industry, such disposal in recent years is becoming increasingly difficult. Changes in the pattern of chemical manufacture due to this cause are to-day world wide. The papers in this issue include "Predicting Coke Strength"; the theme here is that ". . . it would be of immense assistance to coke-oven operators to be able to make accurate predictions of coke strength based solely on the characteristics, as measured in the laboratory, of the coal being carbonized. This article suggests a basis for making such predictions". Another paper, on improving the flow properties of road tars, describes how, by suitably treating coal tars, ". . . relatively low-cost road binders can be prepared which in certain important respects are at least equal to the petroleum bitumens now being used as binders in the construction of all-weather sealed roads". D. J. Williams contributes an article on oxidation of sulphur dioxide in combustion processes, wherein it is shown that in the oxidation of sulphur dioxide to the trioxide in combustion gases, of the active intermediates involved, the role of atomic oxygen appears to be the most important. The porous structure of solid fuels influences their behaviour in combustion and gasification; an article on surface-area measurements describes a method of surface-area determination based on measurements of the adsorption of xenon and krypton. An article on the structure of low-temperature tars deals with tars derived from low-temperature fluidized carbonization of coal, for which, up to now, there has apparently been little commercial outlet. This article discusses an investigation involving both physical and chemical techniques, ". . . whereby existing knowledge of the average compound-type composition of such tars has been extended further into the high-molecular-weight range, thus bringing one stage nearer the possibility of utilizing tars for a wider variety of purposes than at present". The issue concludes with a short paper on tars from the Onia-Gegi gasification process. The inclusion of abstracts of some recent C.S.I.R.O. research papers is a useful guide to other work on coal utilization carried out by this Organization.

Plants of the Scottish Lower Carboniferous

Transactions of the Royal Society of Edinburgh, **66**, No. 1: "On the Structure of Some Petioles Associated with *Rhetinangium* Gordon" represents one of a series by Mr. A. G. Long on the plants of the Scottish Lower Carboniferous (Pp. 8+2 plates. Edinburgh: The Royal Society of Edinburgh, 1964. 6*s*.). We formerly knew very little about plants of this age, but now, thanks as much to Long as anyone, we have something like half knowledge of a considerable number, and very strange some of these plants are. In this paper, Mr. Long describes some petioles and petiole branches as a new species, but he thinks they belong to the stem *Rhetinangium* described fifty years ago. What is perhaps of more significance, he finds that the peculiar seeds *Genomosperma* are repeatedly associated with the petiole fragments. Thus, something approaching a whole plant begins to emerge, though still obscure and indeed insecure. Even such a synthesis is very remarkable with the infrequent and scattered fragments that constitute this flora.

Orientation of Stone Monuments and Buildings of Antiquity in the Near East

RECENTLY it has been suggested that many of the great stone monuments and buildings of antiquity were erected as calendars, being carefully orientated in respect to the Moon or some other heavenly body. A study of certain *Ziggurats* by G. Charrière, in the near east, would appear to confirm this suggestion (*Revue D'Assyriologie et Archéologie Orientale*, "L'Orientation de Quelques Ziggurats et Sanctuaires du Moyen-Orient" *RA* 58—1964). The article in question gives a detailed account of a number of examples such as the Moon palace of Sargon, the Ziggurat of Ur and certain Egyptian sanctuaries. Among an agricultural people the relationship between the calendar and religion is necessarily close, and this is often reflected in the orientation of their monuments.

Perkin Centenary Awards, 1965–1966

APPLICATIONS for the award of Perkin Centenary scholarships are invited by the Perkin Centenary Trust. Awards, tenable for one or two years and renewable for a further year at the discretion of the Trustees, are offered to candidates employed in an industrial firm or other institution concerned with the manufacture or the application of colouring matter, for study at a university or technical college. Two types of award are available, some to the value of £100, which are to be used in conjunction with a local education authority grant, and others to a value of £400, which are intended for students whose normal grant, inclusive of fees, would be less than about £300. Perkin travel grants are also available to teachers concerned with the study of any aspect of the manufacture or the application of colouring matter. Applications for the scholarships must be made by May 1, 1965, and for the travel grants by December 1, 1964. Further information can be obtained from the Secretary, Perkin Centenary Trust, c/o The Chemical Society, Burlington House, London, W.1.

The Night Sky in November

NEW moon occurs on Nov. 4d 07h 17m U.T. and full moon on Nov. 19d 15h 43m. The following conjunctions with the Moon occur: Nov. 13d 02h, Saturn 3° N.; Nov. 19d 04h, Jupiter 2° N.; Nov. 26d 16h, Mars 3° S. Mars is in conjunction with Regulus on Nov. 4d 01h, Mars being 1·3° N., Mercury with Antares on Nov. 14d 12h, Mercury being 2° N., and Venus with Spica on Nov. 18d 08h, Venus being 4° N. Mercury is too close to the Sun for observation. Venus is a morning star, rising at 3h 15m, 3h 55m and 4h 45m on November 1, 15 and 30, respectively. Its stellar magnitude is −3·5; its distance from the Earth increases during the month from 108 to 125 million miles and the visible portion of the apparent disk from 0·765 to 0·848. Mars rises at 0h 05m, 23h 50m and 23h 30m at the beginning, middle and end of the month, respectively. Its stellar magnitude is +1·2 and its distance from the Earth on November 15 is 140 million miles. Mars is in Leo throughout the month. Jupiter is above the horizon virtually all night, rising at sunset on November 12. Its motion is now retrograde near the boundary between Taurus and Aries, it is in opposition on November 13, its stellar magnitude is −2·4, and its distance from the Earth at opposition is 372 million miles. Saturn sets at 0h 15m, 23h 20m and 22h 20m on November 1, 15 and 30, respectively. It is stationary in Aquarius on November 2, after which it resumes its eastwards motion among the stars. Its stellar magnitude is +1·0 and its distance from the Earth on November 15 is 895 million miles. Occultations by the Moon of stars brighter than magnitude 6 are as follows, observations being made at Greenwich: Nov. 9d 16h 46·9m, ψ Sgr. *m*.(*D*); Nov. 18d 01h 41·2m, ξ^1 Cet. (*D*); Nov. 20d 21h 53·0m, ι Tau. (*R*); Nov. 21d 05h 21·5m, 109 Tau. (*R*); Nov. 21d 19h 20·6m, 1 Gem. (*D*); Nov. 21d 20h 11·0m, 1 Gem. (*R*). An occultation by Mars of *B.D.* +19° 1559 (mag. 7·4) has been predicted, Nov. 23d 03h 36m (*D*), Nov. 23d 03h 41m (*R*). *D* and *R* refer to disappearance and reappearance, respectively. The Taurid meteors are active during October 26–November 16, with maximum between November 1 and November 9. The radiant is near R.A. 3h 36m, Dec. +14°, and conditions for observation are favourable. The Leonid meteors are active during November 14–17, with maximum on November 16. The radiant is near R.A. 10h 08m, Dec. +22°, and conditions for observation are favourable.

Announcements

PROF. M. G. KENDALL has been elected president of the Institute of Statisticians in succession to the late Lord Beveridge, who was founder president of the Institute. Mr. R. Brech, formerly honorary secretary, has been elected chairman in succession to Prof. G. A. Barnard, who retired at the end of his term of office. Mr. F. D. K. Liddell has been elected honorary secretary.

MR. AUSTIN BOURKE, assistant director of the Irish Meteorological Service, has been appointed director of the Irish Meteorological Service in succession to the late Dr. M. Doporto (see p. 329 of this issue of *Nature*). Mr. Bourke is at present vice-president of the International Society of Biometeorology.

A SYMPOSIUM on pulse radiolysis will be held in Manchester, jointly by the Department of Chemistry of the University and the Paterson Laboratories, Christie Hospital and Holt Radium Institute, during April 21–23, 1965, immediately preceding the Miller Conference. Further information can be obtained from Dr. M. Ebert, Paterson Laboratories, Christie Hospital and Holt Radium Institute, Withington, Manchester 20.

THE following Acorn Meetings are to be held at the Arthur D. Little Research Institute: "The Interaction of Slow Electrons with Molecules" (November 16); "Organometallic Research in Industry" (December 7); "The Sleeping Brain" (January 11, 1965); "Solar Radiation—A Meteorological Cinderella" (February 8, 1965); "The Matchbox Pole-balancer—A Computer Programme which Learns by Trial and Error" (March 22, 1965). Further information can be obtained from Dr. R. E. Lister, Arthur D. Little Research Institute, Inveresk Gate, Musselburgh, Edinburgh.

CORRIGENDUM. In criticizing the illustrations in *Animal Worlds*, by Marston Bates (*Nature*, **203**, 907; 1964), Dr. L. Harrison Matthews stated that a photograph labelled "living zoo-plankton" is manifestly a preserved dead sample. The sample was, in fact, merely narcotized and was thus still living.

INDUSTRIAL RESEARCH IN BRITAIN

*RESEARCH for Industry, 1963** reports briefly on the progress made by nine research associations the grants of which were reviewed during the year. The British Coke Research Association's investigations of the conditions necessary for improving the quality of coke from a medium-quality coking coal, such as will be carbonized in increasing quantities, have already led to improvements in the consistency and quality of the coke by controlling the bulk density of the charge, adjusting the size and water content of the coal, and admixing small amounts of oil. By adding three different materials to poor coking coal, the physical properties of the coke have been improved sufficiently for it to be suitable for use in blast furnaces. Investigations on foundry coke, in collaboration with the British Cast Iron Research Association, have indicated procedures extending the range of coke suitable for foundry use, and a major achievement in the domestic field has been the demonstration that certain additives give a coke of improved reactivity. Fundamental investigations of the thermal behaviour of coal and the fissuring of coke promise to illuminate the process of fissure formation during carbonization of coal in coke ovens.

Fundamental work by the Research Association of British Flourmillers has included investigations of the amino-acid composition of the total protein of different types of flour, comparison of the individual fractions of the protein and an examination of the interrelation of the disulphide and thiol groups. A new family of at least six anti-oxidants has been isolated from oats and wheat, and development of the air classification of flour continued. Replacement of at least 25 per cent of the malted barley by a low-protein English flour has given beer with a better head and keeping properties. The Fruit and Vegetable Canning and Quick Freezing Research Association has continued its investigations of the heat-resistance of micro-organisms which might be present in the canned products before heat processing. Its work on the chemistry of processed peas in relation to texture is nearing completion, and a survey is being made of the extent of contamination of quick frozen foods by various types of bacteria.

The Gelatin and Glue Research Association has initiated an investigation of the fine structure of collagen and has prepared many derivatives of gelatin and animal glue by substitution of the amino-groups present. Methods have been evolved for determining the degree of substitution, and also for increasing the cross-linkability of certain bone glues. Extra-mural support is being given to an investigation at Cambridge on the ultra-centrifugation of gelatin gels, and there has been further investigation of the effect of maturing conditions on their melting-point and rigidity. As a result of work by the Heating and Ventilating Research Association, together with that of other organizations, the factors conducive to large fuel savings in modern light-weight structures are now known at least qualitatively, and a simple approximate means of sizing the plant is being developed. A simple method of noise-rating has been specified which will enable manufacturers of ventilating and air-conditioning equipment to give the fan-noise output in identical form. The Association is also examining the ventilating and air conditioning of operating theatres.

Research by the British Hydromechanics Research Association on fluid sealing is concentrated on the mechanical or face seal, on moulded seals for reciprocating shafts, and on soft packings for stuffing box glands. Work on centrifugal pumps has involved use of a mathematical model in clarifying the nature of the fluctuating forces and of electrolytic tank methods for determining perfect fluid flow patterns in pumps, while a digital computing method has been used as an alternative to a graphical one for the analysis of complicated pumping systems. The basic engineering principles in the use of oil hydraulic machinery for transmitting mechanical power are being investigated and further model tests have been made on hydraulic problems in the pipe-work systems of the cooling-water circuits of large power stations. In a different field of industry, the 'Lacra' brass bobbin winder developed by the Lace Research Association is being satisfactorily used in Leavers lace factories, and a method has been devised to speed up that part of the process of removing floats or 'clips' from lace which is still carried out by hand, and to mechanize the final removal of the floats.

The Rubber and Plastics Research Association has made fundamental contributions to the theory of polymer molecule distribution and network formation and is able to record noteworthy progress in its investigations of the effect of water on unstrained glass-reinforced plastics. The British Ship Research Association, which for five years from April 1, 1963, was awarded a minimum grant of £300,000, subject to a maximum of £500,000 with a further £200,000 on the basis of £1 per £1 on special contributions from ship owners and managers, continued full-scale trials of turbine machinery and investigated in several ships the cause of the deterioration in performance with time in service. Investigation of the increased wear of cylinder components associated with burning heavy residual oils indicates that an increase in cylinder liner temperature is most beneficial if the temperature is increased at the coolest portion of the liner. Problems of nuclear ship design, construction and operation are being investigated by a special team.

* Department of Scientific and Industrial Research. *Research for Industry, 1963*; a Report on work done by Industrial Research Associations in the Government Scheme. Pp. v+54+19 plates. (London: H.M. Stationery Office, 1964.) 5*s*. 6*d*. net. (See also p. 307 of this issue of *Nature*.)

THE ROCKEFELLER FOUNDATION

THE statement on future programme and policy issued last autumn by the Rockefeller Foundation described five principal interrelated areas in which the Foundation expects to concentrate its efforts during the immediate future. It is with these five areas that the first part of the president's review for 1963 "Promise and Progress" is mainly concerned, and, dealing first with the conquest of hunger, Dr. J. C. Harrar, recognizing that private philanthropy can only play a modest part, points out that the Foundation has chosen to assist this effort through co-operative efforts in the interrelated fields of education and the medical, agricultural and social sciences*. Moreover, it not only seeks to strengthen education in

* The Rockefeller Foundation Annual Report for 1963. Pp. 319+xviii. (The Rockefeller Foundation, 111 West Fiftieth Street, New York, N.Y., 1964.)

all these sciences but also participates in rural health programmes, so that advanced agricultural technologies will accompany better health protection. The Foundation has its own programme in the agricultural and health sciences, within which it has attempted to encourage the development of extension methods and organization, but Dr. Harrar stresses the need for closer union between the social, agricultural and health sciences if the problems of backward areas are to be attacked successfully.

The Foundation's work in the agricultural sciences, which began as a single centre in Mexico directed toward improving the amount and quality of that country's basic foods, has now been extended to include similar centres in Colombia, Chile and India. In addition, two international centres have been established at strategic locations, designed for basic and applied research on food problems of major importance over a wide region. The International Rice Research Institute is a co-operative venture of the Government of the Philippines and the Ford and Rockefeller Foundations, while the International Centre for Corn and Wheat Improvement at the National School of Agriculture, Chapingo, Mexico, is operated by the Government of Mexico and the Rockefeller Foundation.

In the second field, the population problem, the Foundation will assist research in fields relating to human fertility, such as the physiology of reproduction, endocrinology, human genetics, the biochemical effects of diet, etc., as well as in demography and cultural attitudes. It will also support pilot operations in areas where population density is an especially difficult problem and where there is a desire for help. In 1963 the Foundation contributed substantially to the establishment of a Centre for Population Studies at Harvard University, and its interest in rural health centres includes investigation of family planning.

In the third area, that of strengthening emerging centres of learning, Dr. Harrar exemplifies the Foundation's policy by the support given to the University of Valle, which is already internationally important in several areas of medicine, and which in 1963 the Foundation assisted with investigations of the business and financial aspects of the University's administration, the provision of basic teaching materials, and the construction of a new building for the humanities and linguistics departments. The University of East Africa received grants for research and training in economic development, and for the Faculty of Agriculture: the University of Ibadan was helped in the creation of a Department of Psychiatry, Neurosurgery and Neurology, and also in establishing a virology research unit to study the tropical fevers of West Africa which had not been diagnosed, while the University of the Philippines received substantial grants for faculty research in the Department of English and Comparative Literature, History, Political Science and Sociology and for training and research in economics and agriculture.

In the fourth area, the Foundation is making Negro education a main programme, with considerable emphasis on higher education. A substantial contribution to the United Negro College Fund will help to strengthen the faculty and improve the equipment of 32 predominantly Negro colleges, while besides appropriating funds to assist qualified undergraduates at Duke, Emory, Tulane and Vanderbilt Universities, the Foundation has made a grant to the Woodrow Wilson National Foundation to enable Fellows to teach for a year at some 40 southern colleges, mostly predominantly Negro. In the fifth area, aiding the cultural development of the United States, Dr. Harrar cites two grants made during the year to help raise standards of excellence in the theatre.

In the second part of his review "People for Progress", Dr. Harrar describes the way in which the Foundation uses its fellowship programme as part of a training programme, linked with the establishment or reinforcement of local institutions of education and research, and the strengthening of national agencies responsible for putting new methods and materials to use. The 276 new awards in 1963 brought the total of current fellowships and scholarships to 709, of which 218 were in agricultural sciences, 191 in humanities and social sciences, and 200 in medical and natural sciences. The Fellows and Scholars came from 52 countries and an additional 93 fellowships from Foundation funds were administered by other organizations, including 12 by the Medical Research Council, 22 by the Population Council and 59 by the Social Science Research Council. Appropriations for these activities totalled 3·525 million dollars, and 3·625 million dollars has been appropriated for fellowship awards in 1964.

Considering first the provision of trained man-power for health services, Dr. Harrar notes that in Latin America the supply of physicians is reasonably adequate and the output sufficient to establish a reasonable cadre of doctors for medical care and health services. Nurses, sanitary workers and technicians are scarce and are likely to remain so. Accordingly, at the University of Valle a programme for training auxiliary community nurses is under way and in the Candeleria pilot centre comparative studies are being made to determine how much care and preventive service one resident can provide when supported by a first-class medical centre within reasonable distance. In Africa, however, the number of indigenous physicians and auxiliaries is very small and the task is to maintain the high-level clinical training while inserting into a crowded curriculum sound field training in running a dispersed health service largely staffed by sub-professionals. The Foundation is attempting to help by providing staff and economic assistance to centres based on the medical faculties of the University of Ibadan and the University of East Africa, while in India the Foundation is supporting the All India Institute of Medical Science at New Delhi, with both funds and staff in a key scheme to prepare academic personnel for the burgeoning network of medical schools. The Foundation believes that it should undertake to study the problems of the proper utilization of sub-professional staff and to provide guidance for the advanced nations in their efforts to assist the development of health services in countries which need them.

Similarly, Dr. Harrar shows how the 50 per cent increase in 1963 in the funds for supporting scholarships and fellowships in agriculture to a total of 1·5 million dollars—supporting 318 students from 27 countries at 46 institutions—is directed to the conquest of hunger. Another 1 million dollars over four years has been appropriated for the International Centre for Corn and Wheat Improvement; the International Rice Research Institute, now fully staffed, expects to have 60 graduate-trainees by the end of 1964 and already has a comprehensive and integrated programme of basic and applied research on all aspects for rice production. The Foundation has also assisted the establishment of the graduate school of the National School of Agriculture, Chapingo, Mexico, and the postgraduate school of the Indian Agricultural Research Institute, New Delhi, and already the increased resources of trained man-power have permitted progress in the structure and functioning of agencies concerned with agricultural advance. Dr. Harrar also reviews briefly the way in which the support of the Foundation is enabling the University of East Africa and its constituent units to respond to the call for development of leadership in Africa.

Besides the president's review, the annual report includes notes on the regional programmes, and lists the grants and study awards made during the year, together with the financial statement showing appropriations during the year totalling 37 million dollars, of which 7·3 million dollars was for the agricultural sciences, 10·3 million for humanities and the social sciences, and 6·3 million dollars for medical and natural sciences.

SPECIAL CERAMICS

THE third international symposium on "Special Ceramics" was held at the British Ceramic Research Association during July 8–10, 1964. It was organized by Mr. P. Popper and followed the pattern of the 1959 and 1962 symposia. It is interesting to note the change in emphasis of the subject matter as progress in the nuclear power industry increases the demand for ceramic fuels and as advances in aircraft engine design lead to the search for new materials to withstand more rigorous operating conditions. The four half-day sessions of the symposium dealt with the preparation of non-oxides; pyrolytics and inorganic polymers; oxides and their electrical properties; and novel fabrication techniques and prospective applications as engine components. Ample time was allowed for discussion among the 170 delegates, who came from seven countries.

The symposium was opened by Dr. N. F. Astbury, director of the British Ceramic Research Association, who briefly reviewed the activities of the Association and its history, starting from an interest in technical refractories for the steel, glass and gas-making industries, later joining with the group working on pottery research, including electrical ceramics and chemical stoneware, and incorporating a building materials division. The youngest division, established to carry out investigations on special materials for future application in high-temperature engineering, was set up some ten years ago under the leadership of Mr. Popper. The visitors were invited to tour the Association's laboratories, where the many instruments in use included an electron microscope, electron probe microanalyser, direct reading spectrograph and mass spectrometer.

The first two papers, from the Atomic Energy Research Establishment, Harwell, considered the preparation and sintering of uranium nitride as a potential reactor fuel. Its preparation direct from the oxide would have economic advantages over its preparation from uranium metal or hydride, and Dr. J. B. Morris described work that he had carried out in co-operation with K. R. Hyde, D. A. Landsman, W. E. Seddon and H. C. J. Tulloch on the carbothermic reduction of uranium dioxide in a fluidized bed, in nitrogen, the carbon being either intimately mixed with the oxide or introduced as a hydrocarbon gas. The sintering to 92 per cent of theoretical density of uranium mononitride prepared by the reaction of nitrogen with uranium powder and the effects of oxidation, compaction, etc., on the sintering were described by Messrs. J. R. McLaren and P. W. M. Atkinson. General discussion of the relative merits of nitrogen and ammonia for nitriding various elements arose. Miss S. R. Billington (Morganite Research and Development, Ltd.) spoke of the investigations that she and Messrs. J. Chown and A. E. S. White had made of the sintering of silicon carbide and the possible mechanisms for transfer of material during this process, concluding that boundary-flow is operative. Messrs. J. Henney and J. W. S. Jones (Atomic Energy Research Establishment, Harwell) reported that investigations of zirconium carbide and the Zr–C–O system, preliminary to an investigation of the U–Zr–C system, had shown the existence of a single phase from $ZrC_{0\cdot6}$ to ZrC and had revealed two distinct oxycarbide phases, occurring at compositions approximately $ZrC_{0\cdot45}O_{0\cdot55}$ and $ZrC_{0\cdot5}O_{0\cdot5}$, with closely similar face-centred cubic crystal structures and no observed superstructure.

Pyrolysis has proved a useful method of making materials with negligible porosity and therefore high strength and chemical resistance and very low permeability. The method is well suited to the production of protective coatings and can also be used to provide bulk specimens. Pyrolytic silicon carbide was discussed in two papers. Dr. I. Mohyuddin reported that his investigation with Mr. P. Popper of the conditions for deposition of thick layers (3 mm) had shown that fine-grained silicon carbide of almost theoretical density and modulus of rupture 100,000 lb./in.^{2} could be deposited on a graphite substrate at 1,400° C from methyl trichlorosilane/hydrogen mixtures. Mr. R. Moreton (Ministry of Aviation, Farnborough) found that thick coatings of silicon carbide on molybdenum and tungsten rods tended to spall or crack due to a mismatch of thermal expansion coefficients, whereas thin coatings afforded protection against oxidation. Reaction between the silicon carbide and molybdenum was the cause of eventual failure, and Mr. Moreton suggested that a molybdenum/tungsten alloy of the correct expansion coefficient would allow thicker adherent coatings and greater protection.

There is an overlap between the usual definitions of 'inorganic polymers' and 'ceramics', and the relation between these was examined by Mr. P. G. Chantrell and Mr. P. Popper in considering the potentialities of inorganic polymers (classified here as chain compounds of high molecular weight) as electrical materials of moderate temperature stability and as intermediates in the preparation of refractory materials. That this classification was not generally accepted was demonstrated by a paper by Drs. P. Eckerlin, I. Maak and A. Rabenau (Philips, Germany), entitled "$LiPN_2$: A New Type of Inorganic Polymer". Dr. Eckerlin described a crystalline compound, derived from phospham, stable up to 1,000° C in vacuum and having a tetragonal structure formed of PN_4 tetrahedra which bears some resemblance to cristobalite. Dr. B. J. Aylett (Westfield College, London) discussed silicon–nitrogen polymers similar to the well-known polymers containing –Si–O– backbones and the problems of preparation to avoid the hydrolytic instabilities normally ascribed to them. He also mentioned some of the physical and chemical properties of the polymers produced.

Methods of quoting purity figures, as well as accuracy of analysis, came under discussion when three papers on high-purity ceramics were read, the point being made that many elements are not looked for in the analyses. Messrs. K. S. Mazdiyasni and C. T. Lynch (U.S. Air Force Materials Laboratory) advocated the use of organometallic compounds for the production of ceramic powders of ultra-high purity and small particle size, films and fibres, by thermal decomposition of the vapour, stressing the wide variety of compounds available. Fine zirconia powder and adherent refractory coatings on graphite have been produced from alkoxides. The process adopted by Messrs. G. M. Garner and F. C. Cowlard (Plessey-U.K., Ltd.) for the production of high-purity alumina was to prepare aluminium isopropoxide, purify this by vacuum distillation and convert it to alpha alumina. Mr. H. Bennett (British Ceramic Research Association) reviewed the difficulties associated with analysis of alumina ceramics and gave details of possible analysis procedures for different levels of impurity.

The contribution by Dr. L. S. Williams (Commonwealth Scientific and Industrial Research Organization, Australia) on infiltrated oxide cermets was read in his absence by Mr. D. E. Lloyd. Further progress had been made in the infiltration of alumina and other oxides with silver and its alloys and in the combining of impact strength and creep resistance with thermal shock resistance, for possible application as gas turbine components. Prof. W. W. Kriegel (University of North Carolina) described how he, Hayne Palmour III and D. M. Choi had produced and

varied the properties of pore-free polycrystalline magnesium aluminate spinel, which he considered as a possible high-performance structural ceramic on account of its chemical and thermal stability and multiple {111} <110> slip systems.

Three of the papers discussed the electrical properties of alumina. Drs. T. Matsumura and M. Laubitz (National Research Council, Canada) had calculated the ionic transport number from E.M.F. measurements on an $O_2(p_1)$, $Pt/Al_2O_3/Pt$, $O_2(p_2)$ galvanic cell at temperatures 700°–1,200° C and pressures 10^{-2} – 1 atm. Dr. Laubitz reported that their results were in agreement with the work of Schmalried but disagreed with some other diffusivity measurements. This type of experiment can throw considerable light on the mechanism of grain boundary diffusion. Dr. V. Daniel (Electrical Research Association, Leatherhead) described her work with Mr. M. Rogers on the degradation of ceramic insulators at high temperatures and the effects of various electrode materials, with particular reference to the rectifying barriers formed at electrode–ceramic interfaces. Dr. A. J. Moulson had also considered, with Messrs. W. R. Phillips and P. Popper, the degradation of alumina ceramics, concentrating on measurements of the diffusion of the two commonest heater-current metals, nickel and chromium, into various grades of alumina and the effect of their oxide additions on the resistivity. Some discussion of methods of measuring high-temperature resistivity to avoid surface and apparatus effects ensued. In an investigation of the effect of heat on pure single crystals of rutile, Mr. M. G. Harwood (Mullards, Ltd.) found that the d.c. stability at 150° C was raised whereas the a.c. losses were not correspondingly improved, indicating that different centres were responsible, and that only some tri- and tetra-valent cations improved the d.c. stability.

Thermal conductivity and emissivity are of importance in high-temperature applications, and materials of low friction are required for high-temperature bearings. Dr. R. W. Powell presented some results which he and Mr. R. P. Tye had obtained at the National Physical Laboratory, using both the longitudinal heat-flow and thermal comparator methods for measuring the thermal conductivity of carbides and borides, and suggested that these should be considered as metallic alloys rather than covalent compounds. Mr. J. C. Titus-Glover described emissivity measurements at 1,500°–2,500° C made with Dr. V. Croft at the University of Sheffield using a double parabolic arc image furnace and low-resolution spectrograph with a silicon cell radiometer. At the Cavendish Laboratory, Cambridge, Drs. C. A. Brookes and M. Imai had investigated the frictional properties of reaction-sintered silicon nitride and silicon carbide. These compared well with metals and the coefficient of friction was independent of load. Surface finish was important for wear resistance.

In the session on fabrication techniques, Dr. S. Scholz described a neat hot-press that he and Dr. E. Roeder had used at the Philips Laboratory in Germany. It reached 3,200° C and 1,000 kg/cm² and accurate densification/time measurements on the small samples were recorded. Some of the limitations to hot-pressing for industrial applications have been overcome by Dr. G. S. Gruintjes and Mr. G. J. Oudemans (Philips Research Laboratories, Eindhoven), who described a new technique for continuous hot-pressing that had produced long rods of both metals and ceramics. Intense collimated plasma-electron beams of powers up to 5 kW had been obtained by Messrs. J. W. Isaacs, C. W. A. Maskell and J. D. L. Harrison at the Atomic Energy Research Establishment, Harwell, from robust, hollow cathodes without filaments, and Mr. Harrison discussed methods of controlling the energy and size of the beam and applications to the melting of ceramics.

Turning to future applications, Dr. E. Glenny's paper "Ceramics and the Gas Turbine", read by Mr. R. Smith (National Gas Turbine Establishment), attributed lack of early success in applying brittle materials in gas turbines to inadequate effort, arising from insufficient incentive, and to the failure to re-design components to suit the new materials. The usefulness of materials at present available for heat exchangers, nozzle guide-vanes and coated turbine blades was discussed. Mr. N. R. Hemming (Bristol Siddeley) spoke of the need for refractory materials combining strength, toughness, lightness and oxidation resistance for use in air-breathing ramjet engines, which could advantageously be used in hypersonic aircraft capable of speeds of Mach No. 7, where wall temperatures would rise to 2,100° C.

On behalf of the visitors, Dr. W. W. Shaver of Corning's, New York, thanked the British Ceramic Research Association for its hospitality. Following Dr. Astbury's formal closure of the symposium, the visitors were able to inspect some of the work being done at the Association's laboratories.

The *Proceedings* of the symposium will be edited by Mr. P. Popper and published by Academic Press, in the spring.

S. N. RUDDLESDEN

PHENOLICS IN NORMAL AND DISEASED FRUITS AND VEGETABLES

THE phenolic compounds of fruits and vegetables, their relationship to browning, their changes when plants are infected, and their role in resistance of plants to disease constituted the symposium topic for the fourth annual meeting of the Plant Phenolic Group of North America, at the Central Research Laboratories of the United Fruit Co., Norwood, Mass., during July 23–24.

The symposium opened with three papers on dopamine (3,4-dihydroxyphenylethylamine) based on research by the United Fruit Co. Dopamine, a major phenol in banana, is readily oxidized to form melanin. E. H. Buckley discussed the periods of dopamine accumulation, content, and distribution in banana peel and its biosynthesis via tyrosine and tyramine throughout the plant. J. K. Palmer outlined the enzymatic oxidation of dopamine to melanin by banana polyphenoloxidase (PPO) and the unique substrate specificity spectrum of banana PPO, with dopamine the most reactive by far. The inhibition of banana PPO *in vitro* was stressed. Reducing agents apparently worked by three different mechanisms, while the varied inhibition patterns of chelation agents indicated that other mechanisms were involved as well as chelation. Finally, M. E. Mace discussed the relationship between *Fusarium* wilt infection in banana roots and the phenolics present. Anatomical and histochemical investigations, as well as experiments *in vitro* and *in vivo*, were presented in support of the hypothesis that, in infected banana roots, oxidized dopamine mediates the conversion of tryptophan to indolyl-3-acetic acid. The IAA then induces localized tyloses that block the vascular elements and entrap spores, and thereby localize infection.

The first session was completed by a discussion of the anthocyanins of grapes by A. D. Webb (University of California, Davis). Only since the advent of paper chromatography has the complexity of anthocyanins in grapes been appreciated. Completely satisfactory methods for isolating the pigments in natural form are not yet available. Wines made from hybrids of *Vitis vinifera* with disease- and cold-resistant native American species of grape can be detected by chromatography; this test is of legal impor-

tance in France. *V. vinifera* fruits contain principally monoglucosides; the American varieties have, in addition, diglucosides (no other sugars have been detected). Acylated pigments are often present, with caffeic and *p*-coumaric acids as the combined acids.

A paper on enzymatic browning by J. W. Corse (Western Regional Research Laboratory, U.S. Department of Agriculture, Albany, Calif.) opened the second half of the symposium. The enzymes involved are copper-containing oxidases which are difficult to isolate and often appear to be mixtures. Molecular weights are of the order of 120,000 with perhaps four copper atoms; in some cases, the copper appears to be cuprous. The browning reaction occurs stepwise. After formation of a catechol group by introduction of a hydroxyl group, oxidation to *o*-quinones occurs. Finally, the latter polymerize non-enzymatically to dark substances, but the structure of the products is still not clear. Substrates in deciduous fruits include chlorogenic acid and related compounds. In attempts to control browning, physical methods and chemical agents have been abandoned in favour of a biochemical approach. *O*-Methyl transferase has been found to convert the catechol group to the browning-resistant guaiacyl group.

The role of phenolics in disease resistance in plants was discussed by J. Kuc' (Purdue University, Lafayette, Ind.). Increased production of chlorogenic and caffeic acids and a variety of aromatics occur both in response to disease and to wounding, the actual amount produced varying with the agent causing injury. These higher-than-normal concentrations, which are present especially on plant surface or at infection sites, inhibit microbial growth. The mechanisms by which micro-organisms bring about this increased production are varied and not yet completely understood. In general, they appear to be due to specific alterations in the metabolic pathways of both host and micro-organism. Resistance may then result from a shift in the very delicate metabolic balance.

The symposium concluded with a paper by T. Kosuge (University of California, Davis) outlining the present state of knowledge of the biosynthesis of the phenolic acids and coumarins which have been shown to accumulate in diseased tissue. Very few investigations of their biosynthesis in diseased plant tissues have been made, but considerable work has been done on healthy tissues. The acetate and shikimic acid pathways, which occur in higher plants, appear to be involved in the production of such compounds. The *Proceedings* of the symposium will be published in due course.

The officers for the Plant Phenolics Group of North America elected for the present year are: *President*, Dr. Margaret K. Seikel; *Vice-president*, Dr. Bernard J. Finkle; *Secretary–Treasurer*, Dr. V. C. Runeckles (Imperial Tobacco Co. of Canada, Ltd., Montreal, Canada).

Margaret K. Seikel

PROBLEMS OF NOISE

As long ago as 1948 the British Standards Institution published a code of practice entitled *Sound Insulation* (*Houses, Flats and Schools*) (*CP* 3. Ch. 3; 1948). It had, of course, been a problem long before this, but since the Second World War the incidence of noise, in the home, in industry and out of doors, on the human being, has grown to be a matter not only of psychological but latterly of medical concern. That code tackled the problem of noise of both indoor and outdoor origin. It was largely concerned with means of reducing noise by appropriate siting, positioning and screening of windows in buildings. It included data of noise reduction values in 'phons' for different kinds of windows, open or shut. A revised B.S. Code (Ch. 3; 1960), entitled *Sound Insulation and Noise Reduction*, followed. In the same year came the official *Noise in Factories* (*Factory Building Studies*, No. 6, H.M. Stationery Office, 1960); L. L. Beranek's *Noise Reduction* (McGraw-Hill, 1960), and a paper by D. E. Broadbent on "Effects of Noise Reduction in a Work Situation" (*Occupational Psychology*, April 1960). The last-mentioned author, who is director of the Medical Research Council Applied Psychology Research Unit, is also responsible for a more recent booklet, *Noise in Industry*, No. 6 in the series *Ergonomics for Industry*, prepared jointly by the Department of Scientific and Industrial Research Information Division and Warren Spring Laboratory*.

Undue noise is a matter which, in these modern days, affects us all in a great variety of ways, wherever we may be and whatever we are doing. On relaxed occasions it may be encouraged by certain members of the younger generation, abhorred by others more mature. But in day-to-day industrial occupations, it is quite another matter. There are three main reasons, as the author shows: ". . . people do not like it, it damages their hearing and it has a bad effect on their working efficiency". These three effects are not necessarily interrelated. As he says: ". . . a noise which is very annoying may not be loud enough to damage hearing, or make people work less well. On the other hand, there is the rather alarming possibility that noises which do not annoy people at all may quite often damage their hearing or impair their efficiency. It is important therefore to distinguish the various effects from one another and not to assume that all is well just because the workers in a noisy factory seem to be contented". Thus the theme of this booklet is to alert us to the effects, direct or more dangerously insidious, which noise may have on people at work and, it may be added, by inference in social environments where sometimes noise is cultivated for its own sake.

In modern measurements of sound, two technical terms are at present used. 'Cycles per second' (c/s) is the unit used to measure frequency. The 'decibel' (dB) measures the intensity. "The loudest sounds we may meet have an intensity of more than a million million times the intensity of the faintest sound we can hear. A scale of decibels takes this into account." The scale is logarithmic. Proportional changes in intensity are covered by the same number of units. Thus a ten-fold increase, 1–10, 10–100, 100–1,000, etc., is represented by a change of 10 dB. Impressive examples are given: 135 dB hydraulic press at 3 ft.; 105 dB train whistle at 50 ft.; 95 dB automatic lathe at close range; 75 dB office machines between desks; 65 dB speech at 3 or 4 ft.; 20 dB whisper at 4 ft.; and 0 dB threshold of hearing at 1,000 c/s. "It should also be noted that the smallest change appreciated by the ear is about 1 dB where the change is in a faint sound or in a loud sound. dB figures are usually quoted with reference to an arbitrary zero—actually 0·0002 dynes/cm²—which is approximately the faintest sound we can hear at 1,000 c/s."

This publication, although relatively brief, is convincing in its argument, supporting data, and message. The serious effects of noise on the ear, and on work efficiency, under manifold conditions, are incontrovertible. Some far-reaching conclusions from results of investigations into the hazards of noise are, cautiously, drawn by the author; among these may be cited: "The level of 90 dB is not too far from the level which has to be regarded as a threat to hearing; . . . if precautions are taken to prevent deafness, they will also tend to prevent effects on working

* Department of Scientific and Industrial Research. *Ergonomics for Industry*, No. 6. *Noise in Industry*. By D. E. Broadbent. Pp. 16. (London: H.M. Stationery Office, obtainable (free) from D.S.I.R. Warren Spring Laboratory, Stevenage, Herts, 1964.)

efficiency." "This level—90 dB—is not at all unusual in industry, but it is very much higher than the level which people often complain about in offices and other places away from the factory floor." As is common experience, undue noise makes normal conversation impossible; it is in fact difficult unless the noise is reduced well below the 90-dB level. 60 dB is considered a more reasonable level, but in an office environment, where ordinary speech is involved, the limits of noise should be much lower.

The question of noise in all its aspects, but particularly as a hazard in industry, is now a matter of national importance. It is indeed gratifying to learn that the Ministry of Pensions and National Insurance has sponsored a large-scale research project into certain aspects of occupational deafness. This is the joint problem of the Medical Research Council and the National Physical Laboratory. "The purpose of this study is to monitor hearing of people exposed to industrial noise . . . to establish the relation between noise and damage to hearing . . . to make recommendations concerning hearing-conversation measures." When it is commonly realized that effects of prolonged noise, in whatever environment, above a certain level, are real, and provocative of permanent deafness, then people may begin to take those precautions which common sense (and this booklet) suggest. Apart from this, undue and unwanted noise, especially when it intrudes into social life, public or private, without let or hindrance, is a form of annoyance which many people will not tolerate. The recent ban on transistor radio sets operating in some of Britain's royal parks is a step in the right direction, but only a beginning.

H. B. MILNER

THE LISTER INSTITUTE

THE annual report of the Governing Body of the Lister Institute* is necessarily a greatly condensed account of the varied activities of 'The Lister', and it is impossible to indicate in a brief notice the scope and detail of the research involved. The report summarizes the Institute's work under several headings.

In the field of microbiology, work continues on the genetics of bacteria, particularly *Salmonella* spp., with special regard to the transmission of drug resistance; on the specificity of the antigens of *Trypanosoma brucei* and *T. vivax*, and on the conditions governing the formation of capsules by the anthrax bacillus *in vitro*. The Institute's field studies on trachoma in the Gambia continue, together with work on the epidemiology and pathology of pleuropneumonia-like organisms and of the Shope fibroma virus in cell culture. There has been continued progress in making a non-infectious smallpox vaccine and in work on the purification of vaccinia virus and antibody immunity in vaccinia and variola infections.

Among the epidemiological studies was an important investigation, carried out in co-operation with the Malaria Eradication Division of the World Health Organization and the Veterinary Services of Southern Rhodesia, of identification tests of the blood meals of tsetse flies and *Anopheles* mosquitoes.

Immunopathological investigations are being carried out on a factor in anaphylactic tissues which induces eosinophilia; on the local production in the cervix uteri of antibodies lethal to spermatozoa, and on improvement of therapeutic antitoxins to scorpion bites.

T. brucei, which had not been previously cultured for any prolonged period, has been successfully established in a liquid medium which makes it available for immunological and biochemical studies. Other work includes investigations of the immunology of whooping cough and typhoid and the mechanism of infection with actinomycotic dermatitis of sheep. The section devoted to the physiology of bacteria includes studies of the somatic polysaccharides of S. and R. *Salmonella* and the chemistry of the flagellar proteins of *Salmonella*.

Biophysical work includes work on human plasma proteins and on the molecular characterizations of blood-group substances. The latter has led to extension and modification of existing theories for interpreting sedimentation and viscosity data. The Blood Products Laboratory continued to prepare dried plasma and plasma fractions for the National Health Service, and the Blood Group Research Unit has continued to study the Xg and the Rh systems. The Blood Group Reference Laboratory continues its work in its new building north of the Biophysics Department.

The report records the conferment of a baronetcy on the chairman of the Institute's Governing Body, Sir Charles Dodds, and the elections of Lord Iveagh and Lord Brain, both members of the Governing Body, to fellowship of the Royal Society. The death during the year of R. St. John Brooks, curator for more than 20 years of the National Collection of Type Cultures, is a great loss to the Institute and to microbiology in general. The director of the Institute, Prof. A. A. Miles, has been appointed Biological Secretary and a vice-president of the Royal Society of London. During the year the Guinness chair of microbiology was established at the Institute and Dr. B. A. D. Stocker was appointed to it.

A gift of £30,000 from the Fleming Memorial Fund for Medical Research will enable the Institute to establish its own electron-microscope unit. The new Virus Laboratory at Elstree was completed during the year and occupied by the Virus Research Unit, and the Library at Chelsea was enlarged by the addition of another room.

The report also records the lectures given by the staff in Europe and America, and concludes with a list of their numerous publications.

For details of the biochemical work on blood-group substances, cellular phospholipids and glycogen the report itself must be consulted.

G. LAPAGE

* *The Lister Institute of Preventive Medicine.* Report of the Governing Body, 1964. Pp. 37. (London: The Lister Institute of Preventive Medicine, 1964.)

MOUSE ANTIBODY PRODUCTION TEST FOR THE ASSAY OF THE MOLONEY VIRUS

By DR. EVA KLEIN and PROF. GEORGE KLEIN

Department of Tumour Biology, Karolinska Institutet Medical School, Stockholm

INOCULATION of homografts, sub-threshold isografts or tissue homogenates derived from mouse lymphomas induced by the Moloney virus leads to specific transplantation resistance against subsequent Moloney lymphoma isografts[1]. The serum of resistant mice contains humoral antibodies, demonstrable in three different ways: by passive transfer of transplantation resistance, by cytotoxicity for Moloney lymphoma cells *in vitro* in the

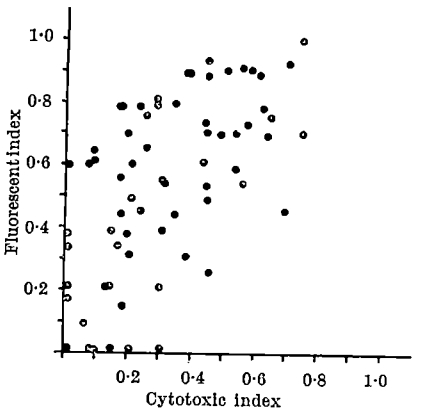


Fig. 1. Correlation between the cytotoxic and the fluorescent index given by *YAC* target cells exposed to different antisera. Only such tests are included where both tests were performed simultaneously with the same sample of target cells. Correlation data: $r = 0{\cdot}61$, $P < 0{\cdot}001$, $Z = 0{\cdot}62$, $\sigma_z = 0{\cdot}12$, $P < 0{\cdot}001$

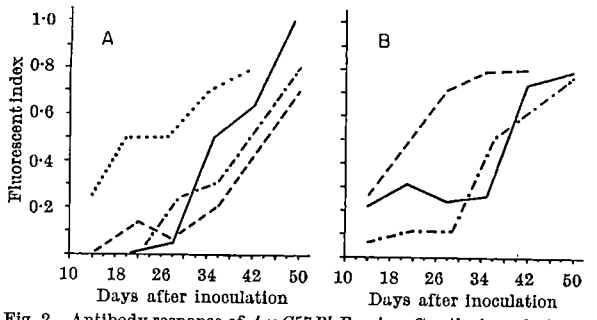


Fig. 2. Antibody response of $A \times C57Bl$ F_1 mice after the inoculation of 5×10^6 irradiated Moloney lymphoma cells, detected by the fluorescent method. *A*, ——, *YAC* sixth transfer; –·–·–·–, *YAC* eighteenth transfer; - - - - - -, *YAC* twenty-first transfer; · · · · · · · · ·, *YDAB* first transfer—Target cells *YLD*. *B*, ——, *YAA* sixth transfer; –·–·–·–, *YAA* twenty-first transfer; - - - - -, *YHA* eighth transfer—Target cells *YAA*

presence of complement, and by the indirect fluorescent antibody reaction.

The detection, identification and quantitative titration of mouse leukæmia viruses have been largely dependent on biological induction tests. The difficulties encountered with the leukæmia viruses contrast sharply against the relative ease of virus assay in systems where suitable *in vitro* techniques are available, such as, for example, in the case of polyoma. Since humoral antibodies have been demonstrated in the Moloney system by the methods mentioned here, it should be possible to develop a mouse antibody production (MAP) test for virus assay. The usefulness of such tests has been demonstrated, for example, in the polyoma system[2], and the sensitivity was comparable with the plaque assay[3]. So long as the formation of the antibodies reacting with the target cells in the cytotoxic or the indirect fluorescent antibody test is induced by the virus, it is immaterial whether they are antiviral in nature or directed against virus-induced new cellular antigens, a question that has not been clarified for this system so far[1].

The work recorded here represents an attempt to examine the feasibility of working out a MAP test of this type for the assay of the Moloney lymphoma agent.

Sera of mice inoculated with materials to be assayed for their ability to induce specific antibody formation were tested each week by the indirect fluorescent antibody method and, in a number of cases, by the cytotoxic technique as well[1]. Moloney lymphomas were used as target cells; *YAC* of strain *A* origin most frequently (4th–20th passage in ascites form), *YAA* of strain *A* origin, *YDAB* of $A \times DBA$ F_1 origin, *YHA* of C_3H origin, and *YLD* of *C57* leaden origin in a number of cases. The proportion of target cells killed in the cytotoxic test or stained in the indirect fluorescent test was expressed as a quantitative index in relation to corresponding controls incubated with control serum, as described in a previous publication[1]. Cytotoxic indexes $\geqq 0{\cdot}2$ and fluorescent indexes $\geqq 0{\cdot}3$ were regarded as positive. When the two types of tests were performed under standardized conditions, the cytotoxic and the fluorescent index increased as a function of time after the inoculation of virus-containing material. The scatter diagram in Fig. 1 shows the correlation between the cytotoxic and the fluorescent index obtained by exposing the same target cells to the same immune serum and indicates that the fluorescent method was more sensitive. A similar relationship was found when the titres given by the two methods were compared. For example, one serum that gave a cytotoxic titre of 1 : 20 against *YAC* had a 1 : 320 titre in the fluorescent test. Furthermore, while all Moloney lymphomas tested were suitable targets in the fluorescent test, some were not sensitive to the cytotoxic effect. The sensitivity of a given leukæmia varied only slightly between different cell pools in the fluorescent test, while the cytotoxic reaction was more variable. Critical comparisons between different sera of a given experimental series were always performed on the same cell suspension and at the same time. Apart from this relatively minor, uncontrollable variation, repeat tests with a given set of sera, a given target cell type, and a standard fluorescent serum or complement, resp., gave reproducible overall results in the fluorescent and the cytotoxic test as well.

Antibody response. The antibody response following the inoculation of a standard dose of X-irradiated Moloney lymphoma cells was determined for different lymphoma lines. The cells received 6,000 r. *in vitro* in order to prevent their proliferation *in vivo* which would bias the quantitation of the inoculum. Some typical experiments are shown in Fig. 2. In each group, 3 $A \times C57Bl$ F_1 male mice were inoculated with 5×10^6 irradiated cells. At different intervals, serum was collected and pooled from all three mice in each group and tested against Moloney target cells. Fig. 2*A* shows the antibody response by the fluorescence test after the inoculation of *YAC* cells from different passages and *YDAB* cells of the first passage, while Fig. 2*B* represents the reaction induced by *YHA* and *YAA* cells respectively. It appears that the time-period preceding the appearance of detectable antibody showed certain differences between the different lymphoma lines. Potent antibodies appeared earlier after the inoculation of *YHA* and *YDAB* (20–28 days) than after different transfer generations of *YAA* and *YAC* which were quite similar to each other, with antibodies appearing after

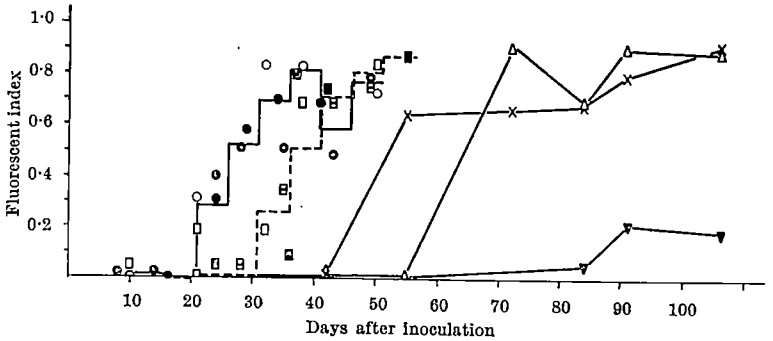


Fig. 3. Antibody response of $C3H \times C57Bl$ F_1 mice after the inoculation of virus containing homogenate, as detected by immunofluorescence. In four different experiments 0·1 ml. of 10^{-1} or 10^{-2} diluted homogenate was inoculated on day 0. In exps. 2, 3, 4 the same virus preparation was used (of $A \times DBA$ F_1 origin). In exp. 1 a different virus preparation was used of *C57* leaden origin. The mean value of the fluorescent indexes for a given virus dilution was calculated for 5 days' intervals for dilutions 10^{-1} and 10^{-2}. In addition, dilutions 10^{-3}, 10^{-4} and 10^{-5} have been included in exp. 1. These are shown in the right. Symbols: circles, 10^{-1}; quadrangles, 10^{-2}; crosses, 10^{-3}; open triangles, 10^{-4}; closed triangles, 10^{-5}. Different shadings represent different experiments

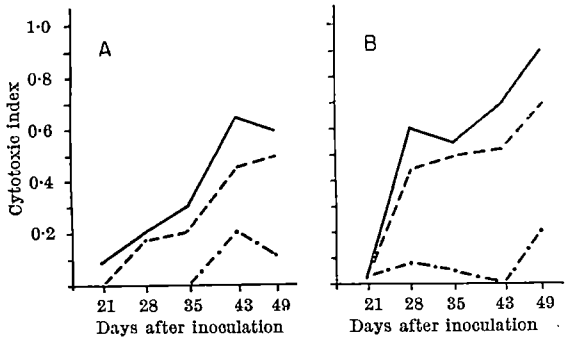


Fig. 4. Antibody response of groups of 3 *C3H* × *C57Bl* F_1 females, inoculated on day 0 with a standard virus containing homogenate (dilution: 10^{-1}). The serum pools were tested with *YAC* target cells. ——, no serum admixture; - - - - -, admixture of serum of untreated *C57Bl* × *DBA/2* F_1 mice; -·-·-·-, admixture of serum of *A* × *C57Bl* F_1 mice immunized with *YAA* Moloney lymphoma cells. *A*, cytotoxicity of serum pools in the presence of complement. *B*, fluorescent staining

35–42 days. A fifth lymphoma, *YLD*, not shown in the figures, behaved similarly to *YAC* and *YAA*. In order to examine the persistence of antibodies, mice inoculated with the same standard dose of irradiated cells from the five different lymphomas were also tested after 103–123 days. A high fluorescent index (range: 0·57–0·85) was found to persist in each case.

The maintenance of a high antibody-level during long periods of time after the inoculation of a single standard dose of irradiated, non-reproductive cells can be best interpreted by assuming that virus release and subsequent virus multiplication are responsible for the induction and persistence of antibodies. On this interpretation, all five tumour lines tested release virus, including those that have been carried through 18–22 passages, and two lines (*YHA* and *YDAB*) release larger quantities than the rest.

If the foregoing postulate is correct, inactivation of the virus should prevent antibody formation. This was tested by inoculating 5 × 10^6 cells of the *YHA* and *YLD* lines after they had been exposed to hydroxylamine[4] in order to inactivate the virus. The cell suspensions were mixed with equal volumes of 4 M hydroxylamine (*p*H 7) and incubated at 22° C for 15 min. The material was diluted 1 : 5 with phosphate buffered saline and dialysed against saline for 24 h. After inoculation, serum was collected after 7, 14, 30 and 75 days. There was no measurable antibody response.

Whether the antibodies detected are induced directly by the multiplying virus particles released by the tumour cells or by new cellular antigen(s) induced by the virus infection in the host cells cannot be decided on the basis of the present evidence.

The antibody response induced by different concentrations of the same virus preparation, preserved in the frozen state, was compared by the fluorescence test. Lymph nodes, spleen and liver of an *A* × *DBA* F_1 mouse with a primary Moloney lymphoma were homogenized in a glass homogenizer in 10 vol. of phosphate buffer and centrifuged for 20 min at 2,300*g*. The supernatant was centrifuged at 10,000*g* for 20 min and 0·1 vol. of the final supernatant were inoculated in 10^{-1} and 10^{-2} dilutions to each of 3 *C57* × *C3H* F_1 females. Three experiments were performed with the same virus pool. In a fourth experiment another homogenate, prepared from a primary lymphoma, induced in a *C57* leaden mouse, was inoculated in 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} dilutions. The pooled sera of the three mice in each experimental group were tested against Moloney target cells by the fluorescent method at different times after inoculation. The serum samples of each experimental series were tested on two or three different occasions against different samples of target cells. The mean fluorescent index was calculated for each dilution and each point of time. After the inoculation of 0·1 ml. 1 : 10 diluted virus, antibodies were detectable after 3 weeks (Fig. 3). A ten-fold dilution of the inoculated virus delayed the response by approximately 10 days.

Neutralization tests. Experiments were also made in order to detect virus neutralizing antibodies in the serum of resistant mice by testing the effect of serum on the immunogenic ability of virus-containing homogenates. A standard homogenate was diluted 10 and 100 times and 0·1-ml. vol. were inoculated to groups of 3 *C3H* × *C57Bl* F_1 females either alone, or after the admixture of 0·05-ml. serum. The serum has been derived from untreated *C57Bl* × *DBA/2* F_1 mice or from a pool collected from 16 *A* × *C57Bl* F_1 mice immunized 6–10 times with 10^5 or 10^6 viable Moloney lymphoma cells of strain *A* origin (lymphoma *YAA*). Specific antibodies against Moloney lymphoma cells could be demonstrated in this antiserum pool by the cytotoxic and the fluorescent test as well. The virus–serum mixtures and the untreated homogenates were incubated at room temperature for 30 min before inoculation. Antibody formation is shown in Figs. 4*A* and 4*B*. The immune serum neutralized the immunogenicity of the virus-containing homogenates. Comparison with the immunogenicity of different dilutions of the same standard homogenate indicates at least a hundred-fold neutralization. Analogous findings were obtained after the inoculation of the higher virus dilution. After the admixture of immune serum there was complete inhibition of antibody formation during the entire observation period of 66 days. The virus-neutralizing ability of anti-Moloney sera was also evident when oncogenic activity was used as test criterion. In this experiment new-born *A* × *DBA/2* F_1 mice were inoculated with 0·05-ml. virus containing homogenate each, mixed with 0·025-ml. serum. The results are shown in Table 1 indicating a clear-cut neutralizing effect.

Table 1. Leukæmia induction in *A* × *DBA* F_1 mice inoculated as new-borns (< 24 h) with virus preparation treated with serum from mice inoculated with Moloney leukæmia cells. Observation period 9 months

Dilution of virus	Virus preparation mixed with Control serum: Incidence*	Control serum: Latency period (days)	Immune serum: Incidence*	Immune serum: Latency period (days)
10^{-1}	2/7	155, 192	1/23	226
10^{-2}	8/23	136, 210 208, 214 214, 218 218, 234	0/22	
10^{-3}	3/17	207, 234 239	0/16	

* Pooled serum from 15 *A* × *DBA* F_1 mice which received 5–8 inoculations of *YAA* Moloney leukæmia cells.

A number of other sera were also tested in the same way for a possible neutralizing effect. Results obtained with the 1 : 100 dilution series are exemplified in Table 2. Only the sera of mice resistant against Moloney lymphoma cells were able to neutralize the immunogenic effect of the inoculum, while anti-Gross, anti-6*C3HED* and anti-polyoma sera had no detectable effect. This is in accor-

Table 2. Effect of different antisera on the immunogenicity of Moloney-virus containing homogenates, as tested by the fluorescent antibody method. Three *C3H* × *C57Bl* F_1 mice were inoculated with the serum-homogenate mixture in each group

	Donor of serum admixed to homogenate: Genotype	Pretreatment	Fluorescent index: 21	31	40 Days after inoculation
Exp. 1	*C57Bl* × leaden F_1	—	0·12	0·35	0·55
	C3H	3 inoculations of 10^6 Moloney lymphoma cells, line *YHA*	0·09	0·00	0·03
	C3H	4 inoculations of 10^5 Gross lymphoma cells, line *GHA*	0·00	0·35	0·41
	C3H × *C57Bl* F_1	11 inoculations of 10^5 6*C3HED* lymphoma cells	0·32	0·44	0·41
	C3H	Inoculation of polyoma virus (HI titre of serum 1 : 1,840)	0·29	0·62	0·73
Exp. 2			16	24	37 Days after inoculation
	A.CA	—	0	0·07	0·68
	A.CA	10 inoculations of liver, spleen, lymph nodes of *A* animals (hæmaggl. titre: 1 : 10^4)	0	0	0·41
	A	6 inoculations of liver, spleen, lymph nodes of *A.CA* animals (hæmaggl. titre: 1 : 64)	0	0·03	0·91

dance with the findings obtained with the same types of sera when tested directly against Moloney cells[1].

The virus homogenate derived from $A \times DBA/2\ F_1$ lymphoma was also treated with *H*-2 isoantiserum against the virus-donor cell genotype on the assumption that host cell components may be incorporated into the coat of the virus particle. The results for the 10^{-2} dilution series are exemplified in Table 2, Exp. 2. There was no detectable neutralizing effect.

This work was supported by research grants from the Swedish Cancer Society, Damon Runyon Memorial Fund (*DRG*–598*A*), Lotten Bohmans Fund, and by grant *C*–4747 from the National Cancer Institute, U.S. National Institutes of Health, Public Health Service.

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MECHANISM OF ENZYME CATALYSIS

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ALTHOUGH numerous detailed investigations of enzymatic systems have been carried out, a basic understanding of enzyme catalysis is still lacking. In particular, two fundamental questions are still unanswered: (1) Why are enzymes such efficient catalysts? (usually at least a factor of 10^6 better than the corresponding synthetic catalysts); (2) Why is a macromolecule required? The two questions, of course, are not unrelated and numerous speculations have been made. The present belief is that the macromolecular conformation is all-important, although very few specific suggestions have been put forth of a quantitative nature. The idea that conformation can control enzyme specificity is easy to understand qualitatively and was embodied long ago in the 'lock and key' hypothesis of Emil Fischer[1]. Several variations of the 'lock and key' idea are now in vogue. One school of thought believes that only a few side-chain groups are really important, and that by bringing these critical groups together in a stereospecific fashion, efficient catalysis ensues. A different variation on the same theme is embodied in the 'induced fit' theory put forth by Koshland[2], who suggests that the substrate induces changes in the protein conformation which aid in the substrate binding and catalysis. In this case the protein conformation changes in a dynamic manner throughout the course of the reaction. Eyring and Lumry[3] have suggested that the protein may serve as a 'rack' which distorts the critical substrate bonds thereby promoting catalysis. Unfortunately, none of these models can really be tested experimentally in a critical fashion. In a few cases (for example, chymotrypsin[4]) the available evidence suggests that the protein conformation does change during the course of the reaction; however, this fact is consistent with virtually all present proposals concerning the mechanism of enzyme catalysis.

The purpose of this article is to put forward some general ideas which might be of possible relevance in understanding enzyme catalysis.

In order to put the ideas presented in some sort of framework, I shall use the language of transition state theory and recall that the overall standard free energy change for a given reaction is unaffected by the presence of catalysis. Obviously, this same statement is also valid concerning the standard enthalpy and entropy changes for a particular reaction. The catalyst, however, does significantly lower the standard free energy of activation since this quantity directly reflects the efficiency of the catalytic process. Let us now consider how the enthalpy and entropy of activation may be affected by an enzyme. Both factors are undoubtedly influenced by the catalytic process although present evidence suggests that entropic effects may predominate.

Enthalpy effects. Enzymes can bring about a lowering of the activation energy in several ways. One of the most probable methods is a 'compensation' effect. By forming intermediate compounds the breaking of a bond can be facilitated; a possible way of accomplishing this is by simultaneously breaking and forming bonds. Several clear-cut examples of this phenomenon as found in elementary gas phase reactions are assembled in Table 1. For example, the activation energy for decomposition of H_2 by an inert body is greater than 100 kcal/mole, while that for the reaction $H_2 + I_2 \rightarrow 2HI$ is about 40·7 kcal/mole. Thus the activation energy for the second reaction is smaller even though an H_2 bond is still broken in the overall process. In general, the greator the energy involved in the bond formed, the more the activation energy is lowered. An analogous possibility for enzyme catalysis is that side-chain interactions such as H bonds, hydrophobic bonds, etc., are involved in the formation of 'compensating bonds'. A schematic representation of this idea is shown in Fig. 1. Such effects could involve co-operative phenomena so that protein groups not directly at the active site could promote catalysis. Of course, we must ultimately pay for this by breaking the side-chain bonds to get the catalyst back in its original state. However, such processes have small activation energies so that overall the activation energy of the reaction would be lowered. For example, a hydrogen bond can be broken in about 10^{-9} sec[6], solvation structure can be altered in about this same time[7], and the helix–coil transition in polyglutamic acid occurs in less than 10^{-5} sec[8]. Another more obvious example of this type of compensation occurs when part of the substrate molecule forms a covalent intermediate with the enzyme (for example, chymotrypsin[9], phosphoglucomutase[10], etc.).

Table 1. Examples of Energy Compensation in Elementary Gas Phase Reactions*

Reaction	Bond broken	E_a(kcal/mole)
$H_2 \rightarrow 2H$	H–H	104·0
$H_2 + I_2 \rightarrow 2HI$	H–H	40·7
$H_2 + Cl \rightarrow HCl + H$	H–H	4·6–6·1
$H_2 + Br \rightarrow HBr + H$	H–H	17·6
$Cl_2 \rightarrow 2Cl$	Cl–Cl	58·0
$H + Cl_2 \rightarrow HCl + Cl$	Cl–Cl	2·4
$Br_2 \rightarrow 2Br$	Br–Br	92·0
$H + Br_2 \rightarrow HBr + Br$	Br–Br	0·9
$CH_4 \rightarrow CH_3 + H$	C–H	102·6
$CH_3 + C_2H_6 \rightarrow CH_4 + C_2H_5$	C–H	10·4
$H + C_2H_6 \rightarrow C_2H_5 + H_2$	C–H	6·4

* Compiled from ref. 5.

One of the most commonly accepted ideas concerning the role of the enzyme in catalysis is that it rigidly holds the substrates in place so that proton or electron transfer can occur readily. This is undoubtedly at least partially true and may be predominantly an entropic effect. How-

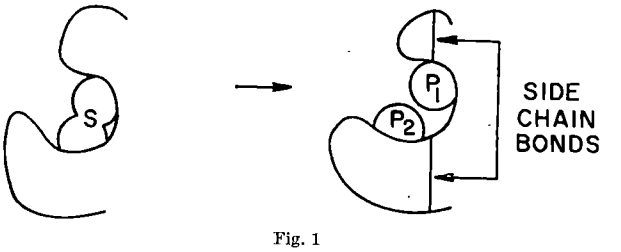


Fig. 1

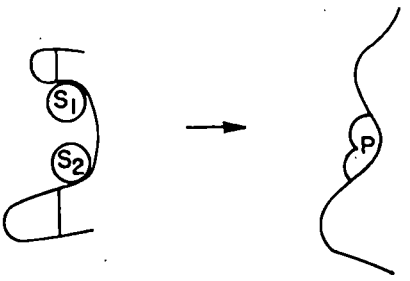


Fig. 2

over, an important consideration is that under ordinary conditions, substrates are very poor proton and electron acceptors and donors; therefore one of the functions of the enzyme may be to polarize the substrate bonds in a manner to facilitate the reaction. This process would involve short-range forces so that positioning would be very critical and might help to explain partially the stereospecificity of enzyme reactions. Possible short-range interactions are ion-induced dipole, ion–dipole, dipole–dipole, etc. As an example of the magnitude of these interactions, consider an ion and an induced dipole where the interaction energy is given by:

$$U(r) = -\frac{\alpha z^2 e^2}{2D^2 r^4}$$

Here α is the polarizability, z is the ionic valence, e is the electronic charge, D is the dielectric constant, and r is the radius of interaction. Taking typical values of α = c.c./mole, $D = 2$ (assuming the protein is the effective media) and $r = 2{\cdot}5$ Å, U is about -5 kcal/mole—a not negligible lowering of the free energy. A related, but by no means identical, idea to this is the previously mentioned 'rack' model where an actual physical strain is exerted on the substrate bonds by the macromolecule[3].

Entropy effects. Entropy effects have been discussed recently by several investigators[11–14]. The basic ideas stressed are the conception of the enzyme as an entropy 'sink'[11], the advantage of putting several catalytic groups together rather than requiring them to come together to form an intermediate complex[12,13] and the advantage of holding the substrate rigidly in place so that the entropy of activation is more favourable in the catalytic steps[14]. Of course, in the latter case the standard entropy of formation of the intermediate is quite unfavourable although the overall effect is probably still to promote the catalysis. The idea of compensating activation entropies may be of importance. A schematic representation of this analogous to the energy compensation previously discussed is shown in Fig. 2. Unfortunately, no good examples of this type of compensation in model systems can be cited. A comparison of entropies of activation for reactions of different molecularity or order is handicapped by the fact that the choice of standard state is completely arbitrary and yet can profoundly affect the numerical results.

Although a detailed interpretation of thermal data for enzyme reactions obtained from steady-state kinetics is not possible, present results suggest that the entropy of formation of enzyme-substrate complexes is often more positive than expected on the basis of simple hydration and electrostatic considerations. Moreover, measurements of volumes of activation suggest that the enzyme structure is loosened when the enzyme-substrate complex is formed[15]. Some experimental results taken from the review of Laidler[15] are given in Table 2. The Michaelis constant in these cases is assumed to be a simple equilibrium constant. This is certainly not correct, but hopefully the results are at least of qualitative significance. The suggestion that 'compensation effects' may be of importance in entropic considerations is certainly consistent with these findings. The nature of the data, coupled with the large spread, of observed values, however, precludes drawing any general conclusions. More recently a kinetic and equilibrium investigation of enzyme-product binding has been carried out with ribonuclease utilizing relaxation methods[16]. These results are also included in Table 2 and again suggest that some sort of entropic 'compensation' occurs.

Table 2. THERMODYNAMIC PARAMETERS OF ENZYME SUBSTRATE COMPLEX FORMATION

Enzyme	Substrate	*p*H	ΔH^0 (kcal)	ΔS^0 (E.U.)
* Chymotrypsin	Methyl hydrocinnamate	7·8	−5·3	−11·3
* Myosin	ATP	7·0	8·0	49·0
* Urease	Urea	7·1	−2·9	0·9
† Ribonuclease	Cytidine-3′-phosphate	‡	> 12·0	> 64·0

*Ref. 15.
† Ref. 16.
‡, *p*H independent.

Conclusions. Many of the foregoing ideas have been put forth previously, but the idea of compensation of activation parameters in the sense discussed here has not been previously stated explicitly to our knowledge. Although this requires that the protein conformation changes continuously throughout the course of the reaction, this is by no means identical with the 'induced fit' theory (although it is certainly not inconsistent with it). Unfortunately, until experiments become more refined, the precise dynamic role of the protein molecule in enzyme catalysis must remain a matter of speculation.

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GENESIS AND BIOGENESIS OF ETHYLENE

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IN a previous report[1] we showed that ethane could be produced in a particulate system from apples. The components of the system were linolenic acid, thiomalic acid or any acid with an SH on the α-carbon, and cytoplasmic particles in an acetate buffer of *p*H 4·5. We suggested that a relationship may exist between this system and that which produces ethylene in cells. Ethane production in a non-enzymatic system, in which the

Table 1. Ethylene and Ethane Production by a Non-enzymatic Model System in the Presence of Copper or Iron, Reducing Agent, and 'Activated' Linolenic Acid

System*	μl. ethane per 30 min at 30° C	μl. ethylene per 30 min at 30° C
Cupric-ascorbic	1·4	16·0
Ferric-ascorbic	8·0	0·4
Cupric-thiomalic	0·05	0·3
Ferric-thiomalic	7·2	0·2
Cupric-hydroquinone	0·03	0·9
Cupric-hydroxylamine	0·06	0·6

* Flask content: 18 mg of activated linolenic acid; 5 μmole of copper sulphate or ferric citrate; 50 μmoles of reducing agent–ascorbic acid, thiomalic acid, hydroquinone or hydroxylamine hydrochloride; 400 μmoles acetate buffer *p*H 4·5 in a total volume of 5 ml. This mixture was incubated with shaking at 30° C for 30 min in the dark. Linolenic acid was activated by exposure to light at room temperatures for 9 days. Ethylene and ethane were determined by gas chromatography using a 2-ft. by ¼-in. diameter glass column packed with alumina. The detection system was as previously described[4]. The reaction mixture was incubated in 25-ml. stoppered flasks containing a capillary tube clamped with rubber tubing. Gas samplings were made with gas-tight syringes connected to the rubber tubing.

Table 2. Production of Ethylene and Ethane in Cuprous or Cupric Model System in the Absence of a Reducing Agent

System*	μl. ethane per 30 min at 30° C	μl. ethylene per 30 min at 30° C
'Activated' linolenic acid + cuprous ion + acetate buffer *p*H 4·5	0·4	4·2
'Activated' linolenic acid + cupric ion + acetate buffer *p*H 4·5	0·01	0·2

* Flask content: 18 mg active linolenic acid; 5 μmoles of cuprous chloride, dissolved in deoxygenated saturated potassium chloride; or 5 μmoles copper sulphate, and 400 μmoles of acetate buffer, *p*H 4·5. Total volume of liquid in flasks was 5 ml. Linolenic acid was activated by exposure to light at room temperature for 9 days.

Table 3. Effect of 'Activation' of Linolenic Acid on Ethylene Production in the Copper–Ascorbic Acid Model System

System*	μl. ethane per 30 min at 30° C	μl. ethylene per 30 min at 30° C
'Fresh' linolenate + copper + ascorbate + acetate buffer *p*H 4·5	0·02	0·02
'Activated' linolenate + copper + ascorbate + acetate buffer *p*H 4·5	1·1	16·7

* 18 mg of methyl linolenate, 5 μmoles copper sulphate, 50 μmoles ascorbic acid, 400 μmoles acetate buffer in 5 ml. total liquid. Fresh linolenate was obtained from a vial recently opened and stored in nitrogen at −20° C. It showed very little triene conjugation and gave no Kries test. 'Activated' linolenic had been exposed to light and stored in air at room temperature (*c.* 20° C) for approximately 2 weeks. This sample showed a very marked spectrum for triene conjugation and gave a strong Kries test. Methyl linolenate (99 per cent + pure) was obtained from the Hormel Institute, Austin, Minn.

reaction of linolenate and an α-SH acid was catalysed by iron, has also been reported[2]. This article describes an analogous non-enzymatic system which produces predominantly ethylene, rather than ethane. This system differs from its predecessor in two respects: it requires copper rather than iron as a catalyst, and ascorbic acid, rather than thiomalic, serves as the reducing agent for the metal.

Activation of linolenic acid is a critical preliminary requirement for operation of the non-enzymatic model systems, producing either ethylene or ethane. Activation involves conjugation of the three double bonds, followed by some degree of oxidation. The extent of activation was evaluated by: (1) increase in the absorption spectra of the triene conjugated system; (2) by the Kries test for peroxidation[3]; (3) by the oxirane test[3]. These estimates of activation correlated well with ethylene production in the reaction.

Three different procedures were used to activate the fatty acid. In the first, linolenic acid was activated by exposure to air, visible light, and room temperatures (20°–25° C), for a minimum of 3 days. Longer exposures, up to 3–6 weeks, increased the activation. Secondly, exposure to short-wave ultra-violet light (mean wavelength 253 mμ) for 24–48 h, also served to activate the unsaturated fatty acid. Lastly, linolenic acid was activated by a 30- to 60-min incubation in phosphate buffer at *p*H 7·0 with the enzyme lipoxidase.

When activated linolenic acid, or its methyl ester, was incubated with ascorbic acid and copper in acetate buffer at *p*H 4·5, sizable amounts of ethylene and much lower quantities of ethane were produced (Tables 1 and 3). The ratio of ethylene to ethane evolved in this system ranged from about 11 to 15. The same reaction, carried out with ferric ion substituted for cupric ion, yielded a preponderance of ethane.

Production of ethylene in the copper system was mediated by the reduced form of the cation (Table 2). The function of ascorbic acid is to produce a steady supply of cuprous ions which interact with activated linolenic acid. Other reducing agents, such as thiomalic acid, hydroquinone, and hydroxylamine, when substituted for ascorbic acid, also gave rise to ethylene in the copper system, by virtue of their ability to reduce cupric ions. However, they are considerably less efficient than ascorbic acid (Table 1). Thiomalic acid was relatively ineffective, due undoubtedly to direct reaction of SH with the cupric ion, which, in effect, sequestered the copper. Other metal cations that exist in two oxidative states, such as manganous, stannous, and cobaltous, could not be substituted for cuprous ion in this reaction. Since the reaction producing ethylene can be catalysed by cuprous ion in the absence of ascorbate (Table 2) the ethylene carbons must derive from linolenic acid.

We have also found that ethylene can be formed non-enzymatically from compounds other than linolenic acid. These appear to have in common either an epoxy or ethoxy group in their molecules. Thus, ethylene oxide, diethyl ether, ethanol, and alkyl phenoxy poly-ethoxy ethanol ('Triton *X*-100'), substituted for activated linolenic acid in the copper–ascorbate system, all give rise to ethylene. There is, however, one distinction between epoxy and ethoxy compounds. Ethylene oxide can form ethylene either in oxygen or in its absence; other epoxy compounds, for example, propylene oxide or 1,2-epoxy butane, form only the corresponding propylene or 1,2-butylene in nitrogen, but do form ethylene in oxygen. Ethoxy compounds produce ethylene only in oxygen atmospheres. We believe the ethoxy compounds may give rise to epoxy compounds, as intermediates, and this step requires the presence of oxygen.

Cytoplasmic particles from apples, prepared as previously described[4], also evolve ethylene in the presence of copper and ascorbate. Linolenic acid is known to be present in apple particles. However, ethylene production from particles is very low when compared to ethylene production by intact fruit. The low activity of the particles might be explained by inactivation of either the enzymatic system responsible for activating linolenic acid, or a copper enzyme concerned in catalysing the final reaction with activated linolenic acid. Alternatively, homogenization of the tissue might cause both the release and acceleration of competing reactions, which can more favourably utilize linolenic acid. Investigations to clarify these points are in progress.

The importance of the model system as a guide to the biosynthetic pathway for ethylene production will, of course, depend on whether it is actually analogous to the natural system. We have made some observations on tissue slices and cytoplasmic particles, which appear to relate the two systems as follows:

(1) Ethylene production by apple plugs is severely inhibited by 10^{-4} M diethyl dithiocarbamate, but not by ethylenediamine tetraacetic acid. These findings suggest that a copper protein is involved since many copper proteins behave in this way[5].

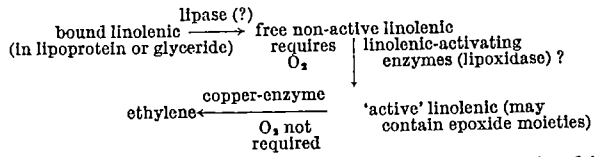
(2) Cytoplasmic particles, with copper and ascorbic acid, will not evolve ethylene in a nitrogen atmosphere, but will produce ethylene in air. Inhibition of ethylene production by nitrogen is characteristic of intact fruit and tissue slices[6].

(3) Cytoplasmic particles from apples can activate linolenic acid for reaction in the copper–ascorbic system. This activation is heat labile and does not proceed in nitrogen.

(4) Ethylene oxide, an inhibitor of ethylene production by intact fruit and occasionally a transient stimulator of ethylene production[4], very readily gives rise to ethylene,

in a nitrogen or oxygen atmosphere in the model system. In the enzymatic system, a specific substrate structure must be required, and linolenic acid with epoxide moieties may provide this requirement. Ethylene oxide, under these conditions, may act as a competitive inhibitor in the enzymatic system by reason of its related structure. In the non-enzymatic system where structural specificity associated with the large fatty acid chain is not involved, the related epoxide moiety can act as substrate for the reaction.

All these factors link the linolenic–copper–ascorbic model system to the natural system for ethylene biosynthesis. Since the components of the model system are known to be present in fruit tissues, a hypothetical scheme may be proposed for the biosynthesis of ethylene, as follows:



Little is known, at present, about the first step in this reaction scheme; but this step may be concerned with freeing bound linolenic acid. After release of the unsaturated fatty acid, various enzymes may compete for linolenic acid, leading to different pathways for its metabolism. The enzymes steering towards the ethylene pathway may activate, by causing triene conjugation followed by peroxidation, in serial steps, which finally lead to the epoxide form. Formation of the epoxide requires oxygen and might be related to the oxygen requirement for ethylene evolution by intact fruit. Lipoxidase might be one of the enzymes involved in this sequence. Once linolenic acid is activated, it can be catalysed very rapidly by the cuprous ion in a copper enzyme. This would then be analogous to the ethylene-producing reaction which proceeds very rapidly, in the model system, with copper, ascorbic acid, and activated linolenic acid, in acetate buffer *p*H 4·5.

Variations of this model system, to be described elsewhere, give rise to saturated hydrocarbons. These reactions require a less-activated (less-oxidized) form of linolenic acid, and can proceed in a nitrogen atmosphere. The genesis of petroleum and hydrocarbon gases from lipids of prehistoric living forms, buried underground, may have arisen anaerobically, via similar reaction pathways.

This work was supported by grant *GB*–1094 from the U.S. National Science Foundation.

Addendum. Since completion of the experiments described we have found that methionine, but no other amino-acid, can also react in the copper–ascorbate system to form significant amounts of ethylene. Unlike the linolenate system, the methionine system produces no ethane and does not form ethylene in nitrogen. This is reminiscent of the natural ethylene system in apple and other fruit. Furthermore, methionine is unique, among the substances which form ethylene in the copper–ascorbate model system, in its ability to stimulate ethylene production in apple tissue slices.

An analysis of the particulate fraction isolated from apples shows that at least two compounds are present which can give rise to ethylene with the copper–ascorbate system. The first is associated with the fat-soluble and the second with the water-soluble constituents of the cell. It therefore appears that there may be more than one ethylene-forming precursor in tissues.

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HISTOLOGICAL REACTIONS OF *Bufo melanostictus* TO CULTURE FILTRATES AND MYCELIAL EXTRACTS OF A PATHOGENIC FUNGUS

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It was previously reported[1] that naturally occurring infection of Malayan toads (*Bufo melanostictus*) by an unidentified fungus produced ulcerated skin lesions and tumour-like nodules on the skin, abdominal wall and internal organs. Pure cultures of the fungus, isolated from diseased toads, when inoculated into healthy toads induced the same histological reactions, that is, nodular formations on the abdominal wall and internal organs consisting of accumulations of monocytes surrounding a central locus of fungal hypha. Attempts were then made to induce similar histological reactions using mycelial extracts and cell-free culture filtrates of the causal organism.

The fungus was grown in 250 ml. aliquots in liquid culture medium consisting of 2·5 per cent glucose, 0·1 per cent yeast extract, 0·2 per cent peptone and mineral salt at *p*H 6·3 and incubated in the dark at 27·5° C. (*a*) The fungus was filtered off through Whatman No. 52 filter paper and the resultant 4 l. of culture filtrate were concentrated under vacuum at 60° C on a 'Rotavapor' to approximately 50 ml. The concentrate was dialysed against running tap-water for 12 h, reconcentrated under vacuum and centrifuged at 3,000 r.p.m. for 10 min and the supernatant filtered through a bacterial membrane filter ('Membran' filter) under vacuum. (*b*) The mycelial mats collected from (*a*) above were alternately frozen for 3 h at −20° C and thawed at 40° C four times and the resultant slurry further disrupted by means of a sonic disintegrator (Branson sonifier U.S. model *L.S.* 75) at frequency 8 for 6 periods of 1 min each alternating with 15-min periods at 0° C. The slurry was centrifuged at 6,000 r.p.m. for 10 min and the supernatant dialysed against distilled water at 0° C for 6 h. Half the dialysate was kept at 60° C for 15 min (solution *b*1), and this together with the non-heated dialysate (solution *b*2) were passed through a bacterial filter and tested against healthy toads.

Spent fungal medium. 1 ml. aliquots of the solution obtained from (*a*) were introduced by means of a sterile hypodermic needle into the subcutaneous cavity and abdominal walls, in the lower abdominal region of healthy toads. Control toads wounded by sterile needles were also established, and these together with the inoculated toads were kept in large aquaria and samples removed for dissection after 8-, 10-, 12- and 14-day intervals. After 8 days, inoculated toads showed marked inflam-

matory reactions 1–2 mm in diameter at the site of skin and abdominal puncture together with zones of inflammation at the xiphisternum. This reaction was in marked contrast to the control toads, where no apparent reaction was seen. Tissue immediately surrounding sites of skin and abdominal puncture was removed, fixed in Bouin's solution, sectioned and stained either with eosin/hæmatoxylin or in Schiff's reagent following pre-treatment with periodic acid. Sections showed that immediately surrounding the site of skin puncture monocytes had accumulated in the poison glands and the dermal layers, while the epidermis had become multiseriate with consequent gross invagination of the tissue. The main migration of the inflammatory cells appeared along channels of connective tissue extending from the subcutaneous cavity through the muscle layer (Fig. 1), suggesting that the initial inflammatory reaction occurred on the inner surface of the skin following subcutaneous injection. Sections at the site of abdominal puncture showed initially an accumulation of monocytes followed by the development of a large mass of inflammatory tissue within the abdominal wall. Thickening of the abdominal wall in this region was brought about by the migration of monocytes in the connective tissue surrounding the myocommata (Fig. 2). At the xiphisternum monocytes accumulated within the thin envelope of connective tissue covering the ventral surface of the cartilage and later within tissues on the dorsal surface. Reactions at this region suggest that the culture filtrate introduced into the subcutaneous cavity in the lower abdomen had migrated along channels in the connective tissue between the skin and abdominal wall.

Mycelial extract. 1 ml. aliquots of the solution obtained from (*b*) here were introduced into the subcutaneous cavity and the abdominal walls of healthy toads. Inflammatory reactions similar to those described here were examined and proved to be histologically identical. One feature of interest was the marked increase in thickness of the epidermal layer 3–4 days after inoculation, that is, before the appearance of any marked inflammatory reaction. In order to examine this phenomenon in more detail 0·2 ml. aliquots of the mycelial extract were placed on the dorsal surface of healthy toads and the skin immediately beneath the drop was scarified by means of a sterile scalpel. After scarification another 0·2 ml. of extract was placed on the scar and the toads were placed in a dry portion of the aquarium to avoid excessive dilution of the extract. Another series of animals were inoculated in a similar way with mycelial extract that had been kept at 60° C for 15 min. Control toads scarified by means of a sterile scalpel were also maintained. Three days after inoculation animals bearing untreated extract exhibited, at the site of scarification, an inflammatory reaction which enlarged during the next 5 days to form an ulcerated zone 1·5–2 cm in diameter. Portions of the skin and abdominal wall at the site of inoculation were removed at daily intervals and examined for histological features. During the first 4 days after inoculation monocytes accumulated in the poison glands, where they divided mitotically (Fig. 3) and passed out along the neck of the gland to lie immediately beneath the epidermal cells.

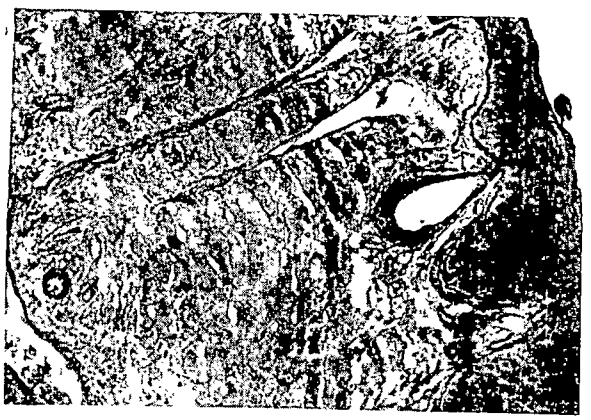


Fig. 1. Section of skin showing thickened epidermis and channels of connective tissue through which inflammatory cells migrate

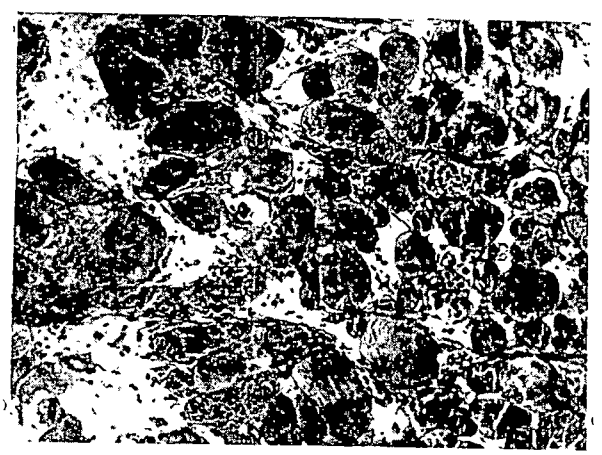


Fig. 2. Monocytes in connective tissue within the abdominal wall



Fig. 3. Mitotic division of monocytes (arrowed) in poison gland. Note the thickened epidermis

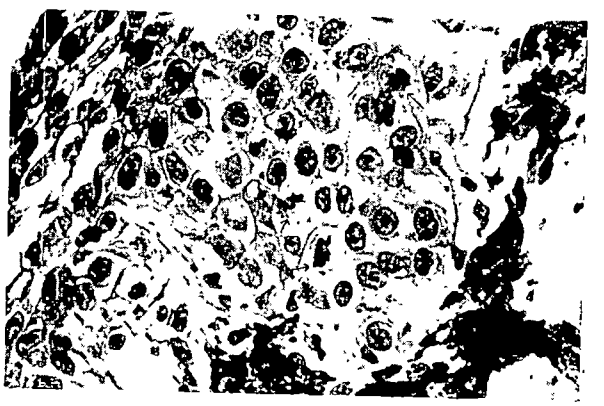


Fig. 4. Divisions in the multiseriate epidermis. (Cells in mitosis arrowed)



Fig. 5. Early stage in division in the epidermal layer

Later rapid divisions within the epidermal tissue (Fig. 4) resulted in a gross thickening of the epidermal layers. During the period of division, that is, the first 5 days after inoculation a mitotic index ranging from 2 to 7/100 cells was frequently observed. In some instances mitotic divisions were induced in the uniseriate epidermal layer (Fig. 5) before migration of monocytes from the poison glands had occurred. Within the abdominal wall monocytes migrated from the blood capillaries into the connective tissue between the myocommata, where rapid mitotic division occurred (Fig. 6). Toads bearing heated mycelial extract, together with toads which had been scarified by means of a sterile scalpel, showed no inflammatory reactions and sections failed to reveal mitotic divisions within any of the tissues.

From these observations it is concluded that the unidentified fungus causing acute lethal inflammatory reactions in toads produces an extramycelial thermostable factor capable of inducing histological reactions



Fig. 6. Mitotic divisions (arrowed) in inflammatory cells within the connective tissue of the abdominal wall

similar to those resulting from fungal invasion, while water extracts of the mycelium contain a thermolabile substance capable of inducing cellular proliferation both in the epidermis and in migrating monocytes within the connective tissues of the abdominal wall. Present work is directed at the establishment of a culture medium from which the factors involved may be isolated together with a biological assay method for determining the relative potency of the substance during extended trials.

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COLLAGEN AND CELL PROTEIN SYNTHESIS BY AN ESTABLISHED MAMMALIAN FIBROBLAST LINE

By Prof. HOWARD GREEN and Prof. BURTON GOLDBERG

Department of Pathology, New York University School of Medicine

We have recently reported that cells of an established mouse fibroblast line (3*T*6) secrete collagen in culture, the collagen precipitating as typical fibres between the cells[1]. Examination of the relation of cell growth to accumulation of collagen in the cell layer showed that collagen appeared after the phase of exponential growth was completed, and accumulated at a constant rate for at least three weeks thereafter[2]. This communication describes experiments on the relation of cell protein synthesis to collagen protein synthesis under different conditions, with the view of determining the factors controlling this differentiated function of the fibroblast.

All cultures were grown in plastic Petri dishes, in the Dulbecco–Vogt modification of Eagle's medium[3]. The medium of all cultures was changed three times weekly. Three replicate cultures of 3*T*6 were allowed to grow to saturation density. At time zero (Fig. 1), one culture was trypsinized and 16 daughter cultures inoculated at about 1/70th of the cell density of the parent culture. Daughter cultures were trypsinized at intervals during the next 10 days and the cells counted. Fig. 1*A* shows the resulting growth curve. At various times, uniformly labelled L-proline-^{14}C was added in fresh medium to one of the daughter or parent cultures. After 12 h incubation with the isotope, activity was stopped with trichloroacetic acid. The contents of the cultures (cells plus medium) were exhaustively dialysed, first against unlabelled proline and hydroxyproline, then against several changes of dilute phosphate buffered-sodium chloride solution. The non-dialysable portion was then hydrolysed in 6 N hydrochloric acid at 120° for 12 h, a known amount of carrier proline and hydroxyproline added and these two amino-acids re-isolated by thin-layer chromatography, according to the method of Myhill and Jackson[4]. Their isotope content was determined and the total radioactivity incorporated into protein as proline and as hydroxyproline was calculated for each culture. Assuming that the pool of free cell proline serves as precursor of the hydroxyproline residues of collagen and of the proline residues of all proteins, that hydroxyproline is incorporated only into collagen, and knowing the abundance of hydroxyproline in collagen and of proline in the cell protein, the ratio of collagen synthesis to cell protein synthesis can be calculated (Fig. 1*B*). This ratio can be regarded as an index of differentiation of the fibroblast. The data show that in saturation density cultures 3–3·5 per cent of the protein synthesized is collagen (curve 1, Fig. 1*B*). The daughter cultures do not lose differentiated function immediately; the amount of collagen synthesis relative to cell protein synthesis begins to drop

after inoculation (curve 2) but does not reach the lowest value until after two days, by which time considerable cell multiplication has occurred. At this time about 0·25 per cent of the protein being made is collagen. As the daughter cultures approach saturation density the rate of collagen synthesis relative to cell protein synthesis begins to rise rapidly and soon approaches the values typical of saturation density cultures.

The *p*H of the culture medium, normally 7·2, is not detectably reduced two days after its addition so long as the cell population is lower than 2×10^6 per plate, but at higher cell densities the *p*H drops appreciably between medium changes. In saturation density cultures (10^7 cells) most of the glucose initially present in the medium (4·5 mg/ml.) is converted to lactic acid[6] and the *p*H drops to 6·6. In order to determine if these changes in the medium of saturation density cultures influence collagen production, the effect of addition of lactic acid was investigated in cultures containing less than 2 million cells.

To replicate cultures containing about 10^5 cells each, lactic acid was added in varying concentrations. Thirty-six hours later the medium of each was replaced with fresh medium of the same lactic acid concentration, containing labelled proline. Twelve hours later, cold trichloroacetic acid was added and the products treated in the usual manner. In Fig. 2*A* the total radioactivity incorporated into the proline and hydroxyproline residues of the protein is shown. The addition of lactic acid was

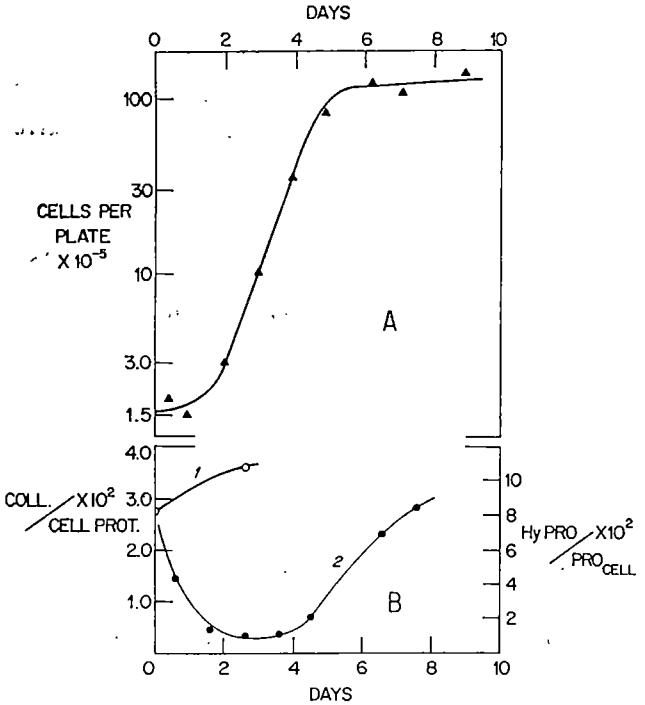


Fig. 1. Synthesis of collagen during growth of 3*T*6. *A*, Growth curve of 3*T*6 following trypsinization of a saturation density culture and inoculation of the daughter cultures at time 0; *B*, Protein synthesis by replicate cultures. Right-hand ordinate gives ratio of counts incorporated into collagen hydroxyproline to that incorporated into proline of 'cell' protein. Left-hand ordinate gives ratio of collagen synthesized to cell protein synthesized. Curve 1, parent cultures allowed to remain at saturation density. Curve 2, daughter cultures the growth of which is shown in *A*

Calculations: (1) It is assumed that all counts incorporated into non-dialysable hydroxyproline (HyPRO) represent collagen synthesis. (2) Proline incorporation is mostly into non-collagen ('cell') protein, but also to a small degree into collagen. Since collagen contains 1·2 proline residues for each hydroxyproline residue[5], 1·2 × HyPRO counts must be subtracted from the total proline counts to give non-collagen protein-incorporated proline (designated PRO_{cell}). This correction is negligible for all but the highest HyPRO values. (3) Since the HyPRO residue content of collagen is 12·2 per cent, while the PRO residue content of cell protein is 4·1 per cent (determined directly on cell hydrolysates by column chromatography), the value $HyPRO/PRO_{cell}$ is multiplied by 4·1/12·2 to give the ratio of collagen synthesis to cell protein synthesis (Coll./Cell Prot.)

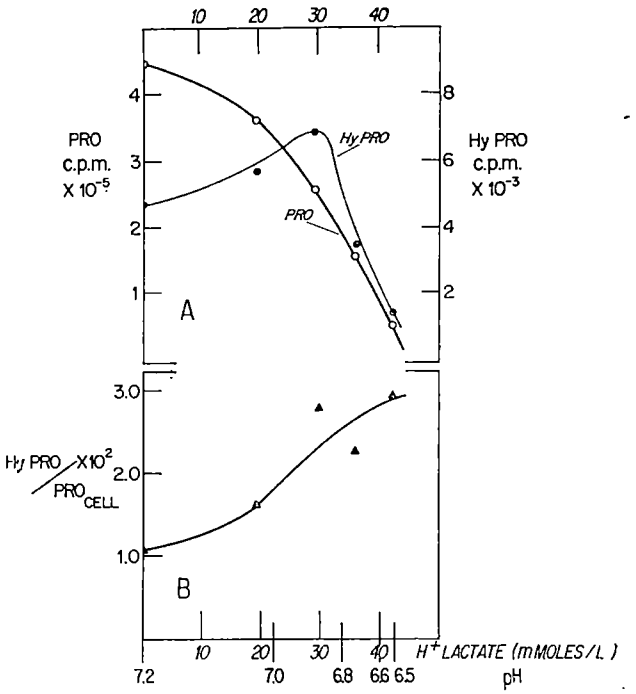


Fig. 2. Effect of added lactic acid on incorporation of proline and hydroxyproline into protein (see legend to Fig. 1)

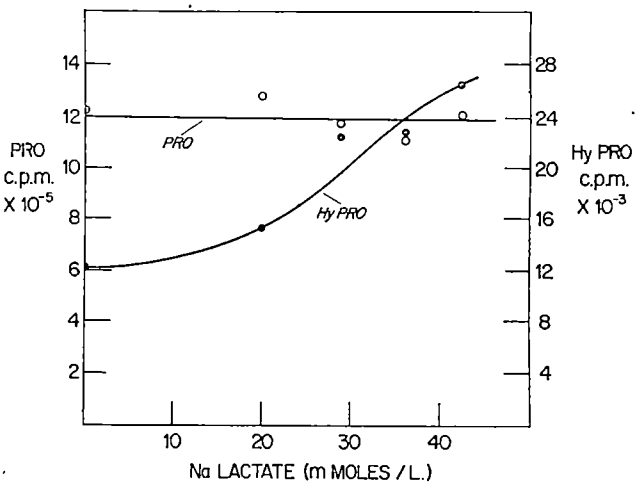


Fig. 3. Effect of added sodium lactate on incorporation of proline and hydroxyproline into protein

inhibitory to cell protein synthesis, as judged by proline incorporation, beginning at the lowest concentration tested, and became much more inhibitory at higher concentrations. Even at the highest concentration tested (43 mM) appreciable growth occurred in the cultures during the first 36 h (nearly 2 cell generations), but by 48 h mitoses were completely absent from the culture. Cultures incubated in the absence of added lactic acid grew slightly over three generations during the 48 h.

The effect of lactic acid on collagen synthesis followed a pattern different from that on cell protein synthesis. In the presence of low concentrations of lactic acid there was an increase in hydroxyproline incorporation, and only at the highest concentrations of lactic acid did the synthesis of collagen decline along with that of the other proteins. The ratio of hydroxyproline incorporation to proline incorporation, plotted in Fig. 2*B*, rose at the higher lactic acid concentrations to about three times that in its absence.

In order to decide which of these effects were produced by hydrogen ion and which by lactate ion, the experiment

was repeated, using sodium lactate instead of free lactic acid, so that the *p*H of the medium was unchanged at all lactate concentrations. Fig. 3 shows the result obtained. Cell protein synthesis, as judged by proline incorporation, was completely unaffected up to the highest concentration tested. This is consistent with the fact that even the highest lactate concentration did not affect the growth-rate of the cells. Collagen synthesis, on the other hand, was stimulated in a manner quite similar to that observed on the addition of lactic acid. It may be concluded that the inhibition of cell protein synthesis following the addition of lactic acid is due to hydrogen ion, while the stimulation of collagen synthesis is due to lactate ion.

These effects are consistent with the changes in collagen synthesis found in cells passing from exponential growth to the stationary phase. However, the magnitude of the lactate effect (2–3 times increase in the fraction of cell protein made which is collagen) is much smaller than the difference observed between exponentially growing and saturation density cultures (up to 15-fold). It may be that the lactic acid produced is only a minor factor influencing the state of differentiation of fibroblast cultures, or that the conditions obtained by adding lactic acid to exponentially growing cultures do not duplicate the lactic acid concentrations existing within the cell layers of saturation density cultures.

These experiments show that the metabolic products of a cell can control, within a range, the expression of its capacity for differentiated function. The level at which this effect is exerted (gene, ribosome, etc.) remains to be determined.

This work was supported by grants and awards from the U.S. Public Health Service.

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TUMOUR SPECIFIC IMMUNITY AGAINST MURINE EPITHELIOMAS INDUCED WITH 9,10-DIMETHYL-1,2-BENZANTHRACENE

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THE presence of tumour-specific antigens in certain chemically induced mouse tumours is now well established[1–5]. There are less data on rat tumours[6–9], but such as there are indicate that these may also possess tumour-specific antigens. The significance of such antigens is naturally of great interest, and for this reason it is important to learn how widespread is the phenomenon of specific antigenicity. The earliest work was performed on murine fibrosarcomas induced with 3-methylcholanthrene. Since then, specific antigens have been demonstrated in tumours provoked by a number of polycyclic hydrocarbons, including 1,2,5,6-dibenzanthracene[10], 3,4,9,-10-dibenzpyrene[5], 3,4-benzpyrene[5], and *p*-dimethylamino-azobenzene (cited in ref. 11). In most series, the tumours tested for specific antigenicity were fibrosarcomas. However, there is evidence that chemically induced mouse breast tumours[12] and hepatomas (cited in ref. 11) possess tumour-specific antigenicity. In the experiments described here, we have examined epitheliomas induced in *C57BL* mice with 9,10-dimethyl-1,2-benzanthracene. Evidence of tumour-specific antigenicity has been obtained in four out of five epitheliomas investigated. In addition, attempts to demonstrate antibody against such antigens have been made.

C57BL or *BALB/c* × *C57BL* hosts were given a preliminary course of immunization. For immunization, either lyophilized tumour or heavily irradiated tumour brei was used. Tumour breis were irradiated by a cobalt-60 source, 15,000 r. being given at a rate of 1,000 r./15 sec. Three injections were given over 6 weeks, each mouse receiving between 25 and 75 mg either of lyophilized tissue or irradiated tumour. In two experiments adoptive immunization was performed using spleen cells from syngeneic tumour-bearing mice. After immunization, the recipients were challenged with increasing numbers of normal tumour cells capable of replication, and growth was compared with that occurring in controls. In many experiments, delay in growth rather than complete inhibition of the tumours was observed, and in these cases the tumours were excised and the mice re-challenged at a later date with a higher tumour dose. Controls were either non-immune mice of the same genotype, or, in about half the experiments, they were 'immunized' with irradiated syngeneic normal tissue. It should be mentioned that histogenetic homogeneity of all strains in our colony is routinely confirmed at least once annually by intra-strain skin grafts made between several offspring of different breeding pairs. Tumour challenge was performed with trypsinized cell suspensions prepared according to Boyse's method[13]. It was necessary to use both *C57BL* and *BALB/c* × *C57BL* hosts due to limited mouse supplies. There is evidence that parental strain parental strain[14,15]. The reason for this is unknown, but tumours grow more slowly in F_1 hosts than in the native we felt that the slower growth of the tumour in F_1 hosts might allow greater opportunities for the recipients to become immunized before being overwhelmed by tumour growth.

However, there is one complication in the use of F_1 hosts. Bailey[16] has recently described an *X* chromosome-linked histocompatibility system. He showed that if paternal strain male skin is transplanted to F_1 males, rejection may occur. Skin grafts from males of the maternal strain survive indefinitely. This problem applies only to male recipients which have a single *X* chromosome. There is no incompatibility in the F_1 females grafted with paternal strain female tissues, as in this case recipients have an *X* chromosome from both parent strains. We have performed appropriate skin grafts on our *BALB/c* × *C57BL* hybrids in order to test for the *X* linked histo-compatibility system. Table 1 shows the results.

Because of the results given in Table 1, it is clearly unsatisfactory attempting to demonstrate tumour-specific antigens in a ♂ tumour by immunizing (*BALB/c* × *C57BL*) ♂ hosts. Any results might equally be due to *X* linked iso-antigenic differences. However, no such incompatibility is involved if *C57BL* ♀ tumours are transplanted to (*BALB/c* × *C57BL*) ♀ recipients. We have therefore used only parental strain male hosts when testing for antigenicity of the epitheliomas *Ep.* 3 and 4, which arose in *C57BL* ♂s; the remaining tumours which originated in *C57BL* ♀s, *Ep.* 5, 6, and 8, were tested in (*BALB/c* × *C57BL*) ♀ hosts.

Table 1. Survival of Paternal Strain Skin Grafts on Hybrid Recipients

Donor	Recipients	Results
C57BL ♂	(*BALB/c* × *C57BL*) ♂	Nine out of 11 hosts grafted showed complete rejection between seventh and fourteenth week. Two animals were regrafted and showed second set responses
C57BL ♀	(*BALB/c* × *C57BL*) ♀	Five animals grafted. All healthy at > 30 weeks

When hosts were challenged with suspensions of the same tumour that had been used for pre-immunization, two types of results were observed. Table 2 illustrates the first type, in which immunization conveyed protection on the host. It can be seen that pre-immunization afforded protection in four out of five tumours investigated. As others have done[4,5], we found immunity to be weak and easily overridden.

Table 2. Growth of Dimethyl-benzanthracene-induced *C57BL* Epitheliomas in Immune and Non-immune *C57BL* or (*BALB/c* × *C57BL*) Hosts

Tumour	Challenge dose (cells × 10^3)	Tumour growth No. of mice with tumours/No. in group: Immune	Non-immune	No. of days between challenge and measurement
Ep. 3	20	3/15	4/4	14
	7*	2/16	4/5†	22
	17*	0/16	4/5†	17
Ep. 5	48	0/15	2/5	28
	93	3/15	5/5	45
	260	4/11	3/4	11
	100	0/16	2/5†	28
	200	4/16	4/5†	22
	285	4/14	6/6†	14
Ep. 6	106	7/14	4/4	33
	148	6/11	4/4	10
Ep. 8	392	10/16	5/5†	10
	339	2/12	5/5†	8
	534	0/5	5/6†	13

* Controls 'immunized' with irradiated normal *C57BL* tissue.
† Experimental mice were adoptively immunized.

However, pre-immunization sometimes had an opposite effect in that it caused increased susceptibility to tumour growth. Table 3 gives details of the experiments in which this was observed.

Table 3. Increased Susceptibility to the Growth of Dimethyl-benzanthracene-induced *C57BL* Epitheliomas in *C57BL* or (*BALB/c* × *C57BL*) Hosts after Pre-immunization

Tumour	Immunization	Challenge dose (cells × 10^3)	Tumour growth (mice with tumours/No. in group) Immune	Non-immune	No. of days between challenge and measurement
Ep. 4	3 injections of irradiated *Ep.* 4 i.p.	6	10/15	1/4	13
Ep. 6	1 injection of normal *Ep.* 6 s.c. No visible tumour ensued	20	5/5	2/6	34
	3 injections of irradiated *Ep.* 6 i.p.	67	7/16	0/5*	28
Ep. 8	3 injections of irradiated *Ep.* 8 i.p., followed by 2 s.c. inocula of normal *Ep.* 8 cells	319	13/15	0/4	40

* Controls pre-immunized with irradiated normal *C57BL* tissue.

The appearance of increased susceptibility to tumour growth followed a variety of immunization schedules. With *Ep.* 6, increased susceptibility was observed in one experiment after a single subcutaneous injection of 5·3 thousand normal tumour cells. However, in most experiments, the schedules were more extensive. The most prolonged schedule was used in the experiment with *Ep.* 8. Here 3 injections of irradiated tumour were followed by 2 subcutaneous inoculations with untreated tumour cells. These doses were insufficient to cause palpable growths except in two recipients, and in these two cases the tumours were excised at an early stage.

The occurrence of increased susceptibility was too erratic for a systematic analysis of the observation to be carried out in these experiments. As yet there are few data to indicate whether it is specific to the tumour used for pre-immunization or not. In only one experiment where increased susceptibility was observed (with *Ep.* 6) did animals of the control group receive a similar schedule of normal tissue. Other workers[4] investigating comparable systems have not observed any non-specific depression of the immune response. It seems unlikely that as little as 5·3 thousand non-irradiated *Ep.* 6 cells, insufficient to produce a visible tumour, were nevertheless capable of inducing a non-specific depression of the host's immune response. However, the possibility does still remain.

If the increased susceptibility is found to be specific to the tumour used for pre-immunization, at least two possibilities must be considered, namely, tolerance and immunological enhancement. It is of interest that the same schedules which induced anti-tumour immunity, in other experiments provoked an increased susceptibility. This is perhaps slightly more suggestive of enhancement than tolerance. Whatever is the explanation of this observation, it is clearly of great practical importance if attempts to immunize human cancer patients against their own tumours are undertaken.

Attempts to detect humoral antibody against tumour-specific antigens have been tried by three different methods.

(1) Sera were prepared in *H*-2 incompatible strains by schedules of 3–5 injections of epithelioma tissue. Afterwards, the sera were tested by passive immunization[15,17,18]. *C57BL* hosts were inoculated intraperitoneally with antiserum, and approximately 2 h later challenged subcutaneously with known numbers of the same epithelioma used in the immunization schedule. Tumour growth was compared with that occurring in control mice which had received tumour only. In this type of experiment, the assumption is made that antibodies against normal tissue antigens are absorbed within 2 h. Antibodies reacting with tumour-specific antigens would, of course, not be absorbed, and hence be free to influence the growth of the tumour later inoculated. Despite doses of 0·5 ml. per mouse of antiserum, there were no significant differences in tumour growth between serum-treated mice and controls.

Sera were also collected from the immunized *C57BL* and *BALB/c* × *C57BL* hosts described earlier. The activity of these sera was tested in two ways.

(2) Cytotoxic tests were performed according to the method of Gorer and O'Gorman[19]. However, none of the sera showed any cytotoxic activity.

(3) Neutralization tests[20,21] were carried out in which aliquots of tumour suspension were incubated *in vitro* either with test antiserum or with a control non-reacting serum. After incubation for 45–60 min at 37°, the cell–serum mixture is inoculated into *C57BL* or *BALB/c* × *C57BL* hosts, and the subsequent tumour growths observed. For maximum sensitivity in this type of experiment, threshold doses of tumour cells should be used. We were unable to detect any differences between control and experimental groups despite the use of threshold inocula of tumour cells, except in two experiments in which very large doses of serum were used. The details of these are given in Table 4. From this Table it can be seen that there was some inhibition of the growth of *Ep.* 3 in mice injected with tumour and anti-*Ep.* 3 serum. In the second experiment in which *Ep.* 6 was used, tumour growth occurred in a larger fraction of the group treated with anti-*Ep.* 6 serum than was seen in the control serum group. Possibly

Table 4. Neutralization Experiments
Tumour cells mixed with serum were incubated at 37° prior to subcutaneous inoculation

Tumour	Serum	Tumour growth: fraction of mice in group with progressive tumours
Ep. 3	0·5 ml. *C57BL* ♂ anti-*Ep.* 3	1/5
42 × 10^3	0·4 ml. *C57BL* ♂ anti-*Ep.* 3	1/3
cells	0·2 ml. *C57BL* ♂ anti-*Ep.* 3	0/4
	0·2 ml. *C57BL* anti-*C3H*	3/5
Ep. 6	0·5 ml. (*BALB/c* × *C57BL*) ♀ anti-*Ep.* 6	4/5
	0·5 ml. (*BALB/c* × *C57BL*) ♀ anti-*Ep.* 6	4/5
	0·2 ml. (*BALB/c* × *C57BL*) anti-*C3H*	2/5

Three different samples of antiserum were used in the first experiment and two were used in the second. *C57BL* ♂ hosts were used in the first experiment, and (*BALB/c* × *C57BL*) ♀s in the second.

this is an example of enhancement. Unfortunately the large amount of serum required has prevented us from repeating these experiments, and their significance is correspondingly in doubt. But it is already clear that the method will not provide a satisfactory basis for biochemical characterization of these antigens.

These results are similar to those obtained with other types of chemically induced tumours. Despite clear evidence of tumour specific immunity, Klein *et al.*[4] were unable to detect humoral antibody activity against methylcholanthrene sarcomas. Old *et al.*[5] describe only one example in their series of dibenzpyrene-induced sarcomas. The general lack of success in demonstrating antibody activity is disappointing, but perhaps only to be expected in view of the extremely weak antigenicity of these tumours. However, if progress in chemical investigations on tumour-specific antigens is to be made, it is essential that reliable *in vitro* detection techniques are developed.

This work was supported by a grant from the British Empire Cancer Campaign.

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FACTORS AFFECTING THE EXPANSION OF THE STEM CELL POOL IN TRANSPLANTED MOUSE BONE MARROW

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SERIAL transplantation of mouse bone marrow in X-irradiated isogenic recipients may result in progressive loss of the marrow's ability to promote survival and hæmopoietic recovery[1,2], of its capacity to proliferate and produce undifferentiated stem cells and differentiating blood cells (that is, depletion of its stem cell pool)[3,4], and of the marrow 'lymphocytes'[3,4]. Although the magnitude of these effects varies with the number of successive transplantations, the impairment of proliferative capacity has also seemed to depend on the number of marrow cells transplanted. Perhaps the hæmopoietic stem cells can be restricted in number by excessive pressure to differentiate when small numbers are transplanted into heavily irradiated recipients, differentiation occurring at the expense of the proliferation of otherwise self-perpetuating cells.

This investigation was concerned with the effects of experimental modifications thought to decrease or increase the need for the production of differentiated hæmic cells by the transplanted marrow. Specifically, it explored the effects of varying the number of marrow cells transplanted, of hypertransfusing the recipients of the marrow with erythrocytes or lymph node cells, and of grafting marrow from female donors into isoantigenic male recipients.

Femoral marrow cells from adult $(BALB/c \times A/He)F_1$ females were suspended in Tyrode's solution and injected intravenously into adult isogenic female recipients which had been exposed earlier on the same day to 850 r. whole-body X-radiation ($LD_{100/30}$). Each recipient received 5×10^5, 5×10^6, or 5×10^7 nucleated cells. Fifty days after injection, 3 chimeras of each recipient dose group were killed, and the competence of their marrow to proliferate on transplantation was assayed in terms of splenic repopulation in irradiated isogenic female test recipients. The 50-day interval for the assay was chosen because this length of time had been found to be adequate for complete regeneration of the marrow proliferative capacity after transplantation of 10^7 cells, but not of 5×10^5 cells[3,4]. The test recipient mice were exposed to 700 r. of whole-body X-rays and were given a standard dose of 10^6 nucleated chimera marrow cells by tail vein, the marrow of each chimera being infused into 5–10 test mice. Five days later, 0·5 μc. of ^{131}I-labelled 5-iodo-2′-deoxyuridine (^{131}IUdR), a thymidine analogue, was injected intraperitoneally into each test recipient to label the cells synthesizing deoxyribonucleic acid (DNA) in the spleen. Each of the test mice was killed 6–17 h after injection of the ^{131}IUdR, and its spleen assayed for radioactivity by crystal scintillation counting. Under the conditions used, the splenic uptake of the DNA precursor, expressed as a percentage of the total radioactivity originally available for incorporation, was linearly related to the donor marrow cell dose, from $0{\cdot}5 \times 10^5$ to 10×10^5 transplanted nucleated cells[3,4] and was, furthermore, a constant function of the number of 'lymphocytes' but not of other types of cells in the donor marrow[5]. As judged by the ability of transplanted cells to repopulate the spleen of test mice, the marrow of chimeras that had been given 5×10^5 hæmopoietic cells remained subnormal at the time of the assay (Table 1). After infusion of larger numbers of hæmopoietic cells the deficiency in proliferative competence of chimeric marrow was less severe or absent (Table 1). In fact, the proliferative capacity of marrow from chimeras infused with 5×10^7 cells overshot the normal (Table 1) as in an earlier experiment[3]. This overshoot, although small, suggests that the pattern of proliferation of transplanted marrow stem cells may be similar to the pattern of marrow regeneration that follows irradiation alone, as was shown by Alexanian *et al.*[6].

In an effort to lower the intensity of stimuli favouring the differentiation of stem cells into erythropoietic and lymphopoietic elements, we hypertransfused isogenic marrow chimeras with erythrocytes and/or lymph node cells, since polycythæmia suppresses erythropoiesis in marrow and spleen[7] and lymph node cells seed the lymphoid structures on transplantation into irradiated marrow-injected mice[8]. Male $(C3H/Anf \times C57BL)F_1$ mice were used as primary marrow recipients for this phase of the experiments. They were exposed to 950 r. of whole-body X-rays ($LD_{100/30}$) and infused promptly thereafter with 5×10^5 nucleated marrow cells from

isogenic female donors. Red blood cells were gathered from male mice of the same strain, pre-exposed to 1,000 r. of X-rays to inactivate the few circulating stem cells that might be present along with the erythrocytes. The red blood cells were centrifuged from heparinized blood, separated from the buffy coat, and given intraperitoneally to the marrow recipients as an 80 per cent suspension in Tyrode's solution. Transfusions of 0·5–1·0 ml. each were initiated one day before marrow transplantation and continued thereafter for 5 weeks, three times a week during the first two weeks and twice a week later. A second group of chimeras was injected with lymphoid cells obtained from mesenteric nodes of isogenic male donors not pre-exposed to radiation. The cells were suspended in Tyrode's solution and injected into the peritoneal cavity of the chimeras in numbers ranging from $3{\cdot}5 \times 10^6$ to $7{\cdot}4 \times 10^6$ nucleated cells per injection. The treatment was initiated on the day of the marrow transplantation and continued twice weekly for 5 weeks. A third group of chimeras received both erythrocytes and lymph node cells, but on different days. A fourth group of chimeras was injected with Tyrode's solution alone. The proliferative competence of the marrow of each chimera was assayed as described here, except that the $(C3H/Anf \times C57BL)F_1$ female test recipients were exposed to 800 r. of X-rays before inoculation of the chimera marrow cells. The values of the marrow proliferative capacity of 7 chimeras injected with Tyrode's solution and of 20 chimeras transfused with erythrocytes and/or lymph node cells are presented in Table 2. It is evident that all the treatments enhanced the proliferative competence of transplanted marrow as compared with the Tyrode's solution alone. It would appear that administration of lymph node cells was less effective than hypertransfusion of erythrocytes. It may be postulated that the red blood cell transfusions exerted a greater effect because the resulting polycythæmia was sufficiently marked and prolonged to suppress erythropoiesis[7], whereas the persistence of the infused lymphocytes may have been relatively transitory[8], because most of them are not endowed with active reproductive capacity[9]. The failure of the two treatments to show greater effects when combined may conceivably have resulted from demands on the marrow stem cell pool for differentiation into immunologically competent cells, that is, the donor marrow cells were of female origin and may have been stimulated to react immunologically against their male hosts by the presence of male isoantigen (*vide infra*).

In an effort to detect stimuli favouring the differentiation of stem cells into immunologically competent elements, we injected female marrow cells into both female and male recipients. Male mice possess a male-specific isoantigen which elicits an antibody response adequate for rejection of male skin or spleen grafted into isogenic female recipients[10–12]. 5×10^5 nucleated marrow cells from female donor mice were infused into whole-body (950 r.) X-irradiated female and male isogenic $(C3H/Anf \times C57BL)F_1$ recipients. To extend the test, 5×10^5 nucleated marrow cells were retransplanted 35 days later from chimeras of both sexes into a second set of recipients of the same sex. The proliferative capacity of the marrow was evaluated 35 days after each transplantation by injection of 10^6 nucleated chimera cells into irradiated test mice. Reduction of the ability of transplanted marrow to promote incorporation of ^{131}IUdR in the spleens of test recipients occurred to a greater extent in female–male grafts than in female–female grafts (Table 3). In both types of grafts, the deficiency in marrow proliferative capacity was greater on the second than on the first transplantation, in keeping with earlier observations[3,4].

From the foregoing experiments, we infer that mouse marrow cells transplanted into lethally irradiated recipients may be unable to regenerate a normal-sized hæmopoietic stem cell population if the requirements placed on them for differentiated cells are too great in relation to the available supply of transplanted stem cells. However, if large enough numbers of marrow cells are transplanted, or if the recipients are supplied directly with differentiated blood cells during the critical stages of hæmopoietic repopulation, reproduction by donor marrow stem cells may be adequate to restore rapidly the pool of such cells to normal levels. Conversely, if the requirements for production of differentiated blood cells are increased, as is the case in isoantigenic recipients, a marginal marrow stem cell pool may be further depleted to meet these requirements. A similar argument has been

Table 1. Influence of the number of marrow cells transplanted into primary recipients on the ability of the transplanted marrow to promote the uptake of ^{131}IUdR in the spleens of secondary test recipients

Number of cells transplanted into primary recipients	Splenic uptake of ^{131}IUdR (%) promoted by 10^6 cells given to secondary test recipients* Mean values of individual marrow samples	Weighted average, with 95% confidence limits	Percentage of normal,† with 95% confidence limits
5×10^5	0·43, 0·56	0·48‡	56
	0·44	0·42–0·54	50–64
5×10^6	0·74, 0·66	0·68	80
	0·64	0·61–0·76	71–91
5×10^7	1·08, 0·93	0·98	116
	0·89	0·90–1·07	103–131

* The uptake values are given as percentages of the total ^{131}IUdR radioactivity administered, in excess of the percentage retained in spleens of irradiated control animals not injected with marrow.

† The uptake of ^{131}IUdR promoted by normal age-control marrow is reported in ref. 4.

‡ Each value represents results for a single chimera donor; the number of test recipients was 5–10. The pooled average values were weighted for the number of test recipients of marrow for each chimera.

Table 2. Influence of erythrocyte and lymph-node cell transfusions on the ability of transplanted marrow to promote the uptake of ^{131}IUdR in the spleens of secondary test-recipients

Treatment to marrow recipients*	Splenic uptake of ^{131}IUdR (%) promoted by 10^6 cells given to secondary test-recipients† Mean values of individual marrow samples	Weighted av., with 95% confidence limits	Percentage of normal,‡ with 95% confidence limits
Tyrode's solution	0·41, 0·40, 0·41, 0·41	0·42§	39
	0·31, 0·65, 0·44	0·33–0·54	32–48
Erythrocytes	0·66, 1·22, 0·81, 0·96	0·90	84
	0·93, 0·81, 1·02	0·75–1·08	69–101
Lymph node cells	0·39, 1·11, 0·42, 0·96	0·73	67
	0·58, 1·08, 0·97	0·47–1·10	52–85
Erythrocytes and lymph node cells	0·53, 0·43, 0·85	0·78	72
	0·74, 1·25, 1·33	0·49–1·24	57–92

* Treatment was initiated at the time of primary marrow transplantation and continued two or three times weekly until the transplanted marrow was tested for proliferative capacity. This was done 35–40 days after transplantation.

† The uptake values are given as percentages of the total ^{131}IUdR radioactivity administered, in excess of the percentage retained in spleens of irradiated control animals not injected with marrow.

‡ The uptake of ^{131}IUdR promoted by normal age control marrow was 1·08 per cent (95 per cent confidence limits 0·92–1·25). This value represents the weighted average of the uptake promoted by the marrow of 8 normal donors; individual values were 0·79–0·87–1·13–1·18–1·19–1·21–1·32–1·37.

§ Each value represents results for a single chimera donor; the number of test recipients was 5 to 7. The pooled average values were weighted for the number of test recipients of marrow from each chimera.

Table 3. Influence of the recipient's sex on the ability of serially transplanted marrow (from female donors) to promote the uptake of ^{131}IUdR in the spleens of test-recipient mice

Transplant generation	Sex of host	Splenic uptake of ^{131}IUdR (%) promoted by 10^6 cells given to test-recipient mice* Mean values of individual marrow samples	Weighted av., with 95% confidence limits	Percentage of normal,† with 95% confidence limits
First	Female	0·72, 0·48, 0·53, 0·54	0·64‡	59
		0·95, 0·72	0·48–0·84	48–73
	Male	0·41, 0·40, 0·41, 0·41	0·42	39
		0·31, 0·65, 0·44	0·33–0·54	32–48
Second	Female	0·68, 0·59, 0·12, 0·34	0·36	33
		0·25, 0·61, 0·71, 0·12	0·22–0·58	24–45
		0·36, 0·48		
	Male	0·24, 0·02, 0·08, 0·37	0·18	17
		0·11, 0·06, 0·42, 0·37	0·09–0·36	11–25
		0·47, 0·21		

* The uptake values are given as percentages of the total ^{131}IUdR radioactivity administered, in excess of the percentage retained in spleens of irradiated control animals not injected with marrow.

† The uptake values for marrow of age control females are reported in the footnote to Table 2. The value for age control males is not significantly different.

‡ Each value represents results for a single chimera donor; the number of test recipients was 4 to 7. The pooled average values were weighted for the number of test recipients of marrow from each chimera.

proposed to account for the decline in antibody-forming ability of spleen cells after their transplantation into lethally-irradiated mice; it has been suggested that the pool of potentially competent donor stem cells is depleted by stimuli to differentiate[13].

This work was supported by the U.S. Atomic Energy Commission under contract with the Union Carbide Corporation.

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RATIO OF POTASSIUM TO LACTOSE IN COWS' MILK AND ITS GENETIC BASIS

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IN an earlier communication[1], the lactose and potassium concentrations in the milk of Friesian heifers free from infections of the udder were shown to be constant from the second to about the fifth month of lactation, and an inverse relationship was demonstrated between the concentrations for lactose and potassium obtained with different animals. A more extensive investigation of this relationship and of how it is altered by infections of the udder, by age or stage of lactation has now been made with a group of Friesian heifers held at the Institute farms under controlled conditions of feeding and management and with animals in commercial herds of Friesian cattle. Our observations suggest that, within the breed examined so far, the potassium to lactose ratio in milk has the characteristics of a genetic parameter.

Lactose and potassium concentrations in the milk of individual animals were determined at intervals from the second to the fifth months of lactation. The frequency of sampling occasions varied from 2 in some animals to 7 in others. On each occasion samples representative of the milk obtained from each quarter at successive evening and morning milkings were prepared and analysed.

Lactose and potassium interrelationships. For animals in their first lactation, the means of the concentrations for lactose (g/100 g milk water) and for potassium (mg/100 g milk water) in the milk of all udder quarters found on all sampling occasions to be free of pathogenic bacteria, negative to the Whiteside test[2] and with a cell count of less than 80,000 per ml. of milk, were taken as an index of the potential of the animal. As will be seen later, present or previous bacterial infection of a quarter, which was excluded by the above tests, is associated with a decrease in the concentrations of lactose and potassium and an increase in the concentration of sodium in the milk of that quarter.

The relationship between such values for lactose and potassium for 20 heifers held at the Institute farms and for 76 heifers from eight commercial herds is shown in Fig. 1. Excluding the values represented by open circles for reasons which are discussed here, regression analysis gave the following highly significant ($P < 0{\cdot}001$) relationship:

$$Y = 7{\cdot}4242 - 0{\cdot}01095X \qquad (1)$$

where Y is lactose (g/100 g milk water) and X is potassium (mg/100 g milk water). The standard error of the regression coefficient is $\pm 0{\cdot}00112$ and the standard error of estimate of lactose at a given value of potassium is $\pm 0{\cdot}144$. This equation differs significantly from that of $Y = 8{\cdot}93 - 0{\cdot}0191X$ obtained previously[1]. The discrepancy probably arose because in the earlier work four heifers that had the lowest milk lactose concentrations were from a single herd which proved to have a high incidence of mastitis, and the determined lactose and potassium contents may not have indicated the potential of the animals; the animals were not infected at the time of sampling, but the possibility of damage due to previous infection or injury of the udder was not excluded since Whiteside and cell count tests were not made. When values for the four heifers are excluded, the earlier regression equation becomes:

$$Y = 7{\cdot}2534 - 0{\cdot}00983X \qquad (2)$$

The standard error of the regression coefficient is $\pm 0{\cdot}00187$ and the equation does not differ significantly ($P > 0{\cdot}05$) from that for the present results.

Of the five animals for which values are represented in Fig. 1 by open circles, the four grouped together were the entire heifer population of one commercial herd, the fifth was purchased in-calf with other heifers and held at an Institute farm throughout the first lactation. All five animals had sodium contents in their milk higher than

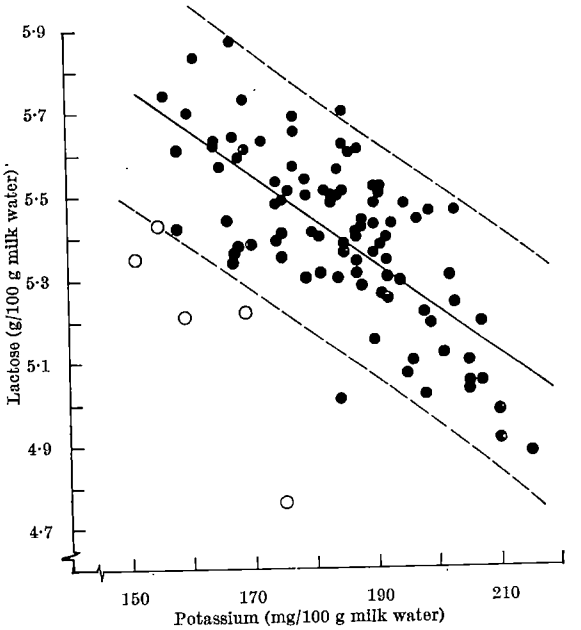


Fig. 1. Relationship between the lactose and potassium contents of the milks of heifers in mid-lactation and free from infections of the udder. The line is $Y = 7{\cdot}4242 - 0{\cdot}01095X$ ($P < 0{\cdot}001$) and relates only to the values represented by closed circles. The broken lines indicate the 95 per cent confidence limits for lactose at given values of potassium. (See text for explanation of values represented by open circles)

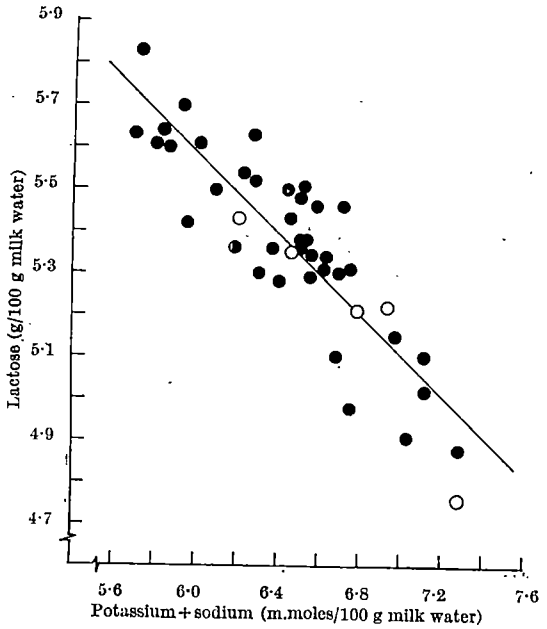


Fig. 2. Relationship between the lactose and the sodium plus potassium contents of the milks of heifers in mid-lactation and free from infections of the udder. $Y = 8{\cdot}456 - 0{\cdot}4785Z$ ($P < 0{\cdot}001$). (See text for distinction between closed and open circles)

was considered normal for heifers in their first lactation, and yet, from inter-quarter comparisons of milk composition and bacterial tests, there was no evidence of udder damage. This apparent distinction disappeared, however, when lactose content was related, not to potassium content but to the equimolar sum of sodium and potassium contents in the milk of these five animals and of other heifers for which information on sodium content was available (Fig. 2). The following highly significant ($P < 0{\cdot}001$) equation was obtained:

$$Y = 8{\cdot}456 - 0{\cdot}4785Z \qquad (3)$$

where Z = potassium + sodium (m.moles/100 g milk water). The standard error of the regression coefficient is $\pm 0{\cdot}0446$.

The empirical relationship between the concentrations of lactose and potassium in milk arises, therefore, because, for the majority of Friesian heifers free from udder disease, variations from animal to animal in the concentration of sodium in milk are small relative to variations in potassium concentration.

Effects of infections of the udder, stage of lactation and age. Lactose and potassium concentrations in the milk from quarters damaged by bacterial infection or physical injury, or in the milk of an animal at an early or late stage of lactation or beyond its first lactation, are seldom the same as those found in the milk from infection-free quarters in the middle part of the first lactation. Each of the effects tends, to varying degrees, to cause a decrease in the concentrations of lactose and potassium and an increase in sodium. Moreover, the resulting changes in the lactose to potassium plus sodium ratio are not distinguishable from the inherent differences in the ratio described by equation (3). The empirical relationship between the lactose and potassium contents of milk secreted by healthy quarters of the udder of cows in the middle part of the first lactation appears to apply, however, to most of the Friesian cattle so far examined. If it could be shown that, while the concentrations of lactose and potassium in milk are depressed by various physiological factors, the ratio of lactose to potassium is unaltered, this ratio would provide a basis for predicting a 'potential' lactose content in cattle of all ages, irrespective of their stage of lactation or presence of infections of the udder.

Barry and Rowland[3] have shown that infections of the udder and advancing lactation cause similar reductions in lactose and potassium contents of milk, but comparable information on the effect of age is not available. We have confirmed that the ratio of potassium content to lactose content in milk is little affected by infections of the udder (Fig. 3), by stage of lactation except during the first few days or the last two months of lactation (Fig. 4), or by age in a single cow in which this effect has been examined.

Prediction of a 'potential' lactose content. In commercial herds of Friesian cattle in which the performance of the heifers is found to conform with the relationship expressed in equation (1), the ratio of potassium content to lactose content in the milk of each of the animals of the herd can therefore be used to predict the 'potential' values for potassium and lactose contents defined by equation (1). Graphically the potential values will be determined by the intercept of the regression line of Fig. 1 with a second line extrapolated from the graph origin, describing the given ratio of potassium to lactose. For lactose content calculation would be made according to the formula:

$$\frac{7{\cdot}4242}{1 + (0{\cdot}01095)R}$$

where R is the ratio of potassium (mg/100 g milk water) to lactose (g/100 g milk water) found in the milk.

Genetic basis of the potassium to lactose ratio. For all animals, including heifers, the ratio of potassium content to lactose content was calculated as the mean for the values of all udder quarters, including those known to

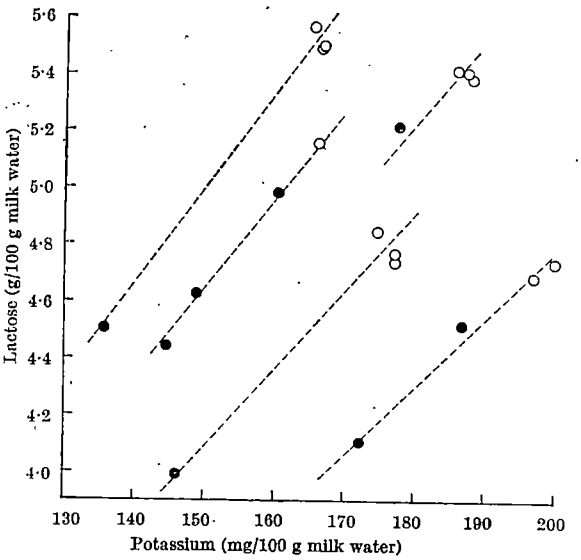


Fig. 3. Relationship between the lactose and potassium contents of the milks from the separate quarters of the udder for five cows in mid-lactation. ○, non-infected quarters; ●, infected quarters. The broken lines describe a constant potassium to lactose ratio

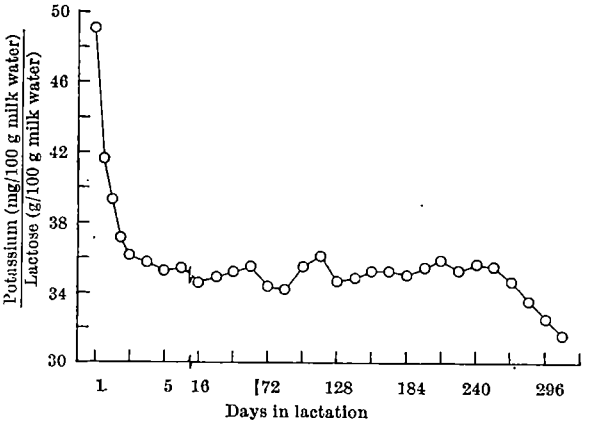


Fig. 4. Changes with stage of lactation in the potassium to lactose ratio in milk. Each value is the mean for six heifers free from udder infections

Table 1. The ratio of potassium (mg/100 g milk water) to lactose (g/100 g milk water) and the predicted lactose potential (g/100 g milk water) in the milk of eight pairs of monozygous (*M.Z.*) cattle twins

M.Z. pair	Breed	Potassium to lactose ratio	Predicted potential lactose content
157	Friesian	33·59	5·428
158		32·98	5·455
165	Friesian	35·39	5·351
166		35·21	5·358
161	Friesian	31·55	5·518
162		33·31	5·440
127	Friesian	40·30	5·151
128		38·41	5·226
015	Friesian	33·20	5·445
016		35·24	5·357
P17	Shorthorn ×	32·91	
P18	Ayrshire	31·54	
067	Ayrshire	37·59	
068		37·89	
037	Jersey ×	34·00	
038	Guernsey	33·09	

have infections, on all sampling occasions. The predicted potential lactose content was calculated using the formula.

The results obtained with eight pairs of monozygous twins held at the Institute farms throughout their first lactation are given in Table 1. The lactose potential is predicted only for the Friesian animals, since it is not yet known whether the relationship expressed by equation (1) applies to animals of other breeds. The heritability of the potassium to lactose ratio estimated from the values for the monozygous Friesian pairs was 0·95. However, this estimate includes correlations between twins due to common pre- and post-natal environment and to dominance and non-additive gene interactions[4].

Additionally, therefore, the records of 29 dams and their 46 daughters present in seven of the eight commercial herds examined were used to estimate the heritability of the potassium to lactose ratio. The results for animals from the eighth farm were excluded since this was the herd that contained the four heifers that gave a characteristically different relationship between lactose and potassium.

A calculation of the within herd regression of daughter group means on the records of their dams gave a heritability estimate of $h^2 = 0{\cdot}95 \pm 0{\cdot}270$. On the assumption that environmental correlation between maternal half-sibs would be small, heritability was also calculated by repeating the record of the dam with each of her daughters in an analysis of co-variance of dams and daughters[5]. The within-herd regression coefficient of daughters on dams was $b = 0{\cdot}38 \pm 0{\cdot}119$, giving a heritability estimate of $h^2 = 0{\cdot}76 \pm 0{\cdot}238$. A comparison of the variance of daughters about their regression on dams with the variance within dams confirmed that there was very little environmental correlation between daughters of the same dam.

The total variance of the potassium to lactose ratio within the population (σ_p^2) is calculated as[5]:

$$\sigma_p^2 = \frac{\text{variance about dam-daughter regression within herds}}{1 - b^2} = 4{\cdot}8$$

These observations suggest that the potassium to lactose ratio and the predicted potential for lactose content in the milk of individual animals are largely independent of the effects of environmental factors and of physiological changes associated with age or stage of lactation, and serve to characterize the genetic potential for lactose content. The use of this concept in examining the relative importance of inherent and environmental factors in determining the milk composition of dairy herds is now being examined.

We thank Dr. R. N. Curnow and Dr. S. J. Rowland for their advice. One of us (J. P. W.) acknowledges a junior research scholarship from An Foras Talúntais, Dublin.

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GALAPAGOS TORTOISE AND MARINE IGUANA DEEP BODY TEMPERATURES MEASURED BY RADIO TELEMETRY

By Prof. R. STUART MACKAY

Medical Physics Division, University of California, Berkeley

As a member of the recent Galapagos International Scientific Project, it was possible for me to make some observations on two unique animals in their natural habitat. Some new types of information were obtained through the use of small radio transmitters which were swallowed by the animals, thus allowing them freedom to move about in a relatively normal physiological and psychological state during the monitoring process. The circuits used will not be given here, since they have already been discussed in the literature, starting with the original article on biological telemetering of internal information[1]. These methods have been summarized[2], brought up to date[3], and methods relevant here for dealing with aquatic animals given[4].

One species examined in this manner was the giant tortoise *Geochelone* of Indefatigable Island (also known as Santa Cruz Island). Particular attention was directed to an animal weighing 65 kg and one weighing 170 kg, each of which was examined for about a week after it had ingested a frequency-modulated temperature-telemetering radio transmitter. These transmitters were approximately spherical and about 1 cm in diameter. Their components were embedded in plastic material and covered with silicone rubber to minimize damage by chewing, or being dropped or walked on; these last factors are important in field work with wild animals.

Two methods of feeding proved effective. One was to push the transmitter into a banana, which was then ingested as a preferred food by the animal during its customary morning feeding. Alternatively, when an animal was placed on its back and a foot tickled, it would hiss, during which time the transmitter could be dropped into its upper jaw, whence it would be swallowed. The animals, which were examined in detail, were free to roam in natural surroundings, but were confined to a general region approximately 100 m on a side by a wall of loose stones. At intervals of 1–4 h they were approached with a radio receiver the signal of which indicated deep body temperature, and simultaneous readings of shell temperature and ambient conditions were made with a thermistor probe. Results for the larger animal are given in the upper part of Fig. 1; those for the smaller one were similar.

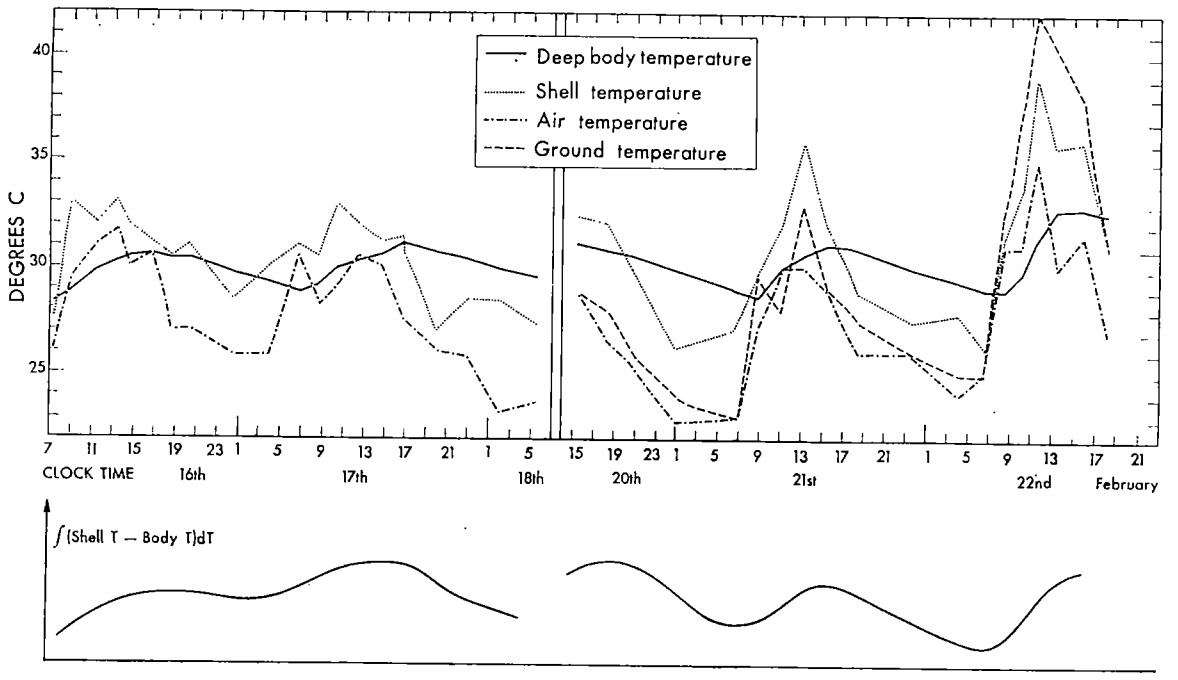


Fig. 1. Temperature cycles in and about a 170-kg tortoise relatively free to move about in or out of sunlight according to its choice. Below is shown the calculated pattern of heat flow which resembles the telemetered core temperature; this suggests that most of the body of the animal is at the same temperature, rather than having heat flowing throughout its mass by diffusion

A few general words about the apparatus might be mentioned. The transmitter circuit was actually the original one tested in human beings[1], with the temperature being transmitted as a rate of arrival of pulses as the transmitter periodically turned itself on and off. The main circuit modification was to use a two-microfarad tantalum condenser in the base connexion of the transistor so that the pulses were generated approximately once a second. Thus they could be timed with a stop watch, as has recently been described[4]. This also led to rather low average current consumption, and continuous transmission for several weeks was demonstrated. Power was low enough to avoid any local diathermy heating. Transmitter calibration by exposure to known temperatures gave temperature as a non-linear function of click rate; a given set of results can be plotted as clicks per second, or as seconds per click, and will generally appear more linear one way than the other, although the overall result is unaffected. An unmodified standard pocket transistor receiver was used to detect the signals, as a further effort towards simplicity and ruggedness. This receiver was selected by entering a radio store with a small transmitter in a pocket, and noting which receivers were most sensitive; cost, size and sensitivity appeared unrelated. The brief transmitted pulses were detected for a wide range of tuning settings, and thus the receiver was simply tuned between commercial radio stations. On the Galapagos Islands where there is no commercial radio, this was only a factor at night when distant transmissions could be detected.

In the upper part of Fig. 1 is a plot of the results from the observations on the larger animal. The air and ground temperatures were similar. Because the region of the experiment was relatively sheltered, the air at the level of the tortoise would be expected to come into thermal equilibrium with the ground. Temperature of the tortoise was usually higher than these temperatures. The shell temperature is seen to cycle up and down about the deep body temperature, sometimes being higher and sometimes lower. The shell temperature shown is an average from several observations taken at different points on the shell. The animals' pattern of activity was usually to spend the night immobile under a bush or tree. When the Sun appeared, they would normally walk around exposed for a few hours while eating, and then return under a bush, from which they would occasionally wander during the rest of the day. The irregular fluctuations in shell temperature reflect this irregular exposure to the Sun and shade. Using the radio receiver in the fashion of a direction-finder showed that, in the several days during which observations were taken, the transmitter did not move significantly within the body of the animal (though after a few weeks such a transmitter can pass normally), and thus the results labelled 'deep body temperature' are consistent. The pattern was similar on a day when no food was ingested, and thus the temperature cycle does not seem due to such things as the fermentation of food in the stomach.

Several general patterns seemed evident. When the animal was free to move about as it wished, its body temperature varied by only a few degrees. These cyclic attenuated temperature fluctuations appear related to changes in temperature on the shell, but with maxima delayed in time by 5–6 h. There are periods when the upper shell temperature is greater than the temperature nearer the animal's centre, which temperature is greater than the ground temperature. At this time, some heat is simply flowing in through the top of the animal and out through its underside. It is clear that blood supply to the limbs did not serve as a heat exchanger to bring the body temperature rapidly to that of the ground or air.

One might expect the temperature of these large animals to remain relatively constant because of thermal inertia associated with their mass, or that, because of their size, temperature changes would take a long time to arrive, if they did at all. Comparison might be made with the heating and cooling of a sphere of water having the same mass. But results recorded here indicate that this could be unrealistic. It does not appear that waves of heating and cooling travel by thermal diffusion through the animal's body, as will be seen from the following. The changes in temperature deep in the animal's body are not abrupt enough to be associated with a simple time delay by diffusion. The results shown are consistent with a representation involving a single time constant. For those who find electrical analogies useful, it is as if

heat were flowing through a resistance to a large central region according to the same equations by which a single condenser charges through one resistor. This is not the way diffusion of heat over relatively great distances would affect core temperature. If heat is thought to flow inward to warm the core of the animal under the 'driving force' of the difference in temperature between shell and core, then the rate of change of temperature deep in the body would be the difference in temperature between surface and core. It would then be expected that the integral of the difference in temperature between inside and outside, with respect to time, would be a curve which resembles deep body temperature. In spite of the limitations of the data, this is seen to be largely true in the lower part of Fig. 1, where the result of a graphical integration is plotted. Also, lack of overall slope suggests that any new outward flow of metabolic heat through the upper shell was relatively small (though some average flow down to the cooler earth must have existed).

These results do not seem to describe a distributed diffusion effect, but rather heat flow through a limited region having a high resistance compared with that of the material absorbing the heat. It seems probable that what happens is that heat flows through the thermal resistance of the animal's shell and into its body tissues, where it is rapidly distributed, all the latter warming and cooling together because of the 'stirring' effect of the flow of blood. A measurement of shell conductivity would allow these same results to be used to calculate that fraction of the total mass of the animal which is involved in this 'stirring'. This particular arrangement of thermal properties would seem to be useful to the animal in that it would allow all its body tissues to be at a 'comfortable' temperature, and would allow temperature regulation by a 'shot' of heat from the Sun or cool from the shade, rather than having a temperature wave moving in. It is interesting to speculate if perhaps dinosaurs were able to use a similar mechanism, possibly involving tissue layers with differing degrees of blood supply.

Temperature cycles also were observed in the marine iguana, a cold-blooded animal which complicates its thermal life by entering the cool ocean to eat seaweed. It seemed important to follow 24-h cycles in a single animal, while interfering with its activities as little as possible, and thus radio telemetry was used. Two transmitter types were used to provide for two types of experiments, and these combined to give an indication of what *Amblyrhynchus cristatus* from Academy Bay can choose to do.

Since Darwin's time, it has been said that marine iguanas do not bite. Though their dispositions seemed better than those of the land iguanas, this is not strictly true. When caught by the tail with the hand, or noosed, they would often attempt one bite and then calmly relax. The method used to feed the same type of transmitter as already mentioned here to these animals was to hold them by the tail and let them bite on a piece of wood held to the side of the head. This was then twisted, without damage to the teeth, and the transmitter dropped into the mouth. Gentle stroking of the throat produced swallowing. Pieces of coral the size of these transmitters were occasionally found in the fæcal matter of these iguanas, and so this experiment was not felt to impose any unnatural or dangerous circumstances. These small transmitters have a transmission range of only approximately one metre, and thus, though they can give information about inaccessible animals, for example, burrowed in crevices in the lava, they are extremely inconvenient to use with an animal that is not confined to some relatively small general area. A 4-kg male was placed in a cage 2 m × 4 m in which there were both exposed regions and shade throughout the day. At the time of the year at which this investigation was made, in connexion with the breeding season, the animals did not enter the water to eat every day. Thus it was felt that the results depicted in the upper half of Fig. 2 are probably representative of the temperature cycle which this animal would have provided for itself if completely unrestrained by any boundaries. At the end of this experiment, this animal was killed in connexion with another experiment. The transmitter was found to have passed just slightly beyond the level of the diaphragm, and the rest of the intestine was still packed with seaweed. Thus the animal could not have been hungry, and the transmitter had remained essentially fixed within the body during the course of these observations. In Fig. 2 also is shown the cycle of air temperature as recorded in a standard way by a weather station a few hundred metres away. This last is suggestive of, but is not completely equivalent to, observation of the immediate ambient conditions.

A second transmitter type having considerably more power was used to make an observation on a completely unrestrained animal. The electronic circuit was quite like that recently described for telemetering from within a dolphin[4], except that a 7-cm diameter loop antenna was used to radiate the signal. Twenty msec pulses of 600-kc/s radio energy were radiated at a rate which indicated temperature. A higher frequency would have carried the signal further with a smaller antenna; but this lower frequency was chosen for its greater ability to penetrate ocean water. These pulses tuned sharply on one of the pocket receivers already mentioned here. A trivial circuit modification was made to introduce a 'local oscillator' which, by beating with the incoming pulses, gave a more noticeable audible signal. The range of this combination was approximately 30 m. At this range, it was possible to track the animal through a mangrove tangle, while simultaneously monitoring its temperature, though it was out of sight.

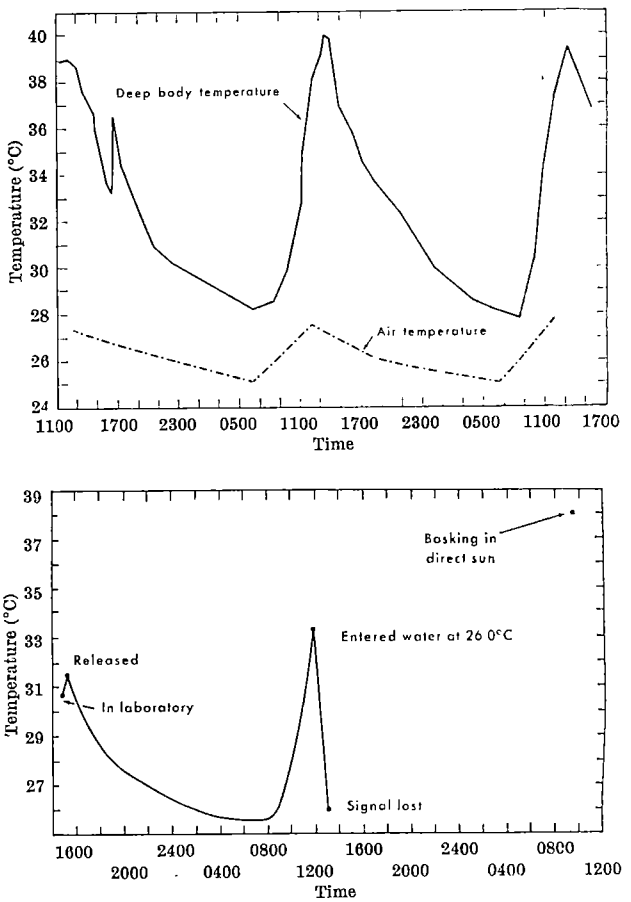


Fig. 2. Internal temperature telemetered from a marine iguana in a large cage (top), and from an unconfined one (below). In this case, air temperature was not taken in the immediate vicinity of the animal

This transmitter was too large to be swallowed, the components occupying about 10 c.c., and so it was attached to the side of the base of the tail, where it would scarcely interfere with swimming or with crawling into crevices. The unit, with a hollow cover to give neutral buoyancy in water, was held in place with two loops of plastic 'Ident-a-Band', over which was placed a layer of ordinary adhesive tape. The loop antenna was held flat across the back of the animal by a band. Temperature was sensed by a thermistor at the end of a catheter, the transmitter itself being insensitive to temperature. Rather than placing this in the usual opening where muscular contractions might have expelled it, it was inserted into the abdominal cavity through a small puncture in the body wall. This wound seemed less severe than some the animals inflict on each other during various disputes. A layer of collodion sealed the probe in place. When the unit was removed a few days later, the animal appeared normal and healthy, and when it was sighted during the next few days, it appeared to remain so. Its temperature history during this period is shown in the lower half of Fig. 2.

When on land, this animal warmed and cooled in an expected way. It might be thought that by sufficient advance warming, it could remain warmer than the ocean during a brief swim, and that holding the head out of the water might supply a bit of extra heat to further delay cooling. However, observation of the actions of these animals suggested that they probably were in the water for longer than any likely thermal time constant, and thus probably cooled down fully. In the case of the foregoing animal, which entered water at 26° C, its telemetered temperature indeed indicated that it had cooled to exactly 26° C while remaining quite active. In the course of this observation, the animal signal was lost; the radio system found it again in an unexpected location the next day.

Thus these two species are seen to act in quite different fashions, the tortoise having a relatively constant temperature while the iguana functions under conditions of relatively large temperature fluctuations during each 24-h period.

I thank the Directors of the Galapagos International Scientific Project for inviting me to attempt these observations. Other scientists there were helpful with their time and advice, and I thank Drs. R. Stebbins, H. Dowling, G. Bartholomew and J. Hendrickson, especially, who were also carrying out investigations on these same animal types. I thank Jean Mackay and Donald Dietlein for assistance in handling the animals and in obtaining the results.

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AGE OF CALEDONIAN OROGENY AND METAMORPHISM IN BRITAIN

THE re-interpretation of British Caledonide chronology by Fitch, Miller and Brown[1] (hereinafter referred to as "the authors") rests on a hypothesis which may be expressed in two parts, namely: (*a*) "A pre-(720–765) m.y. orogeny produced a complex of Moinian, Dalradian and Monian regionally metamorphosed rocks, with migmatites and acid intrusives. On this basement were deposited the Eo-Cambrian and Lower Palæozoic rocks of the Caledonian geosyncline"; (*b*) "The main orogenic event that involved the whole Caledonian system did not begin until late in the Silurian". Both these are open to criticism on purely stratigraphic grounds.

The north Irish–Scottish Lower Dalradian succession contains a well-known boulder-bed, which outcrops at intervals for more than 300 miles along the strike. This formation has many of the characters of a tillite. Elsewhere in the Caledonian province well-developed tillites are known. Harland[2] refers to tillites in the Hecla Hook complex of Spitsbergen as equivalent to the Upper Eleonore Bay tillite horizon of Greenland, and to the Varangian and Sparagmitian tillites of Norway. Troelson[3], discussing the Cambrian of north Greenland and Ellesmere Island, places the tillite horizon at the base of the "Eo-Cambrian". Holtedahl[4] similarly places the lower boundary of the Norwegian Eo-Cambrian below the Moelv conglomerate, considered to be a tillite, and the Moelv sparagmite. Fossiliferous Lower Cambrian here lies conformably above the Eo-Cambrian, as is generally the case in the Caledonian province where both units occur together. Pruvost[5] refers to fossiliferous Middle Cambrian resting, in north-west France, on the metamorphosed, tillite-bearing "Infra-Cambrian" Brioverian complex. It seems probable that all these tillite, or tillite-like, horizons are stratigraphically correlatable, and that they represent a synchronous event, or sedimentary state.

The authors' argument requires the whole of the metamorphic Dalradian to be pre-Eo-Cambrian (which they do not define). This, on the evidence at present available, appears to be unlikely. (It is instructive to note that as long ago as 1930 Peach[6] suggested that part of the Dalradian succession on Islay and the adjacent islands might be Cambrian, and that the base of the Cambrian might well be drawn at the horizon of the Port Askaig conglomerate (tillite). More recently, Hupé[7] has shown that the Lower Cambrian of Morocco contains four trilobite zones below the equivalent of the north-west European *Callavia–Holmia* zones. The Caledonian fossiliferous Lower Cambrian may thus be stratigraphically some way above the base of a notionally complete Lower Cambrian sequence, and the gap could well be filled by part, or all, of the Eo-Cambrian. Rudwick[8] has suggested that the apparently synchronous appearance of skeleton-bearing invertebrates in the Lower Cambrian may be explicable in terms of the climatic amelioration following the Eo-Cambrian glacial episode.)

The Lower/Middle Cambrian trilobite *Pagetia* found by Pringle[9] was ambiguously described by him as from "the Highland Border rocks", and not, as the authors state (*op. cit.* 276–7), from the Highland Border Series, although such an attribution may have been implied in Pringle's report. Stone[10] has shown that the Leny Limestone group, from which the fossils came, is an integral part of the Upper Dalradian succession below the Leny Grits. If the fossil evidence is accepted, and taking into consideration the fact that the whole of the Dalradian is regionally metamorphosed, it follows: (*a*) that some part of the Dalradian is of Lower Cambrian age; (*b*) that at any rate this part of the Dalradian was metamorphosed after late Lower/early Middle Cambrian time, and not, as the authors' hypothesis requires, before the Cambrian and Eo-Cambrian.

Stratigraphic and structural evidence for a pre-Arenigian Caledonian metamorphic/tectogenic episode has been assembled by Dewey[11], with special reference to west Ireland. It is necessary to direct attention to certain misinterpretations which are becoming embedded in the literature as a result of Dewey's conclusions, and which appear to have been accepted by the authors. Dewey

himself states clearly (*op. cit.* 399): "Nowhere in W. Ireland is the contact between the Arenig and the Dalradian seen, and therefore there can be no precise definition of its nature". In fact, the contact is always either faulted or obscured by igneous intrusion, and the significance of this has not yet been elucidated (it might, for example, be a horizon of décollement). The statement by George[12], following Dewey, that "the southernmost tracts of the Connemara schists, continuing towards Galway, carry unwarped Arenig rocks preserved on their surface . . ." is misleading. Dewey's interpretation is based, for this detail, on the work of McKie and Burke[13], who write: ". . . the low degree of deformation, evidenced particularly by the excellent preservation of sedimentary and pillow structures, suggests that the South Connemara Series is unlikely to be pre-Cambrian; it would thus appear to belong to some part of the Ordovician". After making comparisons with the nearest Ordovician outcrops, they conclude by making "a tentative identification of the South Connemara Series with part of the Arenig to Llandeilo stages of the Ordovician". It must also be repeated that the contact between the South Connemara Series and the Connemara schists (? Dalradian) is not seen. The evidence for pre-Arenigian metamorphism of the Connemara schists is thus stratigraphically circumstantial, not direct.

The authors reject the possibility of an important orogenic event having occurred at or about the time of the "Arenig" transgressions of which there are stratigraphic records, because the likelihood that "such an event, accompanied by recumbent folding on the grand scale, could have been closely restricted to the Dalradian belt and have left the rest of Britain unaffected, is to us unacceptable" (*op. cit.* 277). The authors do, however, accept the bone-bed (less than 1 ft. thick) at the top of the Ludlovian Aymestry Limestone of the Welsh Borderland as representing "the initiation of the culmination of the Caledonian evorogeny". It will be useful to compare this type of stratigraphic 'event' with evidence for others tending to support an alternative hypothesis of crustal deformation at other levels within the Lower Palæozoic succession, and, in particular, within the Ordovician.

On the platforms marginal to the Lower Palæozoic mobile belt there is a stratigraphic break within the Cambrian: in north-west Scotland this takes the form of the apparent absence of Middle Cambrian beds; in Shropshire there is a minor unconformity at the base of the Middle Cambrian. In Ireland, slide-conglomerates of pre-*extensus* age (Dewey, *op. cit.*), that is, either early Arenigian or pre-Arenigian, and a widespread pre-Caradocian unconformity[14], indicate crustal instability. A similar unconformity is present within the Scottish Highland Border Series[15]. Farther south, in the Girvan–Ballantrae region, important conglomeratic units lie uncomformably on the Arenigian (in part) Ballantrae Volcanic group[16]. These, and later, Silurian, conglomerates contain large amounts of basic igneous debris which Black[17] considers to have been derived from a northerly extension of the Ballantrae Volcanic group, possibly beyond the Highland Border fault. Black emphasizes the need to postulate severe diastrophism in the Arenigian–Caradocian interval to account for these observations. Bloxam and Allen[18] have shown that the glaucophane-schists and associated rocks of the Girvan–Ballantrae complex suffered the more intense of two deformations in pre-Caradocian, possibly post-mid-Arenigian, time, but not pre-Cambrian as previously supposed.

In north England, the ejection and accumulation of up to 12,000 ft. of mainly andesitic material—the Borrowdale Volcanic group—during the late Llanvirnian–Llandeilian, suggest some local crustal instability, the scale of which is further indicated by the observation that, in places, the whole of the volcanic group, together with part of the underlying clastic Skiddaw group, had been eroded before the Caradocian. George (*op. cit.*), quoted by the authors in support of a late Silurian orogenic event involving pre-Devonian fold-amplitudes of 10–15,000 ft. in the Scottish Midland Valley, writes: "The combined thickness of the Skiddaw and Borrowdale groups is not less than 22,000 ft. and may well exceed 24,000 ft. This gives a measure of amplitude of the pre-Bala [*sc.* pre-Caradocian] folds—*not greatly exceeded in post-Silurian deformation*" (my italics).

In Wales almost the whole of the Ordovician succession is marked by volcanic rocks in one region or another, from the post-Tremadocian pre-Arenigian Rhobell Fawr group to the Ashgillian Cyrn-y-brain rocks, and no fewer than three unconformities can be traced—pre-Arenigian, pre-Llandeilian, and pre-Caradocian. Throughout the succession the pattern of sedimentation is suggestive of severe crustal instability in the source areas[19].

Evidence from the nearest European major part of the Caledonian province is relevant here. In north-west Norway, Kvale[20] refers to deformation and metamorphism of Lower/Middle Ordovician age, followed by uplift and erosion. In central Norway, Strand[21] refers the emplacement of the lower Jotun nappe to a time between mid-Ordovician and early Silurian. The succession in the Trondheim region, described by Vogt[22], shows important conglomeratic horizons, recording diastrophic 'phases', of early/mid Ordovician (Trondheim phase), mid/late Ordovician (Ekne phase), and late Ordovician/early Silurian (Horg phase) ages.

The weight of the stratigraphic evidence thus points to an important tectogenic, possibly metamorphic, event-sequence of post-early/middle Cambrian, pre-Silurian age, which caused widespread sedimentational and structural disturbance. Shackleton[23] remarks on the absence of a molasse (that is, post-orogenic) element in the post-Dalradian sedimentation pattern: it is terminologically improper, but perhaps illuminating, to regard the main part of the British Ordovician and Silurian sedimentary succession—and particularly the post-Llandoverian Silurian—as "molasse in flysch facies".

In conclusion, some comment is offered on the authors' use of Simpson's[24] terms "evorogeny" and "parorogeny", and their derivatives. The authors use these terms in relation to the Caledonian mobile belt, not in Simpson's sense as distinguishing geosyncline from "shelf", but rather as distinguishing the metamorphic from the non-metamorphic part, and treating this latter as if it had "shelf" characteristics. It is, of course, well understood that this part of the Caledonian complex is very far from the accepted notion of the "shelf", except along its outermost margins, and momentarily and over small areas elsewhere. I prefer, and have for some years used, the term "para-orogenic" to label the non-metamorphic part of the British Caledonian complex, which differs from the eu-orogenic part in lacking not only high-grade regional metamorphism, but also a migmatite belt, "alpine" structures, and a serpentine belt in the sense of Hess[25]. The minor diastrophic and sedimentational effects observed on the marginal "shelves"—or, preferably, platforms, since the word "shelf" has more precise geomorphological connotations—have for long been called "epeirogenic", and there seems no need to depart from this. For the case of a region of moderately heavy sedimentation, which later suffers moderately severe deformation, intermediate, that is, between the eu-geosyncline and the platform, I use the term "coelogenic" (basin-producing). This term has the advantage of directing attention to the greater heterogeneity of conditions along the strike, as compared with the greater uniformity in the eu-geosyncline.

Although, no doubt, orogenic, para-orogenic, coelogenic, and epeirogenic deformational events must be linked in some fundamental diastrophic process, there is no justification, as Simpson (*op. cit.*) points out, for Stille's assumption—which has wide acceptance, including, apparently, the authors'—of synchroneity and corre-

spondence between periods of movement and quiescence in the geosyncline and elsewhere. On the contrary, the pattern in most mobile belts appears to have been one of patchiness and migration, both of deformation and metamorphism, in space and time. The Caledonian belt would seem, on the stratigraphic evidence at present available, to conform to this generalization rather than to the "terminal" orogenic pattern proposed by the authors.

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Any hypothesis regarding the age of Caledonian orogeny and metamorphism in Britain can be criticized on "purely stratigraphic grounds" because our stratigraphical knowledge, being incomplete and ambiguous, can be used to support various alternative interpretations. Until stratigraphers can produce convincing definitive evidence regarding the age relationships of British Caledonian rocks, it is necessary to pursue a "multiple working hypothesis" approach to the problems of British Caledonian geology.

We are continuously reviewing the various hypotheses of Caledonian evolution, including that put forward by Dr. T. G. Miller, against the background of a rapidly expanding volume of new isotopic evidence. In our paper[1] we make it clear that the hypothesis discussed is no more than the "working hypothesis at present favoured by us", and that, so far, we have not been able to produce unambiguous isotopic evidence of Pre-Cambrian metamorphism of Dalradian rocks. We are familiar with the evidence that suggests major Caledonian orogeny at the end of the Cambrian or in mid-Ordovician times, and with the stratigraphical evidence in favour of a part Cambrian age for the Dalradian. Unfortunately, the stratigraphical evidence regarding the age of the Dalradian does not enable a convincing conclusion to be reached. The evidence of boulder-beds can never be conclusive: apart from the difficulty of demonstrating whether or not any given boulder-bed truly represents a tillite horizon, in the various late Pre-Cambrian successions mentioned by Dr. T. G. Miller, there are several such beds.

A simple correlation of all of these boulder-beds over the very long distances involved can be no more than tenuous hypothesis, however intellectually satisfying. We feel that the evidence is against such a simple correlation. In this context, it is salutary to recall a more recent opinion expressed by Harland[2], who, after discussing the well-known correlation favoured by Dr. T. G. Miller, said, "However, in addition to one very good double tillite in the Arctic, a lower one has been found, low in the Greenlandian succession, and there seemed evidence of lower ones elsewhere. At the same time, Sutton and Watson have described a boulder-bed from the Banffshire coast much higher in the Dalradian. Since there does not seem to be any well-authenticated tillite in Cambrian or Ordovician times, the serious possibility must be considered that most of the Dalradian is Pre-Cambrian, like the Hecla Hoek and Greenlandian". While agreeing to the plausibility of the correlations suggested by Dr. T. G. Miller, we feel it equally possible that the Moine, Dalradian and Monian rocks of Britain find their equivalents in the Greenlandian of the Carolinides of north-eastern Greenland, and that the Eo-Cambrian (or, more strictly, the sub-Cambrian/post-Carolinide fill of the various Caledonian sedimentary basins along the strike of the mobile belt) is thin or absent in Britain, as the Caledonian rocks are across parts of the older Carolinides belt in east Greenland. There is nothing in the published work of Pringle or Stone that proves conclusively that the fossils found by Pringle came from rocks which form "an integral part of the Dalradian succession". Faulted ground, lithological correlation and structural hypothesis are involved. The continuity and correlation of the various successions within the Dalradian area can also be questioned. If a Cambrian age for a real part of the Dalradian succession could be unambiguously demonstrated, we should most gratefully welcome it.

Dr. T. G. Miller is under the erroneous impression that we reject the possibility of orogenic events at the end of the Cambrian and at other times during the Lower Palæozoic. On the contrary, we are aware of a valid stratigraphical record of numerous episodes of crustal unrest within the British sector of the Caledonian mobile belt, the more important of these occurring in late Pre-Cambrian/early Cambrian, mid-Cambrian, late Tremadocian/early Arenig, mid-Ordovician, late Ordovician/early Silurian, mid-Silurian, late Ludlovian/early Downtonian and mid-Devonian times. We agree that magmatism must be closely related to tectonism, and that the widespread volcanism of the Ordovician must reflect deep-seated crustal instability. What we did suggest, however, as the basis of our working hypothesis, and because we believe that it can be demonstrated from the isotopic evidence, is that the culminating, paroxysmal, Caledonian orogenic/magmatic/metamorphic event in Britain occurred (as in East Greenland[3]) around (420–410) $\pm$ 10 m.y. British Caledonian plutonic magmatism is associated with this event and not with the earlier para-orogenic events. When present dating programmes are completed, it will be possible to suggest dates for some of the earlier Caledonian orogenic events and to discuss alternative interpretations of the overall orogenic time-pattern more conclusively.

We feel that the stratigraphically circumstantial evidence for pre-Arenig metamorphism of the Connemara schists (and therefore of the Dalradian) is more convincing than Dr. T. G. Miller admits. To ignore structural or isotopic evidence unless it confirms or can be confirmed by palæontological stratigraphy is to be unnecessarily cautious. With regard to the evidence of the Ludlovian Bone Beds, it cannot be expected that the sedimentary record outside the mobile belt will be as dramatic as that within it. Our use of the terms evorogenic and para-orogenic is closer to their use by Simpson than to the meanings suggested by Dr. T. G. Miller. We use the term evorogenic when describing the major culminating structural events in the history of a mobile belt and para-orogenic to describe less-important events that may occur either within the constituent geosynclinal basins of the belt or on the adjacent stable or unstable shelves. Where we differ from Simpson is in our interpretation of the

time-sequence of development of the evorogenic structures.

Finally, we would like to point out that our acceptance (as a working hypothesis) of a general contemporaneity between sequences of distinct episodes of increased intensity of crustal instability, both within and along the margins of particular active zones, stems from our attempt at a rational interpretation of the isotopic evidence from the Caledonian and other systems[4], and not from an uncritical acceptance of Stille's at present unfashionable concepts. Dr. T. G. Miller's conclusion, that the pattern of tectonic and metamorphic activity in mobile belts is one of incomprehensible patchiness and migration in both space and time (despite his earlier insistence on the contemporaneity of certain stratigraphical events over great distances), is, in our opinion, a too restricted and defeatist point of view. Episodes of increased crustal instability along one sector of a mobile belt may not produce equal, or indeed any, noticeable increase in the actual intensity of tectonic activity at given localities, but, when viewed as a whole, a pattern of contemporaneous rhythmic increase and decrease of tectonic/thermal activity within the belt may emerge, leading eventually to a widespread paroxysmal event, after which the activity of this particular mobile sector of the crust begins to wane.

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RUBIDIUM/STRONTIUM AGES FROM THE WESTERN TRANSVAAL

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THE older granites and gneisses of the southern and western Transvaal, South Africa, are mainly covered by younger strata, but are exposed in six inliers some hundreds of square miles in extent. In some areas the granites intrude narrow 'Archean' schist belts; in the absence of other field relationships it has not hitherto been possible to demarcate granites of different ages, and the assemblage of granites and gneisses has been termed 'Basement Complex'.

The age of granites as determined by the rubidium/strontium method is critically dependent on the value taken for the primary $^{87}Sr/^{86}Sr$ ratio (R_i), particularly in the case of total-rock samples for which the present-day $^{87}Sr/^{86}Sr$ ratio (R_p) is usually less than about 0·9. In favourable circumstances R_i is determined from an isochron plot of a suite of total-rock samples, but other cases of geological interest occur for which this is not possible, for example, when only isolated borehole cores are available. For such cases R_i might be determined by the investigations of separated mineral fractions, but because of the possibility of misleading results following the re-homogenization of strontium isotopes on a mineral-to-mineral scale this was not attempted. Instead an assumed value of R_i is used, even though the uncertainty in the estimated R_i value is a limitation on the accuracy of the calculated ages. The first part of this article is concerned with the estimation of R_i; following this the ages of a number of isolated granite samples are calculated, and new upper limits are established for the ages of the Ventersdorp, Transvaal and Witwatersrand systems.

The granite which is exposed between Johannesburg and Pretoria was the subject of a previous article[1]; but the R_i value then obtained has since been revised by allowing for mass-spectrometer discrimination. Discrimination in solid-source mass-spectrometers was conclusively demonstrated by Hedge and Walthall[2], who showed that, although repeated measurements of the isotope ratios of a standard strontium sample on different instruments gave scattered results, the variations in the $^{87}Sr/^{86}Sr$ and the $^{88}Sr/^{86}Sr$ ratios were linearly related. An earlier investigation in this laboratory failed to show such a relationship, possibly because imperfect resolution of the strontium-87 isotope by our instruments caused undue scatter of the measurements. Because of this it was felt that the procedure of normalizing[3] all isotope ratios to conform with a particular $^{86}Sr/^{88}Sr$ ratio was not justified. This view is now reversed and all results in this article are normalized on the basis of a $^{86}Sr/^{88}Sr$ ratio of 0·1194. The improvement in the reproducibility of duplicate analyses so achieved is not very marked, possibly because of the imperfect resolution, and for the same reason a small systematic bias may still exist. However, the R_i values deduced here are directly applicable to measurements made on our instruments. The mean $^{86}Sr/^{88}Sr$ ratio as measured on our two single-filament 6-in. mass spectrometers is $0·1182 \pm 0·0002$, and the R_i ratio for the Johannesburg–Pretoria granite is simply reduced by half the ratio of 0·1194–0·1182. The new R_i value so obtained is $0·706 \pm 0·003$.

Table 1. Schweizer Reneke Results

Sample	^{87}Rb (p.p.m.)	^{86}Sr (p.p.m.)	$^{87}Rb/^{86}Sr$ (atomic)	$^{87}Sr/^{86}Sr$ (atomic)
*OG*10	48·32 ± 0·08	17·51 ± 0·08	2·726	0·811 ± 0·002
*OG*12*A*	69·19 ± 0·32	10·66 ± 0·10	6·414	0·941 ± 0·005
*OG*12*B**	67·60 ± 0·67	10·49 ± 0·11	6·368	0·946 ± 0·004
*OG*13	43·84 ± 0·05	15·10 ± 0·05	2·869	0·813 ± 0·005
*OG*14*A*	56·65 ± 0·13	12·58 ± 0·12	4·450	0·864 ± 0·002
*OG*14*B*	56·63 ± 0·26	12·69 ± 0·05	4·409	0·866 ± 0·007
*OG*23	33·27 ± 0·05	24·92 ± 0·23	1·319	0·752 ± 0·002
*OG*26	71·01 ± 0·15	6·72 ± 0·01	10·447	1·112 ± 0·002
*OG*27*A*	32·92 ± 0·51	17·14 ± 0·21	1·898	0·779 ± 0·003
*OG*27*B**	32·52 ± 0·33	16·93 ± 0·17	1·898	0·779 ± 0·003
*OG*28*A*	40·42 ± 0·10	19·75 ± 0·05	2·022	0·788 ± 0·003
*OG*28*B**	41·38 ± 0·41	20·23 ± 0·20	2·021	0·786 ± 0·003

* In these cases only one measurement was made of the concentrations, and an uncertainty of 1 per cent is assumed. In all other cases errors quoted in this article are standard deviations of replicate measurements.

The data set out in Table 1 and Fig. 1 relate to a second granite exposure, sampled randomly over an area of approximately 100 square miles to the south-west of the village of Schweizer Reneke, which is some 200 miles south-west of Johannesburg. The geology of the area was described briefly by Jorissen[4], and in the main the granite is a homogeneous body of medium grain consisting chiefly of quartz, plagioclase, orthoclase, microcline and biotite. It intrudes a complex belt of schists in the west, and in the vicinity of the schist the granite is foliated and occasionally gneissic. Eight total-rock samples were analysed using the usual isotope-dilution techniques. Duplicate dissolutions were made on four of the samples, and on all solutions replicate spiked rubidium-, spiked

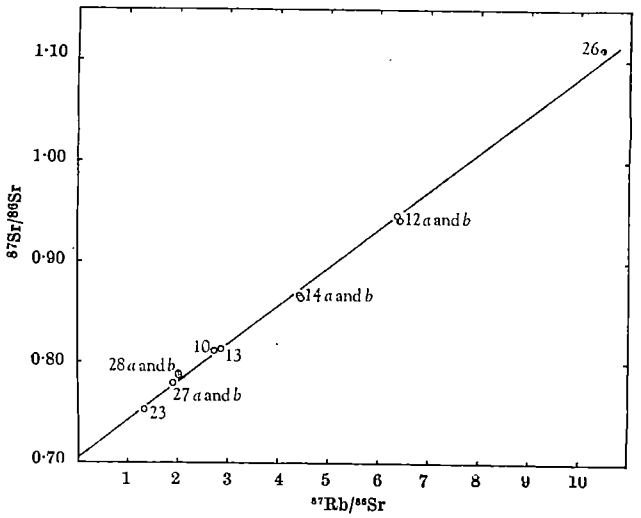


Fig. 1. The Schweizer Reneke isochron; $^{87}Sr/^{86}Sr$ plotted against $^{87}Rb/^{86}Sr$ for all the Schweizer Reneke total-rock samples

and unspiked strontium-aliquots were analysed. In the calculation of R_i and the age by the method of least squares the results of the duplicated dissolutions were treated as independent points, and the result obtained is:

$$\text{Age} = 2{,}700 \pm 55 \text{ m.y.}, \quad R_i = 0{\cdot}704 \pm 0{\cdot}004$$

A further value of R_i was deduced for a rock (*OG*19 from the farm Witklip, 6 miles south of Ventersdorp) from the same area as the samples discussed here. This granite has a very low rubidium/strontium ratio, and by using the measured R_p ratio of 0·718 and an assumed age of 2,700 m.y. (the calculation is relatively insensitive to this figure) a value of R_i of 0·706 ± 0·003 is obtained.

From the Ventersdorp–Klerksdorp area seven rocks of stratigraphic importance were investigated. Some of these were obtained from borehole cores, others from exposures of small expanse. In each case the age of a total-rock sample was calculated assuming $R_i = 0{\cdot}705 \pm 0{\cdot}002$ (the mean of the three values mentioned above) and the data are summarized in Table 2. In the majority of cases the ages agree within experimental error either with that of the Johannesburg–Pretoria granite (3,200 ± 65 m.y.) or with that of the Schweizer Reneke granite (2,700 ± 55 m.y.) and this indicates that there were two distinct periods of granite formation in this region.

Table 2. Ventersdorp-Klerksdorp Results

Sample	^{87}Rb (p.p.m.)	^{86}Sr (p.p.m.)	$^{87}Sr/^{86}Sr$ (atomic)	Age (m.y.)*
*OG*16	77·66 ± 0·40	0·826 ± 0·020	4·17 ± 0·07	2,640 ± 55
*OG*17	66·35 ± 0·35	8·225 ± 0·040	1.050 ± 0·006	3,050 ± 55
*OG*18	97·26 ± 0·45	5·686 ± 0·030	1·335 ± 0·008	2,630 ± 40
*OG*32	31·27 ± 0·15	21·38 ± 0·13	0·772 ± 0·002	3,260 ± 100
*AN*952*A*	27·10 ± 0·20	25·07 ± 0·10	0·745 ± 0·002	2,640 ± 130
*AN*952*B*	26·90 ± 0·10	25·14 ± 0·25	0·747 ± 0·002	2,800 ± 130
*AN*954	62·34 ± 0·35	9·56 ± 0·12	0·955 ± 0·002	2,740 ± 25
RSPIC	25·40 ± 0·20	9·029 ± 0·040	0·816 ± 0·002	2,820 ± 55

* Throughout this article $\lambda = 1{\cdot}39 \times 10^{-11}$ y.$^{-1}$

In the conventional isochron plot of Fig. 1 the Schweizer Reneke results are seen to approximate to a straight line, but the results of the duplicated dissolutions suggest that some points may differ significantly from the best-fitting line. Also some of the Ventersdorp–Klerksdorp results differ widely from the mean ages. These age variations may be genuine; but it is also possible that the samples in question have suffered chemical alteration (this is especially likely in the case of *RSPIC*, obtained only 3 in. beneath the Dominion Reef contact), or that there exist regional variations in R_i. The last-mentioned possibility is quite consistent with the modern view[5] that the basement granites in the Transvaal were formed, at least in part, by the remobilization of older material.

Ventersdorp rocks lie unconformably on the granite at Schweizer Reneke, at the site of *OG*18 and in the borehole *AN*954: the Ventersdorp and Transvaal systems are therefore younger than 2,700 ± 55 m.y. In borehole *AN*952 lower Witwatersrand rocks overlie granite with an age 2,720 ± 100 m.y. and this sets the same upper limit on the age of this system. Similarly, an upper limit of 2,820 ± 55 m.y. is set on the age of the Dominion Reef by the result on the granite from borehole *RSPIC*. The actual ages of the overlying strata mentioned may be considerably younger than the limits given, since a long period must have elapsed between the time of intrusion of the granites and the deposition of sediments on surfaces of the granite exposed by erosion.

The origin of the Witwatersrand gold and uranium deposits has been the subject of much controversy. The age of uranium-bearing minerals found in Dominion Reef sediments is 3,100 ± 100 m.y.[6]. Corresponding data for Witwatersand uraninites give an age of 2,040 ± 100 m.y.[7], but Burger *et al.*[8] observed that the galenas found in veins intruding the Witwatersrand system are enriched in radiogenic lead and reasoned that this came from 'parent uraninites' occurring nearby in the Witwatersrand conglomerates. Assuming that the uraninite age of 2,040 ± 100 m.y. dates the period of lead-loss, Burger *et al.* deduced that the age of the parent uraninite is 2,950 ± 100 m.y. The uranium-mineral ages are compared below with the upper limits given in this article for the ages of the Witwatersrand and Dominion Reef systems.

	Uranium-mineral ages (m.y.)	Limiting sediment ages (m.y.)
Witwatersrand	2,980 ± 100	2,720 ± 100
Dominion Reef	3,100 ± 100	2,820 ± 55

It is unfortunate that the uncertainty intervals attached to the mineral and sediment ages almost overlap; but in both cases the uranium-minerals appear to be older than the sediments in which they are found. This favours the theory of a detrital origin of the uranium and associated gold deposits. It is of interest to note that granites sufficiently old (~3,100 m.y.) to have provided the necessary detritus occur in both the Johannesburg area[1] and the Klerksdorp area (this article).

Samples *AN*952 and *AN*954 were supplied by Dr. Waters of Anglo-American Corporation and *RSPIC* by Dr. Wilson of General Mining and Finance Corporation. I thank Dr. W. Compston and Dr. L. O. Nicolaysen for their advice.

Appendix: Sample Locations

Except where otherwise specified, fresh samples were obtained from surface outcrops. A detailed account of this age survey will be published elsewhere, and will include petrographic descriptions of the samples referred to here.

*OG*10. On Schweizer Reneke townlands; 3 miles from the town along the main road to Vryburg.

*OG*12. On Farm Bellevue 117; 9 miles west of Schweizer Reneke, at level crossing on disused section of road to Amalia.

*OG*13. On farm Seringboomkop 66; outcrops occur to north of road leading from Schweizer Reneke to Amalia, and 7 miles from the former.

*OG*14. On Schweizer Reneke townlands and 2 miles west of it. Outcrops occur south of road to Amalia.

*OG*23. On southern extremity of farm Seringboomkop 66; half a mile south of road at a point 7 miles along road from Schweizer Reneke to Diewedraai.

*OG*26. On farm Delareyskraal 69; outcrops occur to south of road at a point 12 miles along road from Schweizer Reneke to Diewedraai.

*OG*27. On Schweizer Reneke townlands; 1·5 miles north from the junction of the Schweizer Reneke–Vryburg road with the road to Broedersput.

*OG*28. On farm Luciana 43; 3·75 miles south along farm road from its junction with the Schweizer Reneke–Vryburg road.

*OG*16. On farm Vaalbank 66; 3 miles east of Gerdau and approximately 20 miles north of Ottosdal. Outcrops occur close to homestead.

*OG*17. On farm Rhenosterberghoek 88; 3 miles south of Hartebeesfontein on road to Rhenosterspruit.

*OG*18. On farm Brakspruit 5; granite is exposed in dry pan 1·5 miles west of Klerksdorp–Ventersdorp road and approximately 8 miles north of Klerksdorp.

*OG*32. Sample collected at depth of approximately 1,000 ft. in the Rietkuil section of Afrikaner Leases Mine, 7 miles west of Klerksdorp.

RSPIC. Core of borehole situated near southern extremity of farm Rhenosterput 108, 8 miles north of Ottosdal. Sample depth 290 ft., 3 ft. below contact with Dominion Reef quartzites.

*AN*952. Core of borehole situated on farm Makokskraal, 10 miles west of Ventersdorp. Sample depth 10,417 ft., 22 ft. below contact with lower Witwatersrand beds.

*AN*954. Core of borehole situated on farm Ratzegaaiskraal, 6 miles west of Ventersdorp. Sample depth 1,741 ft., 55 ft. below contact with Ventersdorp lavas.

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EUROPEAN MID-DEVONIAN CORRELATIONS

By PAUL COPPER

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A GROUP of brachiopods belonging to the atrypid family Palaferellidae Spriestersbach[1] shows a pattern of muscle platform development which may be of value in the correlation of Middle Devonian sediments in the western hemisphere. A brief description of important features in this rapidly evolving phylogenetic lineage is given.

The family Palaferellidae at present contains only a single genus, *Gruenewaldtia* Chernychev, which is characterized by the presence, in both valves, of muscle platforms which are elevated from the shell floor by a variable number of septa. In a continuous, overlapping and intergrading, morphological series ranging in age from the lower Eifelian to upper Givetian (Middle Devonian), it can be shown that in the Palaferellidae muscle platforms initially were supported by numerous septa and that, with time, these septa decreased in number until support of the platform was reduced, in the case of the pedicle valve, to two septa. The final stage was reached when the platforms became solidly cemented to the shell floor and had only a brief anterior overhang. The end-product of this lineage, forms with cemented platforms, no longer fit categorically into the genus *Gruenewaldtia*, and a new genus, *Desatrypa*, is arbitrarily proposed for these. To avoid subsequent confusion it is described briefly:

'Order' Atrypida. Family Palaferellidae. *Desatrypa* n.gen.

Genotype species. *Atrypa desquamata* Sowerby 1840 (ref. 2), Pl. 56, Figs. 19–22.

Stratigraphic range. Upper Eifelian to upper Givetian, Middle Devonian.

Diagnosis. Biconvex to dorsi-biconvex atrypids with a prominent, wide inter-area and continuous, deep-troughed ribs interrupted marginally by weak, incipient concentric growth lamellæ. No frills or spines developed.

Internally, raised muscle platforms are cemented to the shell floor (a suture line may or may not be visible). Teeth are strong and lack lateral cavities. Massive hinge plates and thick cardinal blocks are typical. Crura are minute, vestigial and structurally different from those in normal atrypids. Some species completely lack dorsally directed spiralia. Deltidial plates are fused medially and are continuous with a pedicle collar or remnant pedicle collar (late Eifelian *Gruenewaldtia* also have monostructural deltidial plates and pedicle collars).

Member species. *Terebratula prisca* var. *flabellata* C. F. Roemer 1844 (ref. 3). *Terebratula insquamosa* Schnur 1853 (ref. 4).

A wide international distribution and long stratigraphic range have commonly been attributed to *Atrypa desqua-*

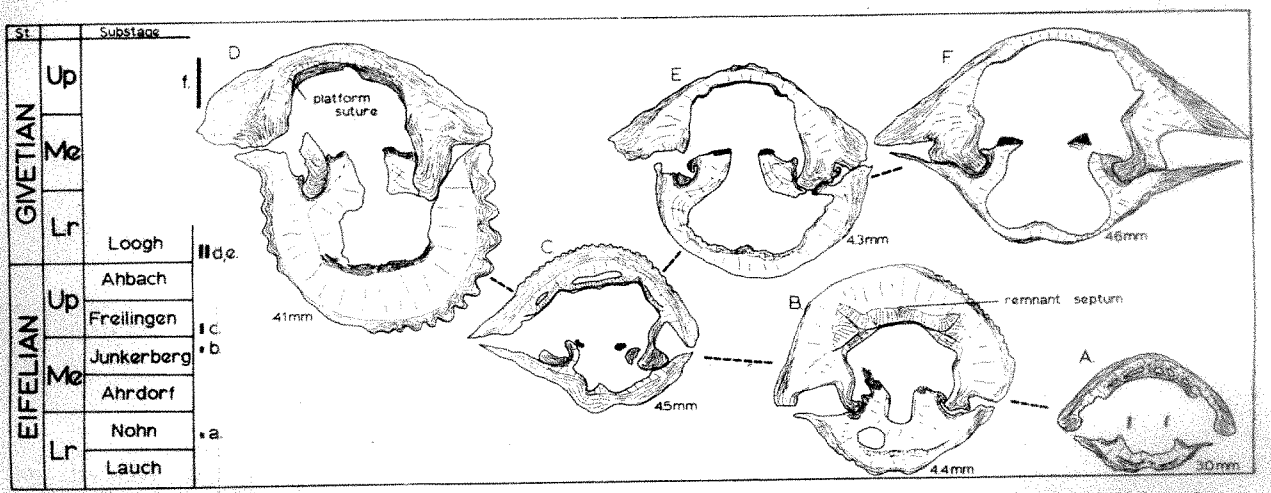


Fig. 1. Transverse sections of different palaferellid atrypids showing the septal development in the pedicle valve. All figures are half-size and are taken at the same stage of crural development. *A*, *Gruenewaldtia* sp. (non *G. rhenana* (Spriestersbach)) from the Schleit horizon, Nohn beds, lower Eifelian; *B*, *Gruenewaldtia latilinguis* (Schnur), upper Junkerberg beds, middle Eifelian; *C*, *Gruenewaldtia* sp. (non *G. apsaklina* Struve), Freilingen beds, upper Eifelian; *D*, *Desatrypta flabellata* (C. F. Roemer), lower Givetian; *E*, *Desatrypa insquamosa* (Schnur), lower Givetian; *F*, *Desatrypa desquamata* (Sowerby), upper Givetian. The transition between the two genera appears to have occurred about late Freilingen–early Ahbach time

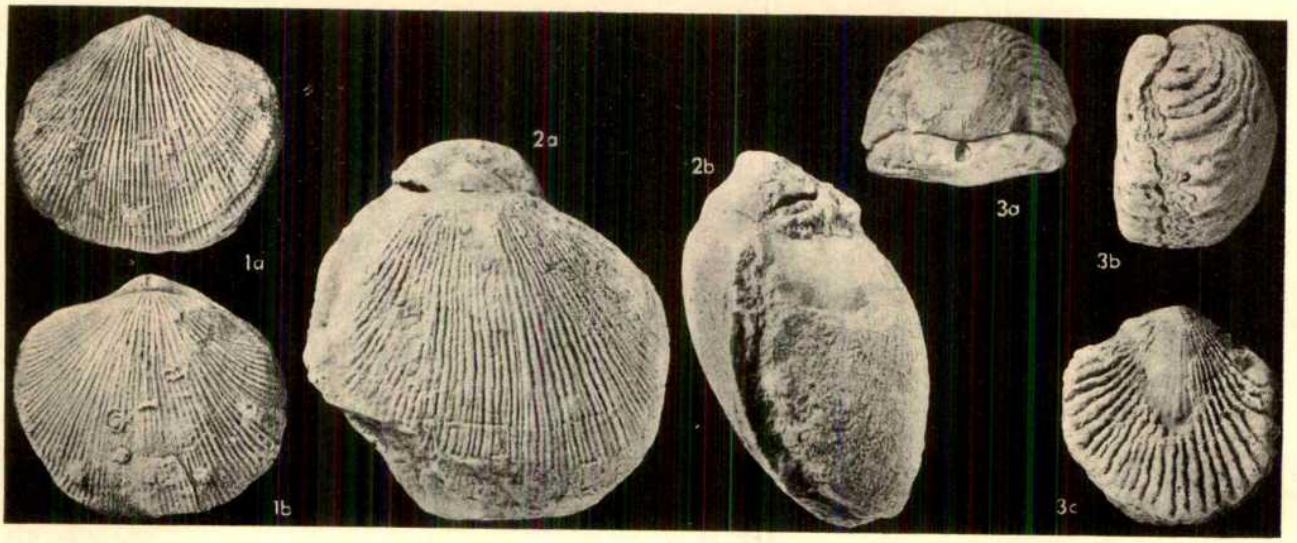


Fig. 2. 1*a* and *b*, *Desatrypa insquamosa* (Schnur), lectotype specimen from Schnur's original collection; 2*a* and *b*, *Desatrypa desquamata* (Sowerby), lectotype specimen, Sowerby collection; 3*a*, *b* and *c*, *Desatrypa flabellata* (C. F. Roemer), holotype, Roemer collection. All figures half size

mata Sowerby, the type species of the new genus *Desatrypa*. Most of the atrypids concealed under this name are, in fact, unrelated to the true *desquamata* of Sowerby, restricted to the upper Givetian. In 1960 Alekseeva[5] established the sub-genus *Desquamatia* and included in this sub-genus many species formerly called "*Atrypa desquamata*". As part of a monographic revision of west European atrypid faunas, I re-examined Sowerby's original material and sectioned topotype specimens of the species *desquamata*. It was then found that the internal structure of *Atrypa desquamata* Sowerby (family Palaferellidae) was very unlike that of the *Desquamatia* (family Atrypidae) described by Alekseeva, although externally they were in part strongly homœomorphic.

The stratigraphical and geographical distribution of the palaferellids is not fully known. Two geographically isolated stocks appear to have existed during late Eifelian–Givetian times. The first, a divergent stock leading to *Desatrypa*, is chiefly concentrated in western Europe and is known from England, France, Belgium and Germany, and probably occurs also in the Carnic Alps of Italy. A second, the root-stock *Gruenewaldtia*, appears to have persisted with little modification to the Upper Devonian at Ober-Kunzendorf near Wroclaw, Poland[6], and is known from the Givetian of the Kutznetsk basin in U.S.S.R.[7]. The emergence, during Givetian time, of massive stromatoporoid-crinoid reefs in north-western Europe coincided with the development of *Desatrypa* n. gen., which is largely restricted to such an environment. It is speculated that *Desatrypa* developed in response to this change in environment. Cementation (and hence strengthening) of the muscle platform probably was advantageous in the more turbid waters of the reefs.

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PRE-PICKWELL DOWN AGE OF THE PLATEAU BEDS (UPPER DEVONIAN) IN SOUTH WALES

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HISTORICAL. The Old Red Sandstone (Devonian) sequence in the Black Mountain, Fforest Fawr, and Brecon Beacons in Carmarthenshire and Brecknockshire comprises the Red Marls—Senni Beds—Brownstones—Plateau Beds—Grey Grits, in that ascending order. The age of the Plateau Beds has remained longest in doubt, and so far there have been several attempts to assess the stratigraphical position of this group. The group is important because of the decisive clues it affords as to the Middle and Upper Devonian history of South Wales.

The term 'plateau-beds' was first used by Strahan *et al.*[1] working in the Brecon Beacons area, for hard pebbly sandstones which gave table-like caps to mountains cut largely from Senni Beds and Brownstones. The plateau-beds were included in the Brownstones referred to the Lower Old Red Sandstone. The Grey Grits, shown to be of Upper Old Red Sandstone (Upper Devonian) age, were considered to overstep or overlap the plateau-beds westwards. Later, from work in the Black Mountain, Strahan *et al.*[2] stated that the Grey Grits overstepped the plateau-beds.

Robertson[3], in revising the account of the Brecon Beacons area, gave the group formational status, writing it as Plateau Beds. From the evidence of a new section in the Abercriban Grit Quarry, Pont-sticill, Brecon Beacons, he concluded that the Plateau Beds graduated up into the Grey Grits. It was suggested that if a stratigraphical break occurred in the Old Red Sandstone sequence above the base of the Brownstones, it could be represented by the coarse strata beginning the Plateau Beds. Such a break, he implied, would if proved probably equate with the Middle Old Red Sandstone unconformity known from the surrounding regions.

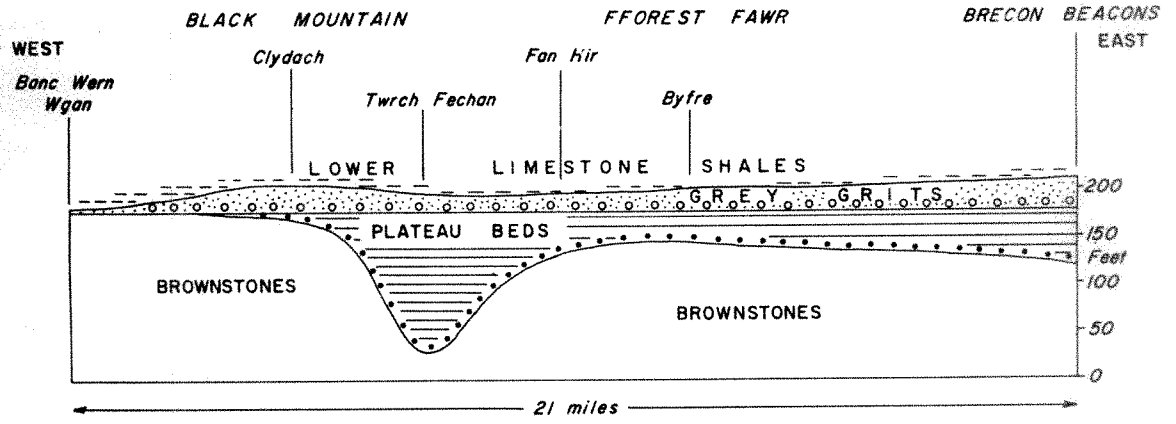


Fig. 1. Relationship of Plateau Beds to Grey Grits and Brownstones in Brecon Beacons, Fforest Fawr, and Black Mountain districts, South Wales

Later, a fauna was reported from the Plateau Beds by Pringle and George[4], Simpson[5] (who acknowledged its partial discovery by Dr. Gwyn Thomas), and Croft[6]. This included the marine brachiopod *Cyrtospirifer verneuili*, marine molluscs, and the vertebrates *Holoptychius*, *Bothriolepis*, and *Sauripterus*, establishing the Upper Devonian, and possibly Famennian, age of the Plateau Beds. Croft[6] also noted the occurrence of *Rhinopteraspis cornubica* (McCoy) in the Senni Beds, which thus emerge as Lower Old Red Sandstone (Lower Devonian) in age.

It would appear that Pringle and George[4] regarded the Old Red Sandstone sequence in the Brecon Beacons area as substantially complete, with the Lower, Middle, and Upper divisions all represented in the sequence Brownstones through to Plateau Beds.

Simpson[5,7] correlated the marine Plateau Beds with the marine strata of late Famennian age at the top of and immediately overlying the Skrinkle Sandstones in Pembrokeshire[8]. This allowed him to postulate a single, "Strunian" marine transgression across South Wales, and he remarked[5] that the first general break in deposition in the region since the close of the Silurian was in the Dinantian (Lower Carboniferous).

Summarizing the position, while the Plateau Beds are established on faunal evidence as Upper Devonian, there is confusion in the literature regarding their relationship to the Brownstones beneath and the Grey Grits above. So far the Plateau Beds have been correlated with the Skrinkle Sandstones of Pembrokeshire.

Stratigraphical breaks. The Upper Old Red Sandstone in Carmarthenshire and Brecknockshire is marked by two stratigraphical breaks: at the very base beneath the Plateau Beds, and higher up between the Plateau Beds and the Grey Grits. Both breaks record erosion after uplift as will be shown, but in terms of angular discordance each is probably a disconformity rather than an unconformity.

Robertson[3] considered the pebbly rocks beginning the Plateau Beds as possibly indicating a break in the Old Red Sandstone. As can be observed repeatedly in the field (for example, National Grid Ref. Nos. *SN* 823180, 742189), a marked change in lithology certainly occurs at this level, for the alternating red siltstones and reddish-brown fine sandstones of the Brownstones are abruptly and erosively overlain by pebbly sandstones or sandy conglomerates of a different shade of red. Since the Plateau Beds yield Upper Devonian faunas, and the Senni Beds a Lower Devonian vertebrate, such a break could mean the absence of a good deal, if not all, of the Middle Devonian.

That an angular discordance between the Plateau Beds and the Brownstones accompanies the lithological break noted can also be seen on the ground, and this important fact was first observed by Dr. Gwyn Thomas many years ago. Plateau Beds above Brownstones are exposed on an inaccessible rock-wall stretching for nearly two miles from Fan Hir (*SN* 833195) to Fan Foel (*SN* 821225) in the Black Mountain. The pebbly sandstones commencing the Plateau Beds form a long, unbroken crag. Sandstones in the Brownstones form less conspicuous crags supporting grassy ledges hiding siltstone ribs. From hills to the east the impression is gained that the Plateau Beds overlie the bevelled edges of sandstones making up the Brownstones. This impression is strikingly confirmed in fore-shortened views of the rock-wall obtainable from the hill-shoulder north-west of Blaen Car (*SN* 835194). The base of the Plateau Beds, tilted gently south, can be seen to transgress sandstone after sandstone in the Brownstones inclined southward a little more steeply.

Evidence for a stratigraphical break below the Grey Grits though not as striking is no less clear, and Strahan *et al.*[2] concluded that in the Black Mountain the Grey Grits overstepped the Plateau Beds westwards. Robertson[3], impressed by the supposed transition from the Brownstones (on his usage with Plateau Beds at the top) to the Grey Grits in the Abercriban Grit Quarry, thought overlap as likely. However, the supposed passage here is found to be illusory.

From the Brecon Beacons to the Black Mountain the Plateau Beds vary irregularly in thickness below the Grey Grits (Fig. 1), pinching out in the extreme west but maintaining over 18 miles a fairly constant development of pebbly rocks at the base. The lithological change from the one formation to the other is even more marked than that from the Brownstones to the Plateau Beds. In the Brecon Beacons and Fforest Fawr the uppermost Plateau Beds are red, fine-grained, silty sandstones and sandy siltstones with much flat- and ripple-bedding. The Grey Grits, in contrast, are pale, medium-grained, clean, pebbly sandstones or sandy conglomerates with a different suite of sedimentary structures. The contact of the two in the Twrch Fechan (*SN* 785208) is sharp, erosional, and without a trace of a passage. A similar contact is found in the Clydach (*SN* 742185), where pebbly, medium sandstones of the basal Plateau Beds lie beneath white conglomerates of the Grey Grits. Further west still undoubted Brownstones occur immediately beneath the Grey Grits. No trace of passage can be found between the Plateau Beds and Grey Grits, and despite the apparent conformity of bedding the field evidence definitely supports Strahan's[2] assertion of overstep by the Grey Grits.

Sedimentological evidence of age. As Strahan *et al.*[2] and Robertson[3] showed, the Grey Grits in the Fforest Fawr and adjoining areas graduate up into the Tournasian Lower Limestone Shales.

The Grey Grits in the same area show a continental facies, closely resembling modern stream channel deposits in grading and sedimentary structures. A similar con-

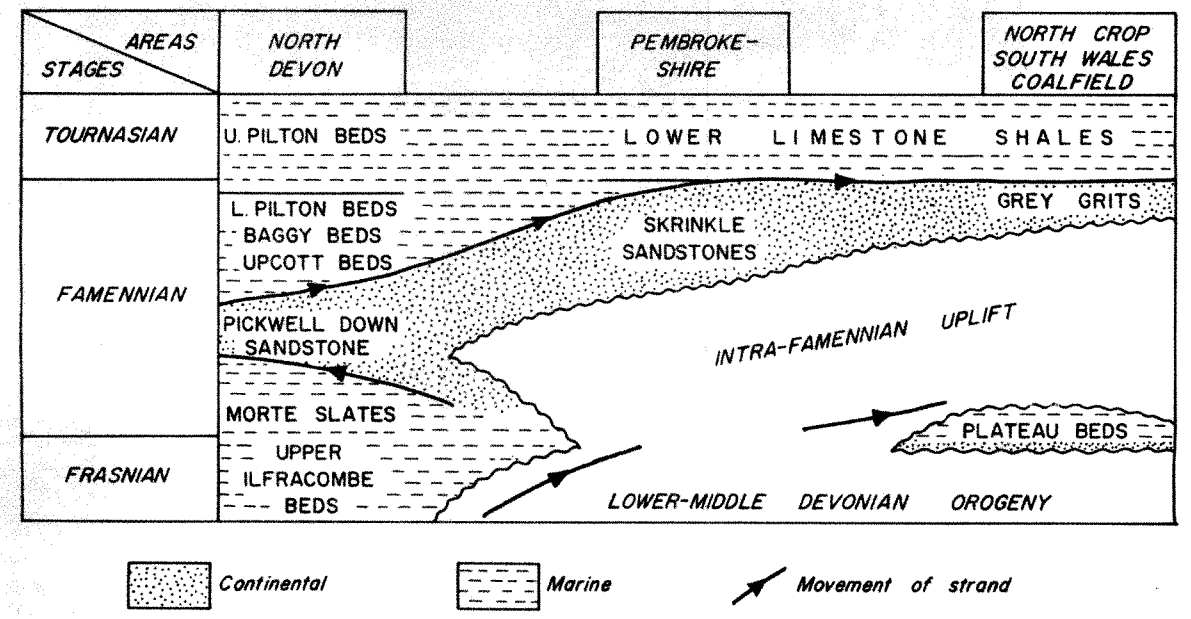


Fig. 2. Upper Devonian correlations in North Devon, Pembrokeshire, and the North Crop of the South Wales Coalfield, to show the probable position of the Plateau Beds

tinental facies—abounding in suncracks, ripple marks, and cross-bedding, and therefore also probably fluviatile—is represented in Pembrokeshire by the Skrinkle Sandstones lying conformably beneath the Lower Limestone Shales[8]. The Skrinkle Sandstones disconformably succeed the Ridgeway Conglomerates and Red Marls. Although because of Lower Carboniferous overlap a connexion between the two areas is not now visible, the Grey Grits and Skrinkle Sandstone must in part be of the same age. Not only do they agree in facies, pointing to an origin during the same depositional episode, but also both pass up into the Lower Limestone Shales.

The largely marine Plateau Beds, separated from the Grey Grits by a disconformity, therefore probably record an event previous to the deposition of the Skrinkle Sandstones. Simpson's[7] inclusion of the Plateau Beds in the same "Strunian" (that is, late Famennian) transgression as the marine, upper portion of the Skrinkle Sandstones[8] is not on these grounds acceptable. The evidence points to there having been two separate transgressions of the South Wales area during Upper Devonian times.

The age of the earlier Plateau Beds transgression hinges largely on a reading of the Upper Devonian sequence in South Wales and North Devon, particularly with regard to the succession and nature of events (Fig. 2). No decisive clue as to age is given by the Plateau Beds fauna, which is certainly Upper Devonian, but not so certainly Famennian.

In South Wales the angular discordance between the Plateau Beds and the Brownstones, and between the Skrinkle Sandstones and Ridgeway Conglomerates or Red Marls, manifests the Lower to Middle Devonian orogeny which strongly affected the more northerly parts of Wales[9]; and the Plateau Beds themselves a northward marine transgression from the Devonian trough in Cornubia. Southward retreat of the shore and uplift followed by erosion is implied by the relationship of the Grey Grits and Skrinkle Sandstones to the Ridgeway Conglomerates, Red Marls, Brownstones, and Plateau Beds. A later marine transgression, oldest in the south and west, is proved by the uppermost Skrinkle Sandstones and the Lower Limestone Shales.

In North Devon the Upper Devonian correlations shown in Fig. 2 are those of Goldring[10]. The Upper Ilfracombe Beds and the Morte Slates with *Cyrtospirifer verneuili* are marine, and a southward retreat of the strand is shown by their transition into the Pickwell Down Sandstone. This is of continental facies, possibly fluviatile, consisting largely of red and grey cross-bedded sandstones. The only fauna is of vertebrates reported as *Holonema*, *Bothriolepis*, *Holoptychius*, and *Polyplocodus*[11]. Following Goldring[10], "the succeeding strata tell an intriguing story of a deepening sea, harmonizing with the marine transgression to the north over the Old Red Sandstone continent".

In South Wales and North Devon, on the northern margin of the Cornubian trough, the sequence of environments represented in the Upper Devonian and earliest Lower Carboniferous strata is from shallow-marine through to continental reverting to shallow-marine. Correlating rocks of like environment to afford a rational picture of events, the Plateau Beds emerge as probably earlier than the Pickwell Down Sandstone, and such a correlation is not inconsistent with the faunas so far obtained from the Plateau Beds and Morte Slates. The Plateau Beds may therefore prove to be of late Frasnian or early Famennian age (Fig. 2).

The pre-Pickwell Down transgression may have entered areas other than South Wales, since at Wyboston, Bedfordshire, a sequence analogous to that of the Plateau Beds and Grey Grits was proved by boring. Upper Old Red Sandstone is reported to overlie marine Upper Devonian rocks unconformable on the Cambrian[12].

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LETTERS TO THE EDITOR

ASTROPHYSICS

Lithium in Capella and Convective Mixing in *F* and *G* Giants

On two spectrograms of α Aurigæ taken on August 25 and 26, 1964 (U.T.), we have found that the lithium line at λ6707·8 is much stronger in the *F*8 star than in the *G*5 component. Henceforth we will refer to the two components as Capella *F* and Capella *G*. One of the spectra is shown in Fig. 1, where lines of both components have been identified. A density tracing is shown in Fig. 2. In this communication we will first present evidence to show that our identification is correct and will derive rough abundances of lithium in the two stars. We will then discuss the implications of this difference with reference to stellar structure and evolution.

According to the orbit of Struve and Kilby[1] and of Wright[2] the radial velocity of Capella *F* was +55 km/sec and that of Capella *G* was +4 km/sec at the time of the observations. Our measurements of the broad feature yield a velocity of +59 km/sec if it is to be identified with λ6707·81 of lithium-7. If it were contributed by the *G* star, the laboratory wave-length would be λ6709·5, at which point we find no reasonable identification. Secondly, the width of the line matches the widths of the *F* star's lines rather than those of any single line in the *G* star[3].

Next we estimate the abundance of lithium in each star. The measured equivalent width of the lithium line contributed by the *F* star is 80 mÅ. Correcting for the fact that the *F* star contributes only 40 per cent of the light at λ6700, the true equivalent width is about 200 mÅ. If the star has the same effective temperature as the Sun, the lithium abundance is at least 100 times the solar lithium content. The exact value depends on the microturbulence and ratio of lithium-6/lithium-7, neither of which can be determined when the lines are broadened by the 10–12 km/sec rotation of Capella *F*. There is no lithium line in the *G* star. The feature shown in Figs. 1 and 2 is the unidentified line which appears in the Sun at λ6707·52. However, it provides a good limit on the minimum visibility of lines so as to estimate the upper limit of the lithium abundance in Capella *G*. We find its equivalent width to be 12 mÅ which is converted to 20 mÅ when account is taken of the fact that the *G* star contributes 60 per cent of the light. Using this as the upper limit on the equivalent width of the lithium line and using Bonsack's data for *G*8 giants[4], we find an upper limit on the lithium content in Capella *F* that is equal to the solar abundance.

We now ask why these two stars separated by about one astronomical unit should differ in their lithium content by a factor of more than a hundred. First, we point out two simple and probably incorrect explanations. The two stars could have formed from material of drastically

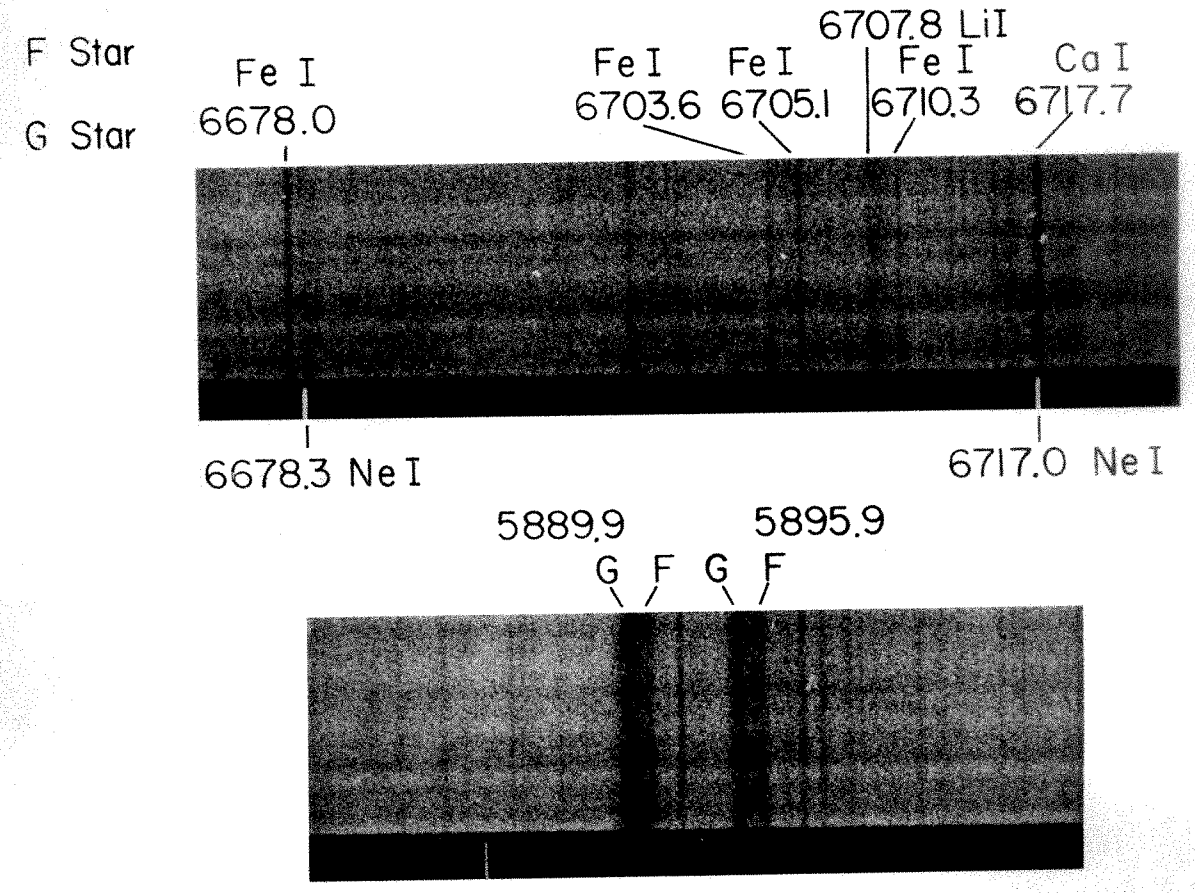


Fig. 1. The spectrum of α Aurigæ at the lithium line and the sodium *D* lines. Wave-lengths and identifications for lines contributed by both the *F* and *G* stars are shown. Two neon comparison lines are also shown in the upper spectrum

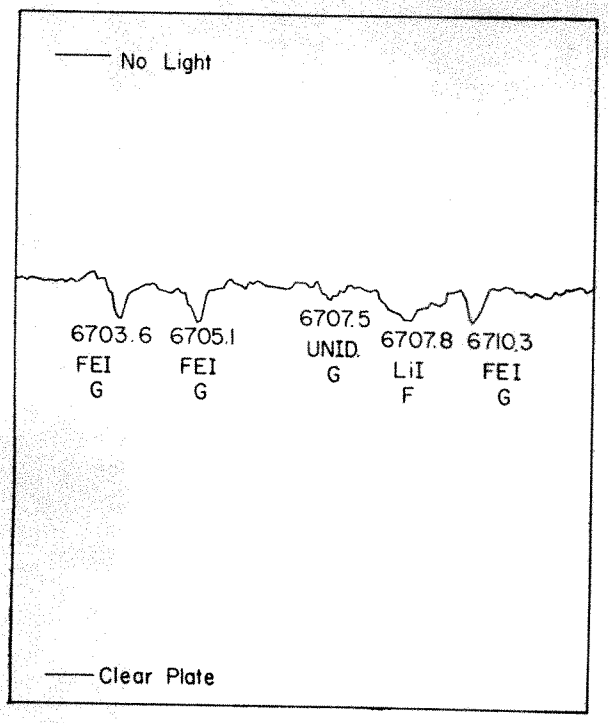


Fig. 2. A density tracing of the region near the lithium line

different chemical composition, or they could have produced different amounts of lithium on their surfaces by spallation reactions. It is difficult to imagine that two stars as close as the Capella pair could have originated from substantially different material. In such binary systems as have been examined, for example, γ Leo[5], 16 Cyg[6] as well as 3 *G* dwarfs and 4 yellow giants in the Hyades[7,8], no differences in chemical composition have been found. Differences in surface production (if that is indeed where the lithium was made) are more difficult to rule out, but we note that for 18 main sequence Hyades stars between types *F*0 and *G*0 the ratio of the greatest to the least lithium abundance is a factor of 6, some (but not all) of which may be accounted for as observational scatter[9].

Table 1

	Capella *G*	Capella *F*
Mass	3·03⊙	2·91⊙
Mv	0·12	0·37
Spectral type	*G*5 III	*F*8-*G*0 III
T_{eff}	4,650–5,000° K	5,300–5,800° K
Radius	14·1–12·2⊙	8·9–7·5⊙

If we are willing to accept that the two components of Capella had about the same lithium content when they were main sequence stars, then we are forced to the conclusion that Capella *G* has destroyed 99 per cent of its original lithium. We suggest that it has done so by convective mixing to regions at which the temperature is sufficiently high (2–3 × 10^6 °K) for the reaction $Li^7(p,\alpha)He^4$. The high lithium content of the main sequence stars in the Hyades[9] and the low lithium abundances in the Hyades *K* giants[4] indicate that a similar effect has occurred there. Thus we suggest that the two stars of Capella can be used as a test of the theories of stellar structure and especially of the theory of convection. The masses and absolute magnitudes are well determined but the effective temperatures are somewhat uncertain because of the uncertainty of the spectral types, especially of the secondary, and the calibration of the spectral type versus effective temperature relations[2,3,10]. The basic data for Capella *F* and *G*, taken largely from Wright's analysis[2], are presented in Table 1.

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RADIOPHYSICS

Signal Strength Variations of Satellite Signals

Recordings of the strength of the 54-Mc/s radio beacon transmission from the satellite 1961 o1, *Transit* 4*A*, have been made at Moore's Valley, Wellington, New Zealand (41° 17′ S., 174° 47′ E.), and at Campbell Island (52° 30′ S., 169° 0′ E.). The signals received on a horizontal half-wave dipole have often been seen to exhibit a quasi-periodic type of fading. This is in addition to the Faraday and scintillation effects normally observed.

The result of an analysis of this fading during seventeen daytime passes recorded at Moore's Valley in April–May 1964 is shown in Fig. 1. Only the near-overhead south-west to north-east passes received during this period have been used, as they give the most consistent recordings of the phenomenon.

The satellite radio signal does not rise appreciably above noise until the satellite zenith angle approaches 70°. At this point the signal suffers large sinusoidal variations in strength at a rate of about 1 cycle in 10 sec. This rate increases steadily as the satellite rises, and reaches a maximum rate of some 2 c/s about 1 min after the satellite crosses the zenith. At this point the amplitude of the fluctuations may be less than 5 per cent of the total signal. Then the rate falls as the satellite progresses northwards, in much the same way as it rose in the south. At Wellington the ray path to the satellite is almost parallel to the Earth's magnetic field lines at the time of maximum fading rate. This may indicate a dependence of the phenomenon on the field direction as well as on the satellite position relative to the observer.

Other variations of the effect are seen in some records, such as two components an octave apart, or two com-

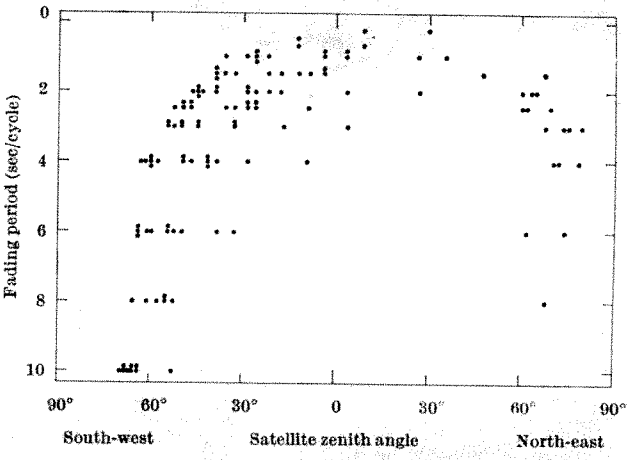


Fig. 1. Signal amplitude fading, 54 Mc/s for daytime overhead passes of Satellite *Transit* 4*A* observed at Moore's Valley, New Zealand, April–May 1964

ponents so close together in rate that beats are observed. There may be 1 beat in 20 sec between two components which have periods of some 2 sec.

The phenomenon is clearly distinguishable from the Faraday effect fading of some 40 sec period which is always seen. It also differs from the random period amplitude fluctuation or scintillation present in some records. This scintillation, with a mean period near 1 sec, is caused by modulation of the phase of the radio waves by irregularities in the ionosphere.

The frequency of the received signal is doppler-shifted due to the motion of the satellite; when it is first received it is about 1,200 c/s higher than the transmitted signal. It is observed to be falling at about 1 c/s/s; when the satellite crosses the zenith the rate of change of frequency is near 10 c/s/s, and for an instant the received frequency equals the transmitted frequency. The rate of change of frequency then decreases as the satellite recedes northwards from the zenith.

The variation with zenith angle of rate of signal fading may appear to be of similar form to the variation with zenith angle of the rate of change of frequency of the received 54 Mc/s wave, but there is no apparent reason for any causative relationship.

The results of a more detailed analysis of this type of quasi-periodic fading, using data from a comprehensive series of observations now in progress, will be reported at a later date.

I thank the Radiophysics Laboratory staff and the Campbell Island observer, Mr. N. McDonald, for their assistance.

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GEOPHYSICS

Palæomagnetic Data and du Toit's Reconstruction of Gondwanaland

In a recent article[1], I demonstrated how palæomagnetic data for South America, Africa, Australia, Europe and North America can be used to reconstruct the positions of these continents in the Palæozoic. Although there are no palæomagnetic data for Palæozoic rocks of India or Antarctica we can investigate whether Mesozoic poles for these continents, listed in Tables 1 and 2, are consistent with du Toit's reconstruction. So far as North America, South America, Europe and Africa are concerned, this is similar to that deduced from the palæomagnetic evidence.

The poles for India and Antarctica were plotted on the 50-cm globe[1] and then traced on to a transparent shell on which the continents had been reassembled as described by du Toit[2]. The polar wandering curves are shown in the oblique azimuthal projection in Fig. 1, of which the pole of the latitude–longitude grid is the point where the Australian, South American and African polar wandering curves separate in the Permo-Triassic (Fig. 2). In Fig. 2, on a similar projection, are drawn du Toit's Gondwanaland and the polar wandering curves for: (1) South America; (2) Africa; (3) Australia; (4) Europe and western Russia; (5) North America; (6) India and (7) Antarctica. The curve for the first 5 continents is the one previously described[1].

Table 1. Palæomagnetic Poles for India

Formation	Place	Pole position Lat.	Long.	Ref.
Rajmahal traps (Jurassic)	Rajmahal	15° S.	112° E.	3
Deccan traps (Cretaceous-Eocene)	Linga	37° S.	97° E.	4
	Khandala	25° S.	101° E.	4
	Nipani (Lr)	35° S.	85° E.	4
	Amba (Lr)	22° S.	103° E.	4
	Nipani (Ur)	52° S.	110° E.	4
	Amba (Ur)	52° S.	116° E.	4
	Pavagadh (Lr)	58° S.	89° E.	4
	Pavagadh (Ur)	75° S.	91° E.	4

Table 2. Palæomagnetic Poles for Antarctica

Formation	Locality	Pole position Lat.	Long.	Mean
Jurassic dolerite intrusions	Ferrar Glacier	58° S.	142° W.	55° S. 136° W.
	Wright Mts.	51° S.	132° W.	
	Theron Mts.	54° S.	136° W.	
Andean intrusive suite	Grahamland	86° S.	2° W.	89° S. 100° E.
Tertiary lavas enozoic volcanics	South Shetland Is.	82° S.	129° W.	
	Cape Hallett	81° S.	94° E.	

Taken from ref. 5.

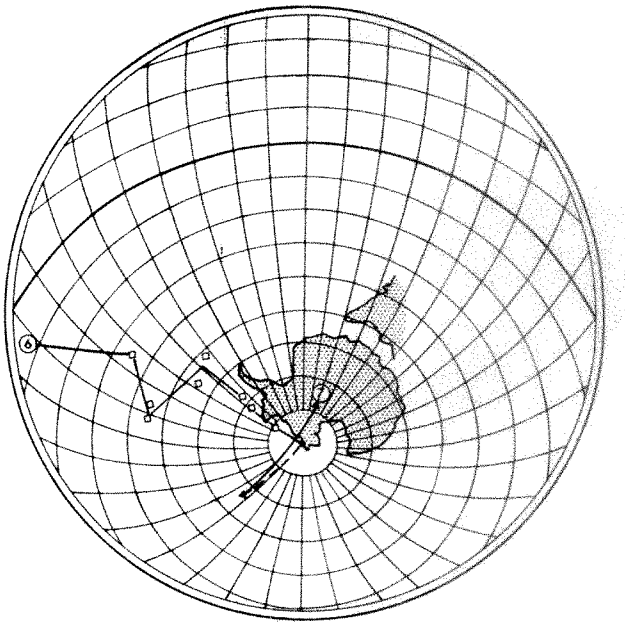


Fig. 1

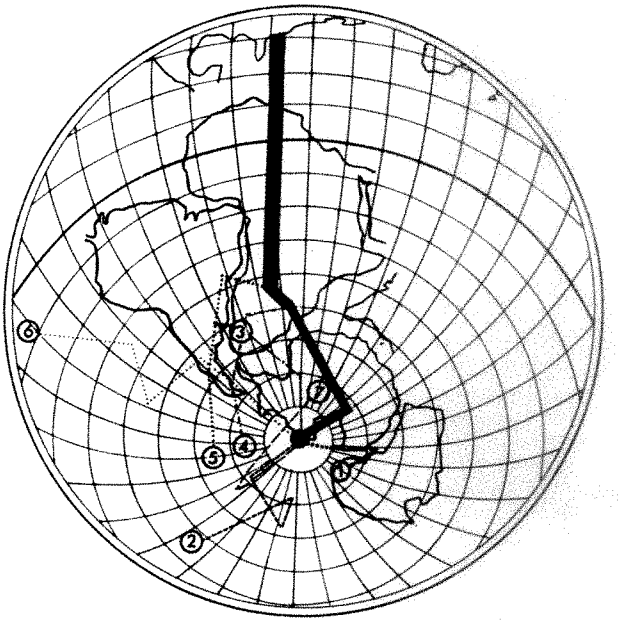


Fig. 2

With the continents so re-assembled we find that (i) the Indian polar wandering curve diverges from the Permo-Triassic point of the South American, African and Australian curves; (ii) the Jurassic pole for Antarctica is close to the Triassic pole on the African curve as though Antarctica and Africa remained together for a short while after South America and Australia had broken away.

One is led to predict that the Triassic pole for Antarctica will lie somewhere along the African curve between the pole of the projection of Fig. 2 and the Antarctic Jurassic pole; (iii) the Triassic poles for Australia and Africa do not coincide—hence these continents must have started to drift apart before the Triassic. There are no Triassic data yet for South America, India or Antarctica; (iv) the positions of all 5 Jurassic poles differ significantly from one another. Hence all five Gondwanic continents had started drifting by then; (v) the positions of the Permian poles for South America, Africa and Australia are not significantly different. There are no Permian data for India or Antarctica. The palæomagnetic data at present available are thus consistent with Gondwanaland having existed in the Permian.

Thus it appears that the Gondwanic continents started to drift apart in the Permo-Triassic. To elucidate in more detail how Gondwanaland broke up we shall have to wait for more comprehensive palæomagnetic data for the Mesozoic. Although those at present available for Australia[6] and Africa[7] are probably adequate, much more data are required for South America, Antarctica and India.

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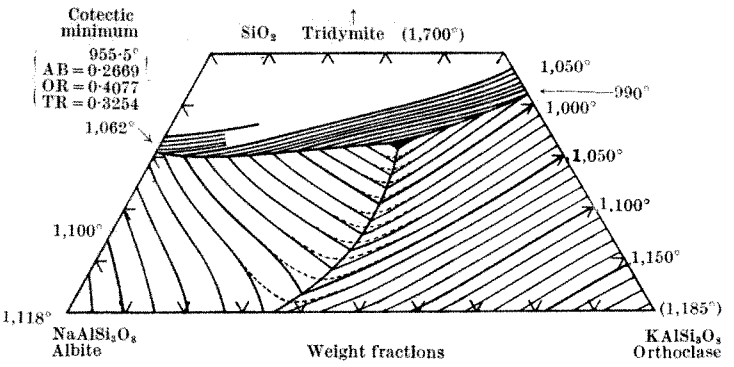


Fig. 1. System albite–orthoclase–tridymite: projected isothermal curves on liquidus surfaces, derived graphically from computed points. The broken curves are estimated positions of the continuous liquidus surface of the alkali feldspars, and the part of the orthoclase liquidus which is known to be metastable is not indicated

Computation of Liquidus Relationships in Multicomponent Silicate Systems

In a paper to be published elsewhere[1], algorithms and *Fortran II* programmes for computing liquidus temperatures and compositions in multicomponent ionic salt systems are discussed in some detail. The following is a preliminary report to the effect that simplified analogous programmes can be used for computing liquidus relationships in multicomponent silicate systems.

The present purpose is entirely practical, the goal being a system of available computer programmes for approximating liquidus relationships in complex systems when data for all the binary constituent systems are known.

We start with the ideal melting function:

$$\ln C = Q(1/T_m - 1/T)$$

in which T is a temperature (° K) on the liquidus of a component whose temperature of melting is T_m and heat of melting is Q, and C is its concentration in the liquid phase. A number of simplifying assumptions are made and the errors due to these and various kinds of non-ideality expected in silicate liquids are accumulated into a second-order polynomial expression for Q. Since it is difficult to retain conventions of molecular or ionic species in systems that involve phases with framework structures, C can be expressed in relative weight, instead of molar, units. Thus we select the following as empirical melting function:

$$\ln x = [a + b(1 - x) + c(1 - x)^2]\,[1/T_m - 1/T]$$

in which x is the weight fraction of a component in the liquid phase (at equilibrium with a crystalline phase in which x is the major component) and the parameters (a, b, c) are constants computed from experimental data on a specific binary system. For higher order systems, we compute the liquidus function of the ith component as follows:

$$\ln x_i = q_i[1/(T_m)_i - 1/T]$$

where:

$$q_i = 1 \Big/ \sum_{\substack{k=1 \\ k \neq i}}^{n} [g_k/(a_{i,k} + b_{i,k}h_k + c_{i,k}h^2_k)]$$

in which $(i = 1, n)$ is the index number of the principal component in the system, $(k = 1, n)$ is the index number of the additive components, $a_{i,k}$, $b_{i,k}$, $c_{i,k}$ are the individual parameters for best fit to liquidus data on the constituent binary systems,

$$g_k = x_k/(1 - x_i)$$

and

$$h_k = g^v_k(1 - x_i) + (1 - g^v_k)x_k$$

The exponent (v) of the weighting constant is included to allow arbitrary non-linearity of combination of the slope expressions: as v approaches zero, each unweighed slope expression approaches the form

$$a_{i,k} + b_{i,k}(1 - x_i) + c_{i,k}(1 - x_i)^2$$

and as v approaches infinity each approaches the form

$$a_{i,k} + b_{i,k}x_k + c_{i,k}x^2_k$$

Results of preliminary testing on data of real silicate systems do not indicate that the best value of v is not 1·0.

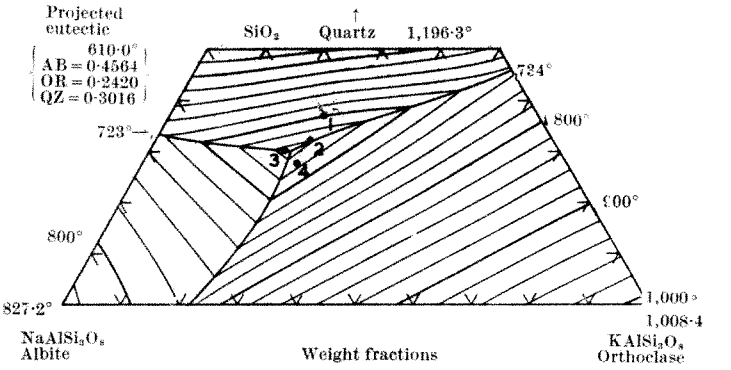


Fig. 2. System albite–orthoclase–quartz–water: projected isothermal curves on liquidus surfaces, derived graphically from points computed for constant water concentration, 0·0967, and constant pressure, 4,000 kg/cm². The numbered points represent published[2] experimental data for these conditions: (1) quartz liquidus at 705 ± 5°; (2) quartz or feldspar liquidus above 660°; (3) feldspar liquidus above 650°; (4) feldspar liquidus above 650°; eutectic below but near 650°. The liquidus surfaces of albite and orthoclase probably are continuous in part, but no estimate of this is shown

Another term which can be added to the net slope expression is one to allow for heat of mixing of components beyond the second:

$$\left(\sum_{k=1}^{n} x_k \ln x_k\right)\left((n-2)/n\right) \cdot T \cdot s$$

where T is the liquidus temperature and s is an arbitrary constant. Preliminary tests do not indicate that the best value of s is not zero.

Points of intersection of liquidus surfaces can be computed by a relatively simple iteration method given in more detail in the earlier paper. Programmes in *Fortran II* language have been written for computing and projecting liquidus points radially from all compositional vertices to one of the three-component planes. Thus the effect of components D, E, F, etc., on the temperature and composition of the eutectic between A, B and C can be investigated. Some of these programmes include the option of computing the temperature at 55 regularly-spaced compositional points on the liquidus of A, B and C at fixed composition of other components.

An example of the results of computations based on the foregoing method is the set of isobaric isotherms on the liquidus surfaces of the ternary system albite ($NaAlSi_3O_8$) –orthoclase ($KAlSi_3O_8$)–tridymite (SiO_2) (Fig. 1).

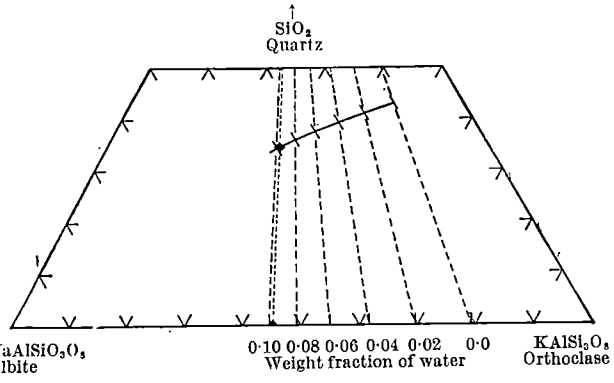


Fig. 3. System albite–orthoclase–quartz–anorthite–enstatite–Fe-pyroxene –Mn-pyroxene–alumina–magnetite–ilmenite–apatite–water: computed projected cotectic composition of the first three components at the indicated weight fractions of water, all other components being constant at normative values calculated from Daly's average composition of granite[3]; also a further projection of the computed cotectic points to the albite–orthoclase join. The normative composition of average granite projected in analogous manner (filled circle) lies very nearly on the curve of the computed cotectic points, and at 0·0970 water

An analogous example in the four-component system albite–orthoclase–quartz–water at a constant-weight fraction of water is shown in Fig. 2.

An example in a 12-component system, albite–orthoclase – quartz – anorthite – enstatite – ferro-pyroxene – mangano-pyroxene – corundum – magnetite – ilmenite – apatite–water, is shown in Fig. 3. In this, the components other than the first three and water were kept constant at values corresponding to those of the normative composition of average granite[3] and the cotectic composition with regard to the first three components was computed at six integral values of water concentration. The figure also shows the normative composition of average granite projected to the same plane. These results constitute a refinement of previous graphical approximation[4].

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PHYSICS

Influence of Structure on Mechanical Properties of Pyrolytic Carbon

Previous work on the mechanical properties of pyrolytic carbon has been confined to massive deposits laid down on stationary formers[1,2]. Such deposits have a limited range of structure. An alternative preparation technique, developed primarily for coating nuclear fuel particles, involves the use of a fluidized bed[3]. Recent work on pyrolytic carbon deposited in a fluidized bed at 1,200°–2,100° C has shown that a very wide range of structures can be prepared by varying the deposition conditions. In general, these deposits are structurally different from massive deposits. The purpose of this communication is to demonstrate how the mechanical properties of pyrolytic carbon deposited in a fluidized bed depend on its structure.

Five different structures were obtained in a 2·5-cm-diam. coater with a 15-g charge of spherical uranium dicarbide particles 150–250μ in diam. The total bed surface area was 400 cm². The gas composition was 11 per cent methane in helium (total pressure = 1 atm), and the bed temperatures were varied from 1,350° to 1,925° C. The average time spent in the bed by the fluidizing gas was about 0·1 sec. The mechanical property specimens were obtained from 100 to 150μ pyrolytic-carbon layers deposited on graphite disks 1/4 in. in diameter introduced into the fluidized bed together with the particle charge.

The structures of the deposits were characterized by measuring the apparent crystallite size, L_c, the density and the preferred orientation. L_c was calculated from the formula[4]:

$$L_c = \frac{0{\cdot}89\,\lambda}{\beta\cos\theta}$$

where λ is the wave-length of the radiation used, β is the half-height width of the (002) peak, and θ is the Bragg angle for the (002) reflexion. The preferred orientation was measured by the Bacon technique[5] and characterized by Bacon's 'anisotropy factor', which is the calculated ratio of thermal expansion coefficients in the parallel and transverse directions at 400° C. Room-temperature, 3-point bending tests on specimens 6 mm × 1 mm × 0·1 mm were used to determine the flexural Young's modulus and the maximum fibre stress and strain at fracture. Deformation was elastic to fracture, and the results are presented in Fig. 1.

There is a remarkable decrease in modulus as deposition temperature is increased. Part of this decrease may be explained by the change in anisotropy. The effect of anisotropy on the modulus was estimated by using the preferred orientation curves in conjunction with the 'Reuss averaging' approach[6]. Elastic constants for graphite crystals[7] were used. Details of the calculation will be published. In general, for anisotropic solids, the flexural modulus is not identical with Young's modulus, but in the present case the anisotropy of the material is too small for the discrepancy to be important. The calculated modulus values are shown in Fig. 1c. It is evident that the change in modulus with deposition temperature is only partially explained by the change in anisotropy, and the bulk of the change is due to other causes. Some preliminary annealing experiments on a 1,350° C deposit obtained in the same coater with a carrier gas containing 40 per cent methane have shown that the modulus can be reduced from $5{\cdot}1 \times 10^6$ to $2{\cdot}2 \times 10^6$ lb./in.² by annealing at 2,300° C for four hours. The annealing treatment caused no significant change in anisotropy but increased the L_c value significantly and, in addition, decreased the X-ray background intensity in the vicinity of the (002) reflexion. This suggests that the abnormally high modulus in low-temperature deposits is associated with their characteristically 'nongraphitic' structure. It is possible that cross-linking between the layer planes in-

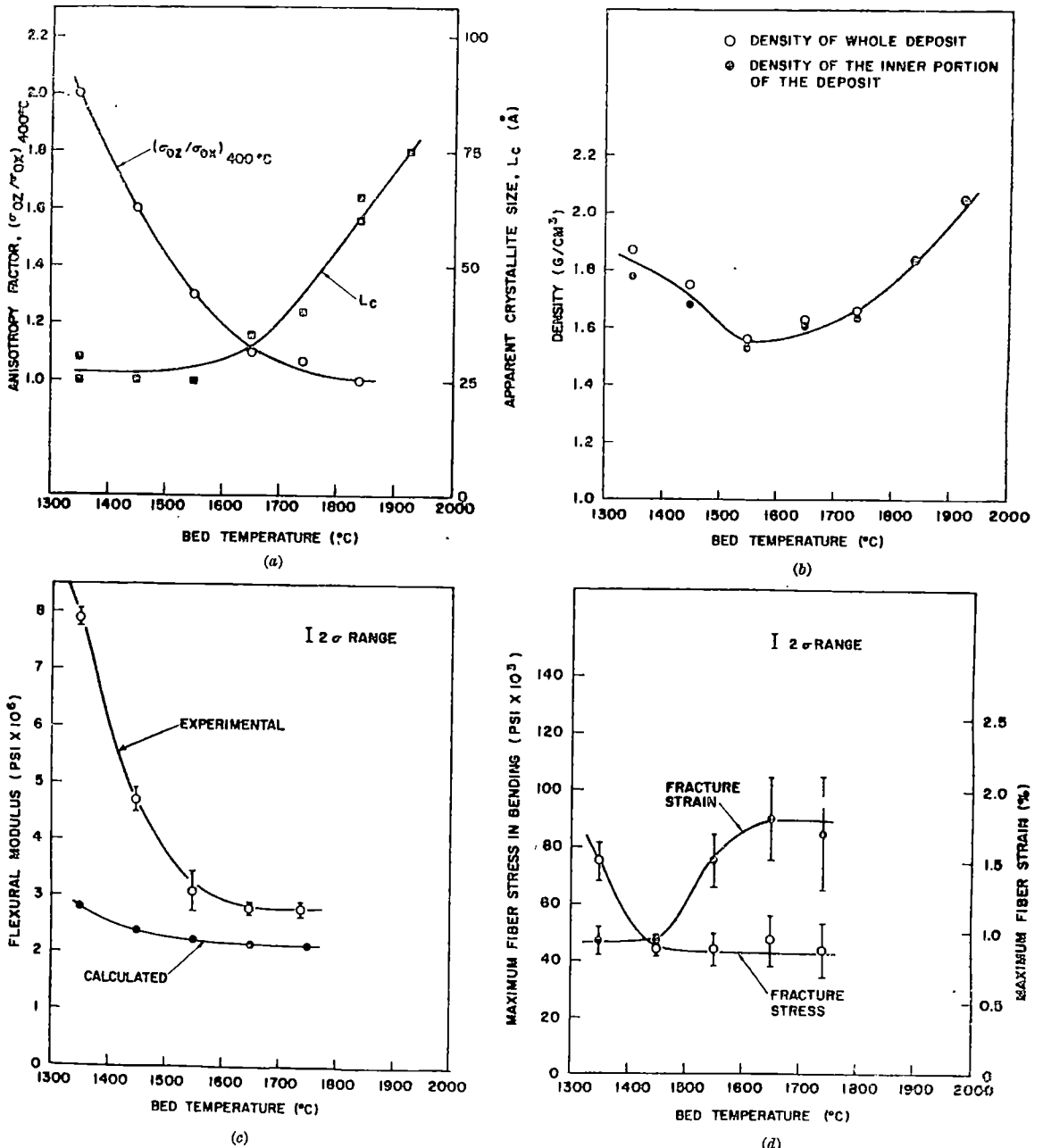


Fig. 1. Dependence of structural parameters and room-temperature mechanical properties on deposition temperature for pyrolytic carbon deposited in a fluidized bed: (*a*) anisotropy factor and apparent crystallite size, (*b*) density, (*c*) flexural modulus, and (*d*) maximum fibre stress and strain at fracture

creases the shear modulus within the crystallites to a value much higher than that of graphite.

The rapid decrease in the modulus, together with the slower decrease in fracture stress as the deposition temperature increases, results in a net rise in fracture strain as the bed temperature increases. Fracture strains of about 2 per cent were observed in material deposited above 1,600° C, which is about 10 times higher than that observed in conventional nuclear graphites. In addition, the fracture strain in the specimen deposited at 1,350° C and annealed at 2,300° C rose from 1·4 to 2·5 per cent on annealing, while the fracture stress fell from 77,000 lb./in.² to 56,000 lb./in.². These results show that more elastic strain can be accommodated before fracture in the high-temperature (or annealed) deposits than in the low-temperature deposits. This result has some practical importance in the design of structures (for example, nuclear-reactor fuel bodies) which utilize pyrolytic-carbon coatings.

This work was supported by U.S. Atomic Energy Commission contract *AT* (04-3)-167.

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Interpolation of Franck–Condon Factors

In recent years, much effort has been expended to provide arrays of Franck–Condon factors $q_{v'\,v''}$ for many molecular band systems, particularly those of astrophysical and aeronomical interest because of the rapid growth of quantitative spectroscopic activity in aerospace and related fields. Many different straightforward analytical and numerical methods have been used in the calculation of q-arrays of various sizes appropriate to a number of molecular potentials of which the Morse potential has been the most popular. The calculation of a $q_{v'\,v''}$ array is in principle a simple task, though often formidable if an electronic computer and appropriate programme are not available.

In spite of the activity, many band systems remain to be treated. Some potential users of $q_{v'\,v''}$ values whose need for the data are, for example, for definitive identification of bands or band systems, or for estimates of radiative heat transfer from hot gases, are often satisfied by order of magnitude estimates of $q_{v'\,v''}$. It would thus be valuable to have a simple means of making such estimates. To this end it is natural to consider whether some form of interpolation could be applied to the $q_{v'\,v''}$ arrays which have already been calculated. A transition parameter t which varies from band system to band system and which may be used for interpolation is therefore required in order that plots of $q_{v'\,v''}$ versus t can be made for each (v', v'') band of interest.

The steady and systematic change of Franck–Condon factor surfaces from a sharp diagonal ($v'=v''$) ridge to broad axially symmetric nested horseshoe-shaped ridges and valleys, as Δr_e (the change in equilibrium internuclear separation) steadily increases[1], implies that Δr_e will be incorporated in any transition parameter. $q_{v'\,v''}$ is the vibrational overlap integral square and is thus controlled by the extent of overlap Δr between the two potentials at v' and v'' respectively. If r_1 and r_2 are respectively the smaller and larger of the classical turning points of the molecular oscillator in the v'th vibrational level, Δr can take the four possible values $r_2''-r_1'$, $r_2'-r_1'$, $r_2''-r_1''$, $r_2'-r_1''$, depending upon the relative position and widths of the two potentials. A 'gross' Δr which might be applied to a whole band system (rather than just to the $v'-v''$ band) is proposed to be the overlap between the two potentials at, say, the D/K height of each, where D is the dissociation energy and $1/K$ is any fraction. A non-dimensional transition parameter $t=\Delta r_e/\Delta r$ is then proposed. It is relatively easy to show that for each of the above four values of Δr, and apart from an arbitrary constant involving the choice of K, that $\beta\ \Delta r_e$ is a very good approximation to t. β is the harmonic mean of the coefficients β' and β'' of $(r-r_e)$ in the two Morse potentials involved. (This is similar to the parameter $\bar{\beta}\ \Delta r_e$ which was suggested some years ago[2] on purely empirical grounds for interpolation of approximate q-arrays. $\bar{\beta}$ is the arithmetic mean of β' and β''.)

The usefulness for interpolation purposes of $\beta\ \Delta r_e$ as a transition parameter has been tested in plots of $\log q_{v'\,v''}$ versus $\log t$ for many (v', v'') combinations using $q_{v'\,v''}$ values taken from the 85 arrays of 'Morse' Franck–Condon factors ($0{\cdot}02 < t < 4{\cdot}7$) which have so far been computed to high quantum numbers using a straightforward method which has been described in detail elsewhere[3]. The plots are smooth and undulating with remarkably little scatter. If the plots are themselves arranged in a $v'-v''$ Deslandres array, it is observed that along the rows and columns $v_t=n$, $v_f=n$, $n+1\ldots$ (v_t or $v_f=v'$ or v'' respectively), each of the plots exhibits n maxima each of which moves to larger t as n increases. The maxima occur where a Condon locus[4] moves through (v', v'') as t increases. A provisional approximate q-array can be read from such plots for an uncomputed band system the transition parameter, t, of which has been evaluated.

This work was supported by research grants from the National Aeronautics and Space Administration (*NsG*–349) and U.S. Air Force Office of Scientific Research (*AF–AFOSR*–62–236 *A*) and by contract *AF* 19(628)–2820 with the Air Force Cambridge Research Center.

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GEOCHEMISTRY

Genetic Type of the Source Material for Common Leads

Recently the following formula was presumed[1,2] to hold in general between the concentration (C_x) of minor element X in a liquid and the corresponding concentration (C_x') in an infinitesimal solid just separated from the liquid:

$$\frac{C_x'}{C_x} = \text{constant} = k_x \qquad (1)$$

where k_x is defined as a partition coefficient of element X. Mathematical development of this general equation and application of its result to a triple combination of lead, thorium and uranium led to[3]:

$$\log(C_U/C_{Pb}) = b\log(C_{Th}/C_{Pb}) + \log(C_U^0/C_{Pb}^0) - b\log(C_{Th}^0/C_{Pb}^0) \qquad (2)$$

$$\log(C_U'/C_{Pb}') = b\log(C_{Th}'/C_{Pb}') + \log\{(C_U^0\,k_U)/(C_{Pb}^0\,k_{Pb})\} - b\log\{(C_{Th}^0\,k_{Th})/(C_{Pb}\,k_{Pb})\} \qquad (3)$$

and

$$b = (k_{Pb} - k_U)/(k_{Pb} - k_{Th}) \qquad (4)$$

where C^0 refers to concentration of subscripted element in the initial material for the Earth's oxide sphere. Equation (2) expresses the change of relative concentrations of the elements concerned in liquid, and equation (3) the corresponding change in solid, with the progress of crystallization of silicate melt. These equations indicate that the relative changes of logarithms of concentration in liquid and solid are expressed graphically by two straight lines running parallel with each other. In turn it is not only possible but also of much significance to decide whether a correlation observed for abundances of some elements represents liquid or solid.

The treatment of data on isotopic analyses of common leads in ores has brought about the relationship[1] written by:

$$\log(C_U/C_{204}) = 0{\cdot}503\log(C_{Th}/C_{204}) + 0{\cdot}231 \qquad (5)$$

where C_{204} stands for the concentration of lead 204, not of whole lead. It was pointed out[3] that in reducing the relevant chondritic abundances to equation (5) gave a result in agreement with the empirical equation (5), in fact suggesting that the source material for common leads is of a liquid type, not solid.

According to our model[1,2] based on the geochemistry of lanthanides, on the other hand the rock of the mantle as a whole is inferred to be a solid-type material which represents the aggregate of crystals segregated from the liquid. The partial melting of the mantle material can

produce a liquid-type material resembling the melt which would have been in equilibrium with the very mantle material of a solid type in the course of formation of mantle. Thus, so far as the directly observed concentrations of minor elements are concerned, we can explain the rise of liquid-type material in terms of the regeneration of liquid-type material from solid-type material by partial melting. However, it must be noted here that the abundance relation obtained from isotopic composition of lead cannot be affected by whether the material of source of lead encountered the partial melting or body melting, but indicates the relationship in the material of the source itself. In addition, it must be realized that the empirical equation (5) was obtained by assuming that the source material for common leads had been segregated and fixed at the time of formation of mantle ($4{\cdot}55 \times 10^9$ years ago). Therefore, the empirical formula implies that the liquid-type material has existed within the upper mantle almost since the formation of the mantle itself. This sequence of isotopes of lead may appear to be inconsistent with our model for mantle. However, an explanation compatible with our model is possible. That is to say, the problem can be solved by assuming that a minute fraction was isolated as liquid within the mantle and then frozen as pockets at or shortly after the time of formation of the upper proto-mantle, and that common leads originated from such a liquid-type relic material. Probably this material may be liquids occluded at the time of formation of upper mantle or those reproduced by partial after-melting shortly after the formation of bulk mantle. The latter possibility is preferable to the former with our model of the mantle, for it demands no modifications of the model.

Moreover, we cannot rule out the possibility that the present crust was generated secondarily as a residual liquid-type material through solidification of re-molten primary liquid-type material which could have thicknesses of, say, 100–400 km.

Moreover, as already stated here, it is essentially important to distinguish between solid type and liquid type for the genetic type of the natural material in hand.

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Composition of the Metallic Phases in some Stone and Stony-Iron Meteorites

Ten chondrites and two siderolites have been investigated, using electron-probe microanalysis to measure the concentration of nickel in the metallic phases. Information about the composition of the metal particles in chondrites is of value in deciding whether they were formed *in situ* or by the accumulation of fragments of large metallic bodies. Conventional chemical analysis is limited to determining the average composition of the metal, owing to the smallness of the particles. Ringwood[1] measured the lattice parameters of the kamacite (α-nickel–iron) and tænite (γ-nickel–iron) in a number of chondrites, and deduced compositions from these measurements. It is preferable to use a more direct method of obtaining compositional information: electron-probe microanalysis has the advantage for this purpose of combining reasonable precision ($\pm$ 1 per cent of the concentration measured) with high spatial resolution (about 1μ).

The results of the electron probe measurements are given in Table 1. The chondrites are listed in order of increasing fraction of metal present. They range from Dhurmsala with 3 per cent of metal[2] to Forest City with 24 per cent[3] and the average nickel content of the metal ranges from 22 per cent in the former to 6 per cent in the latter[4]. The classification used here is as given by Prior and Hey[4]. In general, the relative abundances of kamacite and tænite were found to follow the expected pattern, with the specimens at the top of the list containing mostly tænite with only a little kamacite, and the proportions reversed at the bottom. In some specimens (L'Aigle, Tabor, Limerick and Forest City) the individual kamacite grains were essentially homogeneous, while varying in composition from grain to grain. In Chantonnay the kamacite was homogeneous and did not vary between grains. In other cases the kamacite was appreciably inhomogeneous. The Dhurmsala specimen contained only one grain of kamacite. In Table 1 the kamacite compositions are given in the form of the range of nickel concentrations found. The tænite was generally highly inhomogeneous, as in the iron meteorites: the figures given in Table 1 represent the maximum nickel concentration in tænite in each specimen. In Pultusk and Mauerkirchen a few grains of a different form of tænite were found: these were much less variable in composition than the more usual variety and contained about 50 per cent nickel and 50 per cent iron. In grains as much as 50μ across, the nickel concentration varied only slightly, whereas most of the tænite was highly inhomogeneous over distances of the order of microns. In the two siderolites the variation in kamacite composition took the form of a reduction in the nickel concentration in the immediate vicinity of kamacite/tænite interfaces, similar to that observed in iron meteorites[5]. The distribution of nickel in tænite was found to be essentially the same in these specimens as in iron meteorites: the nickel concentration rises to a maximum at the interface with kamacite. The nickel concentrations in tænite in the siderolites given in Table 1 represent the maximum values obtained.

Table 1

Name	Type	Wt. % Ni in kamacite	Max. wt. % Ni in tænite
Dhurmsala	Intermediate hypersthene chondrite	4·9	48
Knyahinya	Polymict brecciated grey hypersthene chondrite	5·6–7·0	47
Mauerkirchen	White hypersthene chondrite	6·2–6·6	50
Chantonnay	Polymict brecciated grey hypersthene chondrite	7·2	37
Mooresfort	Veined grey chondrite	6·6–7·4	45
L'Aigle	Brecciated intermediate hypersthene chondrite	6·6–7·2	52
Pultusk	Veined grey hypersthene chondrite	6·4–6·8	50
Tabor	Brecciated spherical bronzite chondrite	6·6–7·5	44
Limerick	Veined grey bronzite chondrite	6·4–7·4	50
Forest City	Brecciated spherical bronzite chondrite	6·7–7·3	47
Estherville	Mesosiderite	4·9–7·4	55
Krasnojarsk	Pallasite	6·1–7·4	50

The concentrations of nickel in kamacite in the ten chondrites investigated varied from 4·9 to 7·5 per cent, with the siderolites falling within the same range. This is very similar to the range of compositions found in iron meteorites[6]. The observed tænite compositions are also comparable. Anders[7] has shown that the metal particles in chondrites probably developed their structures *in situ*, as the result of spending a long time in the $\alpha + \gamma$ region of the phase diagram, at temperatures more than 450° C. The results reported here are consistent with this interpretation. The observed kamacite and tænite compositions correspond to temperatures of about 350°–450° C, according to the iron–nickel phase diagram of Owen and Liu[8]. These figures are approximate only, in view of the unknown effect of co-existing troilite and silicates. The inhomogeneity of tænite indicates that cooling from higher temperatures occurred, the observed compositions being those reached at the 'freezing-in' temperature, below which no further diffusion could take place in the time available.

I thank Dr. J. V. P. Long for his advice and help, Dr. S. O. Agrell for supplying the specimens and Dr. G. F. Claringbull of the British Museum (Natural History) for supporting this work.

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CRYSTALLOGRAPHY

Crystal Structure of Lamprophyllite

A specimen of the mineral lamprophyllite (from Kola, the U.S.S.R.) at present being investigated is monoclinic, space group $C2/m$, with unit cell dimensions: $a = 19{\cdot}76$ Å, $b = 7{\cdot}06$ Å, $c = 5{\cdot}40$ Å, $\beta = 96{\cdot}5°$. The unit cell formula is: $4 \times (Ba,Sr,K)Na(Ti,Fe)TiSi_2(O,OH,F)_9$.

Using 174 $hk0$ reflexions, the structure has been determined in projection along the z-axis. The atom positions found are given in Table 1 and the projection along the z-axis is shown in Fig. 1. The short repeat distance of the z-axis enables the main features of the structure in three dimensions to be determined unambiguously.

The structure is closely related to those of the minerals seidozerite[1], rosenbuschite[2] and bafertisite[3]. The basic unit is an infinite sheet of cations, chiefly sodium and titanium, in octahedral co-ordination. To either side of this are attached sheets of silicon–oxygen tetrahedra and titanium–oxygen polyhedra arranged as shown in Fig. 2.

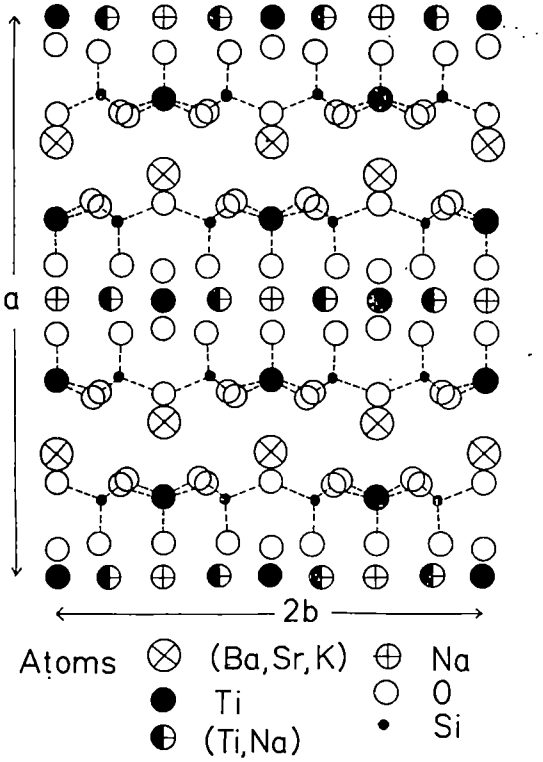


Fig. 1. Lamprophyllite projection along z-axis

Table 1. Atom Co-ordinates

Atom	x	y
(Ba, Sr, K)	0·217	0·000
Si	0·140	0·217
Ti	0·000	0·000
(Ti, Na)	0·000	0·239
Na	0·000	0·500
Ti	0·146	0·500
O	0·056	0·000
O	0·059	0·205
O	0·062	0·500
O	0·165	0·310
O	0·173	0·312
O	0·174	0·000

These composite sheets are separated by barium atoms. The titanium–oxygen polyhedra shown in Fig. 2 are square pyramids, the titanium being in five-fold co-ordination. In this respect lamprophyllite differs from the minerals mentioned above, in which the titanium, or zirconium in some cases, is in six-fold, octahedral, co-ordination.

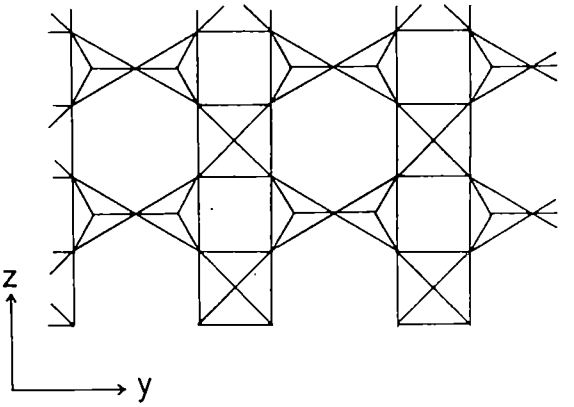


Fig. 2. Arrangement of SiO_4 and TiO_5 polyhedra

Work is in progress to determine directly a second projection of the structure.

This work was supported by a Department of Scientific and Industrial Research research studentship.

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CHEMISTRY

Aluminium Oxyfluoride and the Polymerized Species in AlOCl Solutions

Although the crystalline aluminium oxyhalides AlOCl, AlOBr and AlOI are known[1–3], the corresponding AlOF is not. We have tried to prepare AlOF by the procedure which is commonly used to prepare the other AlOX compounds, namely, the reaction of 3 AlF_3 with As_2O_3 in sealed evacuated glass ampoules at elevated temperatures. However, AlOF was not formed at temperatures up to 480° C, although reaction between AlF_3 and As_2O_3 did occur. Details of these experiments will be published elsewhere. Whereas AlOCl can be prepared by the reaction of steam and $AlCl_3$ (ref. 4), it does not appear that AlOF can be prepared by a comparable reaction with AlF_3. Schober and Thilo[5] found that AlF_3 reacts with steam at temperatures up to 600° C to form a product with a 5·2 per cent fluorine content, and lower fluorine contents at higher temperatures. They formulated their product as $Al_7O_{10}F$. Locsei[6] obtained similar results but preferred to formulate the product as a mixture of Al_2O_3 and AlOF. No reason

was given for the latter choice, but even if it were correct the product would be predominantly Al_2O_3.

We have approached the preparation of aluminium oxyfluoride differently, by the reaction of an aqueous solution of AlOCl with silver fluoride. However, as will be shown, the simple metathetical reaction AlOCl + AgF → AlOF + AgCl does not occur. Instead, equimolar amounts of AlF_3 and an oxyfluoride $Al_4O_5F_2$ are formed. We believe the seemingly unusual course of this reaction provides the answer to a long-standing problem as to the nature of the species present in aqueous AlOCl solutions. That AlOCl in water solution is not simply hydrated AlOCl or AlO^+ and Cl^- has long been known. Kohlschütter *et al.*[7] showed by freezing point depression, specific conductivity and potentiometric titration data that the Al(III) species in aqueous solutions of $Al(OH)_2Cl$ (or AlOCl) must be present in the form of polymerized ions. They predicted the presence of cations containing 3 or 4 aluminium atoms but were unable to deduce the specific formula.

A recent observation[1] that anhydrous AlOCl reacts readily with water at room temperature to form water-insoluble AlO(OH) and HCl is puzzling. Previous work indicates that solutions of $Al(OH)_2Cl$ are quite stable[7], and it is certainly reasonable to presume that solutions of AlOCl in water would be the same as those of $Al(OH)_2Cl$. We have verified this point as follows. Using the same method of preparing anhydrous AlOCl as that of ref. 1, we found that 0·2 M solutions of AlOCl in water are only slightly hydrolysed over the temperature range 25°–100° C. These solutions exhibited a drop in *p*H, compared to that of distilled water, of somewhat less than one-half of a *p*H unit. This corresponds to hydrolysis of AlOCl of the order of 0·1 per cent; insoluble AlO(OH) was not observed. The equivalence of solutions prepared from anhydrous AlOCl with those of $Al(OH)_2Cl$ prepared by other means was also shown. Freezing-point depression and conductivity data for the AlOCl solutions were similar to the data reported by Kohlschütter *et al.* Furthermore, they isolated a hydrate, formulated as $AlOCl{\cdot}2{\cdot}7H_2O$ from aqueous $Al(OH)_2Cl$ solutions by vacuum distillation, while we obtained $AlOCl{\cdot}2{\cdot}5H_2O$ from our solutions.

Aqueous solutions of AlOCl were prepared by adding distilled water to anhydrous AlOCl[1], and filtering off a small non-soluble residue. The concentrations of the latter solutions and of aqueous AgF were each determined quantitatively by precipitation of silver chloride. The reaction between equivalent amounts of aqueous solutions of AlOCl and AgF instantaneously produced a precipitate, which was filtered. Elemental analysis showed that the supernatant contained an F/Al ratio of precisely 3·0, indicating that it was a solution of AlF_3; this was verified by vacuum distillation of the supernatant and X-ray diffraction inspection of the residue. The precipitate was dried to constant weight at 150°–170° C. In addition to a quantitative amount of AgCl, it also contained Al and F, in amounts consistent with the formulation $Al_{4\cdot00}O_{5\cdot04}F_{1\cdot96}$, with the oxygen obtained by weight difference and 7 mg out of a residue of 1·539 g attributed to residual water. The observed ratio of AlOCl consumed to AlF_3 formed was 4·94, while the corresponding ratio for $Al_4O_5F_2$ was 5·10. Within experimental error this is consistent with equation (1). The absence of AlF_3 from the precipitate

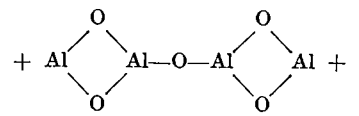
$$5\ AlOCl(aq) + 5\ AgF(aq) \rightarrow AlF_3(aq) + Al_4O_5F_2(s) + 5\ AgCl(s) \quad (1)$$

was evidenced by our inability to extract soluble fluoride from the precipitate despite several extractions with water, the fact that the supernatant contained only 0·00174 g AlF_3/g water, a value considerably below the reported solubility of AlF_3 (ref. 8), and the absence of AlF_3 from the X-ray diffraction spectrum of the precipitate. X-ray diffraction spectra of the latter were characteristic only of AgCl, the oxyfluoride producing no pattern.

$Al_4O_5F_2$ might be formulated as 2 AlOF + Al_2O_3, but this formulation would be inconsistent with the polymerized nature of AlOCl solutions. It would also require that three-fifths of the 5 AlOF initially formed somehow instantaneously disproportionates to AlF_3 + Al_2O_3 at room temperature. It is far more likely that $Al_4O_5F_2$ is a single compound, and that the formation of the latter and AlF_3 in equimolar amounts reflects the existence of the analogous chlorides in the original AlOCl solution. Thus the course of equation (1) suggests that AlOCl dissolves in water according to equation (2). Dissociation of $Al_4O_5Cl_2$ to $Al_4O_5^{+2}$ + 2 Cl^- not only produces a polymeric cation as predicted by Kohlschütter, but also provides the number of particles in solution required by the freezing-point depression and conductivity data. The data for 0·25 M solutions[7] require that each AlOCl unit in solution produce ~0·86 particles; $Al_4O_5^{+2}$ + 2 Cl^- + 1·13 particles per $AlCl_3$ (ref. 7) produces 0·83 particles per AlOCl unit. The agreement is also fairly good at 0·5 M, while the increase in freezing-point depression for 1-M solutions probably reflects an activity effect common for extensively hydrated salts in concentrated solution[9].

$$5\ AlOCl \xrightarrow{HOH} Al_4O_5Cl_2(aq) + AlCl_3(aq) \quad (2)$$

We postulate the following bridge-type structure for the $Al_4O_5^{+2}$ cation (not shown with water of hydration).



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Autoxidation of Carbanions in Azoxybenzene

The unique ability of dipolar solvents to promote carbanion reactions has been the subject of two recent reviews[1,2]. More specifically, the use of a dimethylsulphoxide (80 per cent)–*tert*-butyl alcohol mixture as a medium for the autoxidation of carbanions[3,4] and *N*,*N*-dialkyl amides as media for the autoxidation of mercaptide ions[5–7] has been demonstrated. Zaugg[8] has shown that pyridine-*N*-oxide (PNO) is capable of accelerating the alkylation of enolate anions and other carbanions by alkyl halides in inert solvents. In the present communication, we report the results of our investigations in which we have attempted to use compounds containing an N–O linkage as solvents for base-catalysed oxidation reactions.

All oxidation experiments were carried out in a closed system under one atmosphere pressure of oxygen. Initially, pyridine-*N*-oxide (PNO) and azoxybenzene (AOB) were subjected to stability studies in the presence of base and oxygen. At 80°, a solution of 100 mmoles of potassium

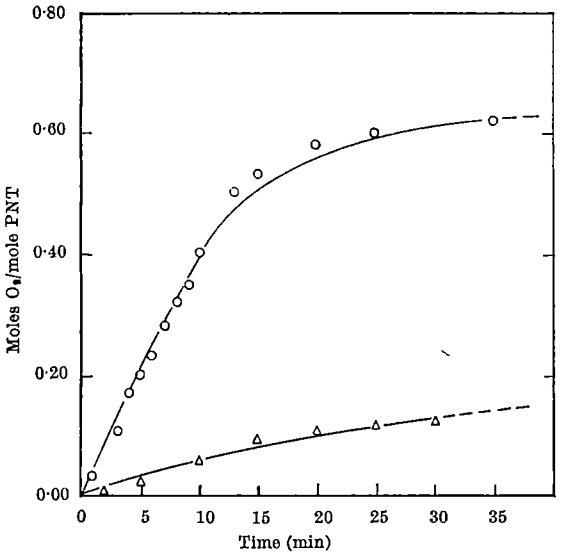


Fig. 1. Oxidation of *para*-nitrotoluene in azoxybenzene. ○, KOtBu −80°; △, KOH −110°

hydroxide in 75 ml. of PNO consumed 18·74 mmoles of oxygen in 68 h of reaction. Thus, it was concluded that PNO was not sufficiently stable to be used as a solvent for base-catalysed oxidations. On the other hand, AOB was found to be stable in the presence of oxygen and potassium hydroxide in the temperature region of 80°–110°. When a stronger base was used, AOB was also found to be highly stable. For example, a solution of 21 mmoles of potassium-*tert*-butoxide (KOtBu) in 150 ml. of AOB consumed only 0·84 mmole of oxygen in 25 h of reaction at 80°. These results prompted us to investigate some typical carbanion oxidations in AOB.

In our carbanion oxidation investigations, a two- to three-molar excess of base to hydrocarbon in 150 ml. of AOB was used. The rates of oxygen consumption were obtained by a wet-test meter and reactions were allowed to proceed until no apparent consumption of oxygen was observed[9]. Under these conditions, the oxidation of 12·5 mmole of *para*-nitrotoluene (PNT) at 80° in the presence of potassium-*tert*-butoxide (KOtBu) proceeded readily and gave essentially a quantitative yield of para-nitrobenzoic acid (1519, 1,710 cm^{-1}, m.p. 242°). Oxidation of PNT in the presence of potassium hydroxide was not possible at 80°. When the temperature was raised to 110°, a 38 per cent yield of *para*-nitrobenzoic acid was obtained. However, as shown in Fig. 1, the rate of oxygen consumption was considerably less than that observed with KOtBu at 80° (k(KOtBu-80°)/k(KOH-110°) = 7·2 based on initial rates). This reactivity sequence is consistent with an equilibrium reaction involving formation of the carbanion (equation 1) and subsequent rapid reaction of the anion with oxygen (equation 2).

$$p\text{-}NO_2\text{-}\varphi\text{-}CH_3 + RO^- \rightarrow p\text{-}NO_2\varphi\text{-}CH_2^- + ROH \quad (1)$$

$$p\text{-}NO_2\text{-}\varphi\ CH_2^- + 3/2\ O_2 \rightarrow p\text{-}NO_2\text{-}\varphi\text{-}COO^- + H_2O \quad (2)$$

The oxidation of other hydrocarbons in AOB has also been investigated. For example, the oxidation of 6·24 mmoles of diphenylmethane in KOtBu/AOB at 80° gave a 15 per cent yield of benzophenone in 21 h of reaction (KOtBu/DPM = 3). No attempt to determine the presence of a coupling product was made. In the presence of KOH, DPM could not be oxidized. Attempts to oxidize hydrocarbons less acidic than DPM in KOtBu/AOB were also unsuccessful. Thus, it would appear that the present system is capable of activating only relatively acidic hydrocarbons, for example the pK_a of PNT is about 20. Recently, we have demonstrated that toluene and ortho-xylene may be oxidized in a KOtBu-diphenylsulphoxide (DPSO) mixture at 100° (ref. 10). The dielectric constant of DPSO at 100° is about 16. At 100° the dielectric constant of AOB is about 4. Since DPSO and AOB have essentially the same stability in the presence of base and oxygen, the present results indicate that the dielectric constant of the solvent medium plays a key part in the initial ionization of the hydrocarbon.

Although no detailed mechanistic investigations were undertaken, it seems probable that the carbanion is initially autoxidized to an unstable peroxidic intermediate that later decomposes to a carbonyl derivative. Such a scheme has been proposed by Russell[11].

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Production of $C_2O_4^+$ in an Ion-molecule Reaction

In an investigation of ion-molecule reactions in carbon dioxide in a magnetic sector mass spectrometer we observed the occurrence of a peak at *m/e* 88. By recording the ionization efficiency curve with the aid of an *XY* plotter, the curve shown in Fig. 1 was obtained. The break in the curve at about 38 eV is to be expected from the production of CO_2^+ by a charge exchange collision in the analyser portion of the instrument, that is, by the reaction:

$$CO_2^{++} + CO_2 \rightarrow CO_2^+ + CO_2^+$$

The portion of the curve above 38 eV follows the ionization cross-section curve obtained for CO_2^{++} (*m/e* 22) in the same instrument.

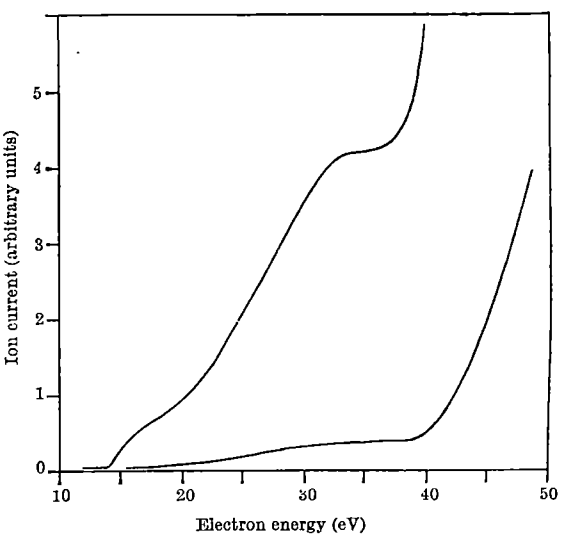


Fig. 1. Ionization efficiency curves for *m/e* 88. The upper curve was recorded with approximately 13 times the gain used for the lower curve

The portion of the curve in Fig. 1 from onset at 13·8 eV up to the break at about 38 eV was found to follow the ionization cross-section curve for CO_2^+ (*m/e* 44). With the use of an ionizing voltage of 35 eV, investigations of the effect of pressure of carbon dioxide in the ion source gave a curve for *m/e* 88 ion current versus pressure which was in excellent agreement with that expected for a three-body process. Variation of the repeller potential in the ion source indicated that, at an ionizing voltage of 35 eV, the ion current at *m/e* 88 behaved in a manner characteristic of an ion-molecule collision process, the current maximizing at a potential below that commonly observed for currents of primary ions. We conclude, then, that the ion current at *m/e* 88 observed in these experiments at ionizing voltages below the appearance potential of CO_2^{++} is due to the formation of $C_2O_4^+$ in the three body ion-molecule reaction:

$$CO_2^+ + 2CO_2 \rightarrow C_2O_4^+ + CO_2 \qquad (1)$$

We have estimated the gas pressure in the ion source by measuring the relative intensities of the CH_4^+ and CH_5^+ ions at several repeller field strengths, using published values[1] for the cross-sections of the reaction:

$$CH_4^+ + CH_4 \rightarrow CH_5^+ + CH_3 \qquad (2)$$

Pressure measurements obtained in this indirect way agree to within a factor of two with those estimated from consideration of the size of the leaks out of the source. In the work recorded here, the molecular number density in the source was about 7×10^{14} cm^{-3}. The rate constant for reaction (1) determined by the method described by Lampe, Franklin and Field[2] is found to be approximately 3×10^{-28} cm^6 sec^{-1} for primary ions of average energy 1 eV.

Polymeric species of carbon dioxide have previously been observed in experiments in which high-velocity streams of gas were sampled into a mass spectrometer[3,4], but these species were probably not the result of ion-molecule collisions. The ion $C_2O_4^+$ has been observed[5] in the negative glow region of a glow discharge in carbon dioxide at pressures of 0·1–0·6 torr. The present work suggests a probable source of this species. We have been unable to observe trimer ions of carbon dioxide in the pressure region up to 40μ.

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Soap Micelles at the Oil/Water Interface

It has recently been shown that unimolecular films of long-chain fatty acids—stearic, behenic, cerotic, etc.—in fact possess a micellar structure[1–3].

The films are deposited on glass or mica slides by dipping them into solutions of the acids in a variety of hydrocarbon solvents. Under the electron microscope the acid molecules appear to be grouped together in micelles, or islands, their thickness being about 20–30 Å, their shapes and diameters varying with the chain-length of the acid and the nature of the hydrocarbon solvent. This is thought to occur because the cross-sectional area of the carboxylic head groups is greater than that of the hydrocarbon tail groups. The latter become tilted from the vertical, the amount of tilt increasing with distance from the centre of the micelle. At the periphery the tilt is so great that it becomes energetically more practicable for additional molecules to form into a new micelle rather than join an existing one[4].

Table 1. Properties of Metal Soaps

Soap	Appearance	Analysis % Metal theory	found
Magnesium decanoate	White solid	6·63	6·60
Magnesium laurate	White powder	5·75	5·80
Magnesium stearate	White powder	4·11	4·24
Copper laurate	Light green powder	13·76	13·69
Copper stearate	Bluish-green powder	10·09	10·16

Table 2. Data at the Benzene/Water Interface at 30° C

Magnesium decanoate				Magnesium laurate			
mg $\times 10^4$	A m^{2}mg^{-1}	Π dyn cm^{-1}	ΠA m^{2}mg^{-1} dyn cm^{-1}	mg $\times 10^4$	A m^{2}mg^{-1}	Π dyn cm^{-1}	ΠA m^{2}mg^{-1} dyn cm^{-1}
2·69	6·44	0·09	0·58	3·54	4·89	0·09	0·44
3·21	5·40	0·15	0·81	5·67	3·06	0·15	0·46
3·64	4·76	0·21	1·00	6·40	2·71	0·21	0·57
4·33	4·00	0·24	0·96	6·69	2·59	0·19	0·49
4·53	3·83	0·30	1·15	7·05	2·46	0·24	0·59
				7·44	2·33	0·27	0·63
	$Y = 0·33$; $n = 20·_1$				$Y = 0·35$; $n = 16·_6$		
Magnesium stearate							
4·01	4·07	0·14	0·57				
5·45	3·18	0·22	0·70				
5·58	3·11	0·18	0·56				
5·88	2·95	0·20	0·59				
6·88	2·52	0·27	0·68				
7·19	2·41	0·29	0·70				
7·74	2·24	0·33	0·74				
	$Y = 0·40$; $n = 10·_3$						
Copper laurate				Copper stearate			
4·33	4·00	0·11	0·44	2·53	6·86	0·07	0·48
6·15	2·82	0·17	0·48	4·00	4·33	0·12	0·52
7·31	2·37	0·24	0·57	5·33	3·25	0·20	0·65
10·20	1·70	0·30	0·51	8·67	2·00	0·31	0·62
11·64	1·49	0·37	0·55	8·67	2·00	0·34	0·68
	$Y = 0·44$; $n = 12·_1$				$Y = 0·42$; $n = 9·_2$		

An investigation has been made recently into the properties of adsorbed films of heavy metal soaps at the benzene/water interface, using the Wilhelmy plate method. In some instances the films were formed *in situ*[5] by dissolving the appropriate fatty acid in benzene and spreading this on to an M/2,000 solution of the metallic chloride. In other cases, preformed soaps in solution in xylene were spread at the interface with the aid of small amounts of iso-propanol. Very pure materials were used (Table 1) and the results obtained are shown in Table 2.

If it is assumed that at the low concentrations used the soap films are gaseous, the equation of state relevant to X g molecules of the soap at 30° is[6]:

$$Y \equiv \operatorname{Lm}_{\Pi \rightarrow 0} \Pi A = XRT = 2{,}519X \times 10^7 \text{ ergs}$$

Expressing Π in dyn cm^{-1} and A in square metres of the interface occupied by 1 mg of the soap,

$$\text{or} \qquad X = 10^{-3}/M \qquad M = 2{,}519/Y$$

where M is the apparent molecular weight of the soap and Y is the intercept on the ordinate of the graph of ΠA versus Π.

It is seen that the apparent molecular weights of the soaps are greater than the formula molecular weights, the aggregation numbers, n, ranging from about 20 for the decanoate down to about 9 for the stearates.

Now it is well known that in organic solvents the soaps of the heavy metals remain in the micellar form down to extremely low concentrations[7,8]. The results recorded here indicate that they are also in the micellar form when spread as a monolayer at the oil/water interface. At this interface the soaps will be slightly ionized: hydroxyl groups will be expected to increase the effective cross-sectional area of the heads and thus favour tilting of the tails.

The number of molecules per micelle, and hence their size, decreases with increasing hydrocarbon chain-length. This is in agreement with the prediction[4] for acid micelles in insoluble monolayers.

It will be interesting to see whether a suitable electron-microscopic technique can be developed for confirming that, at the oil/water interface, these and other films also exist in the micellar form, their cross-sectional areas being between 200 and 400 Å based on the number of molecules per micelle.

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BIOPHYSICS

Diffusion Constants of Proteins determined by a Multi-unit Analytical Method

WHEN optical methods are not available, reliable diffusion constants may be obtained by techniques depending on the quantitative determination of the solute concentration in the diffusate such as the porous disk method of Northrop and Anson[1] (see also ref. 2). For these techniques a substance of known diffusion constant, established independently, is needed for calibration, which is a disadvantage.

An apparatus which requires no calibration and is easy to operate was described by Polson[3,4]. This is referred to as the multi-unit method and enables one to make observations at different time-intervals on the same substance, or different substances may be examined under identical conditions.

The apparatus consists of six independent units, each consisting of four stainless magnetic steel disks; a bottom fixed disk, a 2-cm, 1-cm and 0·5-cm section, the last acting as a lid. Each disk has six cylindrical holes arranged in a circular fashion which may be exactly aligned by proper rotation.

The sample to be examined is introduced into three alternate holes of the bottom disk, filling these completely, the holes in all four sections being in alignment. After filling these, the top three sections are rotated as a unit so that their cavities are shut off from the sample-containing disk. The dispersion medium is introduced into three alternate holes of the top unit filling the two central sections completely. After proper rotation of the lid to seal the units, the apparatus is submerged in a thermostatically controlled water bath to reach temperature equilibrium. The samples and dispersion medium may then be opposed by rotating the top three sections, including the lid, as a unit. The experiment is terminated by similar rotation of the sections at exactly timed intervals. The contents of the top section are withdrawn after adequate mixing and the concentration of the diffused substance determined by suitable means. In the case of proteins, absorption at 280 mμ and a modification of Weichselbaum's biuret procedure[5] have been found satisfactory.

The diffusion constant is calculated from the equation:

$$D = \left(\frac{C_t}{C_0} H\right)^2 \frac{\pi}{t}$$

(for its derivation see Polson[3]).

In this equation C_t/C_0 is the ratio between the concentration of the substance in the diffusate and in the original solution. H is the height of the column containing the diffusate and t is the duration of the experiment in seconds.

By plotting C_t against $\sqrt{t}$ a straight line passing through the origin is obtained. Fig. 1 represents the results obtained in an experiment on γ-globulin prepared by the polyethylene glycol procedure[6].

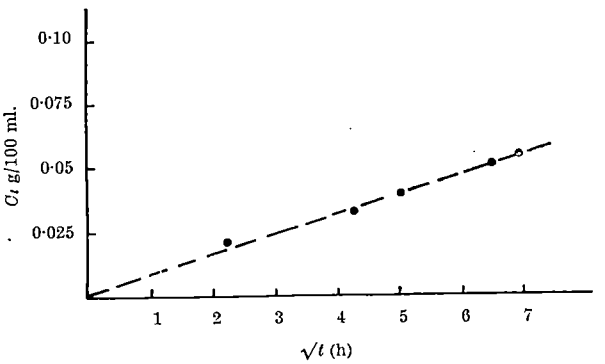


Fig. 1. Diffusion experiment on human γ-globulin. The concentration of protein in the diffusate is plotted against the square root of the time of diffusion $C_0 = 0{\cdot}963$ g/100 ml. Saline $T = 23{\cdot}5°$ C

In Table 1 the diffusion constants of several proteins as determined by the multi-unit technique and the well-known Lamm-scale method are compared. There is good agreement between the two sets of values although the latter method gave slightly higher figures because these were derived by extrapolation to infinite dilution.

Table 1. DIFFUSION CONSTANTS OF SOME PROTEINS AS DETERMINED BY THE MULTI-UNIT APPARATUS AND LAMM-SCALE METHOD

Protein	Concentrations in g/100 ml.	Multi-unit apparatus $D \times 10^7$ cm²/sec	Lamm-scale method $D \times 10^7$ cm²/sec	Ref.
Ovalbumin	0·8	7·60	7·63	8
Human hæmoglobin	1·0	6·60	6·90	8
γ-globulin	0·963	4·80	4·81	7
Jasus lalandii hæmocyanin	1·4	3·39	3·40	9

The discrepancy between the results obtained on γ-globulin and the figure of $4{\cdot}2 \times 10^{-7}$ cm²/sec usually adopted for this protein may be explained by the absence of macroglobulins in our preparations, as shown by ultracentrifugation[6,7].

This method has been used successfully in determinations of diffusion constants on unpurified samples of biologically active material such as the soluble antigens of viruses[10–12].

This investigation was supported in part by a U.S. Public Health Service research grant *AI* 04044–03 from the National Institutes of Health, Bethesda.

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BIOCHEMISTRY

Inhibition by Ouabain of Glycolysis in Ciliary Body

Much recent work has indicated that in tissues with a high secretory or nervous activity the rate of aerobic metabolism may be determined by the activity of the ion transport mechanism. This control is effected through the levels of ADP and ATP in the cell, which vary according to the activity of the sodium–potassium stimulated ATPase, the enzyme which governs sodium transport[1]. It has been shown that ouabain, a specific inhibitor of sodium–potassium ATPase, causes a 40 per cent decrease in the rate of oxygen uptake in kidney[2] and in liver[3] and a 30 per cent decrease in brain[4].

Schatzmann[5], using erythrocytes, had found no effect of ouabain on the glycolytic rate, but during the course of the present work Whittam[6] reported that lactate production by these cells is inhibited to the extent of 16 per cent by ouabain.

Ciliary body, which has a high rate of sodium transport[7] and a high sodium–potassium ATPase activity[8,9], exhibits a high rate of aerobic glycolysis in addition to its respiratory activity[10]. It has been found[11] that the oxygen uptake of the tissue is inhibited in the presence of ouabain by 64 per cent.

To investigate the effect of control of the transport mechanism on glycolysis, strips of pig ciliary body were incubated in Warburg flasks with the addition of ouabain to the medium or the replacement of sodium ions by *tris* or choline. Glucose and lactate were measured at the beginning and end of the experiment by the methods of Marks[12] and of Barker and Summerson[13], respectively.

The absence of sodium ions or the presence of ouabain had identical effects. Lactate production was inhibited 61 per cent when the atmosphere was 100 per cent oxygen and 45 per cent when the atmosphere was nitrogen. Glucose utilization was similarly reduced by 53 and 41 per cent. Under anaerobic conditions there was no appreciable change in the lactate : glucose ratio, 90–95 per cent of the glucose being converted directly to lactate. Under aerobic conditions, however, there was a fall in the ratio from 1·6 to 1·3 and preliminary experiments indicate that there is a relative increase in the amount of glucose metabolized via the pentose phosphate pathway although total oxygen uptake is decreased by 64 per cent.

It is of interest that the inhibition of glycolysis of the ciliary body should be so much greater than that found in erythrocytes, as these cells obtain their total energy requirement for transport from the glycolytic system. Further, the respiratory inhibition is greater than that found in brain, kidney or retina[11]. In the case of the respiratory inhibition it could be argued that in the ciliary body a greater fraction of the available energy supply is directed solely towards sodium transport, whereas in brain, kidney or retina other processes such as synthetic mechanisms are able to maintain a supply of ADP. The sodium–potassium ATPase activity of each of these tissues constitutes a relatively high fraction of 'total' ATPase[8], but under those conditions of assay the indirect sources of ADP were not measured. Thus, in the whole cell, inhibition of respiration would reflect the true fraction of the overall ADP supply which is determined by the sodium-transporting mechanism.

On the basis of the foregoing argument, inhibition of glycolysis in red cells would be expected to be higher than the 16 per cent reported by Whittam[6], as sodium–potassium ATPase comprises about 50 per cent of the total ATPase. However, although there will not be a large yield of ADP from synthetic processes, it is possible that hexokinase and glucose-6-phosphatase might provide an adequate turnover of ADP to relieve inhibition. Alternatively the high inhibition of glycolysis in ciliary body, especially under aerobic conditions, may be dependent on more complex relations with mitochondrial requirements for ADP.

Table 1. Inhibition of Glycolysis of Ciliary Body by Ouabain

	Glucose utilized (μmol./100 mg wet wt.)		Lactate produced (μmol./100 mg wet wt.)	
	Control	+ Ouabain	Control	+ Ouabain
Aerobic 100 per cent O_2 (8)	3·1 ± 0·5	1·5 ± 0·5	4·7 ± 0·9	1·8 ± 0·5
Anaerobic (5)	3·7 ± 0·3	2·2 ± 0·5	6·9 ± 0·6	3·9 ± 0·9

Flasks contained PO_4^{3-} 18 mM, Cl^- 117 mM, Mg^{2+} 1·2 mM, K^+ 30 mM, glucose 3·3 mM. Na^+ or *tris* was 117 mM. Ouabain 0·3 mM. Incubation was for 50 min at 37° C.

The inhibition of glycolysis is presumably effected at one or both of the two sites of phosphorylation, as Whittam has suggested[6], a decrease in the rate of hydrolysis of ATP resulting in a lack of ADP for the phosphokinase reactions. In ciliary body and retina (the only animal tissues, apart from tumour cells, that have a high aerobic glycolysis) transport controls, in part, the energy production from both processes. It thus appears that both glycolytic and mitochondrial ATP may be used as the substrate of the membrane ATPase.

This is supported by the fact that, although aqueous humour formation is oxygen-dependent, under anoxic conditions a fraction of the sodium transport is maintained[14].

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Amino-acid Composition of Prekeratin

In a previous communication[1] a new isolation technique was described by which pure prekeratin can be obtained from the epidermis of the cow's nose. For the extraction of the epidermis 0·1 M citric acid–sodium citrate buffer of *p*H 2·6 and ionic strength of 0·6 was used and prokeratin was isolated from the epidermal extract by serial precipitations in the *p*H range of 4·5–7·0. The prekeratin obtained by this method has a molecular weight of 640,000 and consists of highly asymmetric particles which reveal a typical α-keratin pattern in X-ray diffraction investigation. The chemical composition of this prekeratin was recently determined and the results are reported here.

Prekeratin contains 2·9 per cent lipid that is extractable in hot ethanol, and less than 0·1 per cent carbohydrate as measured by the orcinol test. Its amino-acid composition, as determined by automated columnar chromatography with the Technicon auto-analyser, is shown in Table 1. The amino-acid composition of three different preparations of prekeratin was examined after hydrolysis in 6 N hydrochloric acid at 110° C for 24, 48 and 72 h. Corrections for the destruction of threonine, serine and tyrosine were made by extrapolation to zero time of hydrolysis. The sulphur-containing amino-acids were determined by using performic acid oxidation prior to hydrolysis[2]. The tryptophan content was estimated by the spectroscopic method[3]. The total nitrogen content of the samples was 16·16 per cent as determined by the Kjeldahl method.

Table 1. Amino-acid Composition of Prekeratin

Total N 16·16%	Amino-acid g/100 g protein	Amino-acid residue g/100 g protein	Nitrogen as % of total nitrogen	No. of residues per protein molecule (m.w.: 640,000) Calculated	Nearest integer
Alanine	5·03	4·01	4·90	361·4	361
Arginine	10·54	9·64	17·33	320·2	320
Aspartic acid	10·00	8·65	6·51	481·4	481
Cystine 1/2	1·67	1·42	1·21	75·7	76
Glutamic acid	20·27	18·06	10·64	786·3	786
Glycine	10·59	8·05	12·23	903·8	904
Histidine	1·51	1·38	1·88	46·1	46
Isoleucine	4·57	3·94	3·01	223·1	223
Leucine	9·88	8·52	6·52	482·5	483
Lysine	7·87	7·09	7·46	275·5	276
Methionine	0·29	0·26	0·17	12·7	13
Phenylalanine	5·30	4·72	2·78	205·5	206
Proline	1·57	1·32	1·18	87·1	87
Serine	10·05	8·70	8·29	640·0	640
Threonine	3·92	3·33	2·86	211·0	211
Tryptophan	0·79	0·72	0·67	24·8	25
Tyrosine	3·36	3·03	1·60	119·0	119
Valine	5·06	4·28	3·74	276·7	277
Amide NH_3	1·79	—	9·10	—	—
Total	114·05	97·12	102·08	—	5,534

The amino-acid composition of this prekeratin differs from previously examined keratin[4] and prekeratin preparations[5]. Its most salient feature seems to be a low content of proline and sulphur-containing amino-acids. The low proline content would be expected because most α-proteins contain very small amounts of this amino-acid. The low content of sulphur-containing amino-acids, on the other hand, is unexpected and indicates that epidermal prekeratin may be stabilized only by a small number of disulphide cross-linkages. The calculations indicate that there are only about 76 half-cystine molecules in one prekeratin molecule (Table 1); this would account for only 38 disulphide linkages, which is a very small number for a large molecule with a molecular weight of 640,000.

This work was supported by U.S. Public Health Service research grant *AM* 05924 from the National Institute of Arthritis and Metabolic Diseases.

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Electrophoretic Behaviour of Serum Angiotensinase

When angiotensin is introduced into the circulation, it quickly disappears as a result of degradation by serum angiotensinase, and uptake by the tissues and degradation by their respective angiotensinase activities. To elucidate whether serum angiotensinase activity results from one single enzyme or from non-specific amino-peptidases, its electrophoretic behaviour was compared with that of five other amino-peptidases.

In a first group of experiments, serum was separated by micro-electrophoresis in agar gel according to Scheidegger[1]. 0·03 ml. of the serum was allowed to migrate at room temperature for 100 min in veronal buffer at *p*H 8·6, under a potential of 8 V/cm. The gel was cut into 15 strips, 0·5 cm wide, and each strip was eluted for 12 h at +4° C with 0·6 ml. of 0·9 per cent saline. The eluates, in 0·1-ml. fractions, were then incubated for 24 h at 37° C in 0·1 M phosphate buffer at *p*H 7·0 with amino-peptidase substrates at a concentration of $0{\cdot}35 \times 10^{-3}$; these substrates were: l-alanyl-β-naphthylamide-HCl (ABNA), l-cystine-di-β-naphthylamide (CYSBNA), l-glutamyl-β-naphthylamide-HCl (GLUNA), glycyl-β-naphthylamide-HCl (GLYNA), and l-leucyl-β-naphthylamide-HCl (LBNA). Liberated naphthylamide was determined by colorimetry according to Bratton and Marshall[2]. For measuring angiotensinase, valine-5-angiotensin II, aspartic-β-amide was incubated for 24 h with the eluates, at a concentration of $3{\cdot}9 \times 10^{-4}$; liberated amino-acids were separated by paper chromatography, and valine determined by densitometry according to Klaus *et al.*[3].

Table 1

	No. of cases	Substrate	Hydrolysis in % of the total activity of eluates α_1-	α_2-	β-	γ-globulin	Total activity of eluates	Activity in serum of origin (JE/ml.)
(1) Normal subjects	4	LBNA	88	12	—	—	25·0	29·4
		ABNA	88	12	—	—	35·2	42·6
		GLYBNA	87	13	—	—	11·8	15·1
		GLUBNA	90	10	—	—	8·7	11·4
		CYSBNA	—	—	—	—	—	0·6
		AT II	100	—	—	—	1·0	1·3
(2) Hepato-billary diseases	6	LBNA	69	21	2	8	122·0	134·1
		ABNA	71	19	2	8	158·0	171·1
		GLYBNA	74	20	2	4	50·3	58·1
		GLUBNA	74	19	2	5	51·8	60·7
		CYSBNA	81	19	—	—	3·4	4·1
		AT II	86	14	—	—	4·8	5·9
(3) Pregnancy ninth month	5	LBNA	84	12	1	3	190·0	211·6
		ABNA	84	11	1	5	157·0	168·1
		GLYBNA	87	10	1	2	60·0	69·4
		GLUBNA	89	5	1	5	53·2	63·8
		CYSBNA	86	14	—	—	6·1	6·8
		AT II	100	—	—	—	1·3	1·7

As shown in Table 1, total recovery from the eluates amounted to 70–80 per cent of original activity. Agar-gel microelectrophoresis gives a good separation of α_2-, β- and γ-globulin, but α_1-globulins migrate with albumin. In the sera of four normal subjects (Table 1 and Fig. 1), hydrolysis of ABNA, LBNA, GLUNA and GLYNA appeared as one broad peak in the α_1-region and extended slightly into the α_2-globulins. Angiotensinase was found only under the α_1-peak. CYSBNA hydrolysis was too low, in the sample, to be detected after electrophoresis. In the sera of six patients with hepatitis (Table 1, Fig. 2), aminopeptidase activity for all six substrates was increased. The highest peak of each of these activities migrated with the α_1-globulins; angiotensinase and CYSBNA-hydrolysis extended into the α_2-region; splitting of ABNA, GLUBNA, LBNA and GLYBNA was well extended into the α_2- and γ-globulin regions, and even detectable under the β-globulins. In the sera of five women in late pregnancy (Table 1) all aminopeptidases were increased except angiotensinase. Their electrophoretic distribution was similar to that of the hepatitis sera.

In a second series of experiments, plasma was separated by starch-block electrophoresis according to Kunkel and Wallenius[4]. Five ml. of plasma were dialysed and applied in a slit 20 cm long, and migration was carried out in 0·1 M veronal buffer at *p*H 8·6 at +4° C for 16 h under a potential of 5 V/cm. The block was cut into 25 strips of 1 cm × 20 cm, and eluted with 5 ml. of phosphate buffer 0·1 M at *p*H 7·4 over a fritted glass funnel. One

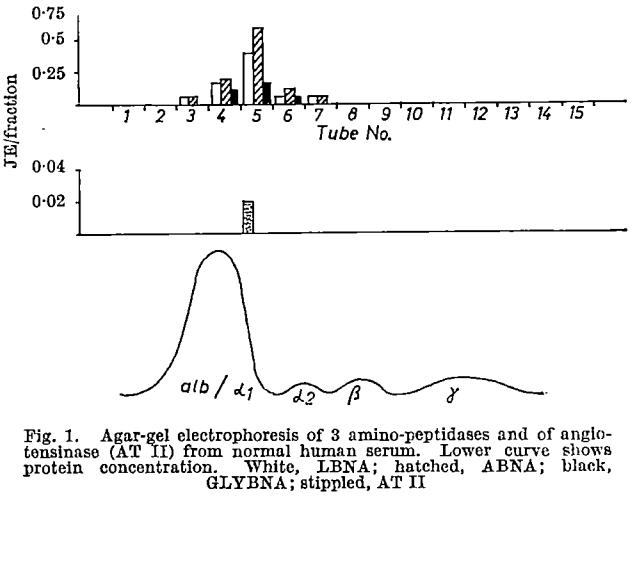


Fig. 1. Agar-gel electrophoresis of 3 amino-peptidases and of angiotensinase (AT II) from normal human serum. Lower curve shows protein concentration. White, LBNA; hatched, ABNA; black, GLYBNA; stippled, AT II

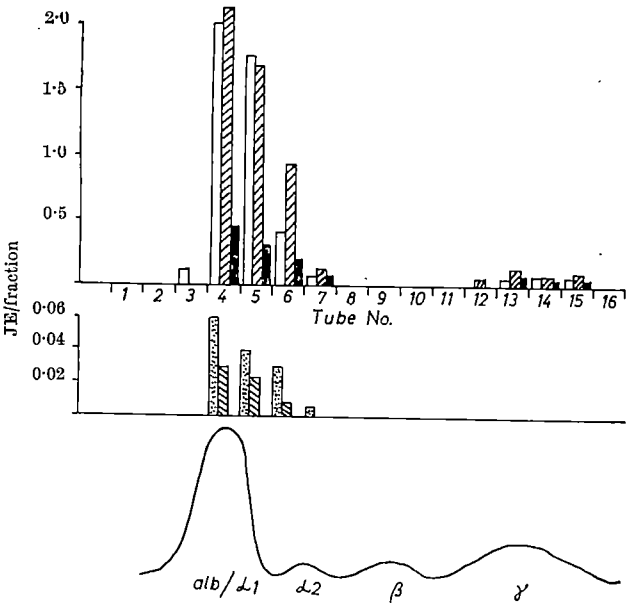


Fig. 2. Agar-gel electrophoresis of 4 aminopeptidases and of angiotensinase, from patients with acute hepatitis. White, LBNA; hatched (left up to right), ABNA; black, GLYBNA; stippled, AT II; hatched (left down to right), CYSBNA

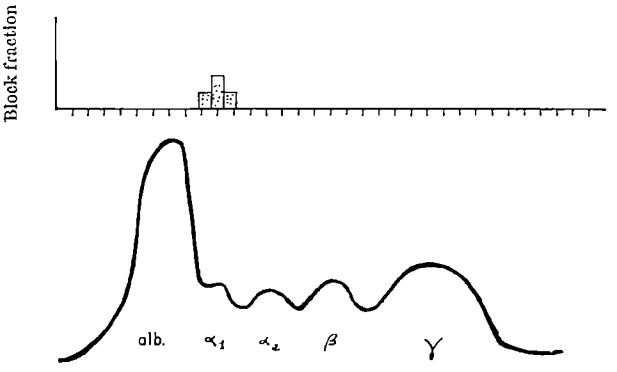


Fig. 3. Starch-block electrophoresis of angiotensinase activity from normal human plasma. Stippled, AT II

ml. of each eluate was incubated for 1 h at 37° C with angiotensin at a concentration of 1 μg/ml.; 2 min boiling stopped the reaction and residual pressor activity of the mixture was assayed in a rat preparation[5]. The results are shown in Fig. 3; in the plasma of four normal subjects, angiotensinase activity was detectable as a single peak in the α_1-region.

There is very little evidence, from the results obtained so far, for the existence of one specific angiotensinase. Although the maximum of the peak was consistently found with the α_1-globulins, activity became detectable in the α_2-region when there was a substantial increase in whole serum activity; this leads us to believe that much more sensitive methods could possibly have detected angiotensinase in other electrophoretic regions. The same reasoning may be applied to the hydrolysis of CYSBNA, the substrate used to determine cystine aminopeptidase or oxytocinase; it was also reported to migrate with the α_1- and α_2-globulins by Riad and Scandrett[6]. The main argument against one specific angiotensinase is that when it increased considerably during hepatitis, there was a comparable increase in all the other aminopeptidases in the same α_1-globulin peak. This main peak, formerly called leucine amino-peptidase, has been resolved by chromatography into several non-specific components by Behal *et al.*[7]. However, there is one of our observations which suggests that angiotensinase may differ somewhat from those other peptidases: the fact that during pregnancy they are all elevated except angiotensinase itself, which is thereby clearly distinguished from oxytocinase. It is interesting that, during pregnancy also, the splitting of LBNA and ABNA was preferentially increased over that of GLUBNA and GLYBNA; normal angiotensinase levels in pregnancy were reported before by Klaus *et al.*[3,8] and Landesman *et al.*[5].

The work of Nachlas *et al.*[9] and the results recorded here would seem to favour the existence, in the serum, of several non-specific amino-peptidases, overlapping in their amino-acid specificity and their electrophoretic mobility.

In normal serum, 2 LAP fractions have been reported to migrate in starch gel with the fast α_2-globulins[10,11] and in pregnancy and hepatobiliary disorders 3–7 additional bands have been described[10–14] in the slow α_2-, β- and γ-globulins. These data are very close to our findings with the substrates LBNA, ABNA, GLYBNA and GLUBNA, normal serum having activity mainly in the α_1-fraction and slightly in the α_2-, and hepatitis and pregnancy sera presenting additional peaks under the β- and γ-globulin areas. Identical results were reported on cellulose acetate by Rutenburg *et al.*[15]. It is conceivable that the β- and γ-globulin portions of these amino-peptidases exist in trace amounts in normal serum but escape detection by our methods; thus Behal *et al.*[16] could detect 4 LAP fractions in normal serum, using DEAE cellulose which can separate greater amounts of serum; in hepatobiliary disease, the increase of these four fractions was not accompanied by the appearance of new bands.

This work was supported in part by grant *HE*–07473 from the U.S. National Institutes of Health, U.S. Public Health Service. We also thank Prof. R. M. Archibald for providing laboratory facilities.

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A New Variant of Transferrin from Greece

The transferrins are a group of iron-binding glycoproteins under genetic control which migrate in the β-globulin region on starch-gel electrophoresis[1]. Commonly there is a single iron-binding band called transferrin *C*, but faster (*B* variants) and slower (*D* variants) migrating iron-binding bands are occasionally present. Sixteen human transferrin variants have been described, which are controlled by the expression of codominant alleles at a single autosomal locus[2–6].

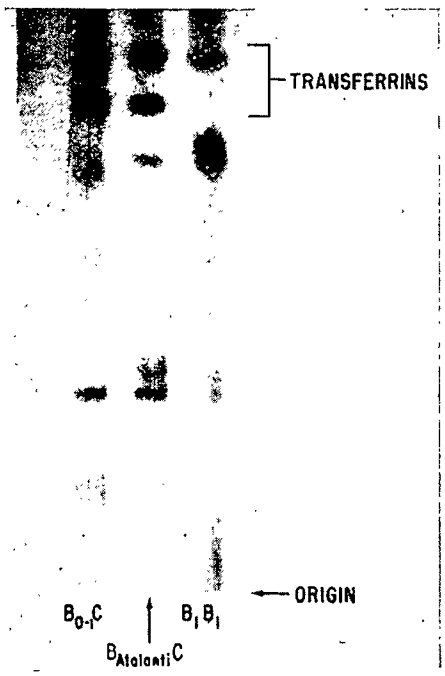


Fig. 1. Starch gel stained with amido black demonstrating that migration of $B_{Atalanti}$ is intermediate between B_{0-1} and B_1

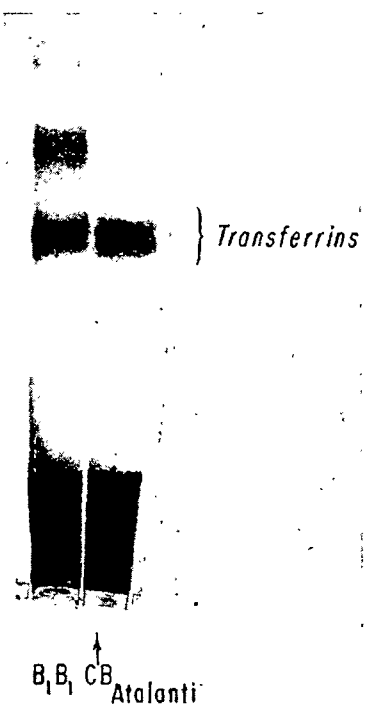


Fig. 2. Five per cent polyacrylamide gel stained with amido black demonstrating further that migration of $B_{Atalanti}$ is faster than B_1

This communication describes a new fast-moving *B* variant discovered during a survey of 709 specimens from ten Greek communities[7,8]. The variant was identified in the serum of a healthy 12-year-old school-boy from the city of Atalanti in Thessaly in Continental Greece and has been designated $B_{Atalanti}$. Its migration is slower than B_{0-1} and slightly faster than B_1 (Fig. 1). This mobility difference was repeatedly demonstrated on two separate serum specimens obtained from the propositus two years apart. The separation of the *B* variants seemed most distinct on vertical starch-gel electrophoresis at 4° C in borate buffer at *p*H 8·0 for 17–19 h at 9 V/cm. Under these conditions the *C* transferrin band migrated 5–5·5 in. from the origin. The mobility difference was also demonstrated on vertical electrophoresis at *p*H 9·0 in 5 per cent polyacrylamide gel (Fig. 2).

The propositus's father is phenotype transferrin *C*. Serum from his mother and sibs could not be obtained.

We wish to thank Dr. E. Giblett for providing the specimens of transferrin B_1B_1 used for comparison.

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Origin of Urinary Resorcinol Sulphate

SOME human subjects have been shown to excrete large amounts of urinary resorcinol monosulphate ester[1]. This appears on paper chromatograms as a magenta spot after spraying with 2 per cent *p*-dimethylaminobenzaldehyde in 5 per cent v/v concentrated hydrochloric acid and heating at 55° C for 12–15 min. (The sensitivity of resorcinol sulphate to this reagent at room temperature is much lower and the resultant colour is grey.) The spot runs rather more slowly than indoxyl sulphate in isopropanol–0·880 ammonia–water ; 20 : 1 : 2 (R_F 0·50, 0·60 respectively), and also in *n*-butanol–acetic acid–water ; 12 : 3 : 5 (R_F 0·42, 0·46).

Meta-dihydroxy benzenoid compounds are unknown in human endogenous metabolism but common in the plant kingdom. Tea is the main dietary source in Great Britain of *m*-dihydroxy compounds, which could give rise to resorcinol *in vivo*. When tea was withheld from the two heavy excretors of resorcinol sulphate found in the original investigation, urinary excretion of this substance decreased to a trace or was undetectable after three days, reappearing as an intense spot on chromatograms after re-introduction of tea to the diet. Another subject who did not normally excrete resorcinol sulphate did not excrete it after drinking 15 cups of strong tea over a period of eight hours or after 10 cups of tea/day for five days. (The urinary chromogen derived from tea described by Fearon and Boggust[2] was excreted in large amount.) Thus urinary resorcinol sulphate appears to be a product of the metabolism of a tea constituent or constituents by certain subjects.

When one of the subjects (a schizophrenic in-patient) was re-investigated after an interval of six years he was found to be still excreting large amounts of resorcinol sulphate. On administration of 500 mg three times a day by mouth of the intestinal antibiotic framycetin (soframycin) together with his usual intake of tea, urinary resorcinol sulphate declined and was no longer detectable after six days. Thus resorcinol, like certain other aromatic constituents of urine including catechol[3] and *m*-hydroxy aromatic acids[4], is formed *in vivo* by intestinal bacteria, both the *m*-hydroxy acids and resorcinol being derived from dietary vegetable matter. Urinary indoxyl sulphate excretion, which gives some measure of the bacterial contribution to the urinary excretion of aromatic meta-

bolites in general[5], declined from 17·7 mg potassium indoxyl sulphate/g creatinine before antibiotic treatment to < 1·0 mg/g after six days treatment with framycetin. (The method of Curzon and Walsh[6] was used.)

Eight weeks after the termination of antibiotic treatment it was found that though 14·5 mg potassium indoxyl sulphate/g creatinine were excreted, resorcinol sulphate was not detectable. Similar observations were made on a urine sample collected 21 months later. Thus, it is suggested that urinary resorcinol sulphate originates from the action on dietary tea polyphenols of particular intestinal bacteria. Though when once established these may persist indefinitely, antibiotic treatment may result in their permanent disappearance. Chromatograms of urines of the father and brother of the subject who was investigated in detail also showed a significant resorcinol sulphate spot. This indicates that there may have been an initial infection of the family group when it was intact.

These findings illustrate that if it is suspected that a constituent of the urine of a particular patient is of dietary origin, the finding that other subjects on similar diets do not excrete it is not necessarily evidence in favour of an endogenous origin as qualitative intestinal bacterial differences may be involved.

We thank the staff of Friern Hospital for their co-operation and also the Joint Research Advisory Committee of the Institute of Neurology who financed some of this work.

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PHYSIOLOGY

Effect of Amphetamine on Spinal Reflexes

L-Dopa (L-3,4-dihydroxyphenylalanine), precursor of catecholamines (adrenaline, noradrenaline and dopamine)[1], and DL-5-hydroxytryptophan (5-HTP)[2], precursor of 5-hydroxytryptamine, have recently been found to influence spinal reflexes. These drugs applied intravenously cause a pronounced increase of the flexor reflex evoked by pinching the skin. An electrophysiological investigation revealed that these drugs depress the short latency transmission of single volleys from the flexor reflex afferents (FRA, that is, Group II and III muscle afferents, cutaneous and high threshold joint afferents) to motoneurones, primary afferents and ascending pathways[3]. The depression of this short-latency FRA pathway accounts for the disinhibition of the long-latency pathway from the FRA to flexor motoneurones and I*a* primary afferents[4,5]. It has been suggested that the effect of L-DOPA and at least in part of 5-HTP is accounted for by increased synthesis and release of monoamines at the monoaminergic synaptic terminals in the spinal cord.

It was a matter of interest to ascertain whether drugs with analogous central effects act on spinal reflexes in a similar way. Amphetamine (*d,l*,β-phenyl-isopropylamine), well known for its central stimulant effects injected intravenously (3–6 mg/kg), has been found to give a pronounced increase of the flexor reflex evoked by pinching the skin in acute spinal cats. The effect appears within 2–3 min, reaches its maximum in 10 min and lasts for about 3–4 h.

'Amphetaminum sulphuricum' (Psychoton-Spofa) has been used in these and subsequent experiments which were carried out in an attempt to analyse further the mechanism of its action.

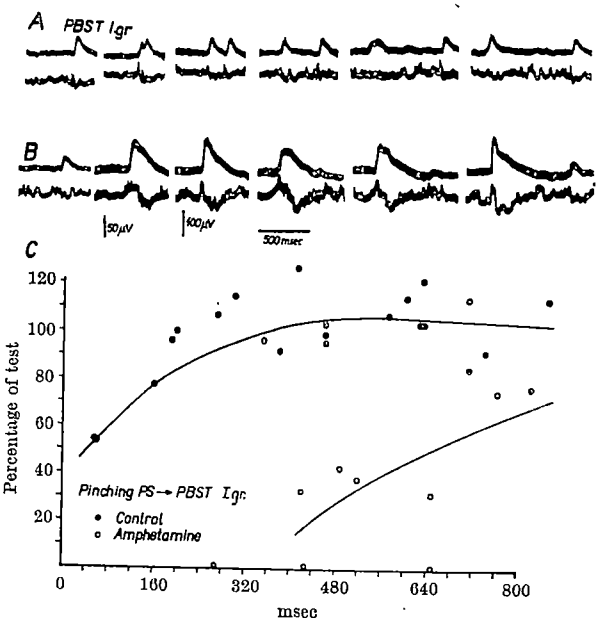


Fig. 1. *A* shows the DRPs evoked by a train of impulses of Group I from the posterior biceps-semitendinosus (PBST) conditioned by pinching the skin in the region of the superficial peroneal nerve (SP) at different intervals, the first record being the test response. *B* shows the effect of 3 mg/kg 'Amphetaminum sulphuricum', intravenous. The time course of the depression is shown in *C*. Calibration 50 μV relates to the upper and 100 μV to the lower beam

In acute spinal cats amphetamine inhibits short-latency transmission from the FRA to primary afferents to ascending pathways and to motoneurones. When this depression is very profound, long-latency discharges in flexor motoneurones with corresponding long-latency PRPs (dorsal root potentials) usually occur to short trains of stimuli. This action can be compared with that of L-DOPA and 5-HTP.

In an attempt to analyse the mechanism by which amphetamine increases the flexor reflex at the time when the short-latency FRA reflex action is depressed the action of amphetamine on spinal reflexes evoked by pinching the skin was examined. Amphetamine was constantly found to prolong the duration of reflex discharges in flexor motoneurones and to increase and prolong the duration of the DRP evoked by pinching the skin. Fig. 1 demonstrates the interaction between DRP evoked by pinching the skin and DRP evoked by a short train of stimuli from Group I primary afferents of the flexor nerve to the biceps posterior and semitendinosus (PBST) at different time-intervals (*A*) before and (*B*) after administration of 3 mg of amphetamine. In (*C*) the depression of the amplitude of Group I DRP evoked by a short train of stimuli to the PBST nerve is expressed in percentage and has been plotted against the time interval between the arrival of impulses evoked by pinching the skin in the region of the superficial peroneal nerve as recorded from the surface of the spinal cord. (All nerves except the cutaneous superficial peroneal nerve were sectioned.) In acute spinal cats sustained pinching depresses the Group I DRP for 200–300 msec (Fig. 1, solid circles). After amphetamine (open circles) the Group I DRP is totally suppressed for more than 400 msec and partially depressed for several seconds afterwards.

In acute spinal cats the depression of DRP from I*a* primary afferents followed by an FRA volley is not caused by their depolarization of the terminals in the spinal cord[6], but by the inhibition of the interneuronal chain between I*a* primary afferents[7]. That the mechanisms of the depression of Group I DRP induced by pinching the skin could be different was already suggested by experiments in which long-latency primary afferent depolariza-

tion after administration of L-DOPA was exclusively exerted on Ia primary afferent teminals[4,5].

Intraspinal excitability measurements from Ia fibres revealed their increased excitability for several seconds during pinching the skin when amphetamine had been given in a dose of 3–6 mg/kg. It can be concluded that amphetamine opens the pathway from FRA to Ia primary afferents by a mechanism which is probably analogous to the action of both L-DOPA and 5-HTP.

The sensitivity to amphetamine is not the same in all cats. In some experiments a dose of 3 mg/kg of amphetamine was sufficient to cause the complete disappearance of DRP evoked by a single volley of FRA. In these experiments spontaneous activity in the ventral roots usually occurred. Only exceptionally did spontaneous activity appear in the primary afferents, which could be registered only in muscle nerves but not in the cutaneous nerves.

In other cats the effect of amphetamine was not so outstanding and an increased dose of 6 mg/kg did not cause any spontaneous activity. Nevertheless, the depression of the DRP evoked by a single volley to FRA was a constant finding and in some experiments a long-latency response similar to that described after L-DOPA and 5-HTP[5] occurred.

It can be concluded that the effect of amphetamine on spinal reflexes is strikingly similar to that of L-DOPA and 5-HTP, which makes it probable that they affect the same interneuronal pathway in the spinal cord. Further facts are required to show whether amphetamine is a drug from which effective monoamine transmitters can be formed or whether it acts by stimulating the monoaminergic terminals inducing a higher release of the transmitter.

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PHARMACOLOGY

Assay of Gonadotrophins by the Deciduoma Method

In this laboratory a method has been developed for measuring the luteotrophic activity of prolactin by the formation of deciduomata in a damaged uterine horn in the hypophysectomized adult mouse[1]. The decidual response was quantal and the percentage of positive responses was transformed into probits. The method was designed as a 4 or 6 point parallel line assay and results were expressed in terms of the 2nd International Standard Prolactin (ovine prolactin, Armour Lab., Lot No. *D* 14083–2*B*, potency 22 I.U./mg).

Human pituitary luteinizing hormone[2] (IRC_2 fraction, potency 1,440 units IRP-HMG/mg by the ovarian ascorbic acid depletion assay (95 per cent fiducial limits 710–2,500 units)) and human chorionic gonadotrophin (CG, Leo, batch No. 162041, 1,500 I.U./mg) induced deciduomata in this assay and their dose-response curves were similar to that of ovine prolactin. Results of assays are shown in Table 1. It will be noticed that both preparations were more active than the reference standard.

Chorionic gonadotrophin, 'Pregnyl' (Organon, commercial preparation, batch No. 9107), was also assayed by the same method and its potency was found to be 62 per cent (95 per cent fiducial limits 44–101) of that of CG Leo when compared in international units CG.

Table 1. Assay of Human Luteinizing Hormone and of Human Chorionic Gonadotrophin by the Deciduoma Assay

Preparation	Result (mg/mg standard with 95 per cent fiducial limits)	Index of precision (λ)
Human pituitary luteinizing hormone, IRC_2	6·92 (4·33–11·80)	0·11
Human chorionic gonadotrophin Leo, 162041	11·39 (6·07–19·31)	0·13

Pregnant mares' serum gonadotrophin ('Gestyl', Organon, Lot No. 9651, 400 units/ampoule) and an extract of the endometrial cup secretion of a pony were assayed by this method and were both found to induce the decidual reaction.

The relative potency of the endometrial cup secretion of the pony, expressed as mg/mg Standard, was 5·24 (95 per cent fiducial limits 2·86–20·96) and the index of precision, lambda, was 0·15. Serum gonadotrophin (PMS) induced the decidual response at 80 units and contained about 14 units of luteotrophic activity per ampoule.

This work was supported by a grant from Organon Laboratories, Ltd., the endometrial cup secretion was kindly supplied by Dr. R. V. Short, University of Cambridge, and the extract was prepared by Dr. W. R. Butt in this laboratory.

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Metabolic Effects of Oxytocin in the Chicken

Oxytocin has been shown to have marked metabolic effects in several species of experimental animals. These effects can be elicited either by administration of exogenous material or by physiological stimuli inducing the secretion of oxytocin from the posterior pituitary gland. A marked species difference may be seen in the effects[1]. In the chicken the occurrence of the oxytocic principle in the hypophysis is established[2] but its physiological significance remains obscure, and the literature contains little concerning the metabolic effects of oxytocin in the chicken. Possible sex difference in the effects was also investigated, as it was recently noted that such difference occurred in the dog[3].

Adult leghorns of local breed were used in this study. After fasting overnight they were bound on the table, unanæsthetized, undisturbed for at least 1 h. Blood samples were obtained through a thin polyethylene tube inserted deep into the wing vein; two control samples and four or five further samples at 5, 10, 20, 40 and 60 min after intravenous administration of oxytocin ('Syntocinon', Sandoz). Blood samples were transferred to iced and heparinized tubes, then centrifuged. The plasma was separated without delay. Plasma glucose was determined by the glucose oxidase method[4] using 'Glucotest' (Boehringer and) plasma free fatty acids by Dole's technique[5].

Oxytocin caused transient but marked increase in the concentration of both glucose and free fatty acids. Glucose reached its maximum value at 10–20 min, whereas free fatty acid attained its peak at 5 or 10 min, that is, always ahead of glucose (Table 1).

Fig. 1 shows dose response curves obtained in the hen. The results are expressed as percentage increments over control levels, and the maximum changes after oxytocin injection were plotted against the log dose scale. Glucose shows 15 per cent increase with 20 mU/kg and slightly more

Table 1. Percentage Changes of Glucose and Free Fatty Acids (FFA) in Plasma after Intravenous Administration of 80 mU/kg Oxytocin (Mean values with the *S.E.* from 5 experiments)

		Control	Changes in percentage			
			5 min	10 min	20 min	40 min
Females (5)	Glucose	229 ± 8·5 (mg %)	+10·4 ± 1·9	+15·0 ± 2·8	+11·2 ± 4·1	+6·2 ± 3·3
	FFA	682 ± 110 (μequiv./l.)	+37·2 ± 9·0	+28·0 ± 6·8	+0·8 ± 3·1	−3·0 ± 9·2
Males (5)	Glucose	225 ± 10·7 (mg %)	+10·2 ± 2·0	+10·4 ± 2·1	+5·0 ± 2·2	+3·4 ± 2·4
	FFA	564 ± 53 (μequiv./l.)	+63·8 ± 10·1	+37·6 ± 11·7	+11·0 ± 9·6	−9·2 ± 3·1

with the increase of the dose, while the free fatty acid curve is steeper, attaining the maximum of about 40 per cent increase with the dose of 80 mU/kg.

Table 1 shows that there is no difference between the sexes either in the response or in the time course. Both cock and hen respond with hyperglycæmia and hyperlipæmia, which decline rapidly. Males show a more marked hyperlipæmia and slightly greater tendency toward a negative fluctuation at 40 min, though all the values are not significantly different from the corresponding values in the hen ($P > 0{\cdot}05$). By 60–90 min plasma levels of both glucose and free fatty acid returned to the pre-test levels. Qualitative differences in response in males and females were not observed (cf. Burt *et al.*[3]); the discrepancy may be due partly to the fact that they collected no sample before 30 min.

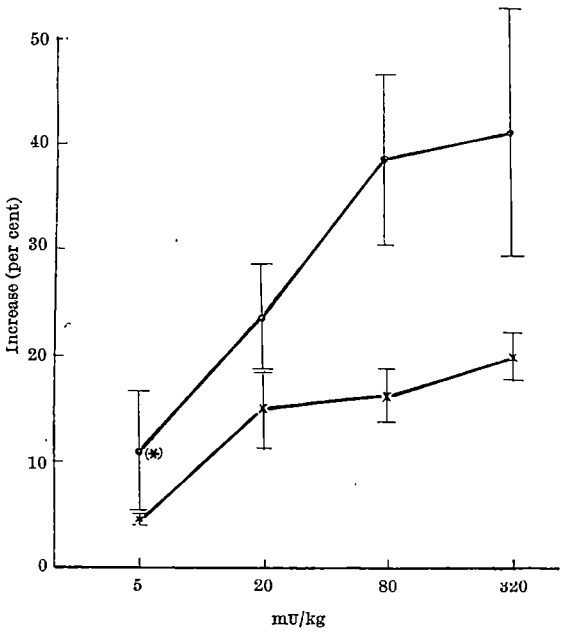


Fig. 1. Dose response curve of oxytocin in the hen. Maximum percentage increases over the pre-test level were plotted against the dose in mU/kg. ●, FFA, ×, glucose concentration in plasma. Each point denotes the mean from 5 hens with the standard error. All values are statistically significant except one (★)

The results of the study presented here are contrary to the findings in the dog, which exhibits marked hypolipæmia and hyperglycæmia when given oxytocin[1,7]. The similarity of its effect to that of epinephrine[5,6] suggests the possibility that endogenous catecholamines may play a part in the metabolic effects of oxytocin. However, in view of the fact that a small dose (5 mU/kg) elicits metabolic effects without any change in blood pressure, that larger doses evoke only transient hypotension without secondary hypertension, and taking into account the promptness of the response, it seems unlikely that the catecholamines are involved. Further evidence was observed in five reserpinized hens (10 mg in two divided doses in successive days and used on the next day) in which oxytocin elicited exactly the same response as in the untreated ones.

Whether these effects are due to increased release of glucose and free fatty acid from the store, or decreased utilization in the periphery, or both, is a matter of conjecture at present, and the biochemical mechanism of these actions, and the species differences, await further investigation.

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Adrenal and Plasma Corticosteroids and Ascorbic Acid Content during Bone Fracture Healing

Adrenal cortical hormones play an important part in recovery from traumatic stress[1]. This applies to the healing of bone fractures, although cortisone and similar substances when given in large doses interfere with fracture healing[2]. These hormones are said to retard healing through inhibiting and delaying the absorption of blood clots, the formation of callus and the production of collagen[3]. Ascorbic acid has a beneficial action on adrenal cortical activity in relation to stress[4]. In addition, ascorbic acid is needed for the biosynthesis of collagen[5], which is indispensable for a normal healing process.

This investigation deals with the changes occurring in the levels of adrenal and plasma corticosteroids, and of vitamin C, at various times after experimental bone fracture. Special attention was directed to changes during the first week.

Ninety adult male white rats, weighing about 220 g, were used. Fractures of the left tibia were induced under ether anæsthesia[2]. Groups of animals were killed at intervals of 6, 12, 18 and 24 h, 2, 3, 4, 5, 6 and 7 days, and 2, 3 and 4 weeks afterwards. The blood of each animal was collected separately. Groups of normal animals served as controls. The adrenal glands were removed immediately after death, carefully cleaned of all superfluous tissue and weighed on a torsion balance to 0·1 mg. Ascorbic acid was extracted from the glands in glass homogenizers containing 10 ml. of 6 per cent trichloroacetic acid. The homogenate was centrifuged and ascorbic acid was determined in the clear supernatant fluid. Adrenal and plasma ascorbic acid were estimated using the dinitrophenylhydrazine method[6].

Adrenal glands for corticosterone estimation were homogenized and prepared according to the method of Guillemin *et al.*[7]. Plasma corticosterone was extracted and prepared as described by Zenker and Bernstein[8], using the modifica-

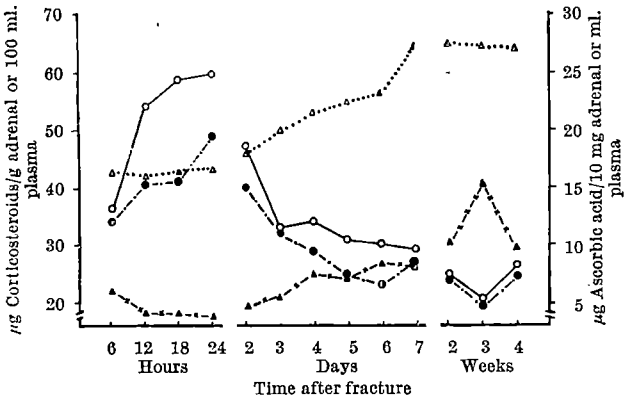


Fig. 1. Corticosteroid and ascorbic acid content of rat adrenal and plasma after tibial fracture; ○—○, adrenal corticosteroids; ●— · —●, plasma corticosteroids; △ . . . . △, adrenal ascorbic acid; ▲— × —▲, plasma ascorbic acid

tion of Dao, Flaxman and Lonergan[8] for the final development of fluorescence. Fluorescence was measured on Dr. Lange's universal colorimeter model *UK* VII equipped with a mercury lamp and two black glass filters slipped on the lens tube with two yellow light filters *GG*11 of 60 mm φ located directly in front of the cell.

The results are plotted graphically in Fig. 1. There was a statistically significant increase in the levels of adrenal and plasma corticosteroids, detected after 6 h, and increasing continuously to a maximum at the end of the first 24 h. The fact that plasma and adrenal steroids increased concomitantly suggests that the synthesis of corticosterone was increased[9]. Plasma and adrenal corticosteroids returned to normal levels after 4 and 6 days respectively.

It is generally accepted that bone resorption occurs at the fracture site before active regeneration leading to callus formation can occur[2]. Using ^{35}S it was possible to demonstrate that, by the end of the first week, the uptake of radioactive sodium by rats' humeri[10] and rats' tibiæ[2] was significantly decreased in the fractured bones compared with the normal bones of the same animals. The incorporation of radioactive sulphur is a measure of the formation of acid mucopolysaccharides which are essential constituents of bone ground substance. In addition, adrenal steroids interfere with cellular activity and depress mitosis[11]. Moreover, cortisol and cortisone were reported to inhibit fibroblastic production of collagen[12]. Clark and Roth[13] showed that the action of hydrocortisone on bone minerals is not mediated through the parathyroid gland but can be attributed to the anti-anabolic action of the hormone.

There was a negative correlation between the levels of adrenal corticosteroids and the ascorbic acid content of the adrenal glands. Although the relationship between ascorbic acid and adrenal steroid synthesis is not yet understood, it has been shown that exogenous ACTH further depressed the level of ascorbic acid in the adrenals of scorbutic animals[14].

Plasma ascorbic acid levels significantly decreased from normal values during the first three days after fracture. This observation is in agreement with the finding of Andreae and Browne[15], who demonstrated a markedly increased catabolism of ascorbic acid in human beings after burns and fractures.

There was no correlation between plasma and adrenal contents of vitamin C. However, there was a significant decrease in the level of adrenal corticosteroids accompanied by a significant increase in the level of plasma ascorbic acid for the values recorded at the end of the third week. An increased level of ascorbic acid favours increased collagen biosynthesis[5]. This is also true during fracture healing since it has been previously shown that maximum production and maturation of callus cellular elements took place during the third week[2].

Finally, the results show that, in rats with experimental fractures, there is a stage of increased suprarenal activity which attains a peak in 24 h, and thereafter declines steadily to normal levels before the end of the first week. The synthesis of ascorbic acid reached its maximum during the third week after the fracture, and this coincides with the period of maximum production and maturation of collagen fibres.

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Exaltation of Toxicity of Sympathomimetic Amines by Thyroxine

Various factors are known to influence the toxicity of sympathomimetic amines in mice, especially ambient temperature[1,2], aggregation[1,2] and psychotropic drugs[2–5]. Halpern *et al.*[6] reported recently that pretreatment with thyroxine considerably aggravated the central nervous stimulation and mortality induced by amphetamine in aggregated mice. Here, evidence is presented to show that pretreatment with thyroid hormone also markedly increased the toxicity of sympathomimetic amines in solitary mice.

Adult 'Swiss' male mice were used, weighing between 20–22 g, obtained from a single breeder. DL-thyroxine was injected intraperitoneally in daily doses of either 200 μg/kg or of 1 mg/kg. DL-amphetamine (sulphate) and ephedrine (hydrochloride) were also injected intraperitoneally in doses and at times after the beginning of thyroxine treatment as indicated in Tables 1 and 2. Throughout the whole experiment, the animals were kept in a room at a constant temperature of 27° ± 1° C. Immediately after the injection of the sympathomimetic amines, the animals were placed in a glass jar (220 mm diameter) divided into equal compartments by an opaque device, each animal occupying an individual compartment in order to avoid the interference of the aggregation phenomenon. The behaviour of the animals was carefully observed and the mortality recorded during the following 24 h.

The results are summarized in Tables 1 and 2. The data reported in these tables suggest the following considerations: Thyroxine considerably enhanced the toxicity of both the sympathomimetic amines studied here. Halpern *et al.* have recently shown that similar enhancement of toxicity by thyroxine was observed with other sympathomimetic substances[7].

The ratio $\frac{\text{toxicity control 60 per cent}}{\text{toxicity } T_4 \text{ 60 per cent}}$ reached a reciprocal value between 15 and 20, which was considerable. There have been few, if any, reported cases in which the

toxicity of a pharmacological agent was increased to such a degree by the administration of a hormone.

The development and the degree of enhancement of the toxicity of the sympathomimetic amines by the thyroid hormone depended on the dose and on the duration of the hormonal treatment. With doses of thyroxine of 1 mg and above, enhancement of toxicity was evident several hours after the injection. The phenomenon increased in intensity as the treatment was repeated and reached a maximum after 3–5 days of treatment. The results were similar for amphetamine and ephedrine. With doses of thyroxine of 200 μg/kg, the effect was similar but less marked. As this phenomenon was demonstrated in isolated mice, we can admit that thyroxine affected some critical mechanisms which were directly implicated in the death of the animal. What may these mechanisms be? The main property of amphetamine and of other drugs belonging to this group is the induction of central and peripheral sympathetic stimulation. According to present views, these effects are likely to be related to the release of catecholamines. It has been recently shown[8] in this laboratory that, in aggregated mice, emotional stress was accompanied by a considerable depletion of brain catecholamines. Moreover, evidence has been furnished that tyramine and other indirect sympathomimetic amines determined a release of noradrenaline from the vascular walls[4]. That the impairment of endogenous catecholamine metabolism may have been directly related to the toxicity of the amines studied here was supported by the recent observation in this laboratory that administration of IMAO equally potentiated the toxicity of amphetamine, ephedrine, etc.

On the other hand, IMAO as well as thyroxine has been shown recently by Halpern *et al.*[9] to be able to annul the protective effect exerted by reserpine against the 'group toxicity' induced by amphetamine in aggregated mice. This similarity of action of IMAO and thyroxine in both circumstances suggests a similar mechanism, which may have been central or peripheral. These problems, the importance of which we wish to emphasize, are now under investigation. The mortality among mice treated with thyroxine and sympathomimetic amines was due to an extensive myocardial necrosis. These observations may also be relevant to the mechanisms by which thyroxine enhanced the toxicity of sympathomimetic amines. Finally we wish to emphasize the potential danger of administering sympathomimetic amines to patients with hyperthyrea, and of drugs associating thyroxine with sympathomimetic agents, as the toxicity has been enhanced by doses which are near the therapeutic range.

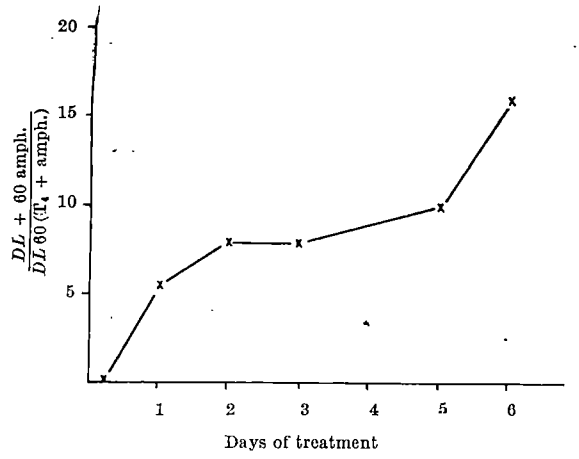


Fig. 1. Ratio of the toxicity of amphetamine (LD > 60) in control and in thyroxine-treated groups of solitary mice as a function of the duration of treatment with thyroxine (dose of thyroxine injected 200 μg/kg/day)

Table 1. Effect of Pretreatment with Thyroxine on Toxicity of DL-Amphetamine (Sulphate) in Solitary Mice

Dose of DL-amphetamine (mg/kg)	Duration of treatment with DL-thyroxine (200 μg/kg) (h)	No. deaths / No. treated	Percentage mortality	
14	—	1/65	1·5	
30	—	2/35	8	
50	—	13/40	32	
80	—	24/40	60	
100	—	27/30	90	
14	4	0/18	0	
30	4	6/10	60	
14	18	7/10	70	
30	18	9/10	90	
8	48	1/10	10	Tox. control / Tox. T_4 = 8
10	48	7/10	70	
8	72	4/10	40	
10	72	14/20	70	Tox. control / Tox. T_4 = 8
20	72	10/10	100	
8	96	4/20	20	
20	96	10/10	100	
4	120	0/10	0	Tox. control / Tox. T_4 = 13
8	120	7/10	70	
4	144	3/10	30	Tox. control / Tox. T_4 > 16
5	144	10/10	100	

Table 2. Effect of Thyroxine on Toxicity of L-Ephedrine on Solitary Mice

Ephedrine mg/kg	Deaths / Treated	Mortality %
280	31/40	77
250	27/58	46
200	22/55	40
100	9/48	18·7
50	1/20	5

T_4 200 μg/kg/day

Days of pretreatment with T_4	Ephedrine (mg/kg)	Deaths / Treated	Mortality %	
3	100	8/10	80	Tox. control / Tox. T_4 > 2·8
5	50	2/10	20	
5	20	1/20	5	

T_4 1 mg/kg/day

Days pretreatment with T_4	Ephedrine mg/kg	Deaths / Treated	Mortality %	
3	100	9/10	90	Tox. control / Tox. T_4 > 2·8
5	50	20/20	100	Tox. control / Tox. T_4 > 5·6
5	20	8/10	80	Tox. control / Tox. T_4 > 14
6	18	19/20	95	Tox. control / Tox. T_4 > 15·5

This work was supported by a grant from the Delegation Générale à la Recherche Scientifique et Technique (No. 61–*FR*–064).

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PATHOLOGY

An Unidentified Compound in the Serum of Children with Kwashiorkor (Protein–Calorie Malnutrition)

When using the rapid chromatographic method recently developed for measuring the balance of amino-acids in the serum of children with kwashiorkor[1] we have found an unidentifiable compound with an R_F value slightly less than that of valine. The solvent used was *A* in Table 1. This communication describes various properties of the compound and the conditions under which it can be demonstrated.

Table 1. R_F values on No. 20 Whatman paper of the unidentified serum compound in kwashiorkor and of piperidine-2-carboxylic acid

	Solvent				
	A	*B*	*C*	*D*	*E*
Piperidine-2-carboxylic acid (authentic)	0·44	0·88	0·45	0·41	0·51
Unidentified compound	0·45	0·86	0·45	0·40	0·50
Co-chromatography	0·45	0·86	0·44	0·40	0·50
Piperidine-2-carboxylic acid (ref. 3)	0·44	0·93	0·45	0·41	—

The solvents were: *A*, butan-1-ol–acetic acid–water (12 : 3 : 5, by vol.); *B*, phenol–water (500 g, 125 ml.); *C*, butan-1-ol–pyridine–water (1 : 1 : 1, by vol.); *D*, ethanol–ammonia–water (18 : 1 : 1, by vol.); *E*, butan-1-ol–water–formic acid (7 : 2 : 1, by vol.).

Unlike the amino-acids that are eluted and estimated for the balance method, the compound did not react with ninhydrin in the cold. On heating to 120° C, however, a purple colour was produced that was more blue than the colour usually formed by amino-acids. The ninhydrin derivative had two unusual properties. In ultra-violet light it had a cherry-red fluorescence, unlike the derivatives of amino-acids, which absorb ultra-violet light and appear black. When the chromatogram was dipped through a solution of ethanolic copper nitrate the colour did not change to the typical pinkish-orange but remained blue-purple. A compound with these properties has been described; it is piperidine-2-carboxylic acid (pipecolic acid)[2,3], a derivative of lysine. The unknown compound gave a strong blue-green reaction with isatin, indicating a relationship to pyrrolidine or piperidine derivatives[4].

Deproteinized samples of serum were prepared and applied without de-salting to Whatman No. 20 paper as previously described[1]. The chromatographic properties of the compound were compared in five solvents with those of a sample of authentic piperidine-2-carboxylic acid obtained from the California Corporation for Biochemical Research, Los Angeles. The results are recorded in Table 1. Colour reactions of the unknown and the known substances agreed closely, and co-chromatography in each of the solvents failed to separate them. The solvent systems used will separate piperidine-2-carboxylic acid from piperidine-3-carboxylic acid, piperidine-4-carboxylic acid, piperidine-2,6-dicarboxylic acid and 5-hydroxy-piperidine-2-carboxylic acid[5].

The unidentified compound is not found in all samples of serum taken from children admitted for the treatment of kwashiorkor. There appears to be no correlation between the presence or absence of the compound and the severity of the kwashiorkor on admission. After a meal containing protein or after a dose of *l*-lysine, it is very often demonstrable. When treatment is proceeding favourably, the compound slowly disappears from the serum and none can be detected after about 10 days. In less favourable circumstances, especially when the concentration of the total serum proteins does not rise satisfactorily, the amino-acid balance ratio rises and the weight gain is poor, the amount of the compound appears to increase even beyond the level found on admission.

Disturbances in the metabolism of histidine, phenylalanine and tyrosine in kwashiorkor have already been described[6,7]. The present finding demonstrates the abnormal metabolism of an additional nitrogenous compound. Confirmation of our tentative identification of the compound as piperidine-2-carboxylic acid would indicate that the metabolism of lysine is at fault.

We have at present no definite information about the source of the unknown compound. It is unlikely to be merely a component of the diet, since it is found in samples of blood taken after an overnight fast, and does not increase in amount towards the end of successful treatment in pace with the improvement of the child's appetite and food intake. The rise in serum concentration after milk protein or lysine has been given suggests, but does not prove, that the source is an amino-acid.

The structure of piperidine-2-carboxylic acid closely resembles that of the amino-acid proline and the vitamin nicotinic acid, and it is possible that the raised concentration of piperidine-2-carboxylic acid in the serum may affect the metabolism of these two substances.

Samples of serum containing the unknown compound are being collected for an elementary analysis and the determination of molecular weight. The collection will take a very long time, because only a small amount of blood can justifiably be taken from the severely ill children in whose serum the compound appears. It is hoped that the publication of this report will encourage other workers to look for the compound. It may have been confused with β-aminoisobutyric acid or ethanolamine, both of which have been reported in the serum in kwashiorkor. The ninhydrin derivatives of these compounds do not fluoresce in ultra-violet light; they give the normal orange colour in ethanolic copper nitrate.

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Influence of Diet and Iodine-131 Injections on the Survival of Dystrophic Mice

Diet has been shown to influence the survival of mice with hereditary muscular dystrophy, since an increased lifespan and an extended period of growth have been recorded when a semi-synthetic diet containing high-quality protein and lipid was used, and the further addition of glycine to the diet increased the growth rate still more[1]. Apparently, the vitamin content of the diet did not produce the effect, since other investigations using massive doses of vitamin mixtures did not prolong the survival of dystrophic mice[2]. Suppression of the thyroid gland activity by ^{131}I (ref. 3), or surgical removal of the gland[4], delayed muscular degeneration in vitamin E-deficient rabbits, indicating an influence of the thyroid hormone on this type of muscle pathology.

Mice with hereditary muscular dystrophy (*dydy*) were obtained by mating *Jax*/129 (*Dydy*) mice from the Jackson Memorial Laboratory, Bar Harbor, Maine, and were maintained in temperature-controlled quarters. Food and water were available *ad libitum*. Mice were weaned at 3–4 weeks, experimental groups were formed by dividing litters, and treatments were initiated at weaning. During the first trial, the diet consisted of 'Purina Laboratory Chow', and treated mice were given a single intraperitoneal injection of 200 mc. ^{131}I as sodium iodide in a small amount of physiological saline at three weeks of age.

During the second trial, the semi-purified diet (Table 1) contained 2 levels of sodium and potassium, and some of the mice were given a combination of the higher sodium level diet, ^{131}I injection as in the first trial, and 5 mg of 17-α-methylandrostene-5-diol-3β-17β-dienanthoylacetate (MADD) in sesame oil in a weekly intramuscular injection, the dose being equivalent to the methylandrostenediol.

Table 1. Composition of the 'Semi-purified' Mouse Diet

Item	Per cent
Vitamin-free casein	40·00
Alphacel (non-nutritive bulk)	3·00
Corn starch	12·00
Sucrose	12·55
Dextrose	12·00
Briggs salt mixture[7]	6·00
Vitamin-free lard	10·00
Potassium acetate	2·50
Magnesium oxide	0·50
Choline chloride	0·30
Inositol	0·40
Ascorbic acid	0·50
p-Amino benzoic acid	0·25
Complete vitamin supplement[8]	—

The diet was mixed in portions sufficient for 5 days and kept under refrigeration to minimize deterioration. In the lower sodium diet, KCl was substituted for NaCl. The higher sodium diet contained 0·583 per cent sodium and 0·400 per cent K, while the lower sodium diet contained 0·237 per cent sodium and 1·862 per cent K.

In the first trial using the laboratory chow (Table 2), the ^{131}I injection prolonged the survival of dystrophic mice approximately two-fold and may have slightly retarded the growth rate of the normal littermates. In the second trial using the semi-purified high protein rations (Table 2), the sodium and potassium levels did not influence survival, but a three-fold increase was noted in dystrophic mice on this diet as compared with those in the first trial receiving the laboratory chow. The growth rate of the normal littermates was retarded by the semi-purified ration. Some benefit from the lower sodium level in the diet might have been expected, since the sodium level of dystrophic mouse muscle is 50 per cent higher than normal[5]. Dystrophic mice receiving the higher sodium level semi-purified diet with injections of ^{131}I and MADD did not survive as long as those receiving the semi-purified diet alone, indicating that the benefits of these treatments were not additive. MADD has previously been shown to prolong the survival of dystrophic mice two-fold[6]. The treatments did not appear to alter the disease syndrome in other ways; both histologically and by gross observation, the pathological processes were similar in all groups.

Table 2. Survival of Dystrophic Mice

Condition and treatment	No. mice	Median survival time and range (weeks)	Av. body-weight (g) at weeks: 3	10–20	20–30	30–40
First trial (commercial pellets)						
Normal						
Males	78	—	10	30	34	33
Females	70	—	9	27	31	31
Normal ^{131}I Inj.						
Males	41	—	10	27	31	33
Females	35	—	10	27	29	28
Dystrophic						
Males	8	6 (4–11)	6	19	—	—
Females	13	8 (4–25)	6	18	18	—
Dystrophic ^{131}I Inj.						
Males	15	14 (9–20)	5	20	21	—
Females	18	18 (7–43)	6	16	17	15
Second trial (semi-purified high-protein diet)						
Normal, Higher Na						
Males	64	—	9	25	29	32
Females	58	—	10	21	26	30
Normal, Lower Na						
Males	48	—	11	23	29	31
Females	44	—	10	21	26	30
Dystrophic, Higher Na						
Males	12	21 (13–28)	6	15	16	—
Females	12	22 (4–26)	7	13	15	—
Dystrophic, Lower Na						
Males	16	22 (7–31)	7	15	13	13
Females	18	20 (8–30)	8	13	13	—
Normal, Higher Na, ^{131}I Inj. and MADD						
Males	12	—	9	25	29	29
Females	12	—	10	21	25	24
Dystrophic, Higher Na, ^{131}I Inj. and MADD						
Males	10	15 (4–34)	7	16	17	16
Females	8	17 (10–22)	6	14	15	—

In summary, the survival of dystrophic mice receiving a commercial laboratory diet was about doubled by the injection of ^{131}I at 3 weeks. A high-protein semi-purified diet prolonged survival approximately three-fold. The level of sodium and potassium in the high-protein semi-purified diet, however, did not influence survival, and a combination of ^{131}I injection, weekly injections of methylandrostenediol dienanthoylacetate and the high-protein semi-purified diet did not prolong survival beyond the time observed with the high-protein semi-purified diet alone. None of the treatments affected the characteristics of the syndrome in any other way.

This work was supported in part by a U.S. Public Health Service research grant, *A*–4273, from the National Institute of Arthritis and Metabolic Diseases. The ethylandroste nedi ol dienanthoylacetate was given by the S. E. Massengill Co., Bristol, Tennessee.

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Intravital Fluorescence Microscopy in the Qualitative Evaluation of the Interaction of Acridine Orange with Nucleic Acid in Intact Living Human Organs*

Observations on living organs excited by ultra-violet irradiations have been almost entirely confined to work on experimental animals. The specific fluorescence method was largely developed through the work of Ellinger and Hirt[1,2], who termed it intravital fluorescence microscopy. The method found use in the investigation of resorptive and excretory functions of different organs, and offered valuable information about the physico-chemical conditions and processes in living cells, in particular the electrostatic phenomena. It also helped in the elucidation of various problems concerning the function of kidneys[3,4], liver[5,6], blood vessels[7], and nerves[8], and other organs of experimental animals.

Although the method proved most valuable, yielding specific information concerning the behaviour of living cells and tissues with minimal interference with their normal physiology[9], and identifying minute concentrations of administered dyes in different tissues[5], no attempt has been reported to apply it widely in the investigation of human biology. One reason is, no doubt, the technical difficulties inherent in the construction and adaptation of the instrument.

In previous investigations[10–12], I demonstrated the affinity of the basic dye acridine orange (3,6-*bis* dimethylaminoacridine) for the nucleic acids of intact human cells and tissues; it forms monomeric, dimeric or polymeric dye complexes[13] within normal, malignant and devitalized tissues, respectively. Identification of tissues by virtue of their differential fluorescence spectra was described, and suggested as the basis for a new clinical cancer test[10–12].

The aim of the present communication is to describe the principles fulfilled in construction of an intravital fluoromicroscopic unit for the excitation and inspection of living human organs fluorochromed with acridine orange, to evaluate the qualitative variations in the interaction of the dye with the nucleic acids of living tissues.

* This communication is a part of an investigation on "A New Cancer Test" awarded the Edgar-Gentilli Prizes for the year 1962–63 by the Royal College of Obstetricians and Gynaecologists.

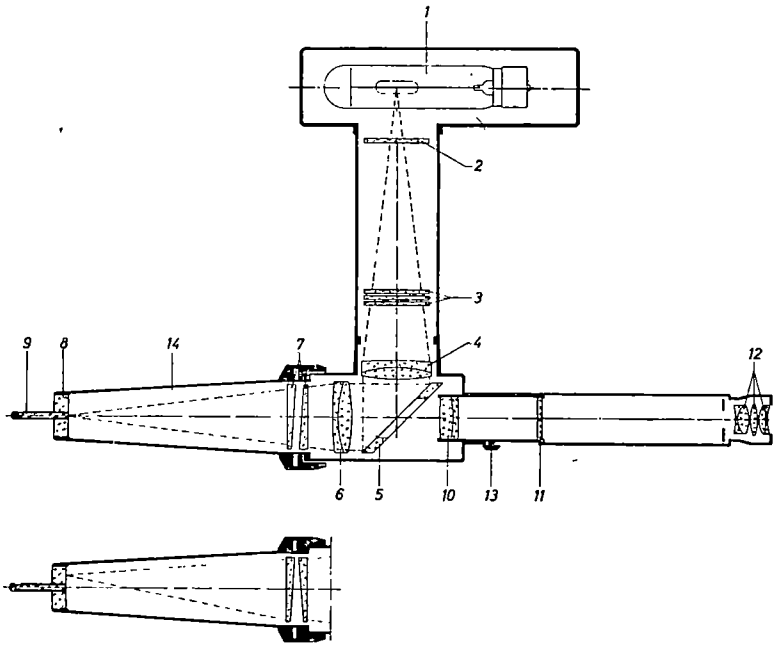


Fig. 1. The design of a fluoro-microscopic unit (the 'Cervicoscope') intended for the examination of the human cervix uteri

An instrument has been constructed (Fig. 1) for the examination of the human cervix†, but the principles are applicable to the design of instruments for the examination of other accessible organs. Incident illumination was used, the ultra-violet beam entering the main tube through a side-arm, to be deflected down to the objectives (Fig. 1, 8 and 9).

To avoid restriction of the objective aperture by the use of a prism or mirror to deflect the beam through 90°, as originally suggested by Ellinger and Hirt[1,2], a dichroic reflector with high ultra-violet reflectance and high transmission of ordinary light was used[5]. This increased the brightness of the image, which is known to vary with $(N.A.)_0^2/M$, where $(N.A.)_0$ is the numerical aperture of the objective, and M is the total magnification of objective and occular combined.

Further increase in image brightness was achieved by using an overall magnification of × 17, reducing to a minimum the distance between source and object, and illuminating a relatively small field with a condenser of higher numerical aperture. According to the fluorometric law of De Ment[14], the fluorescence intensity is proportional to the intensity of the optical excitant over wide ranges, and varies inversely as the square of the distance from the source of irradiation. In the living body, however, possible injury to cell membranes limits undue increase in the source intensity, hence this was kept within the safe range of 150–200 W, supplied by a high-pressure mercury vapour lamp with a specially constructed housing (Fig. 1, 1).

Since the area of the irradiated field at the object is equal to $A\ (N.A.)_s^2/(N.A.)_c^2$, where A is the effective source area, while $N.A._s$ is the numerical aperture of the lamp lens and $N.A._c$ is the numerical aperture of the condenser, it would have been possible to fill a large field by reducing the condenser aperture (Fig. 1, 4). This, however, would reduce the image brightness since the intensity of irradiation is proportional to $B\ (N.A.)_c^2$, where B is the image brightness. Therefore, it was preferable to illuminate a small field and obtain high image brightness by increasing the numerical aperture of the condenser. An alternative system is to fill a larger field by using a light source of large area and a lamp lens of high relative aperture.

To utilize a cross-filter system for excitation and inspection of the fluorescence of acridine orange, which is known to absorb in the region of 4000–4500 Å, a primary filter which cuts out all wave-lengths below 4000 Å was mounted between the lamp lens and the condenser (Fig. 1, 3), protected from the ultra-violet source by heat-resistant filter (2). The secondary filter (11), which cuts out all blue-violet rays (4000–4500 Å), was mounted between the objectives and ocular (12). This limits the fluorescence image to the green, yellow and red, which are the colours of monomeric, dimeric and polymeric acridine–nucleic acid complexes, respectively.

To avoid reflexion loss, the excitation rays are delivered to the object through a metal tube (14), and the fluorescence rays are afterwards transmitted back from the object to the ocular (12). An additional factor in image brightness was the use of a monocular unit, since the brightness is known to be reduced with a factor of more than 2 in all minocular fluorescence microscopes. The wedges (7) and button (13) are mechanical devices, respectively, for the illumination of successive fields and for focusing the fluorescence image.

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† The instrument, termed "The Fluorescence Cervicoscope", was designed by the author and constructed by J. D. Möller, Wedel, Hamburg, West Germany.

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IMMUNOLOGY

Type of Antibody Response in Mice immunized with Transplantable Tumours

Antibody in the serum of several animal species is confined to two main classes of proteins with sedimentation coefficients of approximately 7*S* and 19*S* respectively. The former class comprises molecules with molecular weights of at least 150,000 and includes both the 7*S*γ-globulins and the β_{2A}-globulins. The latter class is made up of β_2-macroglobulins with molecular weights of about 1,000,000. The type of antibody response appears to depend on a number of interrelated factors, probably the most important of which is the physicochemical nature of the stimulating antigen. Thus hæmocyanin[1], *A*2 influenza virus[2], and type III pneumococcal polysaccharide[1] were found to produce 7*S*γ-globulin, while sheep erythrocytes produced predominantly β_2-macroglobulins[1].

Various transplantable plasma cell tumours[3,4] and leukæmias[5] in mice have been described in the literature in recent years. Their availability has allowed a new approach to the study of malignant plasma cells and their effect on the serum protein systems in experimental animals.

An attempt has been made to follow the changes in the mouse γ-globulin system resulting from immunization with transplantable tumours by the use of analytical ultracentrifuge and gel-filtration techniques.

Mouse strains *A*, *C*, *C3H* and *C57BL* were used for these experiments. The transplantable mouse tumours comprised two mouse leukæmias, *AL3* and *EL4*, and two solid tumours, *AMT3* and *EP6*. The original tumours were kindly supplied by Dr. J. R. Batchelor, Guy's Hospital Medical School, London.

Batches of 20–100 mice of each strain were immunized with three injections of each of the heterologous tumours. The first inoculum of approximately 5 million viable cells was given subcutaneously. The second and third injections of approximately 50–100 million cells were given intraperitoneally 4 weeks and 6 weeks respectively after the first inoculum.

Serum was obtained from the mice immediately prior to each successive immunization by bleeding the animals from the tail vein[6]. The mice were finally bled two weeks after receiving the last injection.

Hyperimmune sera from a batch of hybrid mice, designated *PH* mice, and from 129 mice, both immunized with *BP8* tumour, were also kindly provided for examination by Dr. J. R. Batchelor.

The degree of the serum antibody response, as measured by the hæmagglutinating activity against mouse red cells[7], varied considerably in the initial stages depending on the mouse strain and on the tumour type. After the final immunization, the hæmagglutination titres were all of the same order.

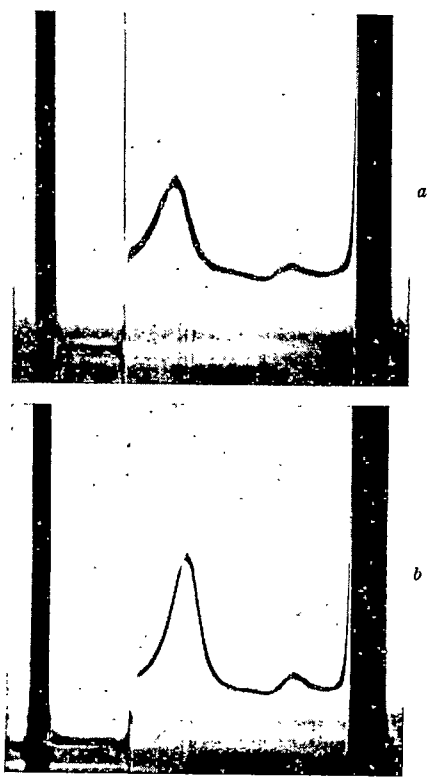


Fig. 1. Schlieren patterns obtained from sedimentation velocity experiments with mouse globulin preparations in the Spinco model *E* analytical ultracentrifuge. Speed, 42,040 r.p.m.; temperature, 20·0° C; photographs taken 52 min after reaching set speed. (*a*) Solution of normal globulins, phase plate angle, 40°; (*b*) solution of immune globulins, phase plate angle, 50°

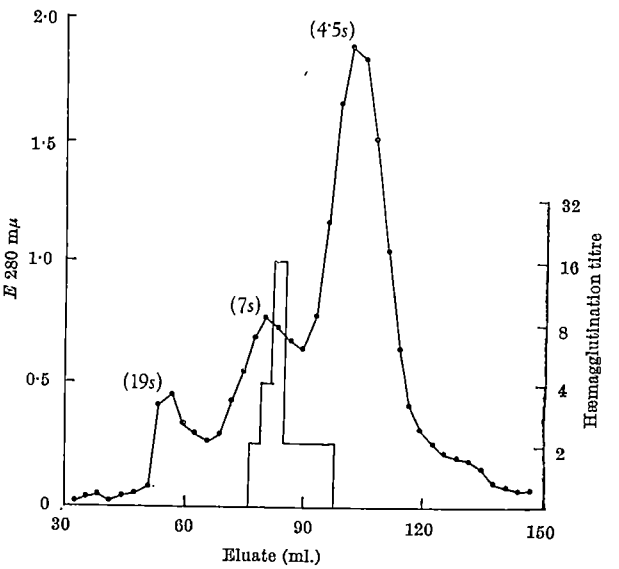


Fig. 2. Gel-filtration of mouse anti-tumour serum on 'Sephadex *G*-200' showing the distribution of antibody activity (open block) related to the eluted protein peaks

Equal volumes of these sera, from which most of the albumin had been removed by fractionation with sodium sulphate, were dialysed against 0·1 M *tris*-hydrochloric acid buffer, *p*H 7·4, in 0·2 M saline, and then restored to equal volumes. These protein solutions were centrifuged at 42,040 r.p.m. in a Spinco model *E* analytical ultracentrifuge operated at 20·0° C. No change in area of the faster sedimenting peak (19*S*) was observed in the Schlieren patterns of the immune sera as compared with the normal sera. However, the area of the slower sedimenting peak, identified as the 7*S* component and which had a sedimentation coefficient of 5*S* under these experimental conditions, was significantly increased in all the hyperimmune sera. This indicates an increase in the relative concentration of the 7*S* component following immunization (Fig. 1).

Samples of serum obtained from the mice 2 weeks after the final immunization were subjected to gel filtration on 50 × 1·8 cm. 'Sephadex *G*-200' (Pharmacia, G.B.) columns. In some cases most of the albumin was removed from the sera by fractionation with sodium sulphate in order to allow greater amounts of the mouse globulins to be loaded on to the columns. 0·1 M *tris*-hydrochloric acid buffer, *p*H 7·4 in 0·2 M sodium chloride, was used to elute the proteins from these columns.

The extinction at 280 mμ and the hæmagglutination activity of the eluted column fractions were measured (Fig. 2). The principal peaks eluted from the column were identified as 19*S*, 7*S* and 4·5*S* proteins in the analytical ultracentrifuge. The proteins contained in these broad zones were further identified by starch-gel electrophoresis. Hæmagglutinating activity was exclusively confined to the 7*S* proteins in all the immune sera examined.

The immune γ-globulin could be isolated by DEAE-cellulose chromatography of the mouse globulin fraction. This γ-globulin was identified by starch-gel electrophoresis and by its behaviour on sedimentation velocity analysis.

These results may be summarized as follows. As judged by hæmagglutinating activity and ultracentrifuge analysis, no evidence was found for the presence of 19*S* serum antibody during the course of immunization of several strains of mice with five heterologous transplantable mouse tumours.

This work has formed the basis for a detailed investigation in mice of the immune response to tumours[8,9]. It was supported by the Cowburn research fellowship,

King's College Hospital Medical School (D. E. H. T.), and by grants from the British Empire Cancer Campaign for Research (J. W. and M. W.).

One of us (D. B. G.) is indebted to the Medical Research Council for providing the analytical ultracentrifuge.

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Observations on Induction of Resistance to Rous Sarcoma Cell Antigens in Hamsters

It has recently been shown that inoculation of mice and hamsters with several oncogenic viruses (polyoma, *SV* 40, myeloid and lymphoid mouse leukæmia) makes them resistant to subsequent transplantation of tumour cells induced by the same virus. Our observations on chickens naturally resistant to the Rous sarcoma virus, or chickens with regressed tumours, suggested that in some instances it was possible to induce resistance to Rous sarcoma cell antigens in chickens. If this were true then the Rous sarcoma is not basically different from the polyoma and other virus tumours. However, certain characteristics of the processes induced by Rous virus (the susceptibility of the adult chicken to this virus, the permanent presence of the virus in tumour cells, the short latent period of tumour induction) make it very difficult to investigate resistance to this tumour cell antigen in its natural host—the chicken. Therefore, it was interesting to investigate the possibility of inducing resistance to the Rous sarcoma cells in a mammal.

Sjögren and Jonsson[8] immunized mice with the Rous sarcoma virus (Schmidt–Ruppin strain) and inoculated the immunized and control animals 5–10 days later with a cell suspension of Rous sarcoma grown on isologous mice. This induced a very weak resistance in virus-immunized mice.

In our experiments adult golden hamsters were inoculated with Rous sarcoma virus (Carr strain). The tumour extract prepared on 0·1 M phosphate citrate buffer was centrifuged at 5,000 r.p.m. for 20 min and the supernatant was stored in sealed glass ampoules at −70° C. The oncogenic virus activity was tested by intracutaneous inoculation of chickens. The supernatant had a virus titre of 10^{-5}/ml. to 10^{-6}/ml. The hamsters were inoculated with the Rous virus 2–3 times. They received every day 1 ml. of the supernatant intraperitoneally and 2–3 ml. subcutaneously. After 7–24 days the immunized hamsters and controls (intact, or immunized with normal hen embryo tissues) were inoculated with sarcoma cell suspension. This tumour was first induced by Rous sarcoma virus (Carr strain) in new-born hamsters and then passaged with cells in adult animals of this species[6]. The cells were suspended in Earle's solution (pH ~8) and counted before inoculation. Each hamster was inoculated with $4{\cdot}5 \times 10^3$ to $4{\cdot}5 \times 10^6$ cells subcutaneously. The animals were observed 2 months after tumour grafting. The tumours were palpated every 2–3 days. The hamsters surviving at the end of the experiment were killed. All animals were autopsied. The results of three experiments are summarized in Table 1.

Table 1. Inoculation of Immunized and Control Hamsters with Tumour Cells

	No. cells in inoculum				Log
	$4{\cdot}5 \times 10^3$	$4{\cdot}5 \times 10^4$	$4{\cdot}5 \times 10^5$	$4{\cdot}5 \times 10^6$	TD_{50}
Immunized	0/27*	4/27 (14·8%)	8/27 (29·6%)	24/27 (88·8%)	5·3
Control	0/22	9/22 (40·9%)	18/22 (81·8%)	22/22 (100%)	4·3

* Figures in the body of the table denote number of hamsters with tumours over total number inoculated. Log TD_{50} denotes log of the cell dose giving 50 per cent positive takes in inoculated hamsters.

These results show that immunization of adult hamsters with Rous sarcoma virus induced some degree of resistance to the sarcoma cell antigens. However, the resistance in this case is not so strong as in the experiments with polyoma or murine leukæmia virus[1–5]. Further experiments are needed to test the specificity of transplantation resistance for Rous tumour and to elucidate the nature of the induced 'new cell antigen'.

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Diagnosis of Auto-immunity

In 1905 Osborne, Mendel and Harris[1] found that the extracts of certain beans had the capacity of agglutinating red blood cells. In 1949 Li and Osgood[2] used the extract of red beans in separating leucocytes from erythrocytes. Rigas and Osgood[3] described a method for the purification of phytohæmagglutinin. This method has been used by Difco Laboratories (Detroit) in preparing this material.

In 1959 Hungerford *et al.*[4] discovered that phytohæmagglutinin has a remarkable ability to initiate mitosis in cultures of leucocytes. Since then it has been extensively used in laboratories engaged in chromosome preparation. The nature of this mitogenic action was obscure until Hastings *et al.*[5] noted that phytohæmagglutinin had a leucoagglutinating activity. Rendon[6], using ^{14}C-labelled amino-acids, demonstrated that the leucocytes in tissue cultures produce a protein which migrates electrophoretically as γ-globulin. It produces fluorescent staining of all cells when incubated with fluorescent anti-γ-globulin. He concluded that the mitogenic action is probably an immune reaction.

This was followed by Pearmain *et al.*[7], who were able to produce mitosis in the lymphocytes from tuberculin-sensitive individuals by adding purified tuberculin to the cultures. Hirschhorn and his colleagues[8] produced the same effect in lymphocytes from individuals sensitized to diphtheria toxoid, pertussis vaccine and penicillin, using the appropriate antigen[3]. Not only microbial antigen but also tissue antigens have been used. Hashem *et al.*[9] found that lymphocytes from patients with infantile eczema were stimulated to undergo mitosis by extracts of human skin.

It seems that the technique of inducing mitosis appears to provide a useful and sensitive method for studying the mechanism of histo-compatibility. This communication describes a simple method which can be used to diagnose auto-immune diseases which have been attributed to release of previously sequestrated antigen (for example,

thyroiditis). This method is derived from the techniques described by Moorhead *et al.*[10], Edwards and Young[11], and Hashem *et al.*[9].

10 ml. of blood is obtained in a sterile heparinized container and left to sediment for ½–2 h. The supernatant layer (buffy coat) is aspirated in a Pasteur pipette and transferred to a sterile bottle. After mild shaking to disperse the cells evenly throughout the fluid, it is divided into three equal parts and put into three sterile universal containers. 'Medium 199' (Glaxo) containing antibiotics is added to each container to make a dilution of 1 part buffy coat to 3 parts of medium. 0·5 ml. of the antigen to be tested is added to the first bottle, an equal amount of phytohæmagglutinin to the second bottle, and of saline to the third bottle. The cells are incubated for 72 h. The slides are prepared as described by Moorhead *et al.*[10]. The results are read by scoring the percentage of dividing cells to non-dividing cells, and comparing the results obtained from the three bottles.

A high percentage of mitoses with antigen and with phytohæmagglutinin, and none with saline, suggests that the patient's symptoms are due to antigen–antibody reaction. A low percentage with antigen, a high percentage with phytohæmagglutinin, and none with saline suggest that there is no antigen–antibody reaction.

We thank Prof. G. M. Bull and Dr. E. O. Bartley for their advice.

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RADIOBIOLOGY

Early Response of *Pinus thunbergii* to Acute γ-Irradiation

While recently there has been much emphasis placed on the examination of the effects of chronic irradiation on intact whole forest ecosystems, the response of individual forest species following acute irradiation has received much less attention. At present only a limited amount of information is available concerning the effects of acute irradiation on the genus *Pinus*. Working at Brookhaven, Sparrow *et al.* have reported on *Pinus strobus*[1], and a second group under Shields has reported on *Pinus monophylla*[2]. The purpose of the work recorded here was to examine the response of *Pinus thunbergii* seedlings to acute γ-radiation under controlled laboratory conditions as opposed to that under the field conditions as examined by other workers[1,2].

One hundred four-year-old seedlings potted in 1-gal. metal cans were obtained from a local commercial nursery. The irradiation dose to be administered was chosen on the basis of Sparrow's work on *Pinus strobus*[1]. One half of the trees were irradiated while in the dormant state on February 21 of this year by means of a 10,000-c. cobalt-60 γ-source. Prior to irradiation the branches of each tree were bundled together loosely about the trunk in order to ensure as uniform a dose as possible to the entire tree. The time of exposure was 21·5 min. The total dose received by the trees was determined to be 715 ± 40 r by means of individual chemical dosimeters attached randomly to each tree. Afterwards, the trees were placed in the glasshouse and arranged in such a manner as to ensure that control and irradiated trees were randomly distributed.

In order that the effects of irradiation on growth of *Pinus thunbergii* be clearly understood, a short description of the normal growth of the plant is given. Longitudinal growth in this species is usually confined to candles laid down in the previous growth period. Development of adventitious buds is rare except in the case of physical injury to the plant, in which case such buds will develop from needle groupings in the area of the stem proximal to the injury. New shoots, or candles, which are laid down in the late summer and the autumn, may vary in length from 1 to 10 cm. Fig. 1 (left) illustrates the normal appearance of dormant candles. Following several months of winter dormancy, growth in the spring begins with the initial elongation of the candle occurring near its base. The zone of elongation then proceeds upwards toward the tip, with the portion of the shoot which has not yet begun to grow being literally lifted up by the elongating tissue beneath it. Such elongation continues, with the new needles being formed about the sides of the newly formed stem, until the zone of elongation reaches the tip of the previous year's meristem, at which time buds are laid down which will develop into candles for the following growth season. Fig. 1 (right) illustrates a group of normal candles in varying stages of development.

Measurements of the length of terminal and selected lateral candles on each tree were made during the first week following irradiation and before any visible growth was evident. In the second week following irradiation, elongation of the candles was evident in the control but not in the irradiated plants. Measurements of candle length were made in the third and sixth weeks on control and irradiated trees selected at random. In the third week the terminal candles of the controls showed an average growth of 3·6 ± 2·1 cm for 16 plants; the lateral candles a growth of 1·1 ± 0·9 cm for 24 plants. Five irradiated terminals and 25 irradiated laterals showed no significant growth. In the sixth week 14 normal terminals

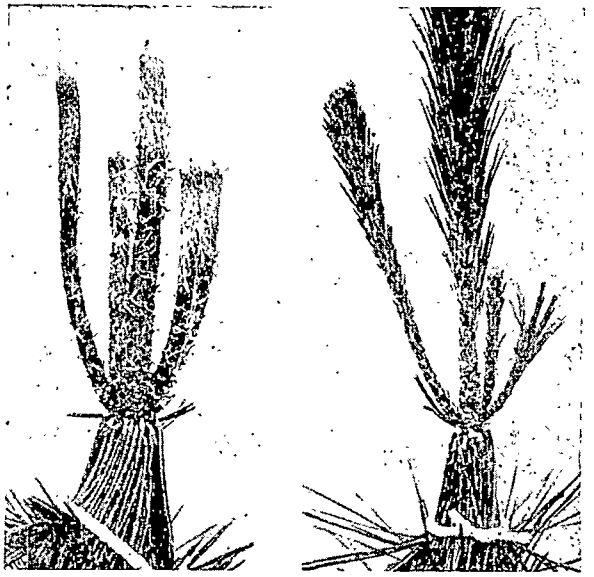


Fig. 1. (Left) Dormant terminal and lateral candles. (Right) Normal appearance of the candles after several weeks of growth. (Needles have been tied back to provide an unobstructed view)

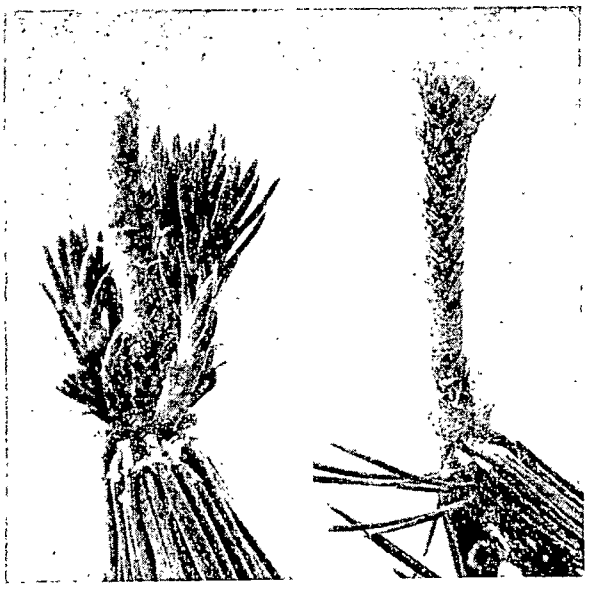


Fig. 2. (Left) Appearance of new buds arising at the base of an irradiated candle. (Right) Appearance of new buds arising at the tip of an irradiated candle. (Needles have been tied back to provide an unobstructed view)

showed a growth of 13·6 ± 5·6 cm; 20 laterals a growth of 6·4 ± 3·5 cm. In the irradiated trees 11 terminals showed a growth of 0·5 ± 0·4 cm, and 30 laterals a growth of 0·5 ± 0·5 cm.

Six of the trees displayed rudimentary male strobili (pollen cones) at the time of irradiation. Three of these trees were placed in the control group and the remaining three in the group to be irradiated. The strobili in both groups began development in the first week following irradiation. In the second and third weeks the strobili reached maturity and those of the control plants released copious amounts of pollen. However, the strobili of the irradiated plants, while reaching the same state of elongation, failed to show any release of pollen whatsoever. This phenomenon is similar to that reported by Mergen and Johansen[3] following chronic γ-irradiation of *Pinus rigida* at dose-rates of greater than 82 r./day.

Female strobili, or seed cones, developed in the control trees in six cases, while in no case did a similar development occur in irradiated trees. This latter phenomenon, however, may have been the result of chance selection rather than irradiation, since the rudimentary female strobili were not readily apparent at the time of irradiation and no attempt was made to ensure equal representation in both groups.

Late in the sixth week following irradiation, adventitious buds began to develop in the region immediately below the candles of the irradiated plants. By the tenth week 36 of the irradiated plants showed this type of development. Fig. 2 (left) shows the appearance of these buds at ten weeks. Brandenburg *et al.*[2] has reported a similar development from basal buds in the pinion pine. In addition to such development from obvious pre-existing basal buds, other buds appeared to develop *de novo* from the stem. Buds also were seen to develop from within needle groups, as commonly occurs when all meristematic tissue is removed from a normal pine. In three cases new buds developed along the length of irradiated candle, even though the particular candle itself failed to show any significant elongation.

In the ninth week following irradiation, at which time most of the shoots of the control plants had reached full growth and new buds were forming at their tips, it was noted that several of the irradiated candles were also in the process of bud formation at the tips, though they had failed to otherwise significantly elongate. Fig. 2 (right) shows the appearance of these buds at nine weeks. By the tenth week 22 irradiated plants exhibited this type of development and only 6 plants had failed to show any type of new growth. Similar bud formation at the tips of irradiated candles has not heretofore been reported in comparable dose ranges.

Such development of buds is of particular significance in that it has not been reported to occur under conditions of chronic radiation. Furthermore, it indicates an ability of the plants to survive and recover even after significant doses of acute γ-radiation. It would therefore be of interest to learn whether or not other species of pine might show a similar response or recovery under similar conditions.

This work was supported by contract *AT*(04-1) *GEN* 12 between the Atomic Energy Commission and the University of California, and by U.S. Public Health Services training grant *5TI-GM-796*.

We thank Mrs. K. Malin, who performed the dosimetry, and Drs. T. G. Hennessy, E. M. Romney and H. Nishita of this laboratory for their advice.

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BIOLOGY

Organization within the Central and Centrifugal Fibre Pathways in the Avian Visual System

Previous experimental studies[1,2] have established the existence of centrifugal fibres in the avian visual pathway and have shown that these fibres arise in the prominent isthmo-optic nucleus in the midbrain, that for a considerable part of their course they run independently of the optic nerve fibres, cross completely at the optic chiasma and terminate principally around the amacrine cells on the inner aspect of the bipolar cell layer as described in normal material[3,4]. The only known source of afferent fibres to the nucleus of origin of these fibres is the optic tectum[5], and there is evidence for some degree of organization within the projection of the tectum on the isthmo-optic nucleus[2]. This observation, when taken together with the electrophysiological evidence for the representation of the retina on the tectum[6], suggested, first, that there may be an ordered representation of the retina on the isthmo-optic nucleus, and secondly, that the nucleus may project in a similarly organized manner on the retina. This would mean that each part of the retina is reciprocally connected (through the tectum) with that part of the isthmo-optic nucleus from which it receives its centrifugal afferents.

This suggestion has been experimentally tested in three stages. In the first, the retinal projection on the tectum has been re-examined using the Nauta technique[7] following small, localized lesions in each quadrant of the retina. The results of this study confirm the pattern of retinal representation on the tectum as defined by Hamdi and Whitteridge[6]: the upper retinal quadrants project to the ventral half of the tectum, and the anterior (nasal) quadrants are related to the posterior half of the tectum. In the second series of experiments the details of the projection of the tectum on the isthmo-optic nucleus have been determined using the same technique after

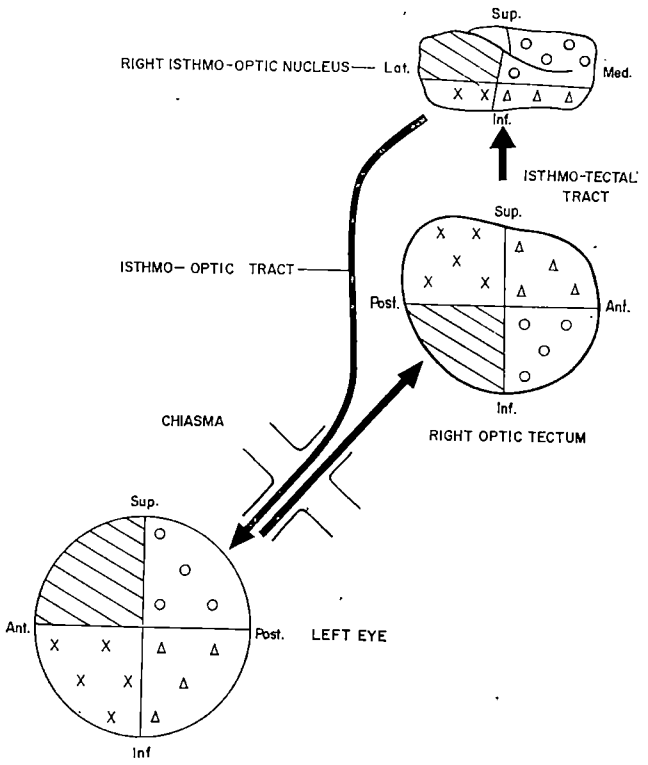


Fig. 1. Organization in the central fibre pathway in the relationship between the retina, optic tectum and isthmo-optic nucleus, and the reciprocal arrangement in the centrifugal projection from the isthmo-optic nucleus to the retina

small lesions of the tectum. The pattern of this projection is such that the dorsal half of the tectum is related to the ventral half of the isthmo-optic nucleus and the anterior half of the tectum to the medial half of the nucleus; that is to say, the retina is secondarily represented on the isthmo-optic nucleus in such a way that the superior retinal quadrants are represented in the superior half of the nucleus and the anterior quadrants in the lateral half of the nucleus. For the third stage in the analysis, it was necessary to determine the projection of the isthmo-optic nucleus on the retina with the retrograde cell degeneration method after lesions of varying size in the different retinal quadrants. The results have shown that there is again a definite organization in the projection of the centrifugal fibres to the retina, at least at the quadrantic level; thus the dorsal half of the nucleus sends its fibres to the superior half of the retina of the contralateral eye, and the lateral half of the nucleus to the anterior retinal quadrants (Fig. 1). It has not been possible to determine the relation of the isthmo-optic nucleus to the retina in greater detail, as small lesions of the retina do not result in detectable cellular degeneration in the nucleus—possibly because of collateral branching of the centrifugal fibres both in the brain and retina. Whether or not each centrifugal neurone influences only those ganglion cells from which it has indirectly received its afferent activation remains to be determined.

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Use of Long Cavities for Aqueous Biological Tissue Samples in an Electron Spin Resonance Spectrometer

A recent communication from this laboratory[1] appears in some ways to have been misunderstood. We did not claim that the machine described was more sensitive than that of other workers, but described a system which we felt was of practical value for the examination of aqueous biological tissues and which, as stated in the communication, was a modified version of one used by Commoner and Townsend.

It has been shown by Stoodley[2,3] that, for a multimode rectangular H_{01n} cavity, when $n = 10$ the optimum thickness of sample to be used is 0·5 mm and that, under these conditions, the product of filling factor (ξ) and Q is $\xi Q \doteqdot 14{\cdot}0$. For a thicker sample of 1 mm in this mode $\xi Q = 7{\cdot}9$ so that, because ξQ is inversely proportional to the minimum concentration of radicals detectable and is a measure of sensitivity, there is a drop in sensitivity of about 2. For a 1-mm sample thickness in a cavity with $n = 2$, ξQ is 8·7, and experiments in this laboratory have confirmed that there is a gain in sensitivity of approximately 10 per cent at $n = 2$, compared with $n = 10$ for 1-mm samples. For the cavity with $n = 2$, at the optimum sample thickness (0·38 mm), ξQ is 35 and, consequently, Stoodley has shown that the sensitivity of our cavity and associated sample cell is some 4·4 times worse than it would be if a short cavity and a thinner sample were used. However, he also recognizes that "biological investigations are hampered by the necessity of using thin samples". We consider that the multimode cavity which enables an increased thickness and volume of sample to be used amply repays in ease and convenience the loss of 4·4 in sensitivity.

In a recently published paper Stoodley[3] gives a graph relating ξQ to sample thickness for $n = 2$ which, as stated above, has a peak value of 35 at sample thickness 0·38 mm. It is immediately apparent that the corresponding curve for $n = 10$ will represent a more slowly varying function since it will only rise to a peak value of 14, as already stated here. As a result, there will be much less dependence of sensitivity on sample thickness and also, it may be inferred, on the exact positioning of the sample at the node of electric field. In fact, the expression for the dependence of ξQ on sample thickness ($2t$) may be written as $\xi Q \propto \frac{t}{n + C_1 t^3}$, where C_1 is a constant, and that on displacement (b) of the centre of the sample from the node as $\xi Q \propto \frac{t}{n + C_2 K^2 t}$ where $K^2 = t^2 + 3b^2$. Substituting numerical values shows that a sample displacement of 0·5 mm in the long cavity ($n = 10$) gives rise to only one-third of the sensitivity change which would occur in the short cavity ($n = 2$).

In the case of those biological measurements where many samples have to be measured as quickly as possible, this represents a very valuable gain in ease of sample placement and measurement. In addition, the use of the thicker sample holder with animal or human tissues enables slices of tissue to be inserted much more easily and quickly than in a thin sample holder and, furthermore, makes it more possible to completely fill the sample holder with tissue in the time available.

Wilmshurst[4] also made several comments on the original paper[1], most of which were valuable but some were based on unjustified assumptions. First, Wilmshurst quotes Commoner[5] as claiming $2{\cdot}5–5 \times 10^{-7}$ M of Mn^{++} as the minimum detectable concentration, and he compared it with the one quoted from this laboratory of 3×10^{-6} M. However, Commoner's sensitivity[5] is based on a signal-to-noise ratio of 1 : 2 whereas our figure is based on a more realistic ratio of 2 : 1. Taking into account this factor of 4, Commoner's sensitivity[5] becomes 1 to 2×10^{-6}, not a

great deal different from our original one. Naturally, since Commoner uses a long cavity technique, of which Townsend was the originator, the factor of 4·4 resulting from the cavity length (slightly modified for the extra length which they use) will also apply to their machine.

Secondly, Wilmshurst assumes that we use a 723*A/B* Klystron giving 20 mW, and compares it with that used in a Varian machine giving 180 mW; as a result he suggests that we are a factor of 3 down in sensitivity compared with the Varian machine arising from the loss of power. This is not the case, since the Klystron which we used is a *KS*20*A* which gives a nominal 35 mW, so that we were only 2·3 down from this point of view. Since the sensitivity is 30 times worse than that of the Varian machine according to Wilmshurst, then this leaves a factor of 13·3 arising from the cavity and preamplifier circuit. Of this, the factor 4·4 arises from the cavity design, leaving a factor of 3 due to the circuit of the preamplifier; this agrees almost exactly with his measured noise figure (7 dB) of the preamplifier rather than with the factor of 4 dB which he calculated.

Finally, Wilmshurst made suggestions to improve the above noise figure and these circuit modifications have proved to be successful; we have used an *E*180*F* in preference to the *EF*89 which he suggested, however, because it has an equivalent noise resistance of only 460Ω which gives a marginal improvement.

Two further improvements have been made to the cavity: first, the tuning paddle method of matching has been discarded in favour of matching screws above the coupling iris to the cavity, and secondly, the modulation coils are now wound with two turns of 18 standard wire gauge bare copper wire instead of enamel-covered wire. This was because a large signal was found to arise from the enamel insulation at about $g = 2$, which was 5–10 gauss wide and which had not been present before and probably arose from low temperature charring of the enamel. Such a signal has also been observed by Assenheim[6].

All these improvements have lowered the minimum detectable concentration to about 10^{-6} M at a signal–noise ratio of 2 : 1.

In conclusion, we support the point of view put forward by Pake[7], who said that it was "advisable to view pontifications on the subject (signal-to-noise)—with vigorous independence of mind"!

This work was supported by the Department of Scientific and Industrial Research.

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Immunological Response to Implanted Adult Dwarf Tapeworms

Earlier immunofluorescent, radioisotopic, and diffusion chamber isolation investigations[1,2] indicated that antibodies, derived as a result of ova-induced *Hymenolepis nana* infestations, combined *in vivo* with adult tapeworms. The authors did not, however, presume that responsible helminth antigens were, necessarily, stage-specific. The work recorded here was carried out to determine whether or not adult cestodes are capable of immunological insult *in situ*. Implantation of adult parasites into the ileum of the mouse host, for various periods of time, was therefore considered. Under these conditions the cysticercoid stage, with tissue phase involvement, could be precluded or controlled without utilization of an intermediate host. This appeared to permit a singular investigation of the lumen stage of the parasitæmia, from the point of view of possible elicitation of a local and/or systemic immunological response.

Initially viable adult specimens of *Hymenolepis nana* were allowed to react *in vitro* against positive antisera in Wilson double-diffusion plates at 37·5° C. The mature tapeworms were maintained in 'CulturStat 199' (Baltimore Biological Laboratory, Baltimore, Maryland) containing Earle's base basal medium, penicillin and streptomycin, following transfers through several rinses of sterile phosphate buffered saline of *p*H 7·2. The 'Plexiglass'-constructed plates were previously subjected to ultraviolet light, and merthiolate was not added to the 0·75 per cent Bacto certified agar used. Rabbit antisera, produced against sonicated worm extracts (intramuscular injection with aluminium hydroxide gel), and antisera from mice, following a 3-week period of infection, were utilized as antibody reactants. In either case two precipitation bands were evidenced within 24 h. With a period of prolonged incubation as many as four bands developed when rabbit antiserum was reacted with secretions and/or excretions from viable hymenolepids as compared with numerous precipitin arcs formed when the same antiserum was reacted on agar slides against electrophoresed sonicated worm extract (Fig. 1).

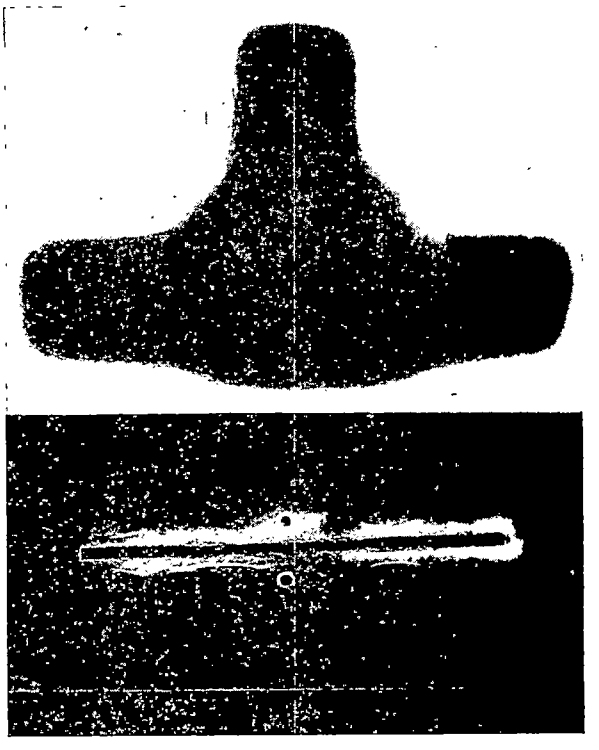


Fig. 1

The implantation procedure[3] was used, following the *in vitro* demonstration of the specificity of hymenolepid secretions and/or excretions, to ascertain whether or not such materials were exoantigenic during the lumen phase of the parasitæmia. Adult worms, obtained from infected *CBA/J* female mice, were transferred through several rinses of sterile phosphate buffered saline of *p*H 7·2, whereon they were implanted into the ileum of non-infected female mice weighing about 23 g. The surgical procedure was performed under 'Nembutal' anæsthesia and aseptic conditions. Following periods of

1, 2, 3, 4, 8 and 12 days, host mice were bled by cardiac puncture, killed and autopsied. Serum antibody was detected and titred by the indirect hæmagglutination method[4]. Formalinized sheep cells (Difco Laboratories, Detroit, Michigan) were tanned and sensitized with hymenolepid antigens. The sensitizing antigens were prepared by sonicating worms at 0° C for 3 min in phosphate buffered saline at 3 amp, followed by centrifugation at 4,340*g* for 30 min at 2° C. The nitrogen value for the supernatant was determined by micro-Kjeldahl analysis according to the Markham modification, adjusted to a value of 650 μg/ml. of antigen and used immediately to sensitize 1 ml. of a 2·5 per cent concentration of tanned cells.

Table 1. Detection of Serum Antibody versus Ileac-implanted *Hymenolepis nana* by Indirect Hæmagglutination

Groups	1	2	3	4	5	6
No. of animals per group	8	8	8	14	17	14
Periods of implantation	1	2	3	4	8	12
Average No. of worms implanted	9·6	8·3	8·5	7·4	7·4	7·0
Average No. of worms recovered	9·3	7·3	7·0	6·5	6·7	4·1
No. of mice in which all implants recovered and have hæmagglutination titres = or > 1 : 96	6	5	4	6	11	5
	+	+	+	+	+	+
Control groups*	*A*	*B*	*C*	*D*	*E*	
No. of animals per group	24	8	24			
Hæmagglutination	1 : 1536	1 : 1536	—	—	—	—

* *A*, Serum (not pooled) from mice infected for a period of 21 days with 5,000 ova; *B*, Serum (not pooled) from mice immunized with sonic worm extract (0·2 mg N) for a period of four days; *C*, Serum from normal mice; *D*, Antigen deficient reaction mixture; *E*, Test serum deficient reaction mixture.

Results (Table 1) indicate that antibody is engendered against adult dwarf tapeworms following implantation in the ileum of the host. The sensitivity of the indirect hæmagglutination procedure permitted detection of a systemic response as early as 24 h after implantation of only 8 worms. It appears, therefore, that hymenolepid exoantigens are capable of villus mediation and elicitation of a generalized immune response. The possibility of a local response is under investigation. Results of the advent of fæcal antibody will be presented following examination of the effect of irradiation of: (1) the ileum; (2) the mouse, exclusive of the ileum, on host response to implanted cestodes.

This work was supported by a research grant, *GB*–1116, from the U.S. National Science Foundation.

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Choanocytes in the Skin of *Harrimania kupfferi* (Enteropneusta)

During an investigation of mucous secretion on the proboscis of *Harrimania kupfferi*[1] ultra-thin sections were made of the epithelium of various parts of the body including proboscis, collar and the anterior parts of the trunk. In the epithelium of the ventral part of the trunk, immediately behind the collar, a distinct choanocyte type of cell was encountered.

Fig. 1 demonstrates the flagellum and the collar in cross-section, that is, a section parallel to the surface of the epidermis. The flagellum in the centre of the ring of microvilli is seen to possess the usual pattern of nine peripheral double filaments and two central single filaments. The collar consists of a ring of microvilli. Each microvillus measures about 500 Å across, and the microvilli are set about 500 Å apart, at least in the basal parts of the complex. Some sections suggest that the collar may be funnel-shaped, the diameter of the collar increasing from the base. Between the microvilli is seen an amorphous or possibly fibrous material, probably mucus. It adheres closely to the surface of the microvilli, and in some cases it is extended between opposed sides of adjacent microvilli (Fig. 2).

It is readily seen that the structure described is morphologically identical with the collar and flagellum of Choanoflagellates[2] and of the choanocytes of fresh-water sponges[3,4].

Some of the sections seem to indicate that some of the material adhering to the microvilli has bent inward. This observation may suggest that by the activity of the flagellum water is drawn into the lumen of the collar through the intervillar spaces. Thus the structure may be functioning in a way similar to that found in the choanocytes of sponges[4].

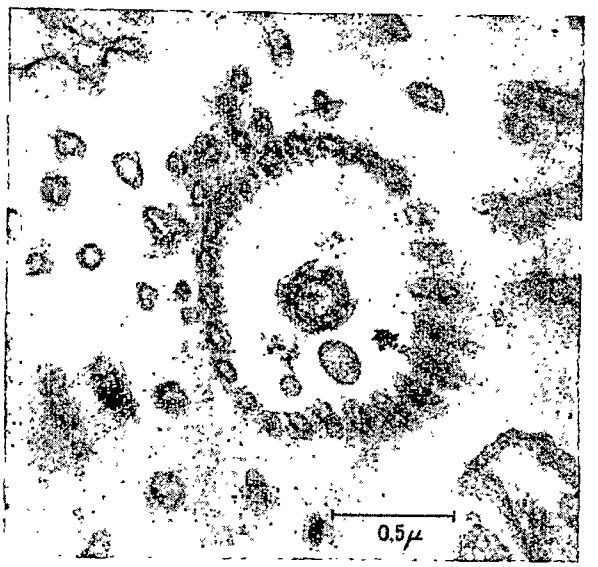


Fig. 1. Electron micrograph of collar and flagellum of the choanocyte in cross-section parallel to the surface of the epithelium. Outside the collar other microvilli are seen. Inside the collar are seen the flagellum, a couple of digitations of the cell surface and a speck of dirt

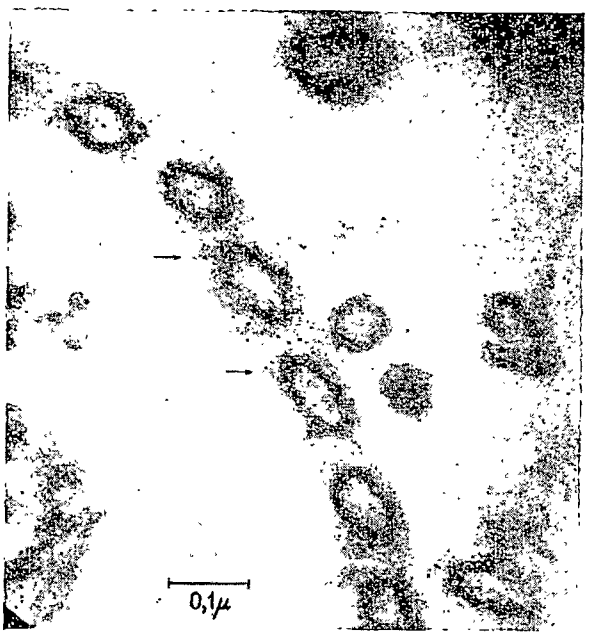


Fig. 2. The microvilli of the collar at a greater magnification. The unit membranes are clearly visible, and so is the amorphous material adhering to the surface of the microvilli. At arrows this material is bent inward (see text)



Fig. 3. Oblique cross-section of the base of a collar and flagellum. *F*, Base of flagellum; *C*, collar (somewhat distorted). The cytoplasm of the choanocyte is indistinguishable from that of the neighbouring cells, *A*, *B* and *D*

It might be suggested that the collar and flagellum belong to some sessile Choanoflagellate, embedded in the epithelium. Although only the outer parts of the choanocytes have been investigated with the electron microscope they evidently are very similar to the ciliated cells surrounding them, containing the same cytoplasmic structures and showing none of the features commonly found in flagellates (Fig. 3).

As will be described elsewhere[1], the other epidermal cells are also provided with a varying number of microvilli. This applies both to ciliated and mucous cells. In the ciliated cells the microvilli are evenly distributed between the cilia, while in the mucous cells the microvilli are often set as a brim along the border of the free cell surface. This brim shows a certain similarity to that of the choanocytes, but the row of microvilli is irregular and the flagellum is lacking. Obviously the function of the microvilli along the border of the surface of mucous cells is to lead the merocrine secretion free of the surface of the epithelium, which seems to be rather uneven, some cells protruding a few microns over the surrounding cells.

Of course, the appearance of choanocytes in Enteropneusta does not imply a close relationship between Parazoa and Stomochordata. It simply means that choanocytes are a more widespread structural feature than formerly believed, and not restricted to sponges and Choanoflagellates. The choanocytes can therefore no longer be used to support a close specific phylogenetical relationship between these two groups.

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Syncephalastrum associated with Bovine Mycotic Abortion

INVESTIGATIONS into an unusually high abortion rate among dairy cattle in Hong Kong have shown that a number of abortions were associated with fungal infections. In one such infection, the fungus has been shown to be *Syncephalastrum racemosum* (Cohn) Schroet. This is an addition to the list of fungi which have been implicated in bovine mycotic abortion, and also increases further the number of phycomycete fungi known to be abortifacients[1,2].

The fœtus, aged 3 months, was examined and cultured within a few hours of abortion. After surface-sterilization of the entire fœtus in alcohol, amniotic fluid was extracted aseptically and streaked on to plates of 'Sabouraud Dextrose Agar' (Oxoid) supplemented with streptomycin at 40 units per ml. Small fragments of cotyledons, fœtal membranes and the embryonic heart, liver, lung and stomach were also surface-sterilized and plated on to the same medium. Plates were incubated at 25° C and at the local ambient temperature (about 30° C), and the cultures were examined after 2–7 days. After 2 days, plates containing amniotic fluid, fœtal membranes or cotyledons all yielded numerous colonies of *S. racemosum*, and the fungus also developed in lesser amounts from the cultured heart tissue. All other tissues remained sterile over the incubation period.

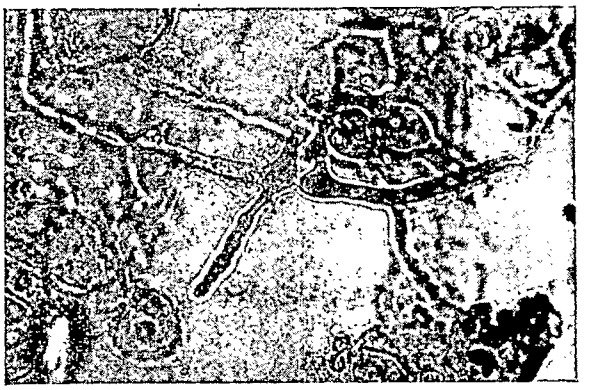


Fig. 1. Hyphæ of *Syncephalastrum racemosum* in the amniotic fluid of an aborted cow fœtus

The amniotic fluid contained small aggregations of mycelium, visible to the naked eye; they were found to be composed of broad, aseptate, hyaline hyphæ which branched and anastomosed (Fig. 1). Similar hyphæ were visible in crush preparations of cotyledonary and fœtal membrane tissue. No superficial development of fungus was seen on pieces of tissue maintained in high-humidity chambers for 3 days, and no lesions were seen on the other organs or on the skin of the embryo.

Syncephalastrum racemosum appears to have been isolated from animals only once previously[3], when it was considered to be an intestinal saprophyte of a pig. The origin of infection in the present instance is not known; *S. racemosum* is, however, ubiquitous in Hong Kong. I have found that spores of this fungus form a regular component of the air spora over the island, and that during the period February–August 1963 they occurred in proportions equal to those of two other phycomycetous fungi associated with abortion, namely, *Mucor* and *Rhizopus*. *S. racemosum* has also been isolated from soil[4], and investigations continue into possible sources of high inoculum.

I thank Mr. Ho Mang Hang for the photograph.

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Gametophytic Plants induced from Single Cells of Moss Callus

Callus and callus-like tissues have been reported in a wide variety of plants. Steward *et al.*[1] have described induced organization of intact plants from 'aggregates' which accumulated from single cells of carrot callus; Bristow[2] has reported induction of discrete fern plants (sporophytic and gametophytic) from his callus cultures under influence of growth substances, and Bauer[3,4] has shown that varied sporophytic and gametophytic forms may be induced from callus of moss. This communication deals with aberrant moss plants, gametophytic in form, regularly induced from single-cell callus plantings of *Polytrichum commune* Hedw.

Diploid callus tissue of gametophytic origin (spore plantings) in this moss has been kept in culture with continuous proliferation through periodic transfers since its isolation[5]. No observable production of organized entities of either gametophytic or sporophytic character has occurred naturally in this period; the callus nature of the cellular aggregates has remained that of the original isolate when maintained on agar *in vitro* with media components favourable to growth of spores and normal moss gametophytes[6].

Recently, organized plants of aberrant form and structure, having limited features of normal gametophytes, have been regularly induced from this callus on media containing growth substances.

Single, isolated cells of the callus (Fig. 1*D*), separated from the callus aggregates by agitation in sterile water, were planted to ensure exclusion of normal protonemata cells which might have accompanied the callus in the original transfers. The basic difference in size and initial division patterns of the callus cells and normal germinating spores in this species is shown in Fig. 1, *A–E*. Substrate for the single cells was media used for routine moss culture with additions of 1 p.p.m. naphthaleneacetic acid (NAA) and 0·25 per cent sucrose. Culture chambers were small Petri dishes sealed with polyethylene film. Glass micropipettes were used to transfer the single cells.

Isolated cells thus planted normally begin divisions within about four days (Fig. 1, *D*, *E*), and the accumulation of an irregular cellular mass rapidly follows. Organized meristematic areas, portending the induced plants, appear at the periphery of the callus aggregate in about two or three weeks. Sections of the small cellular masses show an inner orientation and organization of cells long before emergence of the differentiated plants (Fig. 1*G*). Regularly 100 per cent of the cultures in a series of trials are successful, and numerous plants are commonly induced from the aggregate of a single cell culture.

The new gametophytic-appearing entities have definite organization, large, multicellular apical meristems and predictable patterns of division and differentiation, and they often bear rudimentary leaf-like appendages (Fig. 1*F*). The initial growth is rapid, distinctly exceeding that of the callus aggregate on which they originated. Later (within 3–7 weeks) growth lessens in the absence of isolation and sub-culture, and the callus accumulations may gradually encompass the organized plants. The apical meristematic region of the plants under such conditions ordinarily begins to form callus.

Plants isolated and sub-cultured on media of the type which supported their initiation continue to grow, and limited branching is common (Fig. 1*F*). Growth substance is clearly essential to both formation and maintenance of the organized plants, as also noted by Bristow in similar trials with fern callus[2].

An obvious maturity and subsequent senility are evident in the transplants, whether transferred often or not. This manifestation occurs generally within three months after the original planting under growth conditions maintained for normal moss.

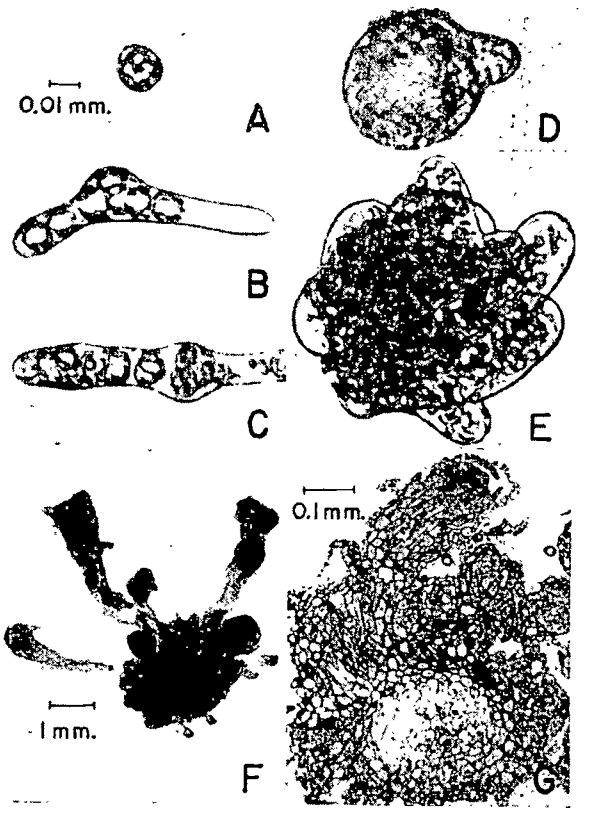


Fig. 1. *A*, *B*, *C*, ungerminated and germinating spores of *Polytrichum commune*, showing patterns of cell division and common orientation of growth in two directions. *D*, *E*, single callus cell, undergoing first division, and several-celled callus aggregate resulting from typically random divisions (scale in *A* is applicable to *A–E*). *F*, organized plants arising from callus aggregate within six weeks after callus was planted on medium containing 1 p.p.m. NAA. Leaf-like appendages are at tip; branches occur at sides. *G*, section of callus aggregate showing internal organization and (at top) detail of young meristematic tip of induced plant

Limited induction of these organized plant entities also results from use of other concentrations of NAA (0·1–10 p.p.m.); 0·1–10 p.p.m. of indole-3-acetic acid; and 2,4-dichlorophenoxyacetic acid in concentrations of 1–100 p.p.m. Gibberellic acid and indolebutyric acid have induced no responses.

The induced plants show essentially no sporophytic characteristics, as found in other mosses by Bauer[3] or by Lal[7] in certain other species, even though the callus in our experiments is determined to be diploid. The general aspect is that of a gametophyte, yet each new plant has a massive meristem and scale-like leaves (or none).

These aberrant plants are uniformly predictable; they can be induced with perfect regularity on appropriate media, and they arise either from single cell plantings on the initiating medium or from small aggregates of callus grown on the appropriate substrate. No special techniques are required to isolate the cells or to promote their subsequent growth.

This work was supported by grant *G*–19369 from the U.S. National Science Foundation. I thank Barbara Moore, Alvin Engelke and Sue Ellen Frederick for their assistance.

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Site of Action of Copper in Photosynthesis

Early observations on the presence of copper in chloroplasts[1] and the inhibition of photosynthesis by several reagents which are regarded as specific copper antagonists[2] established the concept that this element is perhaps a functional component of the photosynthetic system. Recent findings on the presence of a cytochrome photo-oxidase in chloroplasts[3] and the subsequent isolation of a copper–protein (plastocyanin) with characteristics identical to those of cytochrome photo-oxidase[4] provide additional support for a specific role of copper in photosynthesis.

Published experiments of Arnon[5] and Trebst and Eck[6] demonstrated that both photosynthesis and the Hill reaction are sensitive to copper-chelating agents. In addition, Spencer and Possingham[7] showed that chloroplasts from copper-deficient plants had a decreased Hill reaction activity. These findings have been interpreted as indicating a requirement for this nutrient in at least one of the partial reactions in the overall mechanism for oxygen evolution. In contrast to these data, Kok *et al.*[8] reported that the apparent quantum yield of the photo-oxidation of cytochrome, as catalysed by the photo-oxidase system, is increased by wave-lengths above 690 mμ. They concluded that only photochemical system 1 (ref. 9) is functional in this reaction. The present-day interpretation of this finding is that the plastocyanin does not function directly in oxygen evolution but rather in reactions leading to the generation of a strong photoreductant.

Whether or not a certain effect on photosynthesis is restricted to the mechanism of oxygen evolution or reactions culminating in the reduction of carbon dioxide can be tested by examining the capacity for photosynthesis, photoreduction and the quinone–Hill reaction in normal and treated algal cells. In several green algae, containing an adaptable hydrogenase, the photochemistry and carbon dioxide fixation may continue even though oxygen evolution has been eliminated. Consequently, certain specific poisons and manganese deficiencies inhibit the Hill reaction but do not influence photoreduction. On the other hand, cyanide and phosphorylation uncouplers (dinitrophenol, *m*-chloro carbonyl cyanide phenylhydrazone), for example, inhibit both photosynthesis and photoreduction without strongly influencing the quinone–Hill reaction of algae. The usefulness of this technique is apparent when the success of localizing the effects of *o*-phenanthroline[10], manganese deficiency[11] and DCMU[12] on photosynthesis is considered.

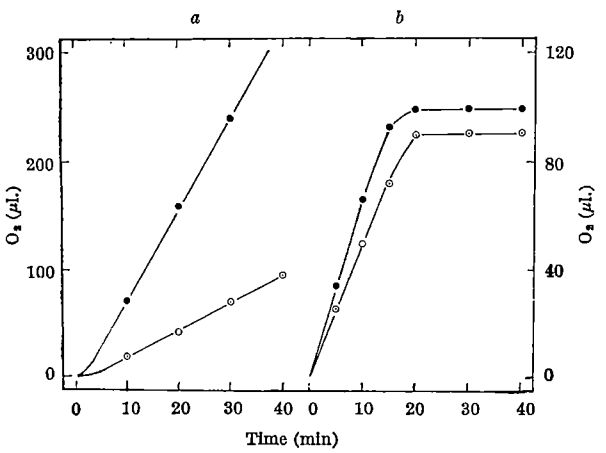


Fig. 1. *a*, Photosynthesis of normal (○) and copper-deficient (●) *Scenedesmus*. Manometric readings (corrected for dark respiration) are for oxygen formation. Experimental conditions: normal cells grown autotrophically with 1 × 10^{-6} M $CuCl_2$ present in culture medium. Copper deficient cells maintained through three transfers and used when six days old in the last transfer. Temperature = 25° C. Buffer = Warburg's No. 9 carbonate–bicarbonate. Gas phase = air. Light intensity = 18 k lux. Chlorophyll concentration = 0·25 mg/vessel. *b*, Quinone–Hill reaction of normal and copper deficient *Scenedesmus*. Reaction conditions were the same as described above except for the following: Buffer = 0·05 M phosphate, *p*H = 6·5. Gas phase = air – 4 per cent CO_2. Quinone concentration = 1 mg/3 ml.

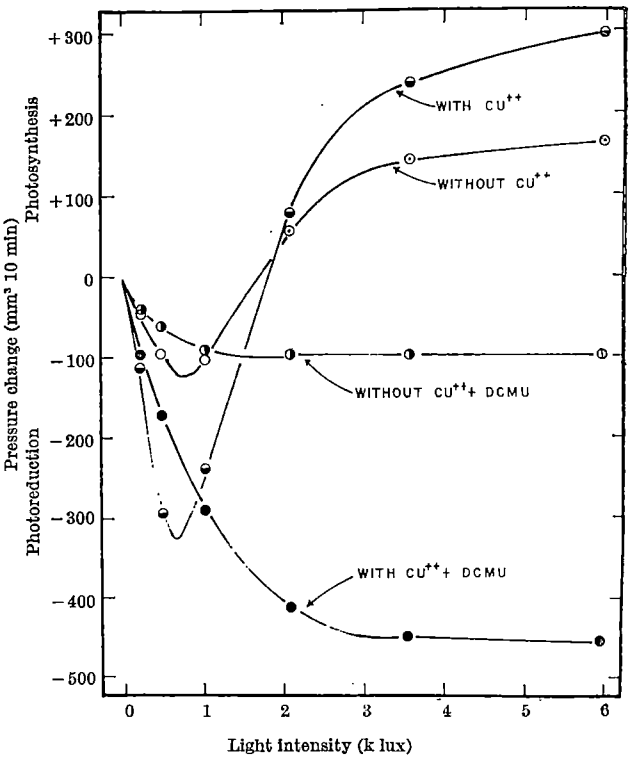


Fig. 2. Rate of photoreduction of normal and copper-deficient *Scenedesmus* at various light intensities with and without DCMU (2 × 10^{-6} M). Reaction conditions same as outlined in legend for Fig. 1*a* except for the following: Buffer = 0·05 M phosphate, *p*H = 6·5. Gas phase = hydrogen – 4 per cent CO_2. ◒, = control; ●, control + DCMU; ○, = copper-deficient; ◑, deficient + DCMU

Cells of *Scenedesmus obliquus* strain D_3 were grown in a medium which had been extensively purified of heavy metals by co-precipitation with calcium phosphate followed by solvent extraction with dithizone dissolved in chloroform. Spectrographically pure solutions of manganese, zinc, molybdenum, cobalt and boron were used for supplying the minor elements required for growth. The capacity for photosynthesis and quinone–Hill reaction of cells grown with and without copper is shown in Fig. 1*a* and *b*. At saturating white light intensity, photosynthesis of deficient algae is reduced by about 65 per cent whereas the Hill reaction rate is only slightly inhibited (maximum of 25 per cent in four separate experiments).

When normal and deficient cells are adapted to an atmosphere of hydrogen–4 per cent carbon dioxide, a pattern of photoreduction is obtained as illustrated in Fig. 2. Cells grown with copper show a normal pattern for photoreduction. Photoreduction by deficient cells is, however, markedly altered. This inhibition becomes more apparent when photoreduction is stabilized with DCMU; in the presence of this herbicide photoreduction by copper deficient cells is inhibited by approximately 78 per cent. From Fig. 2 it is also apparent that the de-adaptation process, that is, the return to normal photosynthesis, is not influenced as would be expected if the capacity for oxygen formation had been altered by the deficiency.

In 1961, Bishop and Gaffron[13] observed that the low apparent quantum yield at 705 mμ for photosynthesis by *Scenedesmus* and *Anhistrodesmus* could be increased by forcing the cells to perform photoreduction. The conclusion drawn at that time was that photoreduction requires the energy derived principally from pigment system I. The parallelism between this finding, Kok's

observation on the quantum yield of cytochrome *c* oxidation, as catalysed by plastocyanin and *P*–700 (ref. 8), and the requirement for copper in photoreduction as indicated by the data presented here suggests that copper is an essential component of the electron transport mechanism of pigment system I.

I recognize that the reduced photosynthetic and photoreductive activity associated with the nutrient deficiency does not demonstrate conclusively the precise function of the element in question. Although the effects observed may be due to a secondary response of the nutrient's direct action, normal rates of photosynthesis and photoreduction are recovered within 24–36 h after addition of copper. This recovery precedes the restoration of the chlorosis which also accompanies copper deficiency.

A more detailed description of these results will be published later. This investigation was aided by the U.S. Atomic Energy Commission (contract *AT*. (45–1)–1783).

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MICROBIOLOGY

Pigment Formation by *Acetobacter acetigenum* in a Lactate-buffered Glycerol Medium

Few observations have been recorded on pigmentation in *Acetobacter* species. Among them *A. melanogenum* forms a black pigment and *A. roseum* a red pigment[1]. Carr and Shimwell[2] have reported the production of dark brown, water-soluble pigment by *A. aceti*. This communication reports the formation of a wine-red pigment by *Acetobacter acetigenum* in a lactate-buffered glycerol medium.

The medium (referred to as Medium *C*) was made up as follows: to 1 l. distilled water was added 2·0 g potassium dihydrogen phosphate, 1·0 g crystalline magnesium sulphate, 0·01 g crystalline ferrous sulphate, 2·5 g ammonium hydrogen phosphate, 0·002 g calcium D-pantothenate, 0·002 g riboflavin, 0·0001 g biotin, 10·2 g lactic acid, and 30·0 glycerol. After dissolving the lactic acid and salts, the *p*H was adjusted to 4·6 with potassium hydroxide and the solution was sterilized at 10 lb./in.^{2} for 15 min. Finally, the vitamins were added in 2 ml. aqueous ethanol (50 per cent by volume). One l. of Medium *C* was dispensed in each of twenty-five 250-ml. conical flasks previously sterilized for 20 min at 15 lb./in.^{2}, and inoculated with an active culture of *A. acetigenum* N.C.I.B. 8132, a cellulose-forming micro-organism. Seven 'Pyrex' tubes, containing exactly 8 ml. of Medium *C* (uninoculated), served as controls. The inoculated conical flasks and the seven 'Pyrex' tubes were maintained at 30° C. At the end of 7, 10, 13, 14, 17 and 21 days, 8 ml. of the culture medium from the conical flasks were carefully released by a sterilized pipette and its optical density was measured against the contents of the 'Pyrex' tubes serving as controls. For this purpose a Hilger Spekker was used fitted with spectrum violet No. 606 Ilford colour filters.

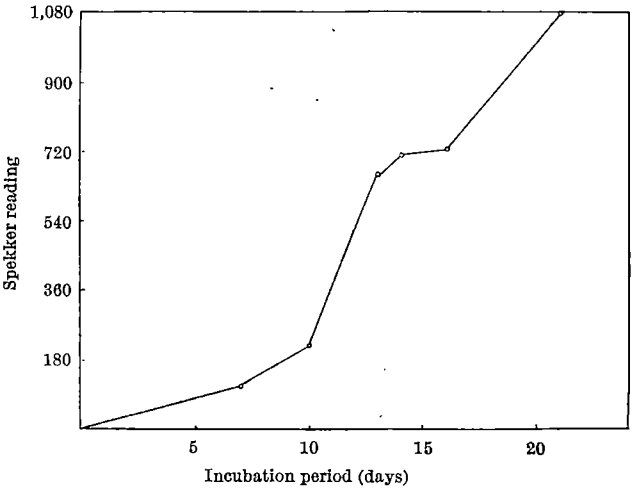


Fig. 1. Pigment formation during the growth of *Acetobacter acetigenum* in glycerol medium

The relationship between the number of days of incubation and accumulation of pigment is shown in Fig. 1; the elaboration of the colouring material seems to increase progressively.

In further experiments 1 l. of Medium *C* was prepared and dispensed aseptically into twenty-five 250-ml. conical flasks and inoculated with a growing culture of *A. acetigenum*. The cultures were maintained statically at 30° C for 21 days. The culture fluid was then pooled, Seitz-filtered and concentrated ten-fold, yielding a wine-red liquid. After preliminary trials with various solvents, isoamyl alcohol was found to be the most promising solvent for the extraction of the colouring matter.

In collateral investigations we have noticed a progressive loss of colour (greenish yellow) of Medium *C* (with ethylene glycol as the primary carbon source) during the dissimilation of ethylene glycol by *A. acetigenum*, ultimately becoming colourless after three weeks. It may be noted that the wine-red colour developed only in static cultures of *A. acetigenum* maintained on glycerol medium and not in the shaken cultures on glycerol. No attempt has been made at isolation, analysis and chemical characterization of the colouring matter, as our efforts were mainly directed towards the isolation of sugars[3] formed by the action of *A. acetigenum* on lactate-buffered glycerol medium, during the synthesis of cellulose. The pigment is presumably a manifestation of some metabolic by-product.

One of us (K. R.) acknowledges the receipt of an Imperial Chemical Industries research fellowship. We thank Prof. T. K. Walker for his advice.

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ENTOMOLOGY

Fossil Diptera and Continental Drift

It is well known that many plant and animal taxa have what has been termed an 'Antarctic' or 'Antarctogaean' distribution, that is, they are mainly or entirely restricted to southern Australia and/or New Zealand, southern South America, and, in some cases, South Africa. This

has been accepted by many biogeographers as evidence of an ancient connexion between these land-masses, probably via the Antarctic continent. In particular, it is seen as strong evidence in favour of Wegener's hypothesis of 'continental drift', a theory now attaining some respectability in the eyes of the geophysicists[1] who were formerly its principal opponents.

Opposition from biogeographers[2,3] has usually taken the form of deriving present 'Antarctic' distributions from earlier Holarctic distributions, the taxa in question being considered terminal isolates of groups which have suffered extinction in northern lands. In opposition to such views, it seems relevant to quote here some comparisons between the Recent dipterous fauna and that known from Tertiary fossils of the Northern Hemisphere.

In a survey of the known Tertiary fauna, one is immediately struck by the absence of genera at present recognized as 'Antarctic'. A good example is furnished by the family of fungus-gnats (Mycetophilidae), of which fossils, principally Oligocene, are reasonably abundant. By my own count, 120 genera are currently recognized in the Recent fauna and, of these, 35 are known from the Tertiary[4,5]. There are at least 11 genera with 'Antarctic' distributions: *Australosymmerus*, *Nervijuncta*, *Paramacrocera*, *Allocotocera*, *Aneura*, *Parvicellula*, *Stenophragma*, *Neoaphelomera*, *Paratrizygia*, *Austrosynapha*, and *Paraleia*; none of these is known from the Tertiary fauna. A simple χ^2 test for independence applied to these figures gives $\chi^2 = 4{\cdot}99$, $P < 0{\cdot}05$. It seems likely that other examples of 'Antarctic' genera will be revealed by further examination of southern faunas; also, the total number of genera under consideration could be reduced by eliminating specialized, endemic forms. Such corrections would reduce still further the likelihood that the observed absence of 'Antarctic' genera from the Tertiary fauna is due to chance alone. It is suggested therefore that their apparent absence represents a historical fact; and that, in accordance with Wegener's theory, they evolved in isolation, during the Mesozoic, on a southern supercontinent.

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GENETICS

Sub-line Differences among C57 Black Mice in Response to Trypan Blue and Outcross

THERE exist many sub-lines of the highly inbred *C57* Black strain of mice. One of the characteristics of the *C57* Blacks, especially noted in sub-lines six and ten, has been the occurrence of sporadic eye defects[1–3], attributed to non-hereditary variability in a genetically determined threshold for abnormality[1]. The anophthalmic phenotype of the mouse has been extensively examined by Chase[1,4] and, more recently in the *ZRDCT–An* line, by Beck[3,5,6]. It is characterized by complete bilateral eyelessness among 90 per cent of the individuals homozygous for the recessive oligogene (*ey*) and most of the polygenic modifiers, and severe microphthalmia in the remaining 10 per cent.

Barber[7], using trypan blue as a teratogenic agent, reported the induction of unilateral eyelessness among fœtuses heterozygous for *ey*, and not among the homozygous normal control population. Gillman *et al.*[8] had discovered the teratogenicityof tr ypan blue, and Hamburgh[9] had shown that the dye was indeed teratogenic in mice. Beck[6], also using trypan blue, demonstrated a higher incidence of eye defects among fœtuses heterozygous for *ey* in crosses of *ZRDCT–N* × *ZRDCT–An* than among homozygous normal pure line *ZRDCT–N* treated in the same way. Additional novel features of Beck's experimental system were the bilateral nature of a number of the defects, and, regardless of whether one or both sides were affected, the eye reduction could be either complete or partial. Beck's findings, in which the principal difference between the two experimental groups of animals was the presence or absence of the mutant *ey* allele on one of the pair of chromosomes, when added to Barber's earlier findings, strongly suggest that *ey* is able, in single dose, to bring animals closer to a threshold for abnormality. If so, then this might be a useful method for detecting subtle differences between groups of animals.

This communication presents the results of an experiment in which the same treatment as has been described[6] was applied to two different sub-lines of the *C57* Black strain. Three intraperitoneal injections, each of 0·25 c.c. of a 0·3 per cent solution of trypan blue in 0·9 per cent sodium chloride, were administered during the seventh, eighth and ninth days of gestation to *C57BL/10 Ch* and *C57BL/6 Fs* females crossed with males of their own or *ZRDCT–An* lines. The treatment was designed to maximize the occurrence of eye defects and minimize

Table 1. ABNORMALITIES AT BIRTH AMONG UNTREATED LITTERS

Cross ♀	Cross ♂	No. of litters	No. of progeny Normal	No. of progeny Abnormal	Description of abnormalities	Frequency (% ± *S.E.*) of abnormalities
C57BL/10	*C57BL/10*	15	85	8	2+/m, m/+, m/m, m/an, s/an, 2s/m	8·6 ± 2·9
C57BL/6	*C57BL/6*	26	138	16	3+m/, 3+/s, 2m/m, 2m/s, 2s/m, 2s/s, s/an, an/s	10·4 ± 2·5
C57BL/10	*ZRDCT–An*	25	177	2	+/m, +/s	1·1 ± 0·7
C57BL/6	*ZRDCT–An*	10	73	2	+/m, +/m	2·7 ± 1·9

In the 'Description of abnormalities' column the character to the left of the stroke describes the left eye and the character to the right of the stroke describes the right eye. + = normal eye; m = medium eye, or slight reduction in size; s = small eye, or severe reduction in size with maintenance of globular shape; an = anophthalmic, or complete absence of the eye.

Table 2. ABNORMALITIES AND MORTALITY AT 18 DAYS *post coitus* AMONG UNTREATED LITTERS

Cross ♀	Cross ♂	No. of litters	No. of implantations Living Normal	No. of implantations Living Abnormal	Dead	Description of abnormalities	Frequency (% ± *S.E.*) of Abnormality	Frequency (% ± *S.E.*) of Mortality
C57BL/10	*C57BL/10*	5	28	7	7	2+/m, m/+, +/s, m/an, an/s, an/an	20·0 ± 6·8	16·7 ± 5·7
C57BL/6	*C57BL/6*	9	66	9	7	4+/m, +/an, 2m/m, s/m, an/an	12·0 ± 3·7	8·5 ± 3·1
C57BL/10	*ZRDCT–An*	5	39	1	7	+/m	2·5 ± 2·4	14·9 ± 4·2
C57BL/6	*ZRDCT–An*	6	42	3	10	3+/m	6·7 ± 3·7	18·2 ± 4·2

For descriptive legend see Table 1.

Table 3. Abnormalities and Mortality at 18 Days *post coitus* among Treated Litters

Cross ♀	♂	No. of litters	No. of implantations Living Normal	Living Abnormal	Dead	Description of abnormalities	Frequency (% ± S.E.) of Abnormality	Mortality
C57BL/10	*C57BL/10*	5	12	4	27	2+/m, 2s/an	25·0 ± 10·8	62·8 ± 7·4
C57BL/6	*C57BL/6*	6	8	2	35	+/m, +/s	20·0 ± 12·6	77·8 ± 6·2
C57BL/10	*ZRDCT–An*	6	37	2	6	+/m, +/s	5·1 ± 3·5	13·3 ± 5·1
C57BL/6	*ZRDCT–An*	9	23	13	29	m/+, +/m, s/+, 2an/+, +/an, m/an, s/an, 5an/an	36·1 ± 8·0	44·6 ± 6·2

For descriptive legend see Table 1.

other abnormalities. Time of insemination was detected by the copulation plug method[10], and the day of insemination was considered day zero. Treated and untreated pregnant females were dissected on the 18th day of gestation. The number of dead and resorbed fœtuses was noted; living fœtuses were examined for eye defects and other gross morphological abnormalities. The results were analysed with respect to frequency and variety of defects and frequency of post-implantational prenatal mortality. Another and larger body of results was collected from untreated litters at birth. The tests of significance applied to the data were the χ-square test of homogeneity and the Student–Fisher grouped-method t-test for the comparison of small samples[11].

At birth (Table 1) there are spontaneously occurring defective individuals in all crosses. There are no significant differences between the pure lines or between the two F_1 samples. Each F_1 does, however, present fewer abnormalities than its corresponding pure line cross ($t = 3{\cdot}1$, $d.f. = 270$, $P < 0{\cdot}002$ for sub-line 10 crosses; $t = 2{\cdot}0$, $d.f. = 227$, $P < 0{\cdot}04$ for sub-line 6 crosses). The defects among the F_1 were, in each case, mild unilateral microphthalmia. A reciprocal outcross of untreated *ZRDCT–An* females mated with *C57BL/10* males, although not specifically a part of this experiment, did produce a group of eight litters containing a total of 61 individuals, all of which were normal.

An examination of untreated litters at 18 days of gestation (Table 2) reveals comparable frequencies of abnormalities to those found at birth. More cases of slight aberration are noted owing to the greater ease of detection by virtue of greater tissue transparency. Examination at this time also provides a measure of prenatal mortality and thus allows the additional criterion of response to the toxic properties of the dye to be assessed. Spontaneous prenatal mortality in all four crosses is about the same. The decreased frequency of eye defects in the *C57BL/10* × *ZRDCT–An* relative to pure line *C57BL/10* crosses is, as in the data at birth, significant ($t = 2{\cdot}5$, $d.f. = 73$, $P < 0{\cdot}02$); the results from the *C57BL/6* matings are not extensive enough to reveal differences, if they exist.

Among the treated litters (Table 3) there are no differences in response between the pure line matings, but the two F_1's do differ markedly from one another both with respect to the frequency of eye defects ($t = 3{\cdot}6$, $d.f. = 73$, $P < 0{\cdot}001$) and mortality ($t = 3{\cdot}8$, $d.f. = 108$, $P < 0{\cdot}001$). The *C57BL/6* × *ZRDCT–An* litters are highly susceptible to the teratogenic and toxic effects of the treatment; their response resembles that of the *ZRDCT–N* × *ZRDCT–An* reported elsewhere[6]. The *C57BL/10* × *ZRDCT–An*, on the other hand, are, of all four crosses, the most resistant to the treatment. They seem to show heterotic vigour.

These results indicate that, by the use of a teratogen coupled with appropriate crosses, one may demonstrate what are probably polygenic differences in two sub-lines of the same inbred strain, exerting modifying effects on the processes of eye development. In the absence of the double treatment (that is, outcross to anophthalmic mice and exposure to the teratogen) the two groups of animals are, with respect to the criteria of classification used, indistinguishable.

This work was supported in large part by U.S. Public Health Service fellowship 10,004 from the National Institute of Neurological Diseases and Blindness. The work was done at the Mammalian Genetics Center, Department of Zoology, University of Michigan, Ann Arbor.

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Preservation of the Heterozygous Diploid Condition in Industrial Micro-organisms

The discovery of parasexuality in *Aspergillus nidulans*[1] has led to the possibility of breeding strains of asexual micro-organisms for increased yields of a desired product[2]. It has been reported that in *Penicillium chrysogenum* a synthesized heterozygous diploid gave a higher yield of penicillin than either haploid parent, perhaps through an effect of heterosis[2]. It might sometimes be desirable therefore to use a heterozygous diploid of *P. chrysogenum* or other micro-organism in an industrial fermentation. A heterozygous diploid, however, does not breed true, but can give rise to segregants as the result of mitotic crossing-over or non-disjunction of chromosomes[3]. The rate of segregation may vary in different organisms, and if it were high then the accumulation of segregants during the course of a fermentation could have an adverse effect on the yield of a product. To prevent this or to avoid the consequences there are two possibilities, namely, to reduce the rate of segregation, or to select against segregants as they are formed.

Chemical and physical agents are known which can increase the rate of segregation[4] and it has also been demonstrated that the effectiveness of some segregation inducers can be obviated by chemical treatments (for example, see ref. 5). So far as is known, however, such treatments do not affect the rate of natural segregation. The second method is therefore suggested as a means of preserving the purity of a heterozygous diploid culture as much as possible during the course of a fermentation.

In *P. chrysogenum*, when segregants are isolated from a heterozygous diploid made between strains which are not closely related, then these segregants tend to be of haploid parental type more often than of recombinant type[6,7]. This process has been termed parental genome segregation[7]. Successive mutagenic treatments of ancestral strains of the haploid parents, for the derivation of isolates of

increased penicillin yield, could have led to different chromosomal rearrangements in each haploid parent which restricted recombination at the diploid stage[6,7]. If, as immediate parents of such a diploid, genetically labelled strains were used which grew poorly in a production fermentation medium, then, because of parental genome segregation, selection would operate against the majority of segregants which arose. The haploid parent strains could be labelled with mutations imposing genetical restrictions on growth—so-called 'dwarf' mutations. A second class of mutants which could serve as parents would be auxotrophs which would grow poorly on complex media but normally on a minimal medium supplemented with their growth factor requirements. Such mutants have been found in *Aspergillus nidulans*[8] and *Neurospora crassa*[9], and presumably their restricted growth on complex media is due to the presence of inhibitory substances in such media. Although in *P. chrysogenum*, mutation to auxotrophy often leads to a reduction in penicillin yield[10], this is usually a pleiotropic effect of the auxotrophic locus, which is recessive[10,11]. Similarly it could be expected that recessive dwarf mutations might not affect penicillin titre in the heterozygous diploid stage if they were non-allelic. If a diploid were made between dwarfs or auxotrophs of the sort discussed, then in a complex production medium segregants of parental type would be contra-selected.

In micro-organisms in which heterozygous diploids have particular advantages over their haploid parents, and segregation occurred at an excessive rate, it may be possible to ensure parental genome segregation if it did not already exist. Heavy doses of X-rays lead to frequent chromosomal rearrangements[12]. The sequence of events would therefore be the induction of parental genome segregation by using X-rays as a mutagenic agent for genetically labelling both haploid parents and the subsequent isolation of dwarf mutants, or suitable auxotrophs, as discussed above, to synthesize the diploid. Multiple labelling by X-ray treatment could be considered as a method for increasing the possibilities of restricting recombination.

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AGRICULTURE

Hydrocyanic Acid Content and Nitrogen-fixing Capacity of Guar

Cyanogenesis has been extensively investigated in many plants chiefly because of the effect that the hydrocyanic acid could have on livestock. Since hydrocyanic acid is very toxic to plants, it does not occur in the free form but combined with sugars to form an insoluble compound, known as cyanogenetic glycoside.

The six cyanogenetic glycosides that have been most investigated are amygdalin, lotusin, dhurrin, sambunigrin, linamarin and lotaustralin. Amygdalin is the best known of these and was first isolated in 1830 from bitter almond and related plants. On hydrolysis in the presence of an enzyme it yields glucose, benzaldehyde and hydrocyanic acid.

Quisimbine[1] listed more than 300 separate species of seed-bearing plants known to contain hydrocyanic acid; including 52 members of the Leguminosae, work in this field has been concentrated mainly on the different species of sorghum, Sudan grass, Johnson grass, white clover and arrow grass. Leeman[2] has given an extensive bibliography on hydrocyanic acid in grasses. Nothing has been found in the literature concerning the content of hydrocyanic acid in guar. The main aim of this communication is to report some of the findings in the preliminary work on guar.

Guar (*Cyamopsis tetragonoloba*) or cluster bean was introduced into Nigeria from India about 1962 and seems to have aroused some interest. It was hoped that it would become an acceptable protein supplement and replace the more expensive imported peas. Guar beans are recommended to be eaten at a young stage when they are soft and succulent. At this stage the taste closely resembles that of lima beans. Matured guar beans are tough, fibrous and less palatable.

In preliminary experiments the guar beans were planted in beds at 3-ft. intervals. The yields in the first crops were most encouraging. Hydrocyanic acid was determined in two categories, the young and the matured beans, using the $AgNO_3$ method[3]. The fresh beans were cut into pieces, distilled water was added and the material was macerated at room temperature for 2 h. Further distilled water was added and the macerate was steam-distilled into 20 ml. of 0·02 N $AgNO_3$ solution, acidified with 1 ml. HNO_3. After collecting 150 ml. the excess $AgNO_3$ was titrated with 0·02 N KCNS using ferric alum indicator.

The HCN content of the young beans ranged from 40 mg to 70 mg, with a mean value of 55 mg HCN per 100 g dry matter, while the matured beans contained only traces which could not be estimated by this method.

This observation is in accordance with the work of other investigators in this field. Swanson[4] found most hydrocyanic acid in Sudan grass when the plants were young and in a vigorous growth. Everist[5] claims that young shoots of Sudan grass and grain sorghum are more dangerous than old ones and that they are most dangerous when young side shoots are plentiful. Others have arrived at the same conclusion[6–9].

The best known cyanogenetic glycoside in Nigeria is linamarin. This is chiefly found in one of our staple foods, cassava (*Manihot utilissima*). Linamarin on hydrolysis yields glucose, acetone and hydrocyanic acid. Dean[10] gives figures of 4–36·5 mg/100 g fresh root for 14 varieties. Corneia[11] found 5–15 mg/100 g and showed that the peel, which is 17·06 per cent of the fresh root, contained 60 per cent of the total HCN and practically all of this was destroyed on boiling.

In Nigeria, cassava is made into 'gari' by finely grating the peeled root, which is then placed in a sack and fermented for about four days. The product is then fried at about 100° C and sieved. The average HCN content of such gari is about 1·5 mg/100 g (1–2 mg per cent). Hence by this process most of the HCN is eliminated. Most of the HCN content of guar beans could be eliminated by cooking. On the other hand, they could be dried before use. Paula and Rangel[12] reduced the HCN content of cassava as follows:

Fresh root	3·9 mg HCN/100 g
Sun-dried root	1·7 mg HCN/100 g
Oven-dried root	0·6 mg HCN/100 g
10 min boiling	0·0 mg HCN/100 g

However, since the bulk of the HCN would be removed in the preparation and it would be unlikely to constitute the principal item of diet, the toxic levels quoted (for example, 24·1 mg/100 g said to have caused two deaths) would not be approached.

However, there are other potential uses to which guar could be put; first, as a protein supplement for cows. In this connexion the conclusions arrived at by Boyd *et al.*[8], from the analysis of nearly 500 samples of Sudan grass, are relevant (Table 1).

Table 1

mg HCN/100 g dry tissue	Toxicity
0–25	Very low (safe pasture)
25–50	Low (safe pasture)
50–75	Medium (doubtful)
75–100	High (dangerous pasture)
>100	Very high (very dangerous pasture)

In comparison, HCN content of guar (55 mg/100 g) may therefore be regarded as being medium. However, guar could be quite safe in pasture if it were grazed at a mature stage, since the content of HCN is then negligible.

The 'apparent yield' of guar from the preliminary experiment exceeded that of any other native beans that have been planted on the University Farm. Analyses of guar beans and native cowpea seeds (*Vigna unguiculata* Walp) are given in Table 2 for comparison.

Table 2. Comparison of Percentage Constituents of Guar Beans and Native Cowpea

	Moisture	Ash	Protein	Crude fibre	Nitrogen-free extractives	Fat
Cowpea seeds	8·70	3·78	22·75	1·81	67·28	2·46
Guar beans	6·9	3·4	25·0	10·0	52·1	2·6

It is obvious from Table 2 that the protein content of guar beans exceeds that of cowpeas and hence guar beans could be used as a source of protein.

This legume might find widespread use as soil-improving (nitrogen-fixing) crop, an annual row crop, a cash crop (for human consumption), and home-grown protein feed for stock.

If the plant should prove satisfactory for all these purposes it would be superior to the legumes now used as fallow crops (cowpea, pigeon pea, Mucuna seed).

Its use in Nigerian agriculture could be justified purely on its nitrogen-fixing capacity if this were found to be superior to other crops. It is this particular aspect which will be the subject of future investigations on guar.

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Biotypes of Potato Wart in Newfoundland

The existence of biotypes of the causal fungus of potato wart, *Synchytrium endobioticum* (Schilb.) Perc., which differ in their ability to attack potato varieties in Europe has been documented by Ullrich[1]. Up to 1962, eight, and possibly ten, biotypes had been distinguished[2].

Potato wart has been known in Newfoundland since 1909 and is now found in all cultivated areas of the Island. It is distinctive in that it attacks a considerable number of varieties[3], such as 'Arran Victory', 'Kerr's Pink', 'Ackersegen', and 'Libertas', which are immune from wart Biotype 1 in Europe and Britain.

Because it was reported that 'Arran Victory' was not susceptible to wart in some districts of Newfoundland, an investigation was commenced in 1959 to see whether potato varieties would exhibit the same reaction to the wart disease in various parts of the province. Since the most significant information was obtained in 1962, the results reported here pertain only to that year.

Eleven potato varieties were planted in wart-infested land at five locations, namely: Bay Roberts, Eastport, Ferryland, Jamestown, and St. Shotts. Eastport was included because farmers in the area had found that 'Arran Victory' always remained free from wart infection. The four other locations were selected to sample representative wart-infested areas in eastern Newfoundland. The varieties used were 'Ackersegen', 'Arran Victory', 'Deodora', 'Katahdin', 'Kennebec', 'Kerr's Pink', 'Long Blue', 'Noordeling', 'Sebago', 'Ultimus', and 'Urgenta'. Each variety was planted in two rows of 10 hills each, randomized throughout the plot. The plants were harvested when growth was complete, and wart development was recorded.

Table 1. Varietal Response to Potato Wart Disease at Five Locations

Variety	Eastport	Bay Roberts	Ferryland	Jamestown	St. Shotts
'Ackersegen'	0	+ + +	+ + +	+ + +	Not included
'Arran Victory'	0	+ + +	+ + +	+ + + +	+ + + +
'Deodora'	+ +	+ +	+ + +	+ + +	+ + + +
'Katahdin'	0	+	+	+	+
'Kennebec'	0	+	+	0	+
'Kerr's Pink'	0	+ + + +	+ + + +	+ + + +	+ + + +
'Long Blue'	+ +	+ + + +	+ + + +	+ + + +	+ + + +
'Noordeling'	0	0	0	0	+
'Sebago'	+	+	+	+	+
'Ultimus'	0	0	0	0	0
'Urgenta'	0	0	0	0	0

0, No infection; +, trace infection; + +, light infection; + + +, medium infection; + + + +, heavy infection.

The results are presented in Table 1.

It is evident that the varietal reaction to wart infection was almost the same at Bay Roberts, Ferryland, Jamestown, and St. Shotts, but not at Eastport. Five varieties which were susceptible at the first four locations remained free from attack at Eastport. The most obvious differences were exhibited by 'Ackersegen', 'Arran Victory', and 'Kerr's Pink'.

The wart proliferations developed on 'Deodora' and 'Long Blue' at Eastport were collected for further study in the greenhouse. 'Arran Victory', 'Deodora', 'Long Blue' and 'Sebago' were planted in 7-in. pots of steam-sterilized soil and inoculated by mixing crushed Eastport wart tissue with the soil covering the tubers. Three pots of each variety were grown for two months.

'Arran Victory' remained free from infection, all plants of 'Long Blue' were heavily infected, wart development in 'Deodora' varied from moderate to heavy, and, in 'Sebago', two plants developed light infection, while one remained healthy. Although they were not specifically included in this series, 'Arran Victory' plants inoculated with wart tissue collected from Bay Roberts have regularly become infected in the greenhouse.

Therefore, it is tentatively concluded from the above results that two biotypes of *Synchytrium endobioticum* are present in Newfoundland.

This investigation will be continued to assess the significance of small differences such as the freedom from infection of 'Kennebec' at Jamestown and the trace infection of 'Noordeling' only at St. Shotts, and to determine the distribution of the biotypes in the province.

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, October 26

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Du Cane Road, London, W.12), at 4 p.m.—Dr. B. Ackner: "Stress and Psychosomatic Disorders".*

MEDICAL RESEARCH COUNCIL (at the National Institute for Medical Research, Mill Hill, London, N.W.7), at 4.30 p.m.—Prof. Sven Gard (Karolinska Institutet, Stockholm): "An Approach to the Study of Virus Synthesis".

INSTITUTE OF NAVIGATION (at the Royal Geographical Society, 1 Kensington Gore, London, S.W.7), at 5 p.m.—Dr. C. Koeman: "The Life and Work of Lucas Janszoon Waghenaer" (The Eva G. R. Taylor Lecture).

UNIVERSITY OF LONDON (in the Botany Theatre, University College, Gower Street, London, W.C.1), at 5 p.m.—Dr. K. L. Manchester: "Some Aspects of the Action of Insulin" (Further Lecture on November 2).*

BRITISH SOCIETY FOR THE HISTORY OF SCIENCE (in the Council Room of the Science Museum, Exhibition Road, London, S.W.7), at 5.30 p.m.—Prof. A. Rupert Hall: "Galileo and the Science of Motion".

INSTITUTION OF ELECTRICAL ENGINEERS, SCIENCE AND GENERAL DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Prof. A. L. Cullen: "Millimetre Wave Examination of Gas Discharges".

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, JOINT I.E.R.E.–I.E.E. COMPUTER GROUPS (at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 5.30 p.m.—Colloquium on "Design Automation".

ROYAL INSTITUTION, LIBRARY CIRCLE (at 21 Albemarle Street, London, W.1), at 5.30 p.m.—Mr. Julian Royle: "A Modern Approach to Colour Printing".

ROYAL AERONAUTICAL SOCIETY, HISTORICAL GROUP (at 4 Hamilton Place, London, W.1), at 7 p.m.—Mr. S. B. Jackson: "The Parachute—an Historical Review".

Tuesday, October 27

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p.m.—Dr. P. J. Newbould: "The International Biological Programme".*

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, London, S.W.1), at 5.30 p.m.—Mr. C. K. Haswell and Mr. Luis Sancha: "The New Buenos Aires Power Station".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. J. R. Squire: "Rheumatoid Factor". (Third of fourteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)*

UNIVERSITY OF LONDON (at the Institute of Archæology, 31–34 Gordon Square, London, W.C.1), at 5.45 p.m.—Prof. Dr. Anders Hagen (Bergen): "Rock Carvings of Norway in the Light of New Discoveries".*

INSTITUTION OF MECHANICAL ENGINEERS, NUCLEAR ENERGY GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "The Achievement of Clean Conditions in Gas-Cooled Nuclear Reactors".

ROYAL AERONAUTICAL SOCIETY, AIR LAW GROUP (at 4 Hamilton Place, London, W.1), at 6 p.m.—Monsieur R. Bulin: "Eurocontrol".

SOCIETY OF CHEMICAL INDUSTRY, PLASTICS AND POLYMER GROUP (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Mr. W. Simpson: "Glass Transitions and Mechanical Behaviour of Film Forming Polymers".

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION, LEA VALLEY SUBSECTION (joint meeting with the Physical Methods Group of the Society for Analytical Chemistry, at the College of Technology, Park Street, Luton), at 7 p.m.—Mr. A. L. Glenn, Mr. A. G. Jones and Mr. F. R. Williams: "Training of the Analytical Chemist in Physical Methods".

Tuesday, October 27—Thursday, October 29

INSTITUTION OF MECHANICAL ENGINEERS, LUBRICATION AND WEAR GROUP (at the College of Advanced Technology, Cardiff)—Meeting on "Iron and Steel Works Lubrication".

Wednesday, October 28

INSTITUTE OF NAVIGATION (at the Royal Geographical Society, 1 Kensington Gore, London, S.W.7), at 4 p.m.—Annual General Meeting. Dr. G. E. R. Deacon, C.B.E., F.R.S.: "Waves and Ships" (Presidential Address).

GEOLOGICAL SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. B. C. M. Butler: "A Comparative Study of the Moine Schists of Ardnamurchan with Moine Schists in other Areas of Scotland"; Dr. J. L. M. Lambert: "The Unstratified Sedimentary Rocks of the Meneage Area, Cornwall".

ROYAL METEOROLOGICAL SOCIETY (at 49 Cromwell Road, London, S.W.7), at 5 p.m.—Mr. P. Graystone: "Dynamics Applied to Weather Forecasting" (Sixth-form Lecture).

FAUNA PRESERVATION SOCIETY (at the Offices of the Zoological Society of London, Regent's Park, London, N.W.1), at 5.30 p.m.—Colonel C. D. Trimmer, D.S.O.: "Wildlife Management in Uganda".

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. C. H. Flurscheim: "Development in Power Engineering" (Chairman's Address).

UNIVERSITY OF LONDON (at Charing Cross Hospital Medical School, 62–65 Chandos Place, London, W.C.2), at 5.30 p.m.—Prof. C. G. Schmiterlöw (Stockholm): "Physiological Disposition and Biotransformation of C14-labelled Nicotine".*

UNIVERSITY OF LONDON (in the Botany Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. Sven Gard (Karolinska Institutet, Stockholm): "Virus-Induced Leukæmia".*

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, JOINT I.E.R.E.–I.E.E. ELECTRO-ACOUSTICS GROUPS (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Symposium of Short Papers on "Magnetic Circuit Design Applied to Electro-Mechanical Transducers".

INSTITUTION OF MECHANICAL ENGINEERS (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Sir Frank Mason: Presidential Address.

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP—NUTRITION PANEL AND FOOD ENGINEERING PANEL (at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Dr. S. K. Kon, Mr. H. A. Burton and Mr. J. Shacklady: "The Effect of Processing on the Nutritive Value of Foods, Part 1—Liquids".

Thursday, October 29

CHEMICAL SOCIETY (at Queen Mary College, Mile End Road, London, E.1), at 2 p.m.—Symposium on "The Chemical Significance of the Mössbauer Effect".

INSTITUTION OF CIVIL ENGINEERS, HYDROLOGICAL GROUP (at Great George Street, London, S.W.1), at 5.30 p.m.—Informal Discussion on "Run-off Measurement" introduced by Mr. J. K. C. Wilson.

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Hollow Beams" opened by Mr. A. H. W. Beck.

GLACIOLOGICAL SOCIETY (in Room 105, Roderick Hill Building, Imperial College, London, S.W.7), at 5.30 p.m.—Mr. John Sheard and Mr. Howard Lovenbury: "The Glaciological Work of the 1963 Imperial College Beerenberg Expedition".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. R. D. Harkness: "Some Aspects of the Metabolism and Function of Collagen". (Fourth of fourteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)*

UNIVERSITY OF LONDON (at the School of Pharmacy, Brunswick Square, London, W.C.1), at 6.30 p.m.—Dr. Harold Blakemore: "Latin America: Past and Present. Indian Civilization: Mexico and the Aztecs".

Thursday, October 29—Friday, October 30

INSTITUTE OF BIOLOGY (in the Lecture Hall, Royal Geographical Society, Kensington Gore, London, S.W.7), at 10 a.m. daily—Symposium on "The Biological Significance of Climatic Changes in Britain".

Friday, October 30

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Prof. R. King: "Radiation 'Damage'".

Saturday, October 31

LONDON COUNTY COUNCIL (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Mr. David Morris: "British Reptiles and Amphibians".*

Monday, November 2

BRITISH SOCIETY FOR THE PHILOSOPHY OF SCIENCE (in the Joint Staff Common Room, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. E. R. Leach: "What Are Social Anthropologists Trying To Do?"

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (joint meeting with the Royal Aeronautical Society, at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. J. I. Kanter: "N.A.S.A. Research and Technology Programmes in Space Navigation".

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (joint meeting with the Science and General Division, at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. A. J. Ellison: "Modern Introduction to the General Theory of Electrical Machines".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 6 p.m.—"The Lost Sahara" (Colour films with commentary and introduction by Mr. A. R. Hanbury-Tenison and Mr. James Wellard.)

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Mr. A. Hart: "Research Project Evaluation".

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (joint meeting with the Woolwich Polytechnical Chemical Society, at the Woolwich Polytechnic, Thomas Street, London, S.E.18), at 7.30 p.m.—Dr. J. Leggett: "The Photochemical Apparatus of the Chloroplast".

SOCIETY FOR VISITING SCIENTISTS (at the English-Speaking Union, Dartmouth House, 37 Charles Street, London, W.1), at 7.30 p.m.—Discussion Meeting on "Computers in Publishing". Chairman: Mr. Richard Clay. Speakers: Mr. C. J. Duncan, Dr. T. A. Margarison and Mr. Fred Simmons.

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT LIBRARIAN (chartered librarian, preferably with some experience in a university or technical college library)—Clerk to the Governors, Woolwich Polytechnic, London, S.E.18 (October 30).

LECTURER (Grade II) IN PURE MATHEMATICS—The Registrar, University of Bristol, Senate House, Bristol, 2 (October 30).

BASIC GRADE PHYSICIST (preferably with previous hospital experience) for duties mainly concerned with physics applied to radiotherapy, and also with the clinical use of radioactive isotopes—The House Governor (A), Charing Cross Hospital, London, W.C.2 (October 31).

LECTURER IN VETERINARY BACTERIOLOGY IN THE DEPARTMENTS OF VETERINARY PATHOLOGY AND VETERINARY BACTERIOLOGY, Royal (Dick) School of Veterinary Studies—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh, 8 (October 31).

SCIENTIFIC OFFICER (with first- or good second-class honours in mathematics with statistics, or a diploma in statistics) IN THE STATISTICS SECTION, for duties mainly concerned with the design and analysis of field and laboratory experiments—The Secretary, The Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen (October 31).

SENIOR RESEARCH ASSISTANT (with a Ph.D. or equivalent research experience) IN BIOCHEMISTRY for work concerned with mucoproteins and their biological degradation products—Dr. F. R. Jevons, Department of Biological Chemistry, The University, Manchester, 13 (October 31).

CHAIR OF MATERIALS SCIENCE within the School of Engineering Science—The Secretary and Registrar, The University College of North Wales, Bangor, North Wales (November 2).

LECTURER IN MECHANICAL ENGINEERING, a LECTURER IN MATHEMATICS, and a LECTURER IN STATISTICS—The Principal, Nottingham and District Technical College, Burton Street, Nottingham (November 2).

RESEARCH DEMONSTRATOR (with a special interest in stratigraphy and/or palæontology) IN THE DEPARTMENT OF GEOLOGY—The Registrar, University College of South Wales and Monmouthshire, Cardiff (November 3).

HEAD (with a degree in horticulture or a degree in science with a diploma in horticulture and considerable experience of horticultural teaching, research and specialist advisory work) OF THE HORTICULTURE DEPARTMENT—The Secretary, The West of Scotland Agricultural College, 6 Blythswood Square, Glasgow, C.2 (November 6).

SENIOR LIBRARIAN (with a good honours degree, preferably in science or engineering, Associate Membership of the Library Association, and preferably experience in a scientific or technical library)—The Academic Registrar, Northampton College of Advanced Technology, St. John Street, London, E.C.1 (November 7).

DIRECTOR (with good experience with scientific digital computers) OF THE COMPUTING UNIT—The Secretary, Battersea College of Technology, London, S.W.11 (November 9).

LECTURER and an ASSISTANT LECTURER IN ELECTRONICS IN THE DEPARTMENT OF PHYSICS—The Registrar, The University, Leicester (November 10).

LECTURER (chemical engineer, physicist or mathematician with interests in mass transfer, chemical thermodynamics or statistical mechanics) IN THERMODYNAMICS—The Registrar, University of Leicester, Leicester (November 10).

SECOND CHAIR OF MATHEMATICS IN THE SCHOOL OF MATHEMATICS AND PHYSICS—The Registrar, University of East Anglia, Earlham Hall, Norwich, Norfolk, NOR 05G (November 16).

READER IN AGRICULTURAL BOTANY AND HEAD OF THE DEPARTMENT OF AGRICULTURAL BOTANY, Makerere University College, University of East Africa—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (November 20).

LECTURER IN ORGANIC CHEMISTRY, and a LECTURER IN PHYSICAL OR ANALYTICAL CHEMISTRY at the University College, Dar es Salaam, University of East Africa—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (November 30).

LECTURER IN PHYSICS—The Secretary of University Court, The University, Glasgow (December 1).

AGRICULTURAL TRAINING OFFICER (with a degree in agriculture, at least five years tropical experience, and preferably a teaching qualification or teaching experience in an agricultural training institute, and a working knowledge of French) in the New Hebrides, to establish and run an agricultural training school teaching the elements of tropical agriculture to junior field staff—The Appointments Officer, Department of Technical Co-operation, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 213/124/01.

ASSISTANT EXPERIMENTAL OFFICER or EXPERIMENTAL OFFICER (with a degree (ordinary or general), Dip. Tech. or H.N.C. with chemistry and/or physics as main subjects) with the Age Determination Unit of the Overseas Geological Surveys, to assist in the determination of the ages of rocks by methods based on natural radioactivity and involving physical and chemical methods of analysis, including mass-spectrometry and high vacuum techniques—Officer in Charge, Age Determination Unit (O.G.S.), Department of Geology and Mineralogy, University Museum, Oxford.

ASSOCIATE PROFESSOR (preferably with research interest in vertebrate biology, ecology or behaviour) IN ZOOLOGY—The Chairman, Zoology Department, McGill University, Montreal 21, Canada.

CHEMIST or BIOCHEMIST (with at least a good honours degree in chemistry or biochemistry with research experience in an appropriate field) IN THE ENZYMOLOGY DEPARTMENT, to work on glycoproteins—The Secretary, Rowett Research Institute, Bucksburn, Aberdeen.

ENTOMOLOGIST (national of the United Kingdom or the Republic of Ireland, with an honours degree in zoology or a relevant subject plus two years postgraduate study in agricultural entomology), in Uganda to carry out surveys and conduct research into pests of crops and stored produce, especially cotton, coffee and cocoa, and to advise on control measures—The Appointments Officer, Room 301, Department of Technical Co-operation, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 213/183/07.

JUNIOR RESEARCH ASSISTANT for fundamental research on sorption by molecular sieves—Prof. R. M. Barrer, F.R.S., Chemistry Department, Imperial College, London, S.W.7.

SOLARTRON RESEARCH STUDENT (preferably interested in dynamic analysis of randomly excited systems) IN THE DEPARTMENT OF ENGINEERING—The Professor of Engineering, The University, Leicester.

TECHNICIAN (with good histological experience) to assist with research on the metabolism of water and electrolytes—Dr. J. M. N. Boss, Department of Physiology, Medical School, University Walk, Bristol.

ZOOLOGIST (national of the United Kingdom or the Republic of Ireland, with an honours degree in zoology, and preferably research or other experience in parasitology or a higher degree in zoology) in Kenya, for the supervision of routine helminthological diagnosis, research on problems of helminthiases of livestock especially the bionomics of nematode and trematode infestations—The Appointments Officer, Department of Technical Co-operation, Room 301, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 217/95/010.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Astronomical Contributions from the University of Manchester. Series III, No. 107: Observations of the Frequency Dependence of Polarisation of the Light of the Moon and of Mars. By David Clarke. Pp. ii+43. (Manchester: The University, 1964.) [318

Royal Observatory Bulletins. No. 86: Time and Latitude Service 1963, October-December. Pp. B243–B258. (London: H.M. Stationery Office, 1964.) 3s. net. [318

Smith Kline and French Foundation. First Annual Report 1963. Pp. 8. (Welwyn Garden City, Herts: Smith Kline and French Foundation, 1964.) [318

International Nickel Company (Mond), Ltd. Ni-Hard Iron Type 4. Pp. 4. (London: International Nickel Company (Mond), Ltd., 1964.) [318

Wye College (University of London). Annual Report of the Department of Hop Research from 1st April, 1963, to 31st March, 1964. Pp. v+55. (Wye, Ashford, Kent: Wye College, 1964.) 6s. [318

British Geological Literature, Vol. 1, Part 1. Compiled by Edward L. Martin and Anthony P. Harvey. Pp. 1–12. Annual subscription: Library edition, with Index, 20s.; Catalogue edition, without Index, 20s. (Bourne End: Coridon Press, 35 Goddington Road, 1964.) [318

B.B.C. Engineering Monograph No. 54: An Analysis of Film Granularity in Television Reproduction. By K. Hacking. Pp. 24. (London: British Broadcasting Corporation, 1964.) 5s. [318

Agricultural Research Council. Report of the Institute of Animal Physiology, Babraham, for 1962–1963. (2nd Biennial Report.) Pp. 86+4 plates. (Babraham, Cambridge: Institute of Animal Physiology, 1964.) [318

The Library Association. Memorandum of Evidence to be laid before the Committee on Libraries, set up by the University Grants Committee, the Ministry of Education and the Scottish Education Department to enquire into the use and needs of Libraries in Universities, Colleges of Advanced Technology, Central Institutions and Training Colleges. Pp. 26. (London: The Library Association, 1964.) [318

Freshwater Biological Association. Thirty-second Annual Report for the year ended 31 March 1964, including Reports of the Council and of the Director, and Accounts for 1963–64, with Lists of Officers, Council, Staff and Members. Pp. 97+1 plate. (Ambleside: Freshwater Biological Association, 1964.) 4s. [318

The Radiochemical Centre, Amersham. Selected References to Tracer Techniques. Pp. 12. (Amersham: The Radiochemical Centre, 1964.) [89

Ciba (A.R.L.), Ltd. Technical Notes, No. 259 (July, 1964): Aerolite-Glued Formwork for Concrete. Pp. 8. (Duxford: Ciba (A.R.L.), Ltd., 1964.) [89

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Bulletin of the British Museum (Natural History). Entomology. Vol. 15, No. 8: Diptera from Nepal. Asilidae. By H. Oldroyd. Syrphidae. By R. L. Coe. Simulliidae. By D. J. Lewis. Pp. 237–294. 22s. Vol. 15, No. 9: A Revision of the Genus *Tylopsis* Fiebèr (Orthoptera: Tettigoniidae). By D. R. Ragge. Pp. 295–322. 11s. (London: British Museum (Natural History), 1964.) [89

Other Countries

Indian Journal of Cancer, Vol. 1, No. 1 (October 1963). Edited by D. J. Jussawalla. Issued twice a year. Pp. xxviii+1–90. Annual subscription: Rs. 6 (post free). (Bombay 12: Indian Cancer Society, 1963.) [318

Federal Radiation Council, Washington, D.C. Report No. 5: Background Material for the Development of Radiation Protection Standards—Staff Report of the Federal Radiation Council. Pp. iii+16. (Washington, D.C.: Federal Radiation Council, 1964. Available from U.S. Government Printing Office.) 20 cents. [318

Geological Survey of Western Australia. Mineral Resources Bulletin No. 8: Copper Deposits of Western Australia. By G. H. Low. Pp. 202+1 map. Bulletin No. 116: The Geology of the Manganese Deposits of Western Australia. By L. E. de la Hunty. Pp. 122+15 plates. (Perth: Government Printer, 1963.) [318

Institut Royal des Sciences Naturelles de Belgique. Mémoires. No. 151: Sédimentologie des Faciès "Marbres Noirs" du Paléozoique Franco-Belge. Par Dr. Barnard Mamet. Pp. 131+5 planches. Mémoires, Deuxième Série. Fasc. 76: Trichoptéres de l'Amazonie: Recueillis par le Professeur H. Sioli. Par Dr. Georges Marlier. Pp. 167. (Bruxelles: Institut Royal des Sciences Naturelles de Belgique, 1964.) [318

International Atomic Energy Agency. Publications in the Nuclear Sciences 1964—a Complete Catalogue of the Publications of the International Atomic Energy Agency. Pp. 87. (Vienna: International Atomic Energy Agency, 1964.) [89

Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Bulletin 113: The Polarographic Determination of Aluminium in Rocks. By Marilyn Levine. Pp. 42. 1.50 dollars. Memoir No. 322: Western Queen Elizabeth Islands, Arctic Archipelago. By E. T. Tozer and R. Thorsteinsson. Pp. xvi+242 (55 plates). 4 dollars. Miscellaneous Report No. 9: Kootenay National Park—Wild Mountains and Great Valleys. By David M. Baird. Pp. vi+94. 1.50 dollars. Paper 63-42: Geochemistry of the Dawson Settlement Bog Manganese Deposit, New Brunswick. By A. C. Brown. Pp. ii+26. 35 cents. (Ottawa: Queen's Printer, 1964.) [89

Annals of the New York Academy of Sciences. Vol. 116, Article 2: Recent Advances in Odor—Theory, Measurement, and Control. By Richard L. Kuehner and 60 other authors. Pp. 357–746. (New York: New York Academy of Sciences, 1964.) [89

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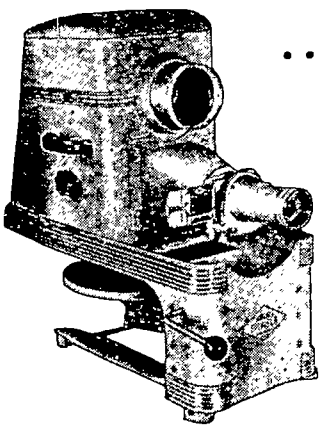
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APPOINTMENTS VACANT

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The University plans to commence graduate and undergraduate teaching on the campus at North Ryde, Sydney, at the beginning of 1967, and to provide for the enrolment of 10,000 undergraduates within a period of ten years thereafter.

It is envisaged that there will be four main components in the academic structure of the University. These will be:

A. A College of Graduate Studies.
B. An Undergraduate College of Arts and Sciences.
C. An organization for Part-time and External studies.
D. Professional schools, for example of Medicine and Agriculture.

As yet no professional schools have been planned. This advertisement relates to responsibilities under the first three headings.

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The initial organization of studies will be in schools. These schools will not be as broad in their scope as those which have been established in some other new universities. The intended scope of schools at Macquarie University is indicated in the following tentative divisions :

1. Mathematics and Physics.
2. Chemistry, Biochemistry.
3. Biological Sciences.
4. Earth Sciences and Soil Sciences.
5. English Studies.
6. Historical Studies, including Philosophy and Political Sciences.
7. Foreign Languages.
8. Classical Studies.
9. Economic and Financial Studies.
10. Behavioural Social Sciences (Psychology, Anthropology and Sociology).
11. Educational Studies.

The University looks for applicants who will be interested in participating in the planning of its activities within the structure stated above. It will commence by filling some six chairs in a range of disciplines which includes Mathematics, Physics, Chemistry and Psychology.

The University reserves the right to fill any position by invitation.

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Detailed applications (eight copies), naming three referees by November 30, 1964, to Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1, from whom further particulars may be obtained.

UNIVERSITY OF SINGAPORE

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Detailed applications (8 copies), naming three referees, by November 30, 1964, to Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1, from whom further particulars may be obtained.

RESPONSIBILITY AND EDUCATION

TOWARDS the close of his presidential address to the British Association for the Advancement of Science (*Nature*, 203, 943; 1964) at Southampton, Lord Brain urged that one of the consequences of the population explosion was that, so far as we could, we should prepare all our people for a new world which would be quite unlike the old. This would mean spending more money and thought on education than we do at present, remembering that the best educational results were unlikely where home conditions were bad. In this context he saw education to-day as having two tasks, one of which was to prepare people for leisure.

First, however, education must concern itself with the whole individual and therefore with behaviour, and here it must largely reflect the views and standards of society. One of our needs as a society, now predominantly secular, is to develop a code of social responsibility which could form an integral part of education. Whether by design or coincidence, this note of responsibility was dominant in the discussions in Section L (Education) at the Southampton meeting of the British Association. The president of Section L, Dr. F. Lincoln Ralphs, dealt first with responsibility and education as the combined mainspring of our continuing evolution (*Nature*, **203**, 954; 1964) Those who followed discussed successively the particular responsibility of the Press, the State, the universities, the parent and the school, the teacher, the Church, the architect, industry and television.

Dr. Ralphs himself was more especially concerned with the changes in our conception of responsibility in relation to education in the past two decades and in the context of the 1944 Education Act. He began with a brief survey of the way in which the sense of responsibility in education had quickened during the previous two generations. Inevitably he touched on fields that were covered by succeeding addresses, but although he found it impossible to consider responsibility and education in the context of the 1944 Act without reference to the remarkable emphasis it placed on religion and worship, there was virtually no overlapping with Mr. Leslie Paul's paper on the responsibility of the Church. Mr. Paul was concerned first with the organizational and social responsibilities of the Church, and then with the spiritual contribution which the Church might make to the tone and content of contemporary education. Dr. Ralphs, however, was concerned with challenging our emphasis on the species rather than the individual, and our failure to develop a society sufficiently inspired by ideals of service and increasingly uncertain about the purpose of life.

Quoting Prof. Butterfield's view in *Christianity and History* that only the Christian view (that each individual soul has eternal moment and has value incommensurate with the value of anything else in the created universe) offered a firm barrier against the emphasis on species, Dr. Ralphs stressed the implications of the religious clauses of the Act. After welcoming their increasing recognition by the Churches, he reminded us that the way in which, in the post-war period, individuals had been ruthlessly urged towards conformity was an affront to human dignity. There was some reason to fear that our barriers against Hitler's prodigality with the individual lives of men and women were down and might not be put up again in time. In our recognition of educational responsibility we needed to realize where we were going.

Dr. Ralphs's address abounded in illustrations of this point. He reminded us once again that intelligence is not increased by legislation, though the opportunity for its development may be. Is this sufficiently appreciated in plans for the expansion of higher education, especially at university level? Or again, is it not true, as he suggested, that we lack imagination in planning new enterprises and tolerate subject specialization leading to academic awards or to apprenticeships that are anachronistic in an age of automation? Failure to think imaginatively and creatively may represent as gross a dereliction of responsibility as flat repudiation.

One of the most serious criticisms of education to-day is that for so many it becomes boring, as do the jobs in which they will have to earn their living. No greater reproach can be levelled against a course or an institution than that it kills the desire to learn, and, as Dr. Ralphs observed, we need constantly to remind those entering the teaching profession that the first function of a teacher is not to instruct but to inspire. This has to be remembered when we tend to measure educational needs in terms of thousands of teachers to be recruited: neither inspiration nor intelligence is mass-produced, though, especially with the former, environment can sometimes be decisive.

Here, however, we must admit that this generation has not been markedly successful in training its adolescents to be aware of the full consequences of their actions. Large classes, and the increasing emphasis on factual knowledge which examinations tend to encourage, are scarcely calculated to inspire the acceptance of responsibility, and Dr. Ralphs believed that irresponsibility has never before been so heavily subsidized. This may be due in part to a confusion in the concepts of discipline, but the traditional belief that education should be endured rather than enjoyed still persists. Dr. Ralphs commented trenchantly that, in education, certificates all too often tend to become documents announcing one's arrival, not single tickets entitling one to travel, or certificates of work-worthiness in responsible enterprise. "If we fail to develop, with our increasing erudition, a matching sense of personal responsibility, the nineteenth century, which died in fear of ignorance, may have done no more than pave the way for the twentieth century to perish in the fear of knowledge".

It is a commonplace to-day that progress depends on the use rather than the possession of knowledge, and while our sense of responsibility demands education for the individual, our education must demand individual responsibility. Whatever criticism may be used of the evidence submitted by the National Union of Teachers to the Central Advisory Council for Education under Lady Plowden's chairmanship, that evidence attests the deep sense of responsibility that pervades the Union: indeed, that is manifest in the very failure to reach common agreement on matters relating to the transition from primary to secondary education. Sir Ronald Gould's address to the Section on August 31 showed this sense of responsibility no less clearly.

Dr. Ralphs then spoke of the responsibility of the State for education. If the individual was to be protected

against all the pressures of a technological society which threatened his individualism, the State's share should be concentrated in efforts to provide for local and individual initiative rather than to prescribe patterns and programmes. Irresponsibility, he remarked, grows all too easily in centralized systems of remote control, whether in industry or in education, and he hoped that in education we would always remain a little suspicious of central control at any level. The point could scarcely be illustrated better than by the past Government's ill-judged and short-sighted attempt at economy in adult education against which the Universities Council for Adult Education protested firmly and with dignity in its statement *Crisis in Adult Education*.

Dr. Ralphs admitted that a more comprehensive view was possible at the centre and that we must accept the responsibility for making the best use of resources, though in the changes we made we must safeguard the effective use of the time and initiative of teachers. Individual initiative arising from personal responsibility, encouraged and accepted, not frustrated by restrictions, was one of our most valuable resources, and, whether from teacher, parent or pupil, flourished best when authority was delegated rather than centralized. Sir Herbert Andrew, in his address on August 28, did not appear to dissent. He thought it inevitable that an increasingly well-informed public opinion would bring more constructive and sympathetic criticism to bear on the educational system, to which the teacher and administrator must respond.

Dr. Ralphs pointed out that the 1944 Act placed the responsibility for the education of children fairly and squarely on the parents, although they could call on the community for help if necessary, and for the vast majority of parents such help is essential. Moreover, in general, the aid is most effective when, as in the public schools, it is sought and seen as a sharing of responsibility and not as a State service provided as of right and on demand. Some parents undoubtedly saw the Education Act as a declaration of rights rather than as a definition of duty, and Dr. Ralphs admitted that there will be differences of opinion as to the extent to which we should, or shall be able to, effectively and properly involve parents in this responsibility for education. Nevertheless, he was firm that we cannot divorce education from responsibility without endangering the community, and to-day it is a world community that is involved.

On these points Dr. Ralphs was firmly supported by Mr. Michael Young in his paper on the responsibility of the parent and the school, for Mr. Young's suggestion for an education welfare service is intended to foster just this sense of responsibility and its discharge. The proposal is tentative and Mr. Young emphasized the need for more evidence of the kind that only research can provide. A main purpose of the education visitors, he suggested, would be to arouse the interest and sense of responsibility of the parent. Whether such association of parents with the work of the school does, in fact, assist the progress of the child at school remains to be established.

Dr. Ralphs recognized that radio and television have already made valuable contributions to the instruction of parents, but the educational potential of mass media was discussed in subsequent papers by Mr. J. Beavan and Mr. Kenneth Aden, and Dr. Ralphs contented himself with the pertinent comment that, as with the teacher, their educational influence extends to all their activities and cannot be restricted to those they choose to describe as educational. Viewed as a whole the mass media seemed to Dr. Ralphs to contribute to the present-day practice of requiring schools to advocate standards the outside world has little mind to honour. We have much more to do than to raise the school leaving age, and Dr. Ralphs did well to remind us that the standards of the street are as influential as the conduct of the classroom.

We cannot ignore the influence of the totality of our environment, and neither teacher nor parent can escape the continuous responsibility that attaches to their private lives. We teach what we are no less than what we know, and if this explains why we tend to prefer instruction to teaching and accept erudition in place of education, it does not excuse either the preference or the acceptance. More or less consciously the issue is linked with the debate over commercial television and broadcasting and with advertising on television; Mr. Beavan at least recognized the implications in discussing the responsibility of the Press. If he was concerned to defend the freedom of the Press, he was equally concerned to maintain that comment must be responsible and proved error admitted promptly and without equivocation.

On the responsibility of the Press in education, which he frankly admitted, Mr. Beavan was concerned particularly with the difficulties that a responsible newspaper encountered in pursuit of its idea that it should help to make good citizenship possible by providing adequate political information, even though it was also an organ of entertainment. He thought the development of the specialist report was a factor making for increased responsibility, but the great problem of a newspaper was to keep its readers interested, and though a sizable minority of the adult population read thoroughly the serious items, most readers either did not read them thoroughly or did not read them at all. Nevertheless, he thought that as more people were educated to a later age, newspapers, aided by television, could hope to increase the following of serious events.

Dr. Ralphs concluded his address on a like note of optimism. The future, he said, is increasingly in the hands of youth, who, in spite of unprecedented inducements to delinquency, were for the most part eminently sensible and imbued with the desire to do their best. Obviously, in his view, youth is responding to the challenge of responsibility so clearly thrown down by the Robbins Report, and a certain note of caution in Mr. H. L. Elvin's address to Section L on August 28 did not conceal the frank acceptance by the universities of their own share of responsibilities. It is indeed only fair to point out that that sense of responsibility is the real cause of some of their reservations and hesitations as to the *tempo* and character of proposed plans for expansion, and what Dr. Ralphs said earlier in his address about compulsion and conformity applies with as much force here as anywhere.

The whole series of papers presented to Section L (Education) at the British Association meeting at Southampton served to display the full range of interlocked responsibilities in education and the need for continuing co-operation, understanding and experiment. Progress is not furthered by unreasonable questioning of the good faith of those who differ as to the timing or magnitude or character of proposed measures, any more than by refusing to recognize difficulties or awkward facts and situations. The whole series of papers represents a material contribution to a wider general understanding and a stimulus to the good will that is equally essential.

CIRCADIAN ORGANIZATION

The Physiological Clock

Endogenous Diurnal Rhythms and Biological Chronometry. By Erwin Bünning. Pp. vi+145. (Berlin: Springer-Verlag, 1964.) 19 D.M.

THIS English translation of the second (1963) edition of "Die Physiologische Uhr" has been brought up to date by the author and is quite indispensable for any biologist interested in the rhythmical aspects of organisms and their manifold theoretical and practical implications. It is more comprehensive and less controversial than other recent books and reviews of the subject. Although it cannot rival the bulk of information presented at three symposia held during the past few years (25th Cold Spring Harbor symposium, 1960; 39th Ross Conference on Pediatrics, Ohio, 1961; Conference on Biological Rhythms of the New York Academy of Science, 1962), it has the advantage of being written by a single author, who has been working on the subject for more than 30 years.

The old-fashioned title of the book should not mislead the reader into believing that endogenous rhythms of approximately 24 h period length can only be produced by highly specialized machinery, such as the neurocrine systems of vertebrates and arthropods or the joints of leaves of higher plants. Bünning makes it abundantly clear that oscillations which Halberg (1959) has named circadian (from circa diem, about a day) occur at most levels of organization, in fungal mycelia and unicellular algae, in birds and flowering plants, and in the social organization of the bees and of man. They affect a multitude of metabolic, locomotor, epigenetic and sexual activities, can be demonstrated by measuring all sorts of parameters and in Nature are synchronized by physical, chemical, biological or social cues, which all derive from the rotation of the Earth. The length of time a circadian rhythm may persist after removal of rhythmic time cues depends on the kind of organism, the function observed and certain environmental factors. According to the different degree of centralization of flowering plants and higher animals a small part of a leaf may show an endogenous rhythm of about 24 h in its CO_2 output, whereas an isolated limb, organ or tissue of a metazoon is unlikely to show any trace of circadian rhythm—with the possible exception of larger parts of the nervous and endocrine systems, which are the 'masterclocks' of these organisms.

The kinds of time cues or synchronizers, the degree of autonomy and interdependence of the various behavioural and organ rhythms, and many interactions of the circadian organization not only with diurnal rhythms but also with the tidal, lunar and seasonal fluctuations, form the principal subject matter of Bünning's book. While to a superficial glance this may appear as somewhat of a medley, it will repay the reader to follow the author's lead when he tries to fit this diverse material into the mathematical, physiological and ecological generalizations which, as his own experimental successes have shown, have considerable predictive power.

Bünning's book indicates one of the profound but nearly imperceptible 'sea changes' in biology; one which is about to sweep away many cherished, and in their time beneficial, concepts of nineteenth-century physiology. One of these is excessive biological atomism, the idea that 'in the end' life will be explicable in those chemical and physical concepts which are derived from the results of isolating and destructive methods. Obviously the study of organization, circadian or otherwise, does not lend itself to this approach. To paraphrase an old gibe: when dissecting an animal a biological clock, like the mind, is just not among the organs, though—again like the mind—time-keeping may have a decided affinity to special organs.

Another change concerns the concept of homœostasis, Claude Bernard's grand idea which in its petrified form has been for half a century a millstone round the neck of physiologists, clinicians and even geneticists. The conviction that 'the organism' strives at maintaining many physiological parameters at a constant level has blinded biologists to the universal existence of oscillations and their functions and also has distorted the aims of some laboratory practices and of therapeutic procedures. Constant temperature or illumination—though compatible with the bare existence of many domesticated strains in insects, rodents or birds—nevertheless introduces strains of their lives and does not produce specimens which are suitable for a host of behavioural experiments. The relative disregard (compared with such obvious factors as heat, hunger or infection) of the importance of the hour of the day for personal habits, shift work, hospital ward practice, or air travel has been at the root of much distress. It all stems from neglecting man's circadian organization, which to such 'quacks' of the eighteenth-century as Hufeland was one of the fundamentals of health. With the improvement of research methods the time aspects of life, and the ever-widening field of their application, are becoming increasingly important; over half an insulin dose can be saved for certain patients by administering it at the right hour: pheromones produced by the females of some moth species attract the males only during the three hours after midnight: the success of growing chrysanthemums and of producing eggs depends to a considerable extent on the light regimen during the day's 24 h—and so on.

It is perhaps paradoxical that the advent of the space age should coincide with the renewed interest of biologists in such old-fashioned terrestrial phenomena as the days, the tides and the seasons; and that some of their work should be supported by funds for space research. But, just as rockets and satellites have revived interest in celestial mechanics, so does the prospect held out by these vehicles of facing life without the accustomed changes of the hours or seasons revive interest in the potentialities and constraints which are the consequence of the circadian organization of ourselves and our fellow creatures.

H. KALMUS

ASPECTS OF CORTICOSTEROID STRUCTURE AND METABOLISM

Structure and Metabolism of Corticosteroids

Edited by Jorge R. Pasqualini and Max F. Jayle. (Proceedings of a Symposium held in Paris, 5–6 July 1963, and organized by the Faculté de Médecine, Laboratoire de Chimie Biologique.) Pp. x+168. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc., 1964.) 35*s*.

THE collection of sixteen papers which constitute *Structure and Metabolism of Corticosteroids* follows no well-defined pattern, and the editors have not found it an easy task in deciding on the best grouping to adopt in preparing it. Part 1 dealing with structural problems has only two chapters, a detailed account by Caspi of work at the Worcester Foundation in which he emphasizes the usefulness of nuclear magnetic resonance spectra, and a contribution by Kellie on the means of differentiating between two types of conjugate, for example, C_3- and C_{21}-glucuronosides.

The largest set of contributions are those in Part 2 entitled "Corticosteroid Metabolism in Vivo and Vitro", where we find much more on the subject of conjugation. Oertel adduces here further evidence to support his previously published work on those non-polar solvolysable steroid conjugates styled "sulphatidyl" steroids. Conjugation in urine and plasma is also the topic dealt with by the joint editors, who have accumulated an impressive array of evidence on which to formulate a set of rules governing the relationship between structure and type of conjugation. Sulphate conjugation is the subject of the paper by Tamm and Voigt, who offer an explanation of the curious

finding that excretion of cortisol 21-sulphate follows the oral administration, but not the intravenous infusion, of large doses of free cortisol. Vermeulen, examining the metabolism of certain anabolic steroids, finds that some testosterone derivatives decrease cortisol metabolism while others do not, and he puts forward the hypothesis that the difference in the two classes might depend on the route of excretion of the administered steroid. The feed-back theory which ordinarily accounts for the homœostatic control of plasma cortisol-levels is seen by James and Landon to be inadequate to explain the elevation of plasma cortisol in conditions of stress. They describe conditions under which stress will release adrenocorticotrophic hormone independently of the circulating steroid concentration. A chromatographic method using glass-fibre paper is described by De Hertogh *et al.* for the analysis of plasma cortisol, corticosterone and cortexolone. It should have a special interest for clinical biochemists.

Among other papers in the same section is one showing a difference between the *in vitro* and the *in vivo* incorporation of corticosteroid in sub-cellular particles of heart and kidney cortex; another indicates a synthetic route to aldosterone starting from the alkaloid holarrhimine. Aldosterone metabolism is dealt with by Pasqualini.

The final part of the volume is devoted to pathological considerations and includes an account of the metabolism of cortisol in Cushing's syndrome and of aldosterone in renal insufficiency. Cost evaluates urinary steroid excretion patterns and re-examines published ideas about the significance of 11-deoxycortisol compounds (compounds S and THS) in relation to the diagnosis of primary adrenocortical carcinoma. Combined histological and biochemical observations are the basis of Grant's paper on transformations accomplished by tissue taken from different zones of the adrenal cortex.

Those specially concerned with corticosteroids are bound to find some, but by no means all, papers in this volume meriting their close attention. J. Patterson

SYNAPTIC PHYSIOLOGY

The Physiology of Synapses

By Prof. John Carew Eccles. Pp. xi+316. (Berlin: Springer-Verlag, 1964.) 36 D.M.

THE understanding of nervous activity depends on our grasp ot two matters, the pattern of interconnexion between cells and cell masses, and functional result in a given cell of the arrival of activity at any particular presynaptic ending. The microanatomy of the nervous system got off to an early and propitious start largely due to the brilliance of Cajal, and although many details of nervous anatomy remain obscure the basic patterns have been known for nearly half a century. On the other hand, the study of synaptic events remained unproductive until the discovery of the intermediary local synaptic potential at the neuromuscular junction. There followed the development of precise biophysical methods for recording synaptic events and for analysing the site and mechanism of action. The main object of this book is to present a critical account of the synaptic areas so far investigated. One of the features discussed is whether transmission is due solely to local current flow or whether it involves the mediation of chemical agents, and Eccles describes the criteria that must be used to make this distinction. While the evidence points conclusively to the chemical nature of the majority of synapses, there is now equally conclusive evidence that some synapses are purely electrical, and there is even an interesting dual mechanism in the ciliary ganglion of the chick in which both processes may play a part. Perhaps one of the most interesting findings in recent years is that, while there is considerable stereotypy in synaptic patterns (for example, the post-junctional effects of all the chemically mediated excitatory junctions appear very similar), there is also available a considerable variety of synaptic types, so that similar final effects may be produced by unrelated mechanisms. For example, inhibition may be produced by a direct action of an inhibitory transmitter on the post-synaptic membrane, causing increased chloride permeability, or it may be produced presynaptically by depolarization of the presynaptic excitatory fibre, thus depressing its ability to release excitatory transmitter. While both these processes will, for example, depress the response of anterior horn cells to primary excitatory afferents, the net result is significantly different. In the first case the inhibiting post-synaptic potential is competitive with all incoming excitatory impulses; but in the second case, because the effect is produced presynaptically, no interference occurs other than with excitation from the presynaptic fibre that is affected. In other words, the second process is a much more selective one. This kind of distinction may have considerable significance in overall nervous integration. One of the most interesting of the newer functional processes is the electrical inhibitory synapse on the Mauthner's cell of goldfish, discovered by Furukawa and Furshpan. In this instance the presynaptic fibre is coiled around the axon hillock of the Mauthner cell and acts as an external source of hyperpolarizing current. In some respects this is reminiscent of the old hypothesis of inhibition by currents generated by Golgi Type II cells. In contrast to the precision with which post-synaptic events can be measured and the mechanisms of the membrane changes delineated by ion injection and by passage of current, our knowledge of presynaptic processes seems meagre. It is probable that transmitter release is highly dependent on membrane depolarization at the terminals, but how this effects release is obscure. The main reason for this deficiency lies in the great technical difficulties in the way of carrying out direct investigations on presynaptic fibres owing to their small size. An even greater disappointment to all interested in synaptic physiology has been the failure to elucidate the nature of any chemical transmitter in the mammalian nervous system other than acetylcholine. It is a sorry situation that despite considerable effort we are totally in the dark as to the chemical transmitter at the primary sensory afferent fibres, and both presynaptic and post-synaptic spinal cord inhibitory systems, not to mention other areas. Sooner or later one hopes that the blunderbuss approach being pursued at present will lead to a breakthrough, but there is really little sign of this so far. Another area in which the result has not been dramatic is the investigation of synaptic plasticity, although this is a vital topic for the understanding of the developing nervous system. The rapid advance in the understanding of synaptic processes has not as yet had much impact on the examination of complex neurophysiological or behavioural phenomena, mainly because there are so many problems of organization still to be solved, but it is surely only the faint-hearted who are not convinced that exact descriptions of such processes are now feasible. Indeed, for all who are interested in the analysis of nervous processes this learned and lucid book is indispensable, and will remain so until superseded by Eccles's next book.

A. S. V. Burgen

CARBOHYDRATE CHEMISTRY AND BIOCHEMISTRY

Advances in Carbohydrate Chemistry

Vol. 18. Edited by Melville L. Wolfrom and R. Stuart Tipson. Pp. xi+456. (New York and London: Academic Press, 1963.) 118*s*.

WHATEVER his or her specialization, every carbohydrate chemist or biochemist will find something of direct interest in the latest edition of this standard

work. The many developments in paper electrophoresis since the first account six years ago are reviewed by H. Weigel, to illustrate this valuable adjunct to chromatography as a means of separation on a micro or preparative scale. Elizabeth F. Neufeld and W. Z. Hassid record for the first time in this series the knowledge of nucleoside diphosphate sugars, now recognized as the major type of poly- and oligo-saccharide precursor in biosynthesis. The discovery of these substances, originating with L. F. Leloir *et al.*, was the foremost event in carbohydrate biochemistry in the past decade, and the impetus of this discovery is still at its height. The article was written before Hassid himself reported the conquering of the plant biochemist's 'Everest', the biosynthesis of cellulose, where the precursor proved to be guanosine diphosphate glucose (Elbein, Barber and Hassid, *J. Amer. Chem. Soc.*, 86, 309; 1964). Having regard to the claims of cellulose to be the most abundant organic compound, GDPG must be one of Nature's most hard-working substances. About 3 kg were required to make the paper on which each copy of Volume 18 of *Advances in Carbohydrate Chemistry* is printed.

C. T. Greenwood, with W. Banks, continues to record the physical aspects of polysaccharides, this time their solution behaviour. D. Horton and D. H. Hutson review thio sugars for the first time since Volume 1 and record another long-sought goal, achieved in two British laboratories in 1961, that of synthesizing sugars with sulphur replacing the ring atom. Other compounds that begin to look less and less like carbohydrates are the amino sugars of antibiotics. J. D. Dutcher reviews this very active field. Two groups of naturally occurring compounds not previously reviewed here are the trehaloses (G. G. Birch) and *C*-glycosides (L. J. Haynes), and the list of articles is completed with photochemistry of carbohydrates (G. O. Phillips) and osotriazoles (H. El Khadem), the latter compounds being cyclization products of osazones. J. E. Courtois contributes an appreciation of E. Bourquelot (1851–1921), and the editors' preface reminds us that we shall expect an obituary notice of J. C. Sowden, for long an adviser to the series.

No sales promotion will be needed to sell this volume. It is as indispensable as its predecessor in its wide-ranging and detailed coverage. M. L. Wolfrom and R. S. Tipson, the editors, themselves responsible for no mean part of the annual output of carbohydrate research, deserve the warmest praise for their efforts in ensuring the prompt annual appearance of these meticulously prepared volumes.

W. J. Whelan

BEHAVIOUR IN INFANCY

Cerebral Function in Infancy and Childhood

By Prof. Albrecht Peiper. (The International Behavioural Sciences Series.) Translation of the third revised German edition by Benedict Nagler and Hilde Nagler. Pp. xii + 683. (New York: Consultants Bureau, 1963.) 25 dollars.

At a time when pædiatricians in Great Britain and the United States have at last become intensely interested in the behaviour of the infant, and especially the new-born infant, the appearance of a translation of Prof. Peiper's monumental account of previous investigations of behaviour in childhood must be warmly welcomed.

Cerebral Function in Infancy and Childhood is a translation of the third edition of the author's *Die Eigenart der kindlichen Hirntätigkeit*, which appeared in 1961 and was based to some extent on his earlier book *Die Hirntätigkeit des Sauglings* of 1928. In it Prof. Peiper gives an account of previous work describing the behaviour of children which he considers to be relevant and scientifically sound. He defines his attitudes to investigations of child behaviour in the prefaces and the first chapter of the book. He is concerned with "the emergence of brain function" in infants and young children as it is manifest in their spontaneous behaviour in test situations. He feels that anatomical investigations and child psychology have been unproductive in examinations of child behaviour, though some physiological animal experiments have given information relevant to the young child, particularly those of Pavlov, Magnus, Radermacker, von Holst and Von Uexküll. He insists on observations being objective and is intolerant of explanations of behaviour in terms of child's consciousness, awareness or emotions. He states on p. 1: "Man knows his own brain functions from inner experience and self-observation, the brain function of others only from observation. As infants and animals are not able to observe themselves and report upon their observations, insight into infant and animal psychology can be gained only by observing their behaviour. This methodology has been accepted in the field of animal psychology and has been found very productive, but it still remains very foreign to the field of infant psychology. Infant psychology should therefore model itself on animal psychology in this respect. 'The soul of infants differs, so to speak, in nothing from the soul of animals'."

There follow fourteen chapters in which a most detailed account is presented of many aspects of child behaviour. The headings of some of the chapters give some idea of the scope of the book. They include: "The Development of Sensory Function"; "Reflexes of Position and Movement"; "Locomotion"; "Movements"; "Reflexes of Clinical Significance"; "The Neurology of Food Intake"; "Conditioned Reflexes"; "Neurological Characteristics of certain Developmental Phases"; "Evolution and Dissolution of Cerebral Function"; and "The Child and his Environment". These give an amazingly full account of 100 years or more of German work in the investigation of child behaviour, together with the highlights of British, French, Russian and American research. Some 1,700 authors are quoted in the author index, and their work is fully described and discussed in the text with a command and sense of proportion which indicate Prof. Peiper's mastery of the subject.

Prof. Peiper's sense of historical perspective enables him to present accounts of the development of ideas as well as to describe actual observations and experiments. The section in Chapter 3 on "Theories of Expression" is a good example of how he is able to show that the ideas of earlier workers, including Spencer and Darwin in this case, were important in determining the course of later research.

Inevitably the book has defects. Many of the controversies he describes are now no longer of interest, and disproportionate attention is directed to observations and experiments which have been superseded by the use of superior modern techniques. The chapters on "The Neurology of Respiration" and "The Neurology of Food Intake" suffer particularly from the author's neglect of recent work, but many examples of this may be given. For example, there is no discussion of the concept of visual fieldedness or of the work of Hildreth in the sections concerned with laterality. Scarcely any non-German references since the Second World War are discussed in the section describing convulsions. The chapter on "The Child and his Environment" gives a fascinating account of wolf children but very little discussion of ideas about deprivation and social environment which have developed since the Second World War. It is apparent throughout the book, however, that Prof. Peiper's knowledge of the world literature since 1939 is limited compared to his encyclopædic knowledge of earlier work. It is sad that a research worker of his eminence should find it difficult to obtain foreign publications.

These are relatively minor criticisms. No one else could have written this book. Prof. Peiper presents a comprehensive account of previous research on child development and

sets it in perspective. It is a book which has long been required and is a completely reliable and knowledgeable description of research work up to the beginning of the Second World War which will remain a uniquely valuable work of reference.

The translation is always adequate though there are a few 'Germanisms', and the book has been very well produced. Inevitably the price is high, but it is likely to be bought by many pædiatricians who until now have had difficulty in finding descriptions of early work on behaviour development.

T. T. S. Ingram

PAPER CHROMATOGRAPHY

Paper Chromatography

A Comprehensive Treatise. Vol. 1, Edited by I. M. Hais and K. Macek. Pp. 955 (1963), 190*s*. Bibliography of Paper Chromatography, 1944–1956, and Survey of Applications, Vol. 2. By Karel Macek and I. M. Hais. Pp. 766 (1960), 171*s*. 6*d*. Bibliography of Paper Chromatography, 1957–1960, and Survey of Applications, Vol. 3. By Karel Macek, I. M. Hais, J. Gasparič, Jan Kopecky and V. Rábek. Pp. 706 (1962), 171*s*. 6*d*. (Prague: Publishing House of the Czechoslovak Academy of Sciences; New York and London: Academic Press.)

THIS work has had a rather complicated history. The first edition was published in the Czechoslovakian language in 1954 and was rightly regarded as the most complete treatise on paper chromatography to be written. The second edition was published in German only four years later, and some time afterwards the Czech version appeared, to be followed by Romanian and Russian translations. The second edition consisted of two volumes: Volume 1 covered principles and applications; Volume 2 was bibliographical, covering the literature from 1944 to 1956. The third edition comprises three volumes. Volume 1 has been brought up to date; Volume 2 is unaltered from the second edition, and Volume 3 has been added to supply bibliography for the years 1957–60. Volumes 1 and 3 are translated into English, but Volume 2 is in Czechoslovakian, although it must have been the intention of the editors to prepare an English translation. There is a separate supplement in English to help the English reader to find his way about this volume.

In both bibliographical volumes, the titles of references are given in their original languages, if in French, German or English—as more than 90 per cent of them are—otherwise they are translated into English. Volume 3 follows the arrangement of Volume 2, the German version of which was reviewed in *Nature* five years ago (184, 1436; 1959), but the editors have been more selective, omitting thin-layer and column chromatography and references in which paper chromatography has not played an important part. Yet the number of references (more than 8,000) for the years 1957–60 compared with some 10,000 for 1944–56 shows that, despite the development of other techniques, paper chromatography continues to hold its own as an analytical tool.

In Volume 1, much of the material of the previous edition has been incorporated unchanged, but there has been some re-arrangement of subject-matter and most of the chapters have been brought up to date. There is a good deal of new material on radioactive substances, peroxides and peroxi-acids, steroid glycosides, terpenes, pesticides, pigments, plastics and intermediates and inorganic substances. The practical notes on detection reagents, solvent systems and quantitation have been usefully extended. Special attention is directed to the relationship between chromatographic behaviour and chemical structure and to the elaboration of solvent systems for the identification of substances on the basis of their chromatographic mobility.

In this volume also, the editors have been more selective than in previous editions. Column chromatography on cellulose derivatives, and electrophoresis, have been omitted, but further pruning would have been desirable, particularly of obsolete material such as the sections on retention analysis, on preparative paper chromatography and on the paper chromatography of proteins. On the other hand, there are a few omissions; for example, the part played by paper chromatography in the discovery of iduronic acid in chondroitin sulphate *B* is not mentioned and the importance of the 'finger print' technique for peptides is not emphasized. The latter is deliberately played down because it involves electrophoresis, yet a fuller account (with photograph) is given of the separation by this means of the amino-acids of blood serum—surely a less important application.

These are, however, comparatively minor criticisms of a work which covers an enormous field with great thoroughness. The English translation is on the whole well done, but the quality of the paper leaves much to be desired. This makes the reproductions of photographs in Volume 1 poor by comparison with the German version, which was printed on good paper. The high price of these volumes will deter many individual prospective buyers, but should not prevent libraries from adding these useful reference books to their shelves.

R. Consden

MATERIALS AT EXTREME CONDITIONS

High Temperature Structures and Materials

Proceedings of the Third Symposium on Naval Structural Mechanics, held at Columbia University, New York, N.Y., January 23–25, 1963. Edited by A. M. Freudenthal, B. A. Boley and H. Liebowitz. (Office of Naval Research Structural Mechanics Series.) Pp. xv+463. (London and New York: Pergamon Press, 1964.) 100*s*. net.

THIS collection of thirteen papers was designed to cover four broad subjects: material aspects of elevated temperature design; effects of high-speed environment; thermal stress analysis; design criteria and reliability. As is stated in the preface: "It was hoped that the collaboration in one conference of material physicists, mechanicians, and structural designers concerned with the problem of elevated temperature effects on the performance and safety of modern structures would at least in part bridge the gaps existing between the three groups". This is a worthy aim, and no doubt much was gained by those who participated in the conference. As a published work, however, the papers cannot be said to carry the bridge-building spirit very far: many of them are fairly specialist reviews, not tailored for general communication (this is particularly true of the more mathematical ones on thermal stresses, and on the thermoelastic boundary problems), while others, good in themselves as technical assessments, fail to carry the arguments to the point where the real nature of the critical problems may be appreciated and discussed in non-specialist terms. Unfortunately it is much harder to write a paper on the lines necessary for a truly interdisciplinary conference than it is to prepare one of the more standard form, as many who have experienced such meetings will know.

Taking the contributions out of this context, one must acknowledge the quality of many of them. The range of subject-matter covered is too wide to be of universal interest for the average reader: it extends from Mason's treatment of the elastic and anelastic properties of solids, involving, for example, discussions on semi-conductor physics, to the crystal mechanics inherent in the paper on creep by Dorn and Mote, and from the applied mechanics approach to thermal fatigue, as exemplified by

the paper by Shuji Taira, to Wolfe's review of design criteria for high-temperature structures.

It is not possible to do justice to the thirteen papers in a review of this kind, but a few might be picked out for special comment. The review by Pask and Copley of the thermo-mechanical behaviour of ceramics is well done; this is, of course, one of the interesting bridge subjects at the present time in the sense, at least, that it concerns the extension of concepts, worked out originally for metallic materials, to the structure and behaviour of non-metallic crystalline materials. The Dorn and Mote review of creep mechanisms is on the lines of the kind of article one associates with *Progress in Materials Science*. Prof. Dorn's own valuable contribution to the rationalization of this somewhat over-ploughed field are well known and well respected. This survey, written in conjunction with his colleague, re-states some of the familiar premises, and one would think that in places it goes beyond the boundaries of a general review, but it is a good contribution to the review literature, and metallurgists, including those involved with teaching, will be very glad to have it. It is interesting to compare Kachanov's presentation of the variational methods of solving non-linear creep problems; the analysis is not related to any experimental results. Mullendore and Grant undertake the subject of the tertiary creep stage, covering in the main the features of boundary sliding and void formation, but at the same time stressing the point that elongation "becomes unstable in second-stage creep" and that tertiary creep need not involve non-uniform deformation and intercrystalline cracking. They also have some faith in a readier predictability of the final stages to rupture from short-term data. The subject of thermal fatigue and creep rupture, reviewed by Shuji Taira, uses as a basis the plastic strain amplitude formulation of Coffin, generalizing first to take account of temperature, and then to cover the case of a mean static stress. The theoretical results are shown to compare well with the experimental findings, but there is no consideration of its metallurgical validity.

The special conditions imposed by space technology projects are represented by papers on high-speed effects (sliding, impact) in both metals and polymers (Bowden and Brunton) and on re-entry problems (Scala). The latter paper outlines some of the materials issues, without dealing very profoundly with any one of them, directing attention to the increasing importance and difficulty of successful non-destructive assessment. The Bowden and Brunton paper draws on many of the results which Dr. Bowden's group has contributed over the years, and in the context of aerospace investigations the beautiful dissection of the problem of high-speed liquid drop impact on polymers and metals is particularly appropriate.

The difficulties inherent in problems of heat flow where phase changes occur are well brought out in the paper by Boley, who has managed to thread his way through some fairly heavy mathematics while at the same time conveying the significance of the results to the behaviour of real materials.

Those who would regard their province as being materials science and engineering will find the book worth sampling.

A. J. KENNEDY

THE GENUS SYNCHYTRIUM

Synchytrium

By Prof. John S. Karling. Pp. xviii+470. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1964.) 125*s*.

THE name of Karling and *Synchytrium* have come to be regarded as almost synonymous, and the choice of John S. Karling, Wright distinguished research professor of Purdue University, Indiana, to undertake the writing of this volume on *Synchytrium* which is dedicated to the memory of Anton De Bary and Michael Stepanovitch Woronin on the occasion of the centennial of the creation of the genus, is a happy one. Founded by De Bary and Woronin in 1863 for the two chytrid species *S. taraxaci* and *S. succisae*, the genus *Synchytrium* now includes more than 200 members; several new sub-genera have been established and almost 100 of the species have been added in the past 30 years.

Sixteen preliminary pages containing the preface, acknowledgements and a list of the herbaria consulted are followed by the introduction and Chapter 1 which provide general descriptions, illustrations of the life cycles and the types of development shown by the six sub-genera. The illustrations are excellent, and after reading this portion of the book the reader feels he has gained a good working knowledge of the genus. Having made it clear that criteria other than those based on morphological differences must be taken into account in any taxonomic investigation of the genus, the writer then proceeds in Chapters 2, 3 and 4 to deal with cytology, sexuality and host reaction respectively. The chapter on cytology is both well written and well illustrated. The considerable disagreement concerning nuclear behaviour, etc., emerges clearly; a truer picture will only result from further intensive investigation, when the employment of modern techniques has eliminated errors and misinterpretations. Sexuality is fully discussed in Chapter 3 and an investigation of host reaction, again beautifully illustrated, is provided in Chapter 4. Chapter 5, which deals with the evaluation of taxonomic criteria, is probably one of the most valuable, pointing out as it does the variability of so many characters in the various species and underlining the danger of using any but the most constant for taxonomic purposes. Indeed, in Chapter 6, which deals with systems of classification, that selected by the author is based entirely on the reproductive organs and the mechanism of reproduction, which form the most reliable guide. Chapter 7 provides full descriptions of the genus, the sub-genera and the species. It occupies 239 pages or just about half the book. The species for each sub-genus are divided very wisely into those described as fully known, those which are incompletely known and those which remain unidentified. Chapters 8–10 deal with relationships and phylogeny, geographical distribution and a host index respectively. References occupy the next 24 pages and, except for the author and subject indexes, the book concludes with a bibliography for *S. endobioticum* and wart disease of the potato which runs to 30 pages.

It seems extraordinary that only one species in this genus of obligate parasitic chytrids should have assumed real economic importance. The species is of course *S. endobioticum*, which causes wart disease of the potato. Its appearance in Europe in 1896, only 50 years after the potato famine caused by late blight (*Phytophthora infestans*), caused great concern; its ravages have been met by the use of resistant potato varieties. *S. endobioticum* is comprehensively dealt with in the text and the bibliography which appears at the end of the book is most valuable.

In his preface the author points out that the genera and species of aquatic chytrids are fairly well known, and described in a series of excellent text-books, but that these investigations do not cover the large terrestrial genera *Physoderma* and *Synchytrium*. The purpose of this book is to fill one of these gaps and to direct attention to the many problems which remain to be solved by the further intensive investigation of individual species. The author can rest assured that his purpose has been fulfilled and that his book might well serve as an example to other classical biologists who have the ability, the good fortune and the patience to pursue the investigation of an individual genus or a group of genera. Perhaps its message is of special import to the classical microbiologist, for the simpler the organism the greater the difficulty of classification—ranging from the microfungi through the bacteria to those nameless

ones—the viruses—which hover between the living and the dead. It is clear that the species concept in microbiology takes on a somewhat different meaning from that which it carries in the case of the higher organisms, and a reference to volume 6 of Rabenhorst's *Kryptogamen-Flora*, where recorded species of *Phyllostica* occupy 155 pages and those of *Phoma* 181 pages, illustrates the point. It is sometimes tempting to wonder whether, in many cases, there is any significant difference other than the host plant on which the species is recorded. It is also tempting to wonder whether a micro-organism, with its habit of physiological specialization and its aptitude for producing physiological races, behaves "as if increase of appetite had grown by what it fed on". If this book does anything further it shows the need for more taxonomy and, in particular, more experimental taxonomy, and its reading will be well worth while for all postgraduate and research classical biologists.

The volume attains the high standard of production expected from Academic Press: the text is extremely free from typographical errors and only two cases of misspelling were detected. Arthur E. Muskett

VARIABILITY OF SPONGES

A Revision of the Classification of the Calcareous Sponges

With a Catalogue of the Specimens in the British Museum (Natural History). By Dr. Maurice Burton. Pp. v + 693. (London: British Museum (Natural History), 1963.) 300*s*.

THE systematics of sponges are notoriously difficult. Breeding experiments for the present are out of the question, so that one's concept of a species must depend on the general form, histology, spicule characteristics, development, and habitat-preferences of the animals concerned. For the great majority of the species hitherto described, however, information is only available regarding the form and skeleton, both of which can show great variation. This is clear from Dr. Burton's book, which is undoubtedly a major contribution to our knowledge of the systematics of sponges.

The book is divided into four main sections. The first includes observations on the range of variation in some common littoral species. Even in the same individual the spicules may vary widely in form and size, while the canal system may assume either the syconoid, grantioid or leuconoid arrangement. Using knowledge of the variability thus gained, Burton examines the genera hitherto recognized and reduces their number from 55 to 22. He then lists the previously accepted species, giving each the equivalent specific name in his revised classification. Of the original 623 species (including 12 varieties) only 47 (48, according to Burton) remain.

Such extensive lumping will be considered by many to be unwarranted at the present state of knowledge. Can one be certain that the range of variation found for one species is equally applicable to another? Might not specimens with roughly similar spiculation and living in the same locality yet be found to differ in their development or habitat-preferences? Is it justifiable to ignore the position of the nucleus (apical or basal) in the choanocytes because there is more doubt about its taxonomic value than with the spicule characters (p. 31)? By so doing, Burton is able to lump together under *Leuconia barbata* several former species with apical nuclei and several with basal nuclei. To what extent does hybridization between species obscure the estimation of their variability? When *Sycon ciliatum*, *Leuconia fistulosa* and intermediate forms were discovered growing closely together, Burton regarded this as evidence for the variability of the former species, yet hybridization might conceivably have been involved. Only by growing regenerated portions of the same sponge in different environmental conditions can one obtain a satisfactory estimate of the possible range of variation for a given genotype.

The second section presents the systematic account of the Calcarea as understood by Burton. Apart from the great reduction in number of species, one can criticize his definition of the Homocoelidae, namely, "Calcarea having the whole endosomal cavity (or cloaca) . . . lined by collared cells". This is inexact, since choanocytes are lacking at the distal ends of the cloacal tubes.

The third and fourth sections will be of great value to future taxonomists. The latter is a catalogue of specimens and slides in the British Museum (Natural History) collection, while the former comprises brief diagnostic descriptions, often illustrated, and references for all the hitherto recognized species, grouped according to Burton's classification. Here, it would have been helpful if the author had indicated the precise source of the descriptions under each species, so that the reader could check and amplify them if necessary. Thus, under *Leucosolenia variabilis*, the spicule characters appear to have been derived at second-hand from Arndt's *Die Tierwelt der Nord-und Ostsee, Porifera* (1935), for the same mistake is found when one compares the widths of the rays of the triradiates with those given in Minchin's original description. Information provided by Minchin on the range of variation in this species has been excluded.

The bibliography is complete up to the year 1953, but suffers from one defect, in that where several publications by the same author appeared in the same year no attempt is made to distinguish between them. Also the reference to Burton (1931) (p. 5) does not appear in the list.

While Dr. Burton's simplified classification will be regarded by many as premature, there can be no doubt that he has produced a remarkable book; one which will be a starting point for future revisions of the classification for many years to come. W. Clifford Jones

AGRICULTURAL PESTS

Pests of Field Crops

By F. G. W. Jones and Margaret G. Jones. Pp. viii + 406 + 32 plates. (London: Edward Arnold (Publishers), Ltd., 1964.) 50*s*. net.

MORE than a century ago, John Curtis wrote the first comprehensive practical book on British farm pests. He dealt mainly with insects but also devoted space, proportionate to existing knowledge, on nematode, molluscan and other pests of farm land and farm stores. In *Pests of Field Crops*, Mr. and Mrs. Jones have produced a present-day 'Curtis'; while their book is intended for students and is concerned mainly with the animals which annoy the British farmer, they draw fully on examples from Europe and elsewhere to illustrate appropriate points. While emphasizing for each species the importance of field ecology and natural control, the authors give full consideration to chemicals and the overall coverage is comprehensive and well illustrated. There the similarity ends, because 'Curtis' was written at a lower level of technical sophistication when man and agriculture were accepted as part of Nature; Jones and Jones, in the second line of their first chapter, define agriculture as "interfering with nature".

So the tone of the book is set: pests are animals in the wrong place and must be eliminated, provided that man is not inconvenienced by pesticide residues in the process. With such an end in view the student must be taught the fundamentals of population dynamics and have a good grounding in ecology. By the time the reader has finished the nine pages of Chapter 1 his appetite is whetted for more information on what constitutes a pest, but instead he is given 24 pages of elementary morphology in Chapter

2. The balance is redressed to a large extent in Chapters 14 and 15, where general relationships between crops and pests are discussed, and consideration is given to problems of pest numbers and crop yield and to various aspects of natural control. Population problems are also discussed in some detail in the chapter of 52 pages on plant parasitic nematodes—the subject of the senior author.

Nearly half the book (195 pp.) is devoted to insects, which are taken in systematic order. Notes on family classification are followed by brief taxonomic descriptions of the more important species which are illustrated with line drawings (which sometimes lack precision). The descriptions lead into biological summaries and notes on crop hosts, damage, and control methods. Sometimes the biological notes are out of date: few applied entomologists to-day would accept that a wireworm population of 5–600,000 per acre does no damage to cereals, or that most crops can be grown with safety on 1 million wireworms per acre (p. 117). Pea moth is said (p. 93) to have become increasingly important in parts of eastern England, when growers generally appear to be much more concerned about crop maturation delays caused by weevil, thrips, or aphid attack than by moth damage. Generally, however, the biological notes are excellent and the authors are to be congratulated on condensing so much scattered information into concise and readable prose.

Four pages are devoted to molluscs, and the section on slugs (1·5 pp.) could with advantage be expanded in future editions, because some modern farming methods (ploughing-in long combine stubbles from successive cereal crops on heavy land, for example) appear to be encouraging slug population growth.

The 29 pages devoted to vertebrate pests form a most useful addition to the book, bringing together in one place much scattered information from journals and bulletins which students probably cannot hunt out individually.

In all, this is a remarkably good book. The authors have taken great pains to cite up-to-date literature, often papers published in the past five years or so, the latest being a report published in March 1964, two months before the book went to press. As an added precaution many of the sections into which the book is divided were checked prior to publication by established specialists, and the acknowledgements in the authors' preface include many respected names. Printing and paper are good, typographical errors minimal, and most of the plates—culled from many sources—are excellent even though they are often badly placed in relation to the text. A. H. STRICKLAND

STYLE AND COMMUNICATION

Science, Humanism and Libraries

By D. J. Foskett. Pp. ix+246. (London: Crosby, Lockwood and Son, Ltd., 1964.) 26*s*. net.

IN *Science, Humanism and Libraries* Mr. Foskett has sandwiched, between two essays based on a thesis dealing with T. H. Huxley as a writer on science, a miscellany of sixteen papers and essays delivered over the years 1951–62. They comprise such themes as special libraries, readers' needs in industrial libraries, documentation in the humanities, in the social sciences and in occupational health and safety, national lending libraries, classification and communication and reference services, comparative and faceted classification, the work of the classification research group, and a historical essay on the pioneer of documentation, Marc-Antoine Jullien. Scattered through them are quotations from the writings of T. H. Huxley, but the thread of communication, while plausible, is somewhat tenuous. Some of the essays, in fact, are rather too technical or specialized for the general reader, who may thus be deterred from pursuing a book which contains much of general interest and goes to the roots of what is involved in the present-day controversy over two—or more—cultures.

This would be unfortunate, for the papers do far more than display the range of Mr. Foskett's interests and the penetration of his thought. Even at his most technical he has shrewd, sound comments to make, though it must be admitted that here and there the essays require to be brought up to date. He pays a generous tribute, well-deserved, to the work of Dr. S. R. Ranganathan, and to the many stimulating comments which should not be overlooked by his professional colleagues.

Of the core or inner layer of the book, it could be said that it is a remarkable demonstration of the feasibility of combining in one person expertise and clarity of exposition, specialized and general knowledge. In other words, the antithesis of the two cultures is a false dichotomy, and from that point of view this part belongs to the outer layers with their dissertation on science, on humanism and libraries. It is only because a thinner sandwich might have attracted a wider circle of readers that there is reason to regret the more technical filling was not curtailed.

Mr. Foskett's study of T. H. Huxley as a writer on science convinces him that it is possible to present scientific results in good English, understandable by all, and that difficulties of communication are due ultimately, not to the material but to lack of good style on the part of the writers. Dullness, he observes, lies in the presentation of the material. A. N. Whitehead, in *The Aims of Education*, asserted that style, in its finest sense, was the last requirement of the educated mind. "The administrator with a sense of style hates waste; the engineer with a sense of style economizes his material; the artisan with a sense of style prefers good work. Style is the ultimate morality." Lewis Mumford expresses much the same idea in the sense of fitness for the purpose which pervades his *Technics and Civilization*, and George Stuart Gordon in *Anglo-American Literary Relations* asserted that "Wherever a man has fairly set down the best that he knows about the thing he knows best, and in words that tell his meaning, there, always, will be literature".

Mr. Foskett's onslaught on this imaginary gap between science and humanism is timely and finds ample support. It is refreshing to find an expert who not only quotes Quiller-Couch on writing but also refers to Pater's essay on style. Curiously enough, he does not quote the most pertinent passage in that essay, the reference to the dependence of style on self-restraint, the skilful economy of means, the choice of the unique word, phrase, sentence, paragraph, absolutely proper to the single mental presentation and vision within. Nevertheless, he reminds the scientist that false reactions in presentation and writing depend not on the qualities of the subject but on factors extrinsic to the subject, and that if the character of scientific material does not properly arouse human feelings, the writer has not presented his material efficiently.

These opening and closing essays of the book deserve to be widely read and pondered, not only in relation to the present-day debate on the two cultures, but in respect of the even wider issues of communication. Communication, he reminds us, is more than merely being told something; a successful lecturer constantly strives to bring his new ideas into relation with what the audience already knows. The primary requisite is to arouse interest, and the writer succeeds in communicating inversely with the effort the reader has to make to establish this bridge of understanding. Mr. Foskett offers no prescription for improving the situation other than his challenge to his fellow librarians to make a particular professional contribution, but what he writes is stimulating and suggestive. Neither the general reader nor the scientist need be at a loss for clues as to what steps he could himself take to remedy a situation that constitutes an indictment of our educational system as a whole. R. BRIGHTMAN

Deltaic and Shallow-Marine Deposits

Proceedings of the Sixth International Sedimentological Congress, The Netherlands and Belgium, 1963. Edited by L. M. J. U. van Straaten. (Developments in Sedimentology, Vol. 1.) Pp. xvi+464. (Amsterdam, London and New York: Elsevier Publishing Company, 1964.) 130*s*.

Deltaic and Shallow-water Littoral Marine Deposits

Edited by V. S. Yablokov and others. Pp. 263. (Moscow: Academy of Sciences, 1963.) 1r. 67k.

DELTAIC and Shallow-Marine Deposits is a compilation of 58 short papers presented to the Sixth International Sedimentological Congress held in 1963. Other than the thought-provoking presidential address by Dr. F. P. Shepard on "Criteria in Modern Sediments useful in Recognizing Ancient Sedimentary Environments", the contributions (in English, French or German) are all research reports, more or less related to the general theme of marine sedimentation under deltaic or shallow-water conditions in Phanerozoic, Recent and modern times. They describe deposits in 20 different countries and are almost as diverse in the nationalities of the authors. In connexion with the same Congress, the Commission on Sedimentary Rocks of the U.S.S.R. Academy of Sciences (Division of Geological and Geographical Sciences) has published the second-mentioned volume of articles, which begins with a useful review by Dr. G. F. Krasheninnikov on "Fossil Deltas of the U.S.S.R.". Unfortunately, only 7 of the 40 Russian contributions appear in translation in the official *Proceedings*, and the small edition (800 copies) of the Moscow publication will make it difficult to procure. However, the high price of the official record will certainly not facilitate a widespread circulation even of the papers printed in Western languages, distinguished though many of them are.

In both books the recent growth of interest in these shallow-water sediments is attributed to the belief that, since such deposits form the host-rocks for many useful minerals, a knowledge of the laws that govern the formation of the strata will be useful in interpreting the genesis of ores and in planning prospecting work. This, however, presupposes that ancient sediments have remained a closed chemical system throughout their history and that pay metals have not been introduced or redistributed by ground-waters. Perhaps the most useful outcome of the actualistic approach to sedimentology, followed by the papers in these symposia, will be a wider recognition of the nature and extent of the post-depositional chemical changes which many older sedimentary rocks have undergone; but this will not come about until more sedimentologists have entered into alliance with geochemists, the assistance of whom they seem at present inclined to eschew.

C. F. Davidson

Advances in Inorganic Chemistry and Radiochemistry

Edited by H. J. Emeléus and A. G. Sharpe. Vol. 5. Pp. ix+429. (London and New York: Academic Press Inc., Ltd., 1963.) 103*s*. 6*d*.

EACH of the volumes in this series has provided articles of general interest to all inorganic chemists—articles which have been, and are, of very great use to research workers in the fields covered. The subjects dealt with in the present volume again range widely over inorganic chemistry and are of general, and particular, interest. R. S. Nyholm and M. L. Tobe discuss the stabilization of oxidation states of the transition metals. The treatment is simple and the chapter could well be required reading for any honours course in chemistry. Another general review is "The Structure and Reactivity of Organophosphorus Compounds" by R. F. Hudson, an article which considers the reactivity of these derivatives in terms of the properties of the phosphorus atom. Of a far more specific nature are the exhaustive reviews on the "Oxides and Oxyfluorides of the Halogens" by M. Schmeisser and K. Brändle, on "The Borazines" by E. K. Mellon, jun., and J. J. Lagowski, and on "Decaborane-14 and its Derivatives" by M. F. Hawthorne. The last-named is a particularly clear and lucid discussion of a subject which is undergoing rapid development at the moment. N. N. Greenwood reviews "The Chemistry of Gallium" with specific reference to the developments made in the chemistry of this element during the past ten years, while O. Glemser and H. G. Wendlandt write on "Gaseous Hydroxides", giving particular emphasis to the transition metal hydroxides. The only article on radiochemistry is "Chemical Effects of Nuclear Activation in Gases and Liquids" by I. G. Cambell. It does not fit in too well with the other articles in this book, as most of the molecules considered are organic in nature.

D. W. A. Sharp

Master Equations and Tables for Symmetrical Component Fault Studies

By S. Austen Stigant. Pp. x+78. (London: Macdonald and Co. (Publishers), Ltd., 1964.) 25*s*. net.

THERE are many similarities between the various conditions of unbalance which can occur in three phase systems, and Mr. Stigant demonstrates the manipulation of the symmetrical component equations in each case to reduce as many problems as possible to basic equations which can then be applied to a given case, by means of tables, in a routine manner. It is of interest to examine this book with regard to its claim "to be of considerable value to H.N.C. students and teachers of electrical system theory, and to system engineers concerned with fault analysis".

There is much more to the application of symmetrical component theory than the manipulation of equations, as the author himself states, and the electrical engineering Higher National Certificate student would do well to achieve a full understanding of the method, from such sources as the adequate list of references given, before turning to the material of this book. Teachers, of course, will be interested in the approach, but practising systems engineers with problems of such complexity that the tables of equations are needed should to-day have access to, or be thinking of, digital computer programmes in which such routine manipulation is included. Simple connexion matrix techniques are described and provide a link with the author's other well-known books, but the possibility of computer application is not introduced. This is disappointing, because I feel that the book could have been valuable in introducing Higher National Certificate students, or the engineer as yet unaware of computers, to the means by which the drudgery of routine calculation is being eliminated in this field as in others, leaving the modern engineer more free for creative effort.

M. N. John

Personnel and Industrial Psychology

By Prof. Edwin E. Ghiselli and Prof. Clarence W. Brown. Second edition. (McGraw-Hill Series in Psychology.) Pp. ix+492. 1955. 45*s*.

Heat and Thermodynamics

An Intermediate Textbook for Students of Physics, Chemistry, and Engineering. By Prof. Mark W. Zemansky. Fourth edition. Pp. xi+484. 1957. 36*s*. (New York and London: McGraw-Hill Book Company, Inc.; Tokyo: Kogakusha Company, Ltd.) International Student Edition.

THE paper-back text-book resolves, to a large extent, the dilemma of the bound science treatise. The binding of a book may cost more than the printing of it, and science books become out of date so quickly. For decades, the French have been aware of this, and book production has been separate from binding. It is not surprising that more publishers are producing solid, academic works in paper-back form. The latest examples are the McGraw-Hill International Student Editions, originally American, and published in England in Maidenhead. They are warmly

welcomed. The saving of cost, by having a paper-back instead of a more substantial binding, is from 30 to 60 per cent. The range of subjects is large and includes sociology, mathematics, mechanics, geography, psychology, medicine, engineering, physics, chemistry, biology and communication theory.

The greater availability of so many text-books and standard works raises an important question. What *is* a student's text-book? Particularly in view of the great extension of university and technical education, with its concomitant increase of examinations, considerations of the nature of the text-book and the purpose and the technique of examination are important and overdue. Some text-books are treatises with notes, others just 'boiled down' treatises, some are mélanges of other treatises and text-books. There is a great spectrum of possibilities in science books for students, from treatises and original papers which make no concessions to the reader, to the programmed or self-help text-book in which every paragraph has been evolved according to the most recent pedagogical and psychological principles. Again, the relations of the text-book to the teacher's work, the student's writing, the recent research, the examination syllabus if any, the maturation level and intelligence of the student are all important. Here is a field which is in urgent need of a thorough investigation.

W. L. SUMNER

Short Guide to Geo-Botanical Surveying

By S. V. Viktorov, Ye. A. Vostokova and D. D. Vyshivkin. Translated by J. M. MacLennan. (International Series of Monographs on Pure and Applied Biology. Division: Botany, Vol. 8.) Pp. xi+158. (Oxford, London, New York and Paris: Pergamon Press, 1964.) 63*s*.

IN any country with large sparsely inhabited regions vegetation surveys are of great importance in assessing natural resources.

Short Guide to Geo-Botanical Surveying reviews the procedures that have been employed in Russia. It is concerned chiefly with the planning of surveys, and discusses the suitability of various methods of ground traversing and aerial interpretation, the setting out of the sample traverses or plots, the data that should be recorded, and how these may be represented cartographically. The practical applications of such surveys are emphasized in separate chapters dealing with forests, peat bogs and grazing land, and with the use of vegetation as an indicator of soil conditions or mineral deposits.

The procedures advocated are mainly of an empirical nature found to be satisfactory by experience, and there is no attempt to estimate the accuracy of different sampling methods. Indeed, with exploratory surveys on a regional scale the choice of method is often dictated more by the nature of the country and the resources available than by academic considerations of sampling density. It is essentially a manual on the organization of survey networks, and strictly ecological topics such as the assessment of botanical composition and the recognition of plant communities are scarcely touched upon.

W. D. CLAYTON

Symposium on Instrumentation for the Detection and Determination of Pesticides and their Residues in Foods

Los Angeles Meetings of the American Chemical Society, April 1963. (Residue Reviews, Vol. 5.) Pp. viii+176. (Berlin, Göttingen, Heidelberg: Springer-Verlag, 1964.) D.M. 26.

THE declared object of Residue Reviews is to make available on an international basis concise, critical reviews of advances in the field of residues in foods, whether in methods for measuring residues, in the understanding of their significance when measured or in methods of controlling their incidence (by legislation or otherwise). This volume differs from the five others so far published in the series in that it is the only one to result from a symposium—organized for the American Chemical Society by the series editor, Dr. F. A. Gunther. It shares in common with the other volumes the fact that for the most part the residues considered are those of pesticides.

With the exception of Dr. H. Frehse, who contributes the opening chapter, all the authors are American. Dr. Frehse considers the problem of residue analysis and food control. He compares the classical, general 'wet' methods of residue analysis (for example, for organo-phosphorus or for chlorinated pesticides) with esterase and bioassay techniques and with the more modern physicochemical methods such as chromatography. And he contrasts the position of the commercial analyst, normally concerned with field trial work in which the identity of the residue is known, with that of the official analyst to whom the field history of the crop or food will probably be quite unknown. There is useful comment on the expression of results in relation to the precision and sensitivity of the analytical method used. Nine of the remaining eleven chapters of the volume are devoted to detailed practical aspects of pesticide residue analysis, ranging from detectors for gas chromatographic work to neutron activation analysis and the automation of 'wet' methods. R. C. Blinn deals with the general subject of residue analysis using infra-red and ultra-violet spectrophotometry, and in the remaining chapter specific consideration is given to polarographic and (despite the title of the chapter) spectrophotometric and gas chromatographic methods for the determination of additives in animal feeding-stuffs.

The volume is primarily of direct interest to practising pesticide-residue analysts: it is at the same time of general interest to those otherwise concerned with the control of residues, and it gives some insight into the complex and varied methods to which residue analysts now resort.

H. EGAN

The Practical Home Gardener

A Guide to the Cultivation of Plants in Australia with special details for the Drier Regions. By T. R. N. Lothian. Second edition, revised. Pp. xix+390+32 plates. (Melbourne: Lothian Publishing Co. Pty., Ltd., 1963.) (A) 97*s*. 6*d*.

IN this recent addition to Australian gardening literature, Mr. Lothian ranges widely, from structural botany to the organization of flower shows. Though writing more particularly for suburban gardeners, he adds useful advice here and there for those struggling with the problems of horticulture in remoter areas.

From his experience of gardening in the extremes of a Mediterranean climate, Mr. Lothian is able to suggest many useful and interesting ornamental trees and shrubs worth trying in other parts of Australia where long dry periods occur, and to this topic he devotes his two longest chapters. Vegetable and fruit growing also receive a fair share of space, but the treatment of other subjects is more sketchy and the touch less sure. An extreme case is the chapter on soils which amounts to only two and a half pages, nearly all of which might have been written for gardeners in Europe. A fuller account of so large a subject, with more reference to Australian conditions, would surely have been useful here.

Throughout the book, much of the information is given in tabular form, whereby space is saved and reference facilitated. The illustrations include a coloured frontispiece and more than sixty black-and-white plates, some rather indifferent in quality. There is also a number of line drawings which will help the reader, though a few of these would have been better if somewhat larger and less confused with shading. Not all spelling mistakes have been detected. Among those which remain are the words *calciphobe* and *calcicole* whose meanings are also transposed.

J. SOUSTER

RADIO ECHO SOUNDING OF POLAR ICE SHEETS

By J. T. BAILEY, Dr. S. EVANS and Dr. G. de Q. ROBIN
Scott Polar Research Institute, Cambridge

FOLLOWING reports by Waite and Schmidt[1] that radio altimeters operating at a frequency of 440 Mc/s could be used to obtain echoes through polar ice sheets up to 300 m thick, equipment was developed in the Scott Polar Research Institute for the specific purpose of sounding cold polar ice sheets. In particular, it was decided that for maximum penetration of ice it was desirable to use the lowest practicable radio frequency. The first set to be built[2] operated at 35 Mc/s and a report on the use of this equipment during 1963 on the Brunt Ice Shelf near the Halley Bay station of the British Antarctic Survey has been prepared by M. E. R. Walford (*Nature*, 204, 317; 1964). Short trials of a second set on the ice sheet of north-west Greenland are summarized in a note by Evans[3]. As part of the same trials, A. H. Waite obtained further measurements with equipment operating on 440 Mc/s and on 110 Mc/s.

An invitation to participate in more extensive trials in the same area during June–August 1964, from the Commanding Officer of the U.S. Army Electronics Research and Development Laboratories, led to further development of equipment during 1963/64. Field operations were supported by the U.S. Army Research Support Group and the general programme of traverse investigations was directed by Dr. J. Rinker of the U.S. Army Cold Regions Research and Engineering Laboratories. As a result of this generous support trials of radar sounding equipment developed at Scott Polar Research Institute and at the U.S. Army Electronics Research and Development Laboratories were made during a journey of 130 miles from Camp *Tuto* (76° 25′ N., 68° 28′ W., elevation 480 m) to Camp *Century* (77° 11′ N., 61° 09′ W., elevation 1,930 m), then to distances 40 miles south and 50 miles north-west of *Century*, and back to *Tuto*. Reports on the performance of the U.S. Army Electronics Research and Development Laboratories equipment will appear separately, but the results were in close agreement with those obtained with the Scott Polar Research Institute equipment and both agreed with preliminary interpretations of results from seismic shooting.

The chief parameters of the Scott Polar Research Institute equipment were similar to those given by Evans[2]: radio frequency 35 Mc/s and energy pass band 14 Mc/s. The main developments were that receiver and control circuits were transistorized and a continuously moving photographic film was used to record echoes displayed on an intensity modulated cathode ray tube. Examples of the records are shown in Fig. 1.

A continuous profile of the form of the bottom surface of the ice was obtained over 97 per cent of the route travelled. The ability to obtain a continuous record will be of great help towards our understanding of the factors governing the surface form and flow of the ice sheet, since our knowledge has been limited hitherto by the difficulty of obtaining a general picture of bottom relief from the spot soundings resulting from seismic shooting or from the areal average figure presented by gravity measurements. The limitations of radar sounding arise mainly from the loss of signal strength by dielectric absorption of the radio wave in the ice. A review paper by Evans (in the press) shows that these losses decrease considerably as the temperature of the ice is decreased. The method is likely to penetrate the full depth of ice sheets over extensive areas of Greenland and Antarctica. During the recent trials, echoes were obtained with the Scott Polar Research Institute equipment through 1,400 m of ice, the maximum thickness of ice which was encountered. The echo strength was then up to 30 dB above receiver noise-level and the mean temperature of the upper part of the ice sheet was −24° C. However, diffi-

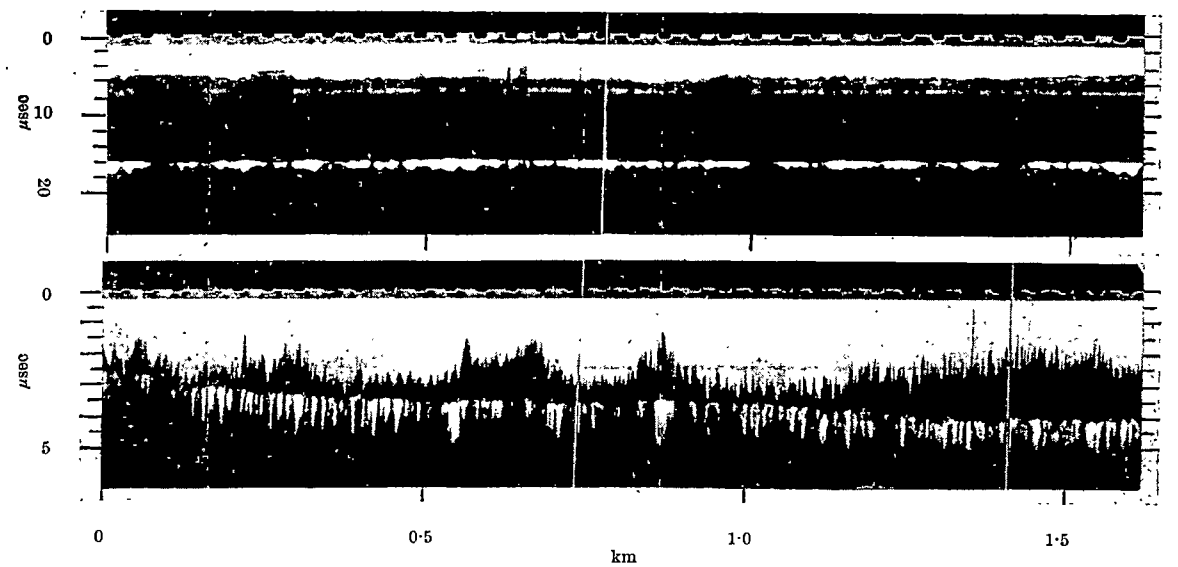


Fig. 1. Continuous recordings of radio echo soundings through a polar ice sheet. The horizontal scale (km) represents distance travelled along the snow surface. The vertical scale (μsec) has an arbitrary zero; the transmitter pulse occurs at the top edge of the broad white band caused by clutter echoes. The upper record shows an echo from an almost horizontal ice rock interface. The echo time delay is 15 μsec corresponding to an ice depth of approximately 1,300 m. Note the weak but continuous echo from a discontinuity in snow structure at a depth of approximately 500 m. The lower record, using a more open time (depth) scale, shows reflexions from an irregular and much shallower bottom surface. Note the variation in echo strength with horizontal position and the occurrence of echoes with longer time delays than that from the normal point

culty was experienced in following a bottom echo through an ice thickness of 500 m when the ice temperature was close to 0° C.

While moving over the ice sheet, the echo from the bottom of the ice increases or decreases in strength as one moves through distances of the order of the radio wavelength. In addition, aerials with a broad polar diagram were used so that strong echoes could be received from angles up to 35° from the vertical. If the bottom surface, though rough, has a slope less than 35° to the horizontal, the minimum time delay echo will arise from a direction normal to the average surface, and echoes at longer time delays (Fig. 1) are supposed to arise from facets or irregularities in the bottom surface at some distance to either side of the normal point. Occasionally the 'normal' echo may be missing, and it is evident that continuous recording is essential to avoid the misinterpretation which would arise if only spot soundings were made.

Within the limitations of many practical compromises the apparatus is given the greatest attainable sensitivity. Since the signal is attenuated as it travels through the ice, the sensitivity required to observe reflexions from the bedrock is much greater than is necessary to observe strong reflectors at short range. For example, a metallic object with effective echoing area 10^{-2} m^2 would be detectable in an otherwise isotropic medium at 250 m depth and a smooth extended surface at the same depth should be detectable if it reflected only 10^{-7} of the incident power. Thus we expect that compacted annual layering of the snow is responsible for the continuous echoes observed from ranges up to a few hundred metres. A change in average specific gravity of the snow from 0·90 to 0·905 taking place in a layer less than 1 m thick will have a power reflexion coefficient of 10^{-6} and should just be detectable down to depths of 500 m. The short range echoes, analogous to 'clutter' from the sea surface in conventional radars, appear as a continuous return signal on the intensity-modulated display. Discrete echoes were observed from layers in the ice down to depths of 500 m over about half the total distance travelled; examples can be clearly seen in Fig. 1. The investigation being undertaken at the U.S. Army Cold Regions Research and Engineering Laboratories of ice samples which have been obtained by thermal drill coring from these depths at Camp *Century* may identify the different physical properties of various layers of the ice sheet which give rise to echoes. We expect that such differences are related to climatic changes during past centuries.

The comparative trials of equipment developed at the U.S. Army Electronics Research and Development Laboratories and at the Scott Polar Research Institute were of mutual benefit in many ways. Special acknowledgement is given here to the advantages of using a balanced-feed aerial supported at least 3 m above the snow surface (Electronics Research and Development Laboratories system) rather than the mechanically simpler system of dragging an unbalanced aerial wire over the snow behind the vehicle (Scott Polar Research Institute system). The efficiency of the Scott Polar Research Institute equipment was increased by adopting the Electronics Research and Development Laboratories aerial which was kindly loaned for this purpose.

The purpose of this article is to direct attention to the potentialities of the new technique. A detailed analysis of the glaciological results obtained in north-west Greenland and a description of the equipment will appear in later publications when comparative figures from seismic shooting and gravimetric measurements are also available. In particular, one method for the determination of the velocity of radio waves in snow of different densities rests on a knowledge of snow depths derived from seismic measurements in areas where the radar showed the bottom surface to be smooth and flat. An independent method, using transmitting and receiving aerials spaced some distance apart on the snow surface, was also used, and it is not yet certain which method will yield the more accurate result.

In addition to the acknowledgements in the second paragraph, we thank the Committee of the R. W. Paul Instrument Fund for the provision of grants to cover the entire cost of the development work at the Scott Polar Research Institute. Special thanks are due to Mr. A. H. Waite of the U.S. Army Electronics Research and Development Laboratories, and Dr. J. Rinker of the U.S. Army Cold Regions Research and Engineering Laboratories, for their help during field operations.

[1] Waite, A. H., and Schmidt, S. J., *Proc. Inst. Rad. Eng.*, **50**, 1516 (1962).
[2] Evans, S., *Polar Record*, **11**, No. 73, 406; and No. 74, 795 (1963).
[3] Evans, S., *Polar Record*, **11**, No. 75, 725 (1963).

WHY SHOULD WE STUDY HIGH-ENERGY NUCLEAR PHYSICS?*

By Prof. C. F. POWELL, F.R.S.
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HIGH-ENERGY nuclear physics raises wide issues of public policy; such questions, for example, as: "What proportion of an economically advanced nation's resources should be devoted to fundamental science?" and, "By what principles should the degree of support for any particular science, in comparison with others, be determined?"

History of the Subject

Throughout human civilization, since the time of classical antiquity, men have speculated about the constitution of all the manifold variety of things which surround us. Until the beginning of the nineteenth century and the development of chemistry, knowledge was fragmentary and even the reality of the existence of the chemical atoms was uncertain. Only with the great advances at the end of the century, especially the discovery of radioactivity and the demonstration of effects due to individual atoms was the question put beyond reasonable doubt.

In the years which followed, the nuclear structure of the atoms was established through the work of Rutherford and Bohr, and the Pauli exclusion principle allowed an explanation of the regularities shown in the Mendeleev Periodic Table of the elements. With the discovery of the neutron it appeared that the model of the constitution of the atomic nuclei as assemblies of neutrons and protons had brought us near to the goal of a description of matter in terms of a very limited number of elementary constituents; nucleons, electrons and the quanta of radiation. Although such a description, guided by the principles of quantum mechanics, provided a sound basis for a detailed understanding of atomic physics, of the principal features of the solid state of matter, and of chemistry, the idea that we were approaching an end to the possibility of a deeper penetration into Nature proved to be illusory. The apparent simplicity was a consequence of our restricted resources for experiment; especially the limited energies of the bombarding α-particles, available for

* Substance of a contribution on "High Energy Particle Physics" delivered before the British Association for the Advancement of Science, Southampton, on September 1.

probing into matter, and the corresponding limits on the closest distance of approach between interacting particles which we could then achieve.

Later in the 1930's, with increasing sophistication in the methods of investigating cosmic radiation, a new sub-nuclear world began to be discovered, and the foundations were laid for the particle-physics of our time. Beginning with the discovery of the muon or heavy electron by Anderson in 1937, and especially after 1945, a large number of entities, the mesons and hyperons were found. They were created as a result of collisions with atomic nuclei of the very energetic protons which, at the time, could only be found in cosmic radiation. All the new particles were very ephemeral, mostly with average life-times between 10^{-6} and 10^{-10} sec, and the detailed examination of their properties could not be carried far in experiments with cosmic radiation because of the very weak flux of bombarding particles which it provides. It was therefore necessary to build large particle accelerators based on new principles so that intense pencil beams, homogeneous in energy, could be generated artificially. It was found essential to provide not only beams of protons and electrons which could be accelerated directly, but also secondary beams of mesons and other particles generated through the impact of the primary protons with suitable targets.

Promise of the Subject

During the past ten years the study of sub-nuclear particles has been rapidly advanced through the work with the great accelerators. The power of experiment has also been greatly increased through the development of powerful new methods of observation, such as the bubble-chambers and the spark-chambers, together with sophisticated computing methods for analysing the significant events which they record in large numbers. Both from the point of view of precision of measurement, and of the statistical weight of the experimental material on rare events which we can accumulate and analyse, our resources have been radically transformed.

As a result of these innovations, a new world of phenomena has again been revealed. They have allowed the existence of an entirely new class of particles or 'resonances' to be established with mean life-times which may be as small as 10^{-22} sec. The immense richness of the recent discoveries, the accelerating *tempo* of discovery, may be seen from the fact that the number of known particles now exceeds one hundred. They have consequences of fundamental importance.

In the first place we begin to believe that the very notion of the world as built up of elementary particles in the Greek sense of 'atom', 'that which cannot be cut', is illusory. All particles including the proton have a structure which we are elucidating. Particles of all types can be created in sufficiently energetic collisions between other particles, and they can be annihilated into other forms of matter and energy when they encounter their own anti-particles. Any particle is not to be conceived as a permanent structure like the Greek atom, but as continually transforming, for brief instants, into other forms, and then rapidly resuming its identity. Most of our familiar world is made up of nucleons, electrons and the quanta of radiation only because they are the most stable of the great family of particles, not because they are the more fundamental, so that at our familiar temperatures it is exceedingly improbable that others will secure even the temporary independent existence which their very short life-times impose. The very notion of 'elementary particle' in the sense in which it has been used hitherto has been undermined.

Secondly, in the description of the behaviour of particles as shown by their interactions with one another, we have been forced to extend widely the conservation laws familiar to us in classical physics. In order to describe the complexities of the behaviour of the particles in their interactions with one another, we are forced to acknowledge that they have attributes for which there are no parallels in classical physics. We ascribe to them new quantum numbers such as those of isotopic spin and strangeness, which are conserved in the interactions between particles, like mass-energy or electric charge.

Elementary particle physics is thus pursuing its familiar role of penetrating ever more deeply into the material universe so that we are forced to introduce basically new concepts for the description of a radically new experience. "Experimental science", said Clerk-Maxwell a hundred years ago, "is continually revealing to us new features of natural processes, and we are thus compelled to search for new forms of thought appropriate for their description."

Thirdly, during the past year, a very important step has been taken, in the recognition of a remarkable degree of order among the particles. Until quite recently the precise masses, spins, life-times and other parameters of the particles appeared as a body of empirical fact and very few significant regularities had been distinguished. We believed that there must be an underlying order which remained to be discovered, but we could not be certain of it. Now, however, it has been shown that the particles can be arranged in well-defined groups, and the validity of the new groupings has been demonstrated by the fact that the existence of missing members and their properties have been predicted and afterwards confirmed. A celebrated example is the discovery of the Ω^- particle, the existence of which was required if the arrangements of some established particles in the Lie algebraic group SU_3 of ten was to be valid, and of which the mass was correctly predicted to 1 part in 1,000.

The analogy of these advances to the recognition of the Mendeleev Periodic Table of the chemical elements seems very close. It is a recognition of regularities which guarantee the existence of an underlying order the fundamental basis of which we may hope to discover. It is not yet the equivalent of the discovery of a new quantum mechanics or of a Pauli exclusion principle; but it gives the assurance that we may, perhaps quite soon, make fundamental discoveries of not less significance.

Parallel with the work on new particles or resonances, great advances have been made in our knowledge of the weak interactions between electrons, muons and neutrinos and other particles. We now believe that there are four fundamental interactions in Nature; the gravitational and electro-magnetic forces long known to us, the very strong forces which operate between nucleons, mesons and hyperons, and the weak forces which are responsible for such phenomena as the β-decay of the neutron and of the muon. It is a recent discovery that all the phenomena involving the weak interactions are governed by the same strength of the coupling constant between them. We can attribute to the particles a weak 'charge' analogous to electric charge which more than a hundred years ago was found to be common to all electrical phenomena; and, just as in the case of the electro-magnetic field we can describe the interaction between electrically charged particles in terms of their mutual emission and exchange of electro-magnetic quanta, and the strong forces between nucleons, mesons and hyperons as due to the exchange of 'heavy quanta', of mesons, so the weak forces may be mediated by other 'field quanta' of which the existence remains to be established.

Technical Needs for the Future

The problems already outlined here are of profound importance for our deeper understanding of the material universe. All our experience of the development of science suggests that there is indeed an order in Nature which we can discover, and that we may hope to resolve our present problems. Such an attitude is very deep in our traditions. A confidence of order in Nature, of the

existence of laws of Nature which men can aspire to elucidate, was an essential condition for the emergence of modern science in Western Europe, rather than in another civilization, such as the Chinese, of which the thought was in some ways more sophisticated, but in which Nature tended to be regarded as inscrutable.

Particle physics is then full of life and vitality and will continue to attract a large fraction of the most gifted of the youth of the world in the economically developed countries who devote themselves to science. It seems probable, however, that the solution of present problems will again require a considerable increase in our technical resources. Thus the 'field quanta' of the weak interactions, if indeed they exist, are of such a nature that experiments with beams of neutrinos much more energetic than those we can generate with our present machines will be necessary for their discovery.

It is for the attack on these problems that we need the great new machines, the storage rings, and the 300-GeV proton accelerator. They and their associated equipment of bubble-chambers, spark-chambers and computers will be very expensive as judged against the scale of expenditure on fundamental science to which we have been accustomed. Thus it is estimated that the 300-GeV machine will take about eight years to build and will cost about £120 million. If constructed in Europe it would clearly need to be supported by a consortium of European states similar to that established at CERN. By way of contrast I would like to direct attention to the *Cambridge Reporter* for November 18, 1929, which gives the expenditure of the Cavendish Laboratory for that year. It is unnecessary to emphasize the eminence of the names of those whose modest salaries are there given, or the fact that this was the period when the experimental and intellectual resources for the great discoveries of the early 1930's were being assembled: of the neutron, the disintegration of the light elements by artificially accelerated particles, and the production of electrons and their anti-particles, the positrons, by γ-rays.

The foregoing comparison of the scale of expenditure in a basic branch of physics for two periods separated in time by less than half a century emphasizes the headlong advance of science and the radically new problems which it brings in its train. The growth of science, the infusion of science into every aspect of our culture, corresponds roughly to a doubling of the content of scientific knowledge every twelve years; in a wide range of disciplines the cost has quadrupled, sometimes more, in the same period. Should we continue to support particle physics in the U.K. and in Europe at the level of expenditure with which we are now faced? What can we expect to gain from it? What shall we lose if we withdraw? By what principles should we be guided in deciding on the appropriate level of expenditure on fundamental science?

Significance of Particle Physics

In approaching these problems we must acknowledge that the share of our resources asked for by science, including fundamental science, is increasing and that it is a process which cannot continue indefinitely. But it could go on for some time at its present rate without making any very great inroads into the economy. Thus, in economically advanced countries, 2–3 per cent of the gross national product now goes into all forms of research and development, and about 2 parts in 1,000 on fundamental science, as compared with 7 per cent on armaments. Such a proportion should not, I suppose, be regarded as unduly favourable to fundamental research in a period of human history in which the development of science is the dominant feature of the times. But why 2 parts in 1,000 towards all fundamental science? Why not ten times more, or ten times less, as the appropriate proportion?

But even 2 parts in 1,000 of our resources is a substantial sum. Pure nuclear and particle physics asks for about a sixth part of it, and other demands on the public purse are pressing. Why should we allocate a considerable proportion of our scientific resources and man-power to particle physics? Although the subject is of immense promise and vitality, we cannot assert precisely how useful it will be in its influence on other sciences and on practice. In the same speech from that I have already quoted Clerk-Maxwell remarked: "For us who breathe only the spirit of our own age, and who know only the characteristics of contemporary thought, it is as impossible to anticipate the general tone of the science of the future as to predict the particular discoveries it will make". All that we can say is that always in the past great advances in natural philosophy have in the long term led to radical changes in all our thinking and eventually, through their influence on the resources and morale of science, on all our practice.

The overall return from investments in fundamental science is difficult to estimate precisely, but it is certainly very high. Thus it has recently been remarked that the total resources spent on fundamental research throughout the whole of history is approximately equivalent to the amount by which this year's total production is expected to exceed that of last. But in different sectors the period from discovery to fruition differs widely. The eventual profitability of particularly penetrating and significant branches of science, such as particle physics or the elucidation of the genetic code in biophysics, is bound to be of a different order of magnitude from that given by less widely ranging investigations, and to take longer to mature.

It is very important not to see the problem too narrowly or to make a judgement on the value of a branch of science solely on the basis of immediate material benefits. The lady in the old story of Benjamin Franklin was right to ask about the use of his new discovery and he was right to emphasize the time-scale to maturity. The discovery of the universal law of gravitation, or of the origin of species, had little direct influence on economic affairs, but their impact on the standing of science and its development was immense. Or again, the development of quantum mechanics appeared as a remote and abstract advance when it first appeared, but in addition to its great importance for physics, it laid the foundations for theoretical chemistry. It was, therefore, an indispensable element in the chain of developments which contributed to the advances in molecular biology. Its ramifications are manifold and priceless.

It is a reasonable speculation that the new advances which now seem to be coming within our grasp will not be less significant and productive in the long term. They may not contribute to the advancement of chemistry, because the methods and theory created by past physics may provide all that is there needed. But they may instead be an essential element in bringing into being whole new fields of human practice, of which at the moment we have no inkling. Nobody in the eighteenth century had any intimation of an electrical industry; or in the nineteenth, of the atomic energy industry; and certainly there will be fields of practice in the twenty-first century of which we in the twentieth will know and anticipate nothing, but the foundations for which will be laid by the pure science of our own times.

We have, indeed, in the past year, had a clear indication that particle physics, in addition to its intrinsic interest, will also be of great importance for another vital branch of science, cosmology. Among some of the radio sources, there are recently discovered visible objects, called quasi-stellars, with a brightness about 10^{12} times that of the Sun, and in which the energy-source is approximately equivalent to the rest-mass of a million suns. Among them are the most remote objects in the universe hitherto identified, and their velocity of recession is sometimes more than half the velocity of light. Their rate of energy generation is so prodigious that it cannot be due to the kind of nuclear processes which provide the energy liberation in ordinary stellar evolution. It appears that for an

understanding of these objects we shall need all the resources of general relativity and of the processes we encounter in particle physics at the highest energy. The complex interaction of different sciences, their mutual influence and support, is well illustrated by this example. Modern particle physics arose out of the study of cosmic radiation, and now the application of our advancing knowledge of particle physics to these new quasi-stellar objects promises to provide a solution for the old problem of the origin of cosmic protons of the highest energy.

It therefore appears that for an understanding of the greatest energy-sources known to us in the universe, two branches of natural philosophy which are commonly regarded as among those most remote from practical affairs are indispensable. In the development of science similar situations have occurred again and again; yet there is an innate intellectual conservatism in us which makes us slow to see that the explosive developments in science through which we have lived are likely to continue, so that, in the absence of a great human catastrophe, the advance of science, the progress of discovery and of technical innovation, knows no limits, and we fail to draw the appropriate conclusions on major problems of policy.

Consequences of Withdrawing Support from the Subject

But why should we continue to support elementary particle physics here and now when the expenses are becoming so heavy and other demands on the public purse are so pressing ? Why should we not leave this field to other countries and hope to share the fruits of their researches when they mature ? It is a question which must be faced; and it should be convincingly answered if we are to proceed.

The tone, the morale, of a laboratory, of a nation, even of a civilization, is a very delicate and impalpable quality, yet of immense importance for the whole organism. The transition from high achievement to decadence has often in the past seemed to rest on a razor's edge. There is a sudden loss of confidence; it is as if the well-springs of human creative power have suddenly been dammed-up, frustrated; and this is followed by a rapid decline.

Ours is an advancing scientific culture, and if in Great Britain and in Europe we are to make an important contribution to it, we must continue to engage in the most advanced science and technique. We must encourage the most gifted of our youth to apply themselves to the most difficult, significant and demanding of the sciences, and at the present time that must include the physics of 'elementary' particles. In the long run it is most painful, and very expensive, to have only a derivative culture and not one's own, with all that that implies in independence in thought, self-confidence and technical mastery.

It is not easy to distinguish all the factors which contribute to morale, but I suppose that it is important for the whole of our educational system that the tradition of penetrating independent enquiry should be kept vigorously alive among us. This is surely especially so in our times when science is beginning to infuse every aspect of education, and the characteristic feature of science is change and development. The continuing theme in human history is the great battle to comprehend better the world around us, and to improve practice in the interests of every aspect of human advancement. People, including children, respect those who are successfully exerting themselves, at all kinds of levels, in this struggle. Immediately we cease to participate, our teaching tends to become stereotyped and pedantic; and in our universities that means that we must continually be involved in significant and essential researches.

So it is most important that we should continue to promote elementary particle physics, in Great Britain and in Europe, effectively. The programme you are to hear about is an important element in providing the necessary support. We all understand that it must be considered taking into account the legitimate claims of other sciences and other public demands on man-power and treasure. We should all be deeply concerned to see a balanced development. Gifted people are always in demand, and they are needed in many fields of technology as well as in fundamental science. But it may be remarked that the great attraction which elementary particle physics has for many of the most able young minds is not due to an arbitrary fashion. They are drawn to the frontiers of knowledge where the intellectual challenge is greatest. On such minds the subject exerts such a fascination that they will not easily be deflected but will go where it is actively pursued, wherever that may be. But if the subject loses its significance they will quickly drift away to other fields.

Particle physics at the present time is a large-scale operation involving thousands of people in the great national and international laboratories, but the number of key people is rather few and they do not seem to emerge in direct proportion to the increasing number of students we educate. It has been remarked that if, in particle physics, we lost a dozen of our best young physicists from Europe, our future contribution to the subject would be gravely prejudiced. A large number of competent scientists would be left with an indifferent leadership, and good generals make a great difference to the fate of armies. We are producing an encouraging number of gifted young physicists in Europe because we have a great tradition in the subject and have given it adequate support since the War, and we must not lose the position we have built up.

What should we do ?

In the future, science may perhaps be organized, not on a regional or continental, but on a world scale. It may become the principal creative activity of mankind utilizing a major part of our growing total resources. We may then be able to plan the general advance of the whole world scientific and technical front in a balanced way on the basis of the internal scientific needs of the different disciplines, with a rational distribution of the total effort between countries or continents, and without first considering their economic costs and returns. But we are clearly far from that situation to-day, and we in the United Kingdom and in Europe must look to our own culture and our own future.

I began by mentioning the present levels of expenditure on all fundamental research, and emphasized that this figure does not result from any clearly formulated policy as to what is the proper proportion to allocate for science in a modern state. So I believe it would be timely for governments and scientists to begin a well-informed enquiry into such problems as those which I posed at the beginning of my remarks. There would be some advantages if the enquiry could, in some measure, be international, for while the situation of each country has unique features and its own special problems, there is much experience which we share and we are in need, in the first place, of principles in the light of which to formulate policies.

In view of the foregoing considerations, I believe that the following propositions which bear on elementary particle physics should receive consideration as part of a wider enquiry:

(1) The present expenditure on fundamental science, about 3 parts in 1,000 of the gross national product, should not be regarded as an upper limit to what is desirable. It would still be reasonable to increase expenditure if the returns were reduced to less than half their estimated present value.

(2) The expenditure on fundamental research should be limited only by the availability of people enthusiastic and competent to do the work and able to justify to a scientifically informed council the promise, feasibility

and scientific significance of their programme. I believe that in the United Kingdom such a principle would not immediately involve us in a drastic increase in research expenditure. There are indications in the United States, whose enterprise and flexibility in the sciences we so much admire, that that limitation may perhaps be approaching. But in the United Kingdom, as in the other States of Europe represented at CERN, it is some way off; we have hidden reserves, a wealth of talent which still waits to be developed. The adoption of such a principle would eliminate any tendency towards a situation in which scientists in different disciplines are forced into a kind of fratricidal competition for limited resources, greatly to the detriment of the body of science.

When a subject or a research programme has become unfruitful, support should be withdrawn in good time, for there is nothing so demoralizing as incompetent research.

(3) While wider forms of international collaboration and exchange should be encouraged, there are good reasons for maintaining, on a world scale, a few main centres of investigation in particle physics at the present time so that there may be variety in the approach and in the ancillary apparatus, and the dangers of a mono-culture may be avoided.

(4) In view of the importance of the subject, we in the United Kingdom and in Europe should continue to give adequate support for particle physics in the high-energy region. It is an essential sector in the front line of advancing science, and is needed not only for its own contribution but also for its influence on neighbouring sciences including cosmology, meteorology, magneto meteorology and space. This will involve us both in improving the technical equipment in the United Kingdom and at CERN, and also in building a laboratory and its accelerator in the 300-GeV region. For such a programme, the time-scale is of great importance. It should clearly not be so fast that the technical and scientific problems involved are insufficiently considered so that we become involved in wasteful expenditure. But there are substantial financial reliefs to be gained from a slower *tempo*, and we shall almost inevitably proceed at a rate less than that which could be technically and scientifically justified. It is therefore appropriate to emphasize what is likely to be a greater danger. At the present time, we have in the United Kingdom, and in Europe, established schools of particle physicists in excellent standing and perhaps the most talented group of accelerator designers and constructors in the world. If decisions are too long delayed, they may lose heart and be dispersed and our effort will be too little and too late. So the correct *tempo* of development should be given careful and early consideration.

MICROBIAL CLASSIFICATION

A CONFERENCE on the classification of micro-organisms was held during August 30–September 5 at Laval University, Quebec City, Canada. It was organized by the Canadian Committee on Culture Collections and the Numerical Taxonomy Subcommittee of the International Association of Microbiological Societies.

The classification of micro-organisms is of practical importance in many fields, such as medicine, agriculture and food science. Good classifications are here required, but microbial taxonomy is notably difficult, because of the simple morphology of micro-organisms and because our knowledge of their genetics is still scanty. Microbiologists have therefore increasingly turned toward biochemistry and molecular biology for the data they require for classification, and toward computers and other data-processing machines for converting the information into workable taxonomies.

Microbial classification can, however, assist other disciplines in another way; those already mentioned—and many others—all have their own problems of classification. The classifications of disease syndromes and of soils are examples. With the advent of computers it is possible to construct taxonomies by automatic procedures, and workers in other fields are keen to learn from the growing experience of microbial taxonomists, who have been among the pioneers in the new subject of numerical taxonomy. In their turn, microbiologists have much to learn from parallel developments in other sciences.

For these reasons this small informal conference was held, which has made a substantial contribution toward the aims outlined here. The inter-disciplinary approach was fostered by including contributors from biometrics, psychology, botany, zoology, genetics and biochemistry. The programme was a wide one, based on the following outline: classification theory, numerical taxonomy and statistics; techniques and cultures for obtaining the primary data; data-processing methods, together with sessions on nomenclature, identification and genetics. Most of the topics related in the main to bacteria. The discussions were notably lively and profitable.

Dr. P. H. A. Sneath (Medical Research Council, University of Leicester) gave an introduction on the general theory of classification in various fields. He explained the use of the technical terms in numerical taxonomy compared the usage in some other fields, and emphasized the logical sequence of steps in the process of classification. He was followed by Prof. L. L. McQuitty (Michigan State University, East Lansing), who contrasted recent work in psychology, and illustrated many fascinating parallels. He showed how different material required the use of different methods of cluster analysis, and described several novel ones. They were followed by Prof. L. G. Silvestri (University of Milan), who discussed the special problems of the numerical taxonomy of micro-organisms. He discussed the way in which phenetic clusters could be tested for homogeneity and some of the automatic methods for producing diagnostic keys. Dr. F. Möller (Bocconi University, Milan) reviewed the statistical bases for numerical taxonomy, with special reference to information theory, and Dr. F. J. Rohlf (University of California, Santa Barbara) described the different methods of cluster analysis and gave illustrations of their relative advantages. Prof. W. R. Lockhart (Iowa State University, Ames) discussed in some detail the Adansonian practice of giving equal weight to all taxonomic characters, and its influence on coding them for computer analysis, which was also briefly commented on by Dr. Sneath. This problem still raises difficulties in certain groups of organisms, and it is clear that more work is needed in this field.

Another session was devoted to the sources of the primary data needed for classification. Dr. J. M. Shewan (Torry Research Station, Aberdeen) described the problems facing curators of culture collections and emphasized the need for workers to deposit important strains in these collections. Dr. E. F. Lessel (American Type Culture Collection, Rockville, Maryland) and Dr. I. Marcovici (International Centre of Type Cultures, Lausanne) then outlined the present position in bacteriological nomenclature. Many bacteria have no type strains, and the whereabouts of existing types is often unknown, so that lists of types and neotypes (which are now being prepared) will be valuable for future work. From this an interesting discussion, led by Prof. J. Liston (University of Washington, Seattle), developed on the way in which numerical taxonomy could provide criteria for choosing typical strains to serve as neotype cultures where the

original type cultures were unavailable. The procedure for proposing and validating neotypes was also considered. The median organism and the centrotype concepts are here suitable, but although they serve to describe the centre of phenetic clusters they have less power to define their borders, and various suggestions were made on how this might be done.

The conference then went on to discuss the kinds of information which can be obtained from biochemical tests and to the problem of standardizing them. This latter problem is a difficult one, and Prof. V. B. D. Skerman (University of Queensland, Brisbane) introduced this. Discussion on this was rather inconclusive, and it is evident that a huge task faces microbiologists if they are to bring order into the field. There seemed to be a general feeling that test methods for each major group of bacteria would need standardizing for that group, but the problem of reliable comparisons between major groups was more difficult. Several speakers emphasized that descriptive taxonomic tests required different treatment from rapid diagnostic tests for identification; the latter were best devised after sound taxa had been established on the basis of the former, and diagnostic tests could and should be devised for their own special purposes. Dr. O. Lysenko (Institute of Biology, Prague) also made a plea for some standardization in the descriptions of bacteria (as is now being considered in the Actinomycetes), which would avoid many of the present difficulties due to inadequate descriptions in taxonomic studies.

Prof. J. De Ley (State University, Ghent) went on to describe the comparative biochemistry of micro-organisms, and pointed out that there are large areas that were untapped for taxonomic work, either because the comparative biochemistry was not yet known, or because the techniques were not familiar to most microbiologists.

Dr. Rita R. Colwell (Georgetown University, Washington, D.C.) and Dr. R. Hugh (George Washington University, Washington, D.C.) described the practical organization of a laboratory for taxonomic studies. They were followed by Prof. O. B. Weeks (University of Idaho, Moscow, Idaho) and Dr. Muriel E. Rhodes (University College of Wales, Aberystwyth), who illustrated the theme with two particular examples—pigment and flagella respectively. Dr. L. Colobert (Centre de Recherches du CRSSA, Lyon) demonstrated a handy device for rapid testing.

The next session dealt with modern data-processing machines, lucidly described by Dr. F. J. Rohlf. Prof. Skerman discussed the setting-up of a world data centre for bacteriology, and Dr. C. Quadling (Biosciences Division, National Research Council, Ottawa) gave details of the punched-card system being introduced by the Canadian Committee on Culture Collections. Merging with this was a session on identification and how data-processing equipment can assist. Dr. K. J. Steel (National Collection of Type Cultures, Colindale, London, N.W.9) reviewed the prospects here, and described the wide range of identification techniques that are now available. Papers were read on behalf of Dr. L. C. Payne and Mr. D. A. Franklin (Elliott Medical Automation, Boreham Wood, Herts.) and Dr. W. Dybowski (Medical Laboratory Centre, Eastcote, Middlesex) describing a computer system that—from the test results on an unknown organism and the accumulated knowledge of bacteria stored in the computer memory—will print out the most likely alternatives together with any new tests that may be needed for confirmation of the identification. Several speakers touched on the central role of computers in the future, whether on-line in hospitals and laboratories for swift diagnosis, or handling the output of the automated testing machines that are being planned, or in the numerical taxonomy of micro-organisms and the production of diagnostic keys and tables for those without ready access to computers. The logical necessity for constructing classification before identification schemes was also emphasized.

The contribution of genetics and molecular biology to taxonomy was a particularly interesting topic, provoking much lively discussion. It was introduced by Dr. M. Mandel (Anderson Hospital, Houston, Texas). He showed how *GC* base ratios of DNA could contribute toward deciding if major groups were homogeneous and could also indicate possible taxonomic affinities; he went on to discuss the practical difficulties of work on nucleic acid homology by base pairing—a field which potentially has great possibilities in taxonomy by revealing fine genetic homology. Dr. Colwell, Prof. De Ley and Dr. Lily Jones (University of Minnesota, Minneapolis) described their recent findings in the field, and an interesting point that emerged was that strains that are phenetically typical of a cluster were also, as far as present experience goes, typical in *GC* content.

The conference ended with a session led by Prof. M. P. Starr (University of California, Davis) and Prof. Liston, in which participants from different disciplines summed up their reactions, and consideration was given to the future support of work in microbial classification. There was enthusiasm for arranging a second such conference in a few years' time. The proceedings of the present meeting will not be published in full; but it is hoped that the original work presented will be published in the appropriate journals. The conference was supported by the National Research Council of Canada, the U.S. National Science Foundation and the American Society for Microbiology.

OBITUARY

Dr. Eric W. Buxton

Eric Buxton's friends the world over will be grieved to learn of his death at Harpenden on August 4, 1964, at the age of thirty-eight years. His illness, which lasted for over a year, cut short a brilliant career in plant pathology.

Eric William Buxton was born at Ripley in Derbyshire on June 9, 1926. He joined the Royal Air Force in 1944. In 1948 he went up to Cambridge where he gained a double first in the Natural Science Tripos, specializing in botany. He was awarded the Aldis scholarship at Trinity Hall in 1950, and later held a College research scholarship and an Agricultural Research Council studentship, during the tenure of which he worked at the Botany School under the supervision of Dr. (now Professor) Noel F. Robertson, with a 6-month interlude while he studied in Glasgow under Prof. G. Pontecorvo. These experiences expanded his early work on diseases of *Gladiolus* into the wider problems of pathogenicity and genetics of the genus *Fusarium*. In 1954 he came to the Plant Pathology Department at Rothamsted Experimental Station, and rapidly gained international esteem for the new ideas he brought to the study of plant pathogenic fungi in the soil. By pairing avirulent mutants, produced by ultra-violet irradiation of virulent isolates of *Fusarium* from wilted peas, he obtained heterokaryons that were as virulent as the wild-type, and he suggested that such heterokaryons might arise naturally in the soil before invasion of a host plant, thus forming, from originally non-pathogenic clones, pathogenic heterokaryons that would break up again when the fungus sporulated on dead host tissue and so might escape detection. Further, he showed that

nutritionally deficient mutants can attack resistant varieties of host if the appropriate nutrient is supplied with the inoculum.

Parasexual recombinations within heterokaryons of pea-wilt and banana-wilt *Fusaria* were indicated as one source of variability. The terminal cell in *Fusarium* was shown to be multinucleate and therefore potentially heterokaryotic, and Buxton suggested that a few pathogenic nuclei might survive in heterokaryons containing predominantly nuclei adapted to saprophytic existence in mycelia growing saprophytically in soil. Avirulent mycelia could thus constitute a reservoir from which pathogenic forms, with mainly pathogenic nuclei, could arise when selectively favoured by the proximity of a host plant. Buxton's work on pea-wilt threw important light on the mechanisms of resistance and susceptibility of plant to fungus by showing that exudates from roots of one variety of pea can inhibit growth of a race of *Fusarium oxysporum* f. *pisi* to which that variety is resistant, but stimulate another race to which it is susceptible. Further, he showed that when conidia of race-1 were held for 14 days in concentrated root exudates from a host plant resistant to race-1, some were modified into race-2 pathogenicity. Genetic variability was Buxton's prime interest, but he was no narrow specialist; his 40 publications show his unusual versatility, as he was equally able and productive in practical work on disease control as in more academic studies. He also served as Secretary to the British Mycological Society's Plant Pathology Committee, and was on the Editorial Board of the *Journal of General Microbiology*.

With the award of a Kellogg Foundation fellowship, Buxton made his first visit to the United States in 1957. In 1958 he returned as the guest of the American Phytopathological Society to contribute to their golden jubilee meetings at Bloomington, Indiana, and in 1960 he went at the invitation of the United Fruit Co. to work for a year in their laboratories at Norwood, Mass., on *Fusarium* wilt of the banana.

His stimulating conversation, his ability to teach and his friendly personality explain why Buxton's laboratory was sought, not only by his colleagues, but also by visitors from other parts of the world. In 1950 he married Doreen Hampson of Ripley, Derbyshire, who survives him with their three sons. He gave to his family life the same zest as his research.

P. H. GREGORY

NEWS and VIEWS

Directorship of the Commonwealth Mycological Institute: Dr. J. C. F. Hopkins, C.M.G.

DR. J. C. F. HOPKINS, who retired on September 30 from the directorship of the Commonwealth Mycological Institute, Kew, a position he had held since 1956 (*Nature*, 178, 1150; 1956), is well known as a versatile tropical plant pathologist. After service in the Royal Air Force during the First World War, he graduated from the University of London and continued his training in Trinidad at the Imperial College of Tropical Agriculture. For the next two years he worked in Uganda, then moved to Southern Rhodesia, where in the course of twenty-eight years he was successively mycologist, senior plant pathologist, and finally chief botanist and plant pathologist. An authority on diseases of tobacco in Africa, and the author of the standard book on this subject, Dr. Hopkins's services have been in much demand, and during recent years he has travelled widely both as a consultant and on behalf of his Institute and the Commonwealth Agricultural Bureaux. Under his directorship, in addition to a general expansion of the work of the Institute, a unit for the identification of plant pathogenic bacteria was successfully initiated. Dr. Hopkins has taken an active interest in the affairs of the Institute of Biology and besides serving on the Council has acted as chairman of the London Branch. In 1962, in recognition of his public services, Dr. Hopkins was appointed a Companion of the Order of St. Michael and St. George.

Dr. G. C. Ainsworth

DR. G. C. AINSWORTH, who has been appointed to succeed Dr. J. C. F. Hopkins as director of the Commonwealth Mycological Institute, obtained a London first-class honours degree in botany in 1930 and was awarded a Ph.D. in 1934. He showed an early interest in medicine, obtaining a Ph.C. in 1929 and was awarded the Pharmaceutical Society's silver medal and the Harrison Memorial Prize. From 1930 until 1939 he studied plant diseases at Rothamsted and Cheshunt, where he specialized in crop diseases, particularly those due to viruses. He was then appointed to the Commonwealth Mycological Institute and helped in the production of the *Review of Applied Mycology*; he also prepared a monograph on the British Ustilaginales. After the Second World War, his earlier interests became evident again and he was appointed head of the Mycological Department at the Wellcome Research Laboratories working on antibiotics and developed research on the mycoses of man and animals. As a natural consequence, he was soon appointed a Wellcome Trustees Research Fellow in medical mycology at the London School of Hygiene and Tropical Medicine, and his insistence on accurate identification and correct nomenclature from that time onwards has had a marked effect on the development of this discipline, especially under the sponsorship of the Mycology Committee of the Medical Research Council, of which he was secretary for ten years. In 1948, he was appointed lecturer in botany at the University College of the South West of England (now University of Exeter), and was promoted reader in 1951. He continued his researches into the mycoses of animals, and the records of a two-year survey, supported by the Agricultural Research Council, of mycotic diseases of farm animals in Britain were transferred to the Central Research Laboratory, Weybridge, as the basis for a new veterinary mycology unit. But the call of the Commonwealth Mycological Institute proved too strong and he returned there to be made, in 1961, assistant director. He has been president of the British Mycological Society, but is probably best known throughout the world as the author of *The Dictionary of the Fungi*, the fifth edition of which has recently been published.

Registrar and Secretary to the Council for National Academic Awards: Mr. F. R. Hornby, M.B.E.

MR. F. R. HORNBY has been appointed registrar and secretary to the Council for National Academic Awards. Mr. Hornby was educated at Heversham Grammar School, Westmorland, and Magdalene College, Cambridge, where he obtained first-class honours in the Natural Science Tripos, Parts I and II. He has taught in Highbury County Secondary School, London, and at Taunton School, Somerset. During the Second World War he served with the Royal Army Ordnance Corps and the Royal Army Education Corps, and attained the rank of Lt.-Colonel and was awarded an M.B.E. for services in North Africa and Italy. Before he became secretary of the National Council for Technological Awards in 1956 he was assistant education officer in the City of Nottingham Local Education Authority.

The charter and statutes of the new Council for National Academic Awards were published on September 25, and

the first meeting was held on September 30. The appointment of Sir Harold Roxbee Cox, formerly chairman of the National Council for Technological Awards, was announced earlier this year (*Nature*, 201, 975; 1964) and the names of the other twenty-one Council members in July. The Robbins Committee in its report on higher education recommended that the National Council for Technological Awards should be replaced by the Council for National Academic Awards. The new Council has a wider role. It is able to award higher as well as first degrees, and in subjects other than technology; it is to provide for part-time, as well as full-time and sandwich course, students. Moreover, it is to be concerned with Scotland as well as England and Wales.

Scientific Research in Belgium

The fourth annual report of the National Council for Scientific Policy, Brussels, covers the year ended November 30, 1963 (Pp. 212. Bruxelles: Conseil National de la Politique Scientifique, 1964). It is in two parts: the first, in six chapters, deals with the activities of the Council during the period and in the perspective of future needs and policy; the second presents a chronological record of the activities of the Council and its constituent organs during the period, and appended are the constitution of the Council, and details of its membership and that of its committees and working groups and study groups. The six chapters of Part 1 deal successively with the development of scientific policy at the national and at the international level, the scientific budget, the organization of research, the expansion of higher education, the scientific establishments, and scientific careers. The scientific budget for 1963 provided credits amounting to 4,539 million francs, an increase of 459 million francs on 1962. This was distributed by ministries as follows (in millions of francs): education, 2,417; economic affairs, 1,432; agriculture, 189; defence, 151; Prime Minister's office, 141; public works, 92; public health, 91; foreign affairs, 12; other departments, 14. By institutions the distribution was: universities and institutions of higher education, 2,539 (of which 666 was for research); scientific establishments of the State, 850; establishments of public utility, subsidized by the State (Study Centre for Nuclear Energy), 792; industrial concerns and co-operative centres, 242; miscellaneous, 116. Expenditure on fundamental research was estimated at 1,594 million francs, of which 461 million francs was for the exact sciences, 292 million francs for the medical sciences, 204 million francs for nuclear science, and 138 million francs for biology and other natural sciences. Of 1,630 million francs for applied research, 359 million francs was for industrial research, 289 millions for agronomic research, 850 millions for nuclear research, and 56 million francs for space research. During the year, four working groups were established for the development of nuclear, medical, advanced technological and space research and of social science, respectively. Annexed to this part of the report is a survey of the national scientific potential in 1961, which estimates the effective scientific manpower in that year as 22,509 full-time workers in 1,644 scientific units. Of these, 6,703 held a university degree, 2,451 an advanced technical diploma, and 13,350 were technicians or craftsmen. Of the university graduates, 3,623 were employed in education, 1,100 in the public sector, 1,437 in private enterprise, 328 in research associations in industry and agriculture, and 143 in interuniversity centres; by sciences, 604 were in physics, 496 in chemistry, 764 in biology, 1,064 in medicine, 255 in geology and Earth sciences, 95 in mathematics and 2,136 in technology, with 821 in the social sciences.

The University Foundation, Brussels

The forty-third annual report of the University Foundation, Brussels, for the year ended September 30, 1963, records grants to new students totalling 94,000 francs, and a further 420,000 francs for study in scientific establishments overseas (Pp. 164. Bruxelles: Fondation Universitaire, 1964). Subsidies to scientific publications totalled 815,000 francs, with a further 2·28 million francs in support of learned periodicals in Belgium and 961,000 francs in subsidies to scientific associations and institutions. The Documentation Service arranged exchanges with 41 overseas institutions, and 255 foreign reviews were deposited in Belgian scientific libraries in exchange for a similar number of Belgian reviews. The *Index of Periodical Publications* now covers 42,000 periodicals, compared with 27,000 in 1935. Besides details of the grants and subsidies awarded, scientific prizes, and other activities of the Foundation, the report includes details of its relations with other bodies, membership of the Council, Secretariat and Commissions, and the text of the rules governing its activities and awards.

The Mauritius Sugar Industry Research Institute

The annual report of the Mauritius Sugar Industry Research Institute for 1963 is a very creditable publication both in contents and form (Pp. 153+xxxii. Reduit: Mauritius Sugar Industry Research Institute, 1964). It reflects the high standard of the Research Institute and, indirectly, also that of the industry as a whole, which has increased its sugar production from 491,000 tons in 1954 to 682,000 tons in 1963. Eighty-four pages are devoted to the cultivation of the cane, twenty-seven pages to the sugar manufacture, and six pages to by-products. Each section describes interesting work of value to the local industry, but little that might be applicable to other sugar cane growing areas.

Skill and Capital in Horticulture

The short article, "Investment in Skill", contributed by Dr. R. R. Folley to the *Westminster Bank Review*, August 1964, is concerned with the effect of the Agriculture and Horticulture Act, which makes grants available to assist horticulturists to improve their capital equipment. He stresses the need to match this increase in capital equipment with a higher degree of individual skill. Skill, he urges, is to be preferred to scale, particularly in the intensive branches of the industry, where the product, whether fruit, flowers or the less common vegetables, is improvable in quality, and where value and price are sensitive to even small improvements. Dr. Folley quotes, as an example, annual savings amounting to £1,558,000 to be achieved by raising the yield of apples from 250 to 350 bushels per acre, with reduction in acreage from 64,000 to 45,500, on an expenditure of £7,651,000 for the lower yield.

Growth of Scientific Literature

Papers presented at the thirty-eighth annual conference of the Association of Special Libraries and Information Bureaux (Aslib) at the University of Exeter, during September 28–October 1, were for the most part somewhat specialized, but Sir Thomas Scrivenor's discussion of the growth of scientific literature is of more general interest. He challenges the validity of some estimates of the rate of growth, pointing out that secondary publications, although included in the *World List of Scientific Periodicals*, should not, strictly speaking, be taken into account in measuring the volume. There is also room for more than one opinion on what should be regarded as a deceased publication. He recognizes that it may not be easy to achieve an agreed definition of what constitutes a scientific publication, but suggests that a modified version of the *World List* would meet the situation. This should be published regularly every five years, titles of all periodicals neither scientific nor technological being eliminated, and all entries numbered in series straight

through to permit accurate reckoning of totals without delay. Sir Thomas also suggests replacing the present arrangement of entries in alphabetical order by one in which they are grouped by subjects and possibly by languages within each subject: such an idea would probably be more acceptable if such a list supplemented the present alphabetical form.

The Indian Journal of Cancer

THE first issue of the attractively produced *Indian Journal of Cancer* appeared in October 1963. Edited by Dr. D. J. Jussawalla for the Indian Cancer Society, Hospital Avenue, Parel, Bombay, and printed in Bombay, the *Journal* is produced on glossy paper, with good illustrations. The editor is fortunate in having the assistance of 57 British, American, Japanese and Indian experts who constitute the international Editorial Advisory Board. The first issue contains ten articles on various aspects of the cancer problem (1, No. 1; October 1963. Issued twice a year. Pp. xxviii+1–90. Bombay: Indian Cancer Society, 1963. Annual subscription, Rs. 6, post free). The future of the *Journal* will be watched with interest by all who are concerned with the world-wide effort to understand the causes underlying the occurrence and development of malignant growths and to prevent their occurrence and mitigate their effects on man and other animals.

National Research Council of Canada

THE *Review of the National Research Council of Canada*, 1964, contains besides the usual reports on the work of the several Divisions, with lists of publications during 1963–64 and of the Medical Research Council, reports from the various Committees of the Council with details of the membership of the Council and its Committees. There is also a directory of staff and a list of staff serving on outside organizations.

Royal Scottish Museum, Edinburgh

THE annual report for 1963 of the Royal Scottish Museum records the urgent need for additional accommodation and the promised reconstruction of the north-east and north-west wings. It has been decided that the greater portion of the reconstructed north-east wing will be given to an introductory Hall of Biology which is being designed by the Departments of Geology and Natural History. Notable purchases included a silver cake basket by William Dempster, Edinburgh, 1747. In the Department of Natural History exhibition work has been concentrated on the reconstruction of the exhibits in the British Animal and Bird Halls. The major exhibition work in the Department of Geology has been the preparation of cases illustrating general mineralogy. Coastal and glacial scenery has also been dealt with in a series of photographs, and the regional geology of the Carboniferous of the Midland Valley of Scotland has been illustrated.

The National Health Service

FOUR surveys conducted by Research Services, Ltd., show that 88 per cent of the general public think that the National Health Service is, on the whole, working well. Seven out of ten believe that treatment in hospital and treatment by the patient's own doctor under the National Health Service compares well with that in other countries. Two per cent take the opposite view. These attitudes have not changed significantly over the past two years (*Attitudes Towards the National Health Service Amongst Family Doctors and the General Public*, 1962 and 1964). In contrast, the proportion of family doctors expressing general satisfaction with the Health Service fell from 79 per cent to 62 per cent between 1962 and 1964. The fall was greater among those under the age of forty-five, for whom the proportion expressing satisfaction dropped from 83 per cent to 59 per cent. There was a big increase between 1962 and 1964 in the number of ways in which family doctors wanted to see the National Health Service improved. In 1964, 21 per cent of doctors wanted improved terms of service, or arrangements for provisions of locums; 13 per cent wanted better hospital facilities; 10 per cent wanted ancillary help; and 5 per cent wanted the provision of health centres or group practice premises. Other improvements suggested in both years included a reduction in the number of patients per doctor, mentioned by 14 per cent, and the need to educate patients to avoid abuse of the service, mentioned by 12 per cent. Despite their present discontent, general practitioners in 1964 seemed to think there was rather less risk of their becoming subordinate medical auxiliaries than they had appeared two years earlier.

International Commission on Zoological Nomenclature

NOTICE is hereby given of the possible use by the International Commission on Zoological Nomenclature of its plenary powers in connexion with the following cases, full details of which will be found in the *Bulletin of Zoological Nomenclature* (21, Part 4; published on October 16, 1964): (1) Suppression of *Mus canguru* Muller, 1776 *et al.*, and *Jaculus giganteus* Erxleben, 1777; validation of *Macropus major* Shaw, 1800 (Mammalia). *Z.N.(S.)* 1584. (2) Designation of a type-species for *Curimata* Bosc, 1817 (Pisces). *Z.N.(S.)* 1590. (3) Designation of a neotype for *Belemnites mucronatus* Link, 1807 (Cephalopoda). *Z.N.(S.)* 1160. (4) Suppression of the specific name *Echinus rosaceus* Linnaeus, 1758, 1764, 1767, and Gmelin, 1788, and of the generic name *Echinanthus* Leske, 1778 (Echinoidea). *Z.N.(S.)* 1616. (5) Suppression of *Nana* Schumacher, 1817 (Gastropoda). *Z.N.(S.)* 1622. (6) Validation of *Sphalerosophis* Jan, 1865 (Reptilia). *Z.N.(S.)* 1627. (7) Emendation to *Astraeus* of the generic name *Asthraeus* Laporte and Gory, 1837 (Insecta, Coleoptera). *Z.N.(S.)* 1628. (8) Validation of *Rhyncogonus* Sharp, 1885 (Insecta, Coleoptera). *Z.N.(S.)* 1629. (9) Validation of *Ambalodus* Branson and Mehl, 1933 (Conodont). *Z.N.(S.)* 1633. (10) Validation of *Cnemidophorus septemvittatus* Cope, 1892 (Reptilia). *Z.N.(S.)* 1634. (11) Suppression of *Procyon brachyurus* Wiegmann, 1837, and *Procyon obscurus* Wiegmann, 1837 (Mammalia). *Z.N.(S.)* 1640. Any zoologist who wishes to comment on any of the foregoing cases should do so in writing to the Secretary, International Commission on Zoological Nomenclature, c/o British Museum (Natural History), Cromwell Road, London, S.W.7, before April 16, 1965.

Texas Instruments

THE annual meeting of Texas Instruments Inc. was held in Dallas on April 15, 1964, and some 800 shareholders were present. The president, Mr. P. E. Haggerty, reported on the company's activities (*Texas Instruments Incorporated, 1963 Annual Report*. Pp. 12. Dallas: Texas Instruments, Inc., 1964). Net sales amounted to 276·5 million dollars, 15 per cent higher than in 1962, and this increase has continued into 1964. Semiconductor components, including transistors and diodes, were again the largest contributor to the profits. One hundred and sixty new products were introduced—in particular, a series of ultra-high-frequency tuner transistors for television receivers, low-cost silicon diodes, and electronic devices for the *Minuteman* intercontinental ballistic missile and the *Polaris* missile. Semiconductor integrated circuits, which the Company was the first to introduce, consist of both amplifier and high-speed computer types, and may include as many as 40 different component equivalents.

The sale of materials and control systems increased. An electronic control system for home clothes-dryers was one of the new products introduced during the year, and

the manufacture of semiconductor silicon material continued on a profitable basis despite considerable market price pressure. The production of nuclear fuel elements and cores, mainly for the Navy's propulsion programme, also continued. Texas Instruments is the main contractor for the electronic equipment of the naval *Shrike* air-to-surface missile, and the programme has now advanced from the development to the manufacturing stage. Prototype advanced radar and infra-red systems for the Air Force's new reconnaissance fighter aircraft *RF4C* were delivered, with production scheduled for 1964. New sales records were established by the Company's petroleum exploration activities and in applications of Earth, ocean and space sciences. Contracts were obtained to develop a major multi-sensor shipboard survey system which will perform and record simultaneously many oceanographic tests such as those for temperature, gravity and magnetic measurements, and to find the best methods to make scientific measurements and geophysical investigations during the Apollo visit to the Moon. Expenditure on development or application of new or modified products, services, equipment or processes totalled about 45 million dollars, of which about 25 million dollars was from outside contracts, mainly with the Government of the United States. Business in Europe exceeded expectations, and was good both in Latin America and Australia. Texas Instruments France moved into a new 60,000 ft.^{2} semiconductor plant near Nice and an additional plant of 59,000 ft.^{2} for metal fabrication was leased in Australia. A new plant was opened at Toronto, Canada, for control device manufacture, and construction will shortly begin on a similar plant near Aversa, Italy. Approximately 18 per cent of the total net sales of the Company were from operations in countries outside the United States.

Preparation and Properties of *bis*-Hydrazides and Related Compounds

C. N. O'Callaghan and Dermot Twomey, of the Medical Research Council of Ireland, Trinity College, Dublin, have described the preparation and properties of *bis*-hydrazides and related compounds (*Proceedings of the Royal Irish Academy*, 63, Section *B*, No. 12: *Anti-tubercular Substances*; 19: *The Preparation and Properties of bis-Hydrazides and Related Compounds*. 1964. 1*s*. 6*d*.). Since the discovery of the anti-tuberculosis properties of isoniazid, considerable attention has been directed to the preparation of compounds containing hydrazide groupings. Apart from mono-hydrazides, anti-tuberculosis properties have also been ascribed to various *bis*-hydrazides and structurally related compounds. One of the latter, cyanoacetohydrazide, has been thoroughly examined; despite conflicting reports, it is now generally considered to be much less active *in vivo* than isoniazid. A feature of compounds such as ethyl malonate, ethyl cyanoacetate and ethyl acetoacetate, which contain a reactive methylene group, is the ease with which they undergo the well-known Knoevenegel reaction with aldehydes and ketones to yield a series of compounds of type I, where R is CO_2Et, CN and $COCH_3$, respectively. The reaction of these condensation products with hydrazine has been investigated.

British Geological Literature

Among the recommendations of the British Commonwealth Official Conference on Geology and Mineral Resources, which met in London in 1948, there is one that "The Geological Survey (or other appropriate authorities) throughout the British Commonwealth should each take steps to publish an annual volume of abstracts of all geological papers published relating to the territories for which they are respectively responsible". In most Commonwealth countries this proposal has long since been implemented; but in Britain not even an annual bibliography of geological works, far less a volume of abstracts, has been available in post-war years. A special welcome must therefore be extended to a new unofficial publication, *British Geological Literature*, compiled by E. L. Martin, of the Geological Survey, and A. P. Harvey, of the British Museum (Natural History), the first part of which lists nearly 300 references published early in 1964. *British Geological Literature* (Coridon Press, 35 Goddington Road, Bourne End, Bucks. £1 per annum) will be published quarterly, with annual author and subject indexes; and a loose-sheet edition for card-index catalogues is also being produced. The compilers are to be congratulated on their initiative, which deserves every support. Perhaps it is not too much to hope that their modest publication may expand into the much-needed journal of abstracts which the appropriate authorities have as yet been unable to produce.

Last Cretaceous Vertebrates

Two papers recently issued as *University of California Publications in Geological Sciences* add much to our knowledge of the vertebrate faunas near the critical Mesozoic–Cenozoic boundary. In a re-investigation of the Lance Formation in Wyoming, Dr. R. Estes reports on the palæoecology and zoogeography with details of the non-mammalian vertebrate fauna and a brief account of the flora and invertebrate fauna (49: *Fossil Vertebrates from the Late Cretaceous Lance Formation, Eastern Wyoming*. Pp. 180 + 5 plates. Berkeley and Los Angeles: University of California Press; London: Cambridge University Press, 1964. 4 dollars). Dr. W. A. Clemens deals with the Multituberculata in the first part of a publication and will deal with the metatherians and eutherians in later parts (48: *Fossil Mammals of the Type Lance Formation, Wyoming*. Part 1: Introduction and Multituberculata. Pp. 1–105. Berkeley and Los Angeles: University of Cailfornia Press; London: Cambridge University Press, 1963. 2.50 dollars). The authors have been fortunate in obtaining extensive new collections by underwater screening of the matrix, so concentrating a microfauna of more than 30,000 specimens. Five multituberculate genera, including a new species, are described and all referred to the Ptilodontidae. The reptile fauna comprises an eosuchian, six turtles, fourteen lizards, a snake, two crocodiles and eight dinosaurs, the latter with theropods, carnosaurs and ornithischians. A good amphibian fauna is represented by both salientes and urodeles. The fish include sharks, sturgeon, amiids and gar. Estes is able to confirm the fresh-water character of the formation investigated, with sub-tropical climate prevalent. The environment envisaged is a warm, humid, coastal plain with abundant riparian habitats and rich vertebrate fauna. The remaining parts of these investigations will be keenly awaited, the rare combination of Mesozoic and modern elements in the biota making it of especial interest.

Locomotion in Antarctic Seals

Fergus O'Gorman, of the British Antarctic Survey and the Anatomy Department, Charing Cross Hospital Medical School (London), has made observations on the modes of terrestrial locomotion among Antarctic members of the Phocidae (*Proc. Zool. Soc. London*, 141, Part 4; 1963). An account is given of the sinuous or 'swimming' type of progression which is particularly developed in the crab-eater seal, *Lobodon carcinophagus* (Hombron and Jacquinot), and is also seen in the leopard seal, *Hydrurga leptonyx* (Blainville). This type of movement has not been seen in the Weddell seal, *Leptonychotes weddelli* Lesson, where the undulatory (caterpillar-like) method is the normal locomotory pattern on land. The fore-flippers are not used by the adult Weddell seal in terrestrial locomotion, but the pup does make some use of the fore-flippers during its first 10–15 days.

A Dangerous New Stonefish from Western Australia

A NEW species of the genus *Inimicus*, belonging to the family Synanceidae from Western Australia, has been described by R. J. McKay, of the Fisheries Department, Perth, Western Australia, and compared with other members of the genus (*Journal of the Royal Society of Western Australia*, 47, Part 1; 1964). *Inimicus cirrhosus* differs from all others of the genus in that it possesses yellow spots on the inner surface of the pectoral fin, which is described as "dark brown, almost black with indistinct pale cross-bars and variable blotches on outer surface. Inner surface brown to blackish, with 20 to 35 white (in life bright yellow) spots of indefinite shape". In larger examples, the yellow spots are more diffuse and frequently in the form of ocelli. McKay adds some notes on the severity of the sting: the species is capable of inflicting an extremely agonizing wound. The following notes were made early in October 1960, after the author was stung on the thumb. "The pain was immediate and intense, the wound bled very freely for a few minutes, then ceased. Bleeding was difficult to restart. Pain became rapidly unbearable. 1145 h. While sorting a trayful of fish emptied from trawl left thumb deeply penetrated by dorsal fin of *Inimicus*. Bleeding freely. 11.50 h. Bleeding stopped. 1155 h. Thumb swollen, area around wound becoming grey-blue in colour, extremities of fingers becoming very painful, pain in thumb almost unbearable. Elbow and shoulder now aching. 12 midnight. Sweating profusely, thumb and hand swollen, wrist very stiff, area around wound bluish, throat and mouth dry. 0005 h. Became delirious, do not remember much, but can remember throwing myself about cabin in fearful pain. Crew members restrained my movements for the next 30 minutes, during which I was screaming and complaining of pains around the neck and chest. 0045 h. Regained consciousness, the pain in arm and shoulder very agonizing. 0115 h. Hand and thumb immersed in hot water and sedatives taken. 0200 h. Pain still present but greatly diminished. Thumb remained stiff and swollen for four days, but the pain had subsided 12 hours after the encounter. Bathing the wound in hot water greatly reduced the pain, but when the water became cool enough to be comfortably borne, the pain returned. A tourniquet was not applied."

Rhizobial Nitrogen Fixation in African Acacias

RHIZOBIAL nitrogen fixation, previously reported in *Acacia cyclops*, has also been shown to occur in *A. cyanophylla* by Prof. E. R. Roux and C. C. G. Marais (*South African Journal of Science*, 60; July 1964). These trees are invasive exotics of Australian origin, now widespread in the Western Cape. Root nodules have so far not been found on any of the indigenous South African acacias examined. However, four species (*A. karroo*, *A. robusta*, *A. sieberiana* and *A. giraffae*) were successfully inoculated with rhizobia derived from nodules on *A. cyclops*. Nodule formation and nitrogen fixation occurred in all cases.

University News :

Aberdeen

THE following appointments have been announced: *Senior Lectureships*, A. E. Bursill and J. J. Cowley (psychology). *Lectureships*, Dr. J. M. Hay, Dr. J. H. Holloway and Dr. G. P. McQuillan (chemistry); J. Henderson (forestry); C. K. Mackay and G. Rochford (psychology); G. A. Myers (statistics). *Research Fellowships*, I. Patterson (natural history); Dr. J. Paul (soil science).

Belfast

DR. I. CAMPBELL RODDIE has been appointed to the Dunville chair of physiology in the Queen's University of Belfast.

Sheffield

DR. J. M. GANI, visiting professor of statistics, Michigan State University, has been appointed to the chair of statistics. The following appointments have also been announced: *Lectureships*, Dr. D. J. Brown (fuel technology and chemical engineering); C. C. Heyde (statistics); Dr. N. Birks (metallurgy); F. Booth (applied mathematics); J. K. Clarkson (psychology); G. J. Roberts (glass technology); G. Sedman (psychiatry); T. Shelley (medical physics in the Department of Obstetrics and Gynæcology). *Research Fellowships*, A. T. Davenport (Ironmongers' Company research fellowship in metallurgy); F. Wallstrom (sociological studies).

Announcements

MR. R. F. LETHBRIDGE, head of the Mining and Exploration Department of the Rio Tinto-Zinc Corporation, Ltd., has been elected president of the Institution of Mining and Metallurgy for 1965–66. Mr. Lethbridge has served on the Council of the Institution since 1955, holding the office of vice-president from 1963.

ENTRIES for the 1965 Maccabaean Prize and Medal of the Worshipful Society of Apothecaries are invited for an essay of 5,000–7,000 words on some aspect of the history of medicine or pharmacy. Intending candidates, who must be under thirty years of age on March 15, 1965, may apply for further particulars to Dr. F. N. L. Poynter, Wellcome Historical Medical Library, Wellcome Building, Euston Road, London, N.W.1, who is honorary secretary of the Faculty of the History of Medicine and Pharmacy of the Society.

THE second Texas symposium on "Relativistic Astrophysics" will be held in Austin, Texas, during December 15–18. Further information can be obtained from Mrs. J. Wardlaw, Department of Physics, University of Texas, Austin, Texas 78712.

THE twenty-second session of the International Geological Congress will be held in New Delhi during December 14–22. Further information can be obtained from the Secretary General, XXII International Geological Congress, Geological Survey of India, 27 Chowringhee, Calcutta 13.

THE third conference on "Performance of High Temperature Systems" will be held in Pasadena, California, during December 7–9. The conference will be devoted primarily to the application of fundamentals to the analysis and computer evaluation of the performance of high-temperature systems. Further information can be obtained from Mr. G. S. Bahn, 16902 Bollinger Drive, Pacific Palisades, California 90272.

THE 1964 Reith Lectures will be delivered by Sir Leon Bagrit in the B.B.C. Home Service on "The Age of Automation". The series will include lectures on: automation, an extension of man (November 8); the range of applications (November 15); education for an age of automation (November 22); some political considerations (November 29); some industrial and economic consequences (December 6); new opportunities for social enrichment (December 13). The Lectures will be repeated in the Third Programme and will also be heard in the Overseas Service. Further information can be obtained from the British Broadcasting Corporation, Broadcasting House, Portland Place, London, W.1.

ADDENDUM. Mr. J. Liebster has supplied the following references which were omitted from his joint communication with V. Kočandrle entitled "Exchange Labelling of 'Lipiodol' Ultra Fluids with Iodine-131 and its Intralymphatic Administration with a Fistula on the Thoracic Duct" (*Nature*, 203, 777; 1964):

[11] Liebster, J., Kácl, J., and Babický, A., *Nature*, 183, 1474 (1959).
[12] Liebster, J., and Andrýsek, O., *Nature*, 184, 913 (1959).
[13] Product of Laboratories André Guerbet et Cie, Saint-Ouen-Paris (Seine).

REGIONAL ORGANIZATION OF RESEARCH IN AUSTRALIA AND SOUTH-EAST ASIA

THE concept of the development of scientific research on a regional basis is one which has long been advocated by Unesco, and followed in the operations of its field science co-operation offices. The South-east Asia Science Co-operation Office has been active in stimulating collaboration and co-operation in a number of scientific fields in its region, and has been responsible for two important meetings—in Bandung in 1959 and in Hong Kong in 1961. Representatives of national research councils in the area met to discuss general problems of organizing scientific research (see *Nature*, 186, 859, 1960; 194, 626, 1962). More recently, in February 1964, a third meeting was held in Canberra, Australia, for a similar purpose, the local arrangements being made by the Australian Commonwealth Scientific and Industrial Research Organization.

The Canberra meeting enlarged the scope of the earlier meetings, as it included representatives of national research organizations from both South and South-east Asia. Delegates came from Ceylon, China (Taiwan), Hong Kong, Indonesia, India, Israel, Japan, Korea, Malaysia, Nepal, New Zealand, Pakistan, Philippines, Thailand, Viet Nam and Australia. There were also present consultants from India, Indonesia, Japan, the United States, the U.S.S.R. and Australia, and observers from the World Health Organization, the Food and Agriculture Organization, the Pacific Science Association, the Pan-Indian Ocean Science Association and the South Pacific Commission.

Following the United Nations conference on the "Application of Science and Technology for the Benefit of the Less Developed Areas" (*Nature*, 197, 1135; 1963) in Geneva in 1963, Unesco made plans to extend considerably its activities in the field of natural science, and the Canberra meeting was looked on as a regional follow-up to the Geneva conference. It was considerably strengthened by the presence of M. Rene Maheu, the director-general of Unesco, for part of the proceedings and a delegation from Unesco headquarters including Prof. V. Kovda, the director of the Natural Sciences Department. Sir Frederick White, chairman of C.S.I.R.O., was elected chairman of the meeting. Prof. Sarwono Prawirohardjo, president of the Indonesian Council of Sciences, was elected rapporteur.

The meeting, which was held in the Australian Academy of Science building, was opened with an address from Senator the Honourable J. G. Gorton, the Australian minister assisting the Prime Minister in education and research. Mr. Gorton referred to the fact that many developing areas could benefit significantly by the application of scientific facts which were already known, so that it was essential for Governments to provide adequate extension services and ensure the supply of trained technicians.

M. Rene Maheu also addressed the meeting and emphasized what he described as the salient features of the Geneva conference: that countries can scarcely hope to obtain economic independence—and political independence without economic independence is little more than a façade—if their own scientific and technical potential remains below a minimum threshold. A far-sighted governmental policy for science will seek two main objectives: to develop scientific and technical potential on one hand, and, on the other, to apply the creative and assimilative capacities of that potential to the cultural, economic and social progress of the country. The purpose of the Canberra meeting was to define fields of application, and methods of formulating and executing such national scientific policies.

The principal sessions of the meeting covered national science policies, relations between science and Government, research and economic development, investment in research, research organization and administration, and regional collaboration in research and training.

Underlying all the discussions was the question raised by M. Maheu: how best to ensure that science plays its most effective part in the processes of economic development. This involves the setting-up of suitable advisory machinery for government. It will vary according to the stage of the political, economic and scientific development of the country concerned, but must bring the possibilities of science to the notice of the economic planners, and also ensure feed-back of economic problems to research groups. Proper organization and management are essential if science is to flourish and to make an effective contribution to development. At the same time it is necessary to plan educational and manpower development and to ensure the availability of trained men, not only to engage in investigation and research, but also to ensure that research results are put to agricultural and industrial use.

As was to be expected, discussion was largely directed towards the role and activities of Unesco in the regions represented at the meeting. It was clear that there was general appreciation of the work which Unesco has been doing in the various countries concerned, though there was by no means an uncritical acceptance of all its policies. The principal help needed from Unesco was towards building up within each country an organization which would enable it to develop internal solutions to its own problems.

G. B. GRESFORD

ASPECTS OF SOIL SCIENCE

THE eighth International Congress of Soil Science was held in Bucharest during August 31–September 9. The official opening of the Congress was held in the Great Hall of the Palace of the Romanian Peoples Republic by President Gheorghe Gheorghiu-Dej. In his speech welcoming the 1,200 delegates, the Romanian President stressed the need for raising the standards of food production in the world. He complimented the International Soil Science Society for its contribution to this vital work, and for the part it played in the development of understanding and peace.

After the necessary business of the Society had been concluded, the programme of scientific papers began with a general session in which the history and progress of the Food and Agriculture Organization/Unesco project for a World Soil Map were described. Good progress had been made, and hence it was possible for several maps to be exhibited at the Congress. Throughout the nine remaining days of the meeting, more than 700 papers were read to the seven Commissions of the Congress, dealing with soil physics, soil chemistry, soil biology, soil fertility, soil genesis, classification and cartography, soil technology, and soil mineralogy. Abstracts of the papers were made available in English, French and German, and the proceedings of the Congress will be published in the near future.

The papers read in Commission 1 (Soil Physics) dealt with such diverse topics as the physical relationships between the solid and liquid phases of the soil and the relationship of tillage problems to physical properties. Several papers were devoted to investigations of the

movement of water in saturated and unsaturated conditions through the soil. Soil chemists in Commission 2 concerned themselves with fundamental problems of research, and in particular research pertaining to the properties and formation of humus complexes. The importance of chemical change, especially exchangeable cation relationships, associated with soil genesis was also discussed. The investigation of the soil solution assumes even greater importance in the semi-arid areas where salt concentrations are sufficient to affect crop production. The influence of irrigation on saline and alkaline soils was reviewed by delegates from Egypt, Israel, the United States, Spain, Tunis, Hungary, Turkey, the U.S.S.R., and Romania. At the other extreme of the *p*H scale, the moderation of acid soils and their improvement for agriculture were the subjects of several papers. In a joint session with Commission 4 (Soil Fertility) the problems of the chemistry and biochemistry of phosphorus in the soil and in plant nutrition aroused considerable interest.

Problems involved in the examination of soil micro- and macro-fauna were discussed, and of special interest to the non-specialist in this field was the section dealing with the possibility of control of the activity of soil organisms. The variation of the natural environment within the soil and its repercussions on populations of organisms enables the soil biologists to gauge the effects of modern pesticides on the beneficial inhabitants of the soil. Interactions of soil micro-organisms and higher plants were discussed as well as the nature and effect of bacterial fertilizers.

The role and efficiency of fertilizers were the province of Commission 4, and the influence of soil conditions on nutrient availability to plants was discussed, as was the part played by organic matter in soil fertility. The section of the programme dealing with the efficacy of fertilizers on genetic soil types with different crops under irrigated or non-irrigated conditions provided an interesting link with the work of those concerned with soil classification. Many problems arising from the deficiency of major plant nutrients are now fairly well understood and have been remedied in advanced countries. However, the minute amount of the minor elements necessary has become an important investigation: papers read included reference to boron, zinc, molybdenum and sulphur, in various soils.

The most extensive programme was that undertaken by Commission 5 (Soil Genesis, Classification and Cartography). In this section alone more than 160 papers were read. As might be expected, the soils of south-eastern Europe were discussed at some length by delegates from the host country, Romania, and from Hungary and Bulgaria. Other sections were devoted to the origin and genesis of soils of the desert and semi-desert, steppe, mountain and cold regions, tropical, Mediterranean and sub-tropical regions. Progress in soil mapping and cartography was outlined by speakers from many different parts of the world.

Soil technology is never far removed from the fundamental investigations of soil chemistry and physics, and it was no surprise to find that there were joint sessions of Commission 6 with Commissions 1 and 2. Soil erosion is always a danger, particularly in semi-arid lands, and methods for its control have been developed from fundamental investigations of the physical and chemical conditions of soils. The improvement of eroded land provides the soil scientists with special problems, and on this point papers were read dealing with wind and water erosion and its control in Europe and in North and South America. Other topics in this Commission were the reclamation of saline and alkaline soils under irrigated and non-irrigated conditions; factors regarding the time and amount of irrigation water; and the influence of tillage depth and fertilizer placement on plant rooting.

Delegates to Commission 7 (Soil Mineralogy) were especially concerned with the genesis and changes that occur in the minerals present in soils; methods of identification of these minerals and research techniques were discussed. Clay mineralogy was extensively dealt with especially from the point of view of the influence of clay minerals on the structure of soils and their chemical and physical properties. Papers were also included on clay migration and the specificity of clay minerals to certain types of soil-forming processes.

Particular interest was stimulated by the general sessions of the Congress when a number of invited lectures were given. The session on the World Soil Map has already been mentioned; other topics were: "The Dynamics of Water in Soils and Plants", by M. B. Russell; "New Methods of Research in Soil Science", by J. J. Fripiat; "Some Aspects of Nitrogen Metabolism in Soils", by G. W. Harmsen; "Tropical Soils", by G. Aubert; and a paper on "The Modern Dokuchaev Approach to Soil Classification" was read on behalf of I. P. Gerassimov, who was unfortunately unable to be present at the Congress.

Three symposia were held outside the scope of the Commissions: these dealt with the fertility of forest soils. the interaction of photosynthesis and mineral nutrition of plants, and metabolism of the primary assimilation of nutritive elements in the absorbent zone of roots.

For the energetic there was the choice of one of three tours, before or after the Congress. These proved extremely successful and were arranged to demonstrate the soils of south-east, east and north-east, and central and west Romania. For the not-so-energetic, profiles of all the major soil groups found in Romania were assembled in a magnificent display which was on view throughout the Congress. The All-Union Soil Science Society of the U.S.S.R. also organized an excursion from Moscow to Kherson, illustrating the soils of European Russia, and providing many interesting comparisons with similar soils on the plains of Romania.

The soil scientists of Romania have achieved a great deal in recent years, and the results of their hard work were well shown in the excellent presentation of the soil landscape of their country during the Congress and its tours. All who attended the eighth International Congress of Soil Science will want to congratulate their Romanian hosts on the smooth way in which the Congress was run and to wish them success in their future work.

E. M. Bridges

USE OF FILMS IN RESEARCH AND TEACHING

A WORKING party, with Dr. W. L. Francis as chairman, formed by the Department of Scientific and Industrial Research in June 1960, following a meeting of those interested in scientific films from universities, industry and Government departments, has now reported on *The Film in Scientific Research**.

The working party was to consider what is needed, in terms of information, research and development, in the field of scientific film and the appropriate functions of a central organization for scientific cinematography. It decided to limit its report mainly to the needs of scientific ciné-photography and photography, and to the uses of research film in research and in advanced teaching. An enquiry made for the working party by Mr. G. E. D. Bouler-Carter of Production Engineering, Ltd., indicated

* Department of Scientific and Industrial Research. *The Film in Scientific Research: Report of a Working Party*. Pp. vi + 67. (London: Department of Scientific and Industrial Research, 1963.)

that existing arrangements for training scientists, technicians and managements in scientific photography are inadequate, and that the low pay offered to scientific and medical photographers does not encourage pursuit of these occupations. Research workers have difficulty in obtaining information which would assist them in applying photographic methods, but certain advisory technical and research facilities could usefully be provided to assist them. Facilities for research photography are unco-ordinated in many universities; there is also lack of co-ordination between users and producers of educational film for universities, and existing distribution facilities for research films are inadequate.

The working party surveyed the demand for research films and their availability, and the use and improvement of scientific photography in research in Britain, as well as its use overseas. Recommendations were adopted proposing the establishment of an information service, preferably in some Government research establishment. The establishment was suggested of a central research photography unit, incorporating the information centre and financed jointly by Government and industry, the main function of which would be to conduct research on the techniques and development of scientific photography, and to encourage its use in industry and the universities. This it would do by rendering technical assistance and consultancy services where these are not readily available. Besides the establishment of a department of scientific photography in a suitable university or college of advanced technology, short advanced courses in scientific photography should be instituted in such colleges, particularly directed to the needs of scientists in industry. Central photography units should be encouraged in universities and, with the help of these units, universities should form their own libraries of 'concept film' as an aid in advanced teaching. The working party considered that the Department of Scientific and Industrial Research should be invited to compile, primarily as an aid in locating research film, a directory of users of cinematography in research in Britain, based on information already collected. The possibilities of making or compiling a film or films illustrating the techniques and applications of scientific photography should also be looked into carefully.

The recommendations relating to a university department of scientific photography, central photography units in universities and a research film library fall within the terms of reference of a Joint Committee of the University Grants Committee, the Department of Education and Science and the Scottish Education Department. It was appointed in 1963 to survey the present use of audio-visual aids in teaching and research in the pure and applied sciences in higher education. It is not expected, therefore, that these particular recommendations will be implemented before that Committee reports in 1965.

OCEANOGRAPHY IN THE CARIBBEAN AND EASTERN ATLANTIC

NO. 2 of the first volume of the *Boletin* issued by the recently established Oceanographic Institute at the Universidad de Oriente, Cumana, Venezuela*, contains five papers, the high standard and choice of subject-matter of which promise well for the future of the subject, in an area where oceanography and the scientific approach to fisheries problems have hitherto received little attention; and that little mainly offshore work during long-range cruises by research ships from the United States and other countries, with all the unavoidable lack of seasonal coverage that this implies.

The emphasis has clearly been on filling the gaps in basic knowledge of the hydrology of the region, both seasonally and into the inshore areas where no extensive observations had hitherto been made; while on the biological side correlation between organic sedimentation and the hydrology of the bay most readily accessible from the new laboratory and the revision of the systematics of a group of fishes important to the local inshore fishery, with detailed description of their osteology, will both help to provide a basis for future research on obviously desirable lines.

For these reasons it would seem that the director of the new institute deserves congratulations on his choice of studies to be encouraged, no less than the individual researchers on the high standard of presentation of their observations. Apart from their local significance the hydrological papers contain much of interest to oceanographers generally.

In the first paper, Herman G. Gade describes oceanographic observations in the south-eastern Caribbean Sea and adjacent Atlantic waters with special reference to the influence of the Orinoco River. Two cruises carried out in August 1960 and April 1961 by the R.V. *Guaiqueri* give adequate coverage for the region northwards from the delta, with a special investigation of the shallow Gulf of Paria between Trinidad and the mainland, and "round the corner" into the Caribbean.

There was great seasonal fluctuation in the volume of the upper water of the region. In the Atlantic the depth of the transition zone varied from 100 m in August to 160 m in April. The offshore surface water changed from less than 34 ‰ to more than 36 ‰ salinity during the same period. The slope of the isopycnals indicated moderate upwelling towards the coast in both Atlantic and Caribbean regions. This was considered to be a result of strong currents parallel to the coast, and was most intense during the dry season. There were indications of a counter current below and within the transition zone in both regions. This seems analogous to the similar effect suspected (though not yet proved) to exist below the upwelling zone in the Benguela current[1]. Here, the maintenance of the sub-surface salinity maximum is attributed to this feature. The negative anomaly of surface temperature in the coastal upwelling zone of the Caribbean region reached as much as 5° C in the dry season. The ocean current entering the Caribbean through Tobago Sound appeared as a strong jet affecting conditions as far west as Curaçao.

Within the Gulf of Paria a western region of low salinity was separate from a region of more oceanic water to the east. The eastern and central waters of the Gulf appeared to be rotating clock-wise. In the north, three layers of water were distinguished: the two upper ones of Gulf water, the lower one of Caribbean origin. While the residual current flows through the Gulf from south to north, Caribbean water may at times penetrate into it at all levels under the influence of tidal currents, but chiefly below the surface layer through the Dragon's Mouth. In the appendix the strength of the anticyclonic circulation in the gulf is discussed on the basis of the deformation of the sub-surface isopycnals. In the theoretical model the water rotates as a rigid body. Applied to the observed

* *Boletin del Instituto Oceanografico*, Volumen 1, Numero 2. Pp. 287–482. Suscripcion: Bs. 10 por volumen de dos numeros; Bs. 6 por numero suelto. (Cumana, Venezuela: Universidad de Oriente, Instituto Oceanografico, 1961.)

data, the corresponding angular velocity was found to be 0·17 radians/h.

The fresh-water discharge from the Orinoco and other Guiana rivers is carried by the ocean current some distance offshore. To the north of Venezuela it is displaced to the north of the upwelling zone and is not found along the coast. It follows the ocean current from Tobago Sound in a west-north-west direction and flows like a river into the central southern parts of the Caribbean Sea. This system appears quite stable except perhaps in September/October when slackening of the trade wind greatly reduces the coastal upwelling.

The Amazon discharge culminates in July and is carried by the Guiana current into the Caribbean, but is sometimes separated from the Orinoco water by a band of higher salinity. The bulk of the tropical low-salinity water (greatly augmented by precipitation at sea, in addition to the effects of Amazon discharge) enters the Caribbean north of 12° N.

Towards the end of the year the Amazon discharge is scarcely detectable in the Atlantic, and the Orinoco discharge becomes the main source of reduced salinities in the south-eastern Caribbean, its effects being limited to the south by the coastal upwelling and to the north by Atlantic water of the main Caribbean current.

In the next paper K. Kato discusses the relationships between organic sedimentation in Mochima Bay and the hydrological conditions. The sediment samples from 35 stations were collected by Dr. Roa Morales in October, and hydrological observations were made in November 1960.

The bay is a small narrow inlet on the East Coast of Venezuela opening into the Caribbean, some 8 km long north and south, and 2·3 km wide (not 23 km as printed). In contrast to conditions in the Gulf of Cariaco, where the prevailing easterly wind carries out the surface waters, setting up a fairly constant circulation as they are replaced by sub-surface movements, the waters of Mochima Bay, surrounded by mountains and with its opening to the north, are little influenced by winds. Here, water transport seems to be mainly due to tidal motion.

On the basis of organic carbon content, the bay was divisible into four sub-areas: (1) The entrance to Punta Garrapata, where the sediments were very poor in organic carbon, $< 0{\cdot}25$ per cent. (2) The narrow channel with carbon content still low, $< 0{\cdot}5$ per cent, but rising near shore to < 1 per cent. (3) The north-eastern part of the interior, carbon content still mainly < 1 per cent. (4) The south-western head of the bay, where there was abundant accumulation of organic matter, more than 6 per cent organic carbon at some stations, with average values of 2·8 per cent. Here also the *p*H value of the mud was appreciably lower than in the other sub-areas.

The attempt to determine possible relations between this disposition of organic sedimentation and the hydrobiological régime involved hydrological and chemical observations carried out at five stations, one to give the conditions in the Caribbean coastal water outside the bay, and one in each of the four sub-areas. Temperature, density, salinity, dissolved oxygen, nitrate, nitrite, phosphate and silicate were determined, following standard methods established by the Japanese Society of Oceanography. The results are described in detail with good graphic presentation.

There were temperatures of 24°–25° C in the upper 5 m of the bay water, where the stability was very high. Subsurface water from the outer Caribbean penetrated below the well-defined discontinuity layer at the flood tide and reached the bottom of the bay about the seaward boundary of the innermost sub-area. Here it came in contact with the interior bay water and this border (or slant plane) extended obliquely towards the surface seawards, nearing the surface in the narrow channel. It is held that appreciable turbulence may develop along the border where there is said to be much biological activity. A most vigorous growth of phytoplankton is said to occur here, but turbulence seems sufficient to prevent oxygen depletion, with comparatively rapid re-cycling of organic constituents and hence comparatively high *p*H values in the bottom muds.

The more stagnant water at the head of the bay seems isolated from the inflowing water. It contained abundant nutrients and a vigorous phytoplankton development in the surface layer, thought to be distinct from the dense population found in the channel area, with super-saturation of oxygen. The bottom water in this interior sub-area, however, showed oxygen depletion, and here there was an abundant accumulation of organic matter, as much as 1·3–6·5 per cent in organic carbon content.

A direct plankton investigation comparing the populations in the two distinct areas of high productivity should not be too difficult, and would go far towards confirming the general picture. This seems exceptionally clear and well presented. No doubt complexities would obtrude if a whole seasonal cycle were investigated, but the prime consideration of the bottom deposits, which might be termed a semi-conservative element, seems to have ensured that the summation effect is fairly represented; that the investigator has, in fact, allowed Nature to do some of his integration for him.

The third paper, by Herman G. Gade, describes the hydrographic observations during January–May 1961, carried out in continuation of his earlier work in the Gulf of Cariaco, and discusses the circulation and water exchange conditions they reveal. The Gulf of Cariaco is a much larger inlet than Mochima Bay, lying south of the Peninsula de Praya with its long axis east and west, and opening to the west. Thus the horizontal shear set up by the prevailing dry-season winds becomes the prime factor in maintaining the circulation within the Gulf. The inflow during these months is derived from the salinity maximum layer and below. Upwelling takes place fairly regularly within the Gulf, but is normally limited to water above 50 m. Below this the water exchange is slow except for a period during the dry season when the external water is heavier and the deeper layers participate in the exchange. Outflow is general above 25 m, and there is a compensation current below that depth. The repeated observations showed that the maximum period of deep upwelling fell earlier than during the 1960 season. This was thought to be connected with variations in the more general upwelling along the northern coast of Venezuela and, consequently, in the thickness of the upper water layer.

There seemed to be an almost permanent circulation of the upper layers inside the Gulf maintained by the wind shear, and this was verified by many drift measurements with current crosses, while the vertical circulation was studied directly by observations with Ekman current meters, in addition to the sections showing isohalines, isopycnals and oxygen isopleths, to obtain what is believed to be a typical profile of the vertical current distribution near the mouth of the Gulf.

The total flushing period of the Gulf was estimated to be 20 days, but in periods when the exchange is limited to higher levels the period would be shorter. The upper layers are much warmer than can be accounted for by the incoming radiation alone, and their salinity also is sometimes higher than the surface evaporation alone could account for. It is suggested that this is due to occasional reversal of the circulation during periods of calm or westerly winds, when the upwelling process must be slowed or stopped. This would be a direct analogy with the larger-scale anomalies that affect the normal régime of upwelling off the western coasts of the continents at times when the wind conditions become atypical[1].

A most detailed description of the osteological characters of eight species of Pomadasyidae is presented by Francis Mago Leccia in the fourth paper. The work was carried out with the definite objective of providing a sound systematic basis for future bionomic research on this family of

Perciforme fishes, known locally as *corocoros*. To further that end the paper is well and copiously illustrated, and convenient tabulations of the characters deemed taxonomically significant are provided. The importance of the group to the local fishery is shown by the fact, mentioned in the introduction, that already by 1959 more than 200 tons of them were being marketed annually.

The illustrations bring out clearly one point, not specifically dealt with by the author, but which is still likely to prove important in any future population studies on the group: whereas the otoliths look unpromising material for age-determinations, some of the other bones provide clear indications of growth zones, notably the supra-occipital crest of *Haemulon plumieri* and several of the opercular bones of *Orthopristis ruber*. Apart from their value in dealing with some fresh-water fishes, exemplified by Le Cren's[2] investigations of perch in Lake Windermere, the use of bones other than otoliths has been found useful in studying the growth of several very diverse species of marine fishes (see Menon[3,4] and Chang[5]).

This number of the *Boletin* is concluded by a short systematic paper by R. A. Curra of the new Institute, and P. de Miranda Ribeiro of the National Museum of Brazil (a pioneer in several lines of aquatic biology in Latin America). This seeks to establish the taxonomic position of a rare species of gymnotid fish first described by the latter in 1920, *Urumara rondoni* Miranda Ribeiro. To further this end, the main diagnostic characters of the three genera at present included in the family Rhamphichthyidae are illustrated and a formal key is provided.

T. J. Hart

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[4] Menon, N. D., *J. Cons. Internat. Explor. Mer.*, **16**, 311 (1950).
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EQUATORIAL UNDERCURRENT IN THE WESTERN INDIAN OCEAN

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FOUR times during March–June this year, the equatorial undercurrent has been observed by the R.R.S. *Discovery* in the course of its participation in the International Indian Ocean Expedition. We have found much higher speeds than those reported by Knauss and Taft[1] in the same season of the previous year, and a brief preliminary account may be of interest although not all the results have yet been processed.

Knauss and Taft found only a weak undercurrent in the western Indian Ocean, with maximum speeds generally less than 50 cm/sec and sometimes scarcely any significant eastward flow at all. In contrast, two of our sections showed speeds exceeding 120 cm/sec, comparable with those found in the Pacific undercurrent, and some subsurface eastward movement was always found.

On the first three crossings of the equator, a buoy was anchored at each of the stations shown (Figs. 1–3). Water movement relative to the ship was measured with direct-reading current-meters[2] and the ship's drift was determined by radar relative to the anchored buoy. Drogues were also used for measuring the near-surface current.

Section 1, 58° *E.*, *March* 18–23, 1964. The undercurrent was centred on the equator, with a maximum eastward speed of 122 cm/sec at 75 m depth, in the thermocline. The meridional component there was 28 cm/sec southward. The eastward speed falls to about half-value at 1° N. and 1° S. and meridional flow in the undercurrent was relatively weak at all stations. Spreading of the thermocline was only slight, the separation of the 15° C and 25° C isotherms being 105 m at the equator, and 80–95 m at 2° S. and 2° N. The water in the undercurrent had a high salinity, the maximum values decreasing from north to south. At the equator the maximum was 35·55 parts per thousand at 100 m, slightly deeper than the high-speed core. Surface currents were north-west, 30–60 cm/sec, at 1° N., the equator and 1° S., and south-west, 50 cm/sec, at 2½° S.

This section would be very similar to typical Pacific sections but for the asymmetry due to the deeper eastward flow at the southern end of the section.

Evidence in support of the persistence in time, and continuity with longitude, of the undercurrent comes

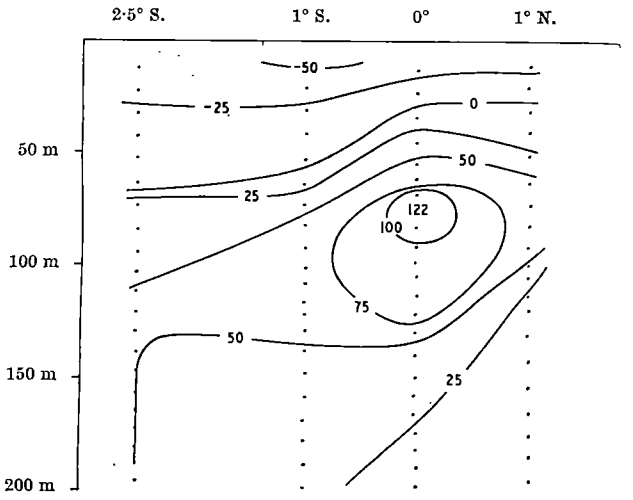


Fig. 1. Section 1, 58° E. March 18–23, 1964. Eastward component of velocity in cm/sec

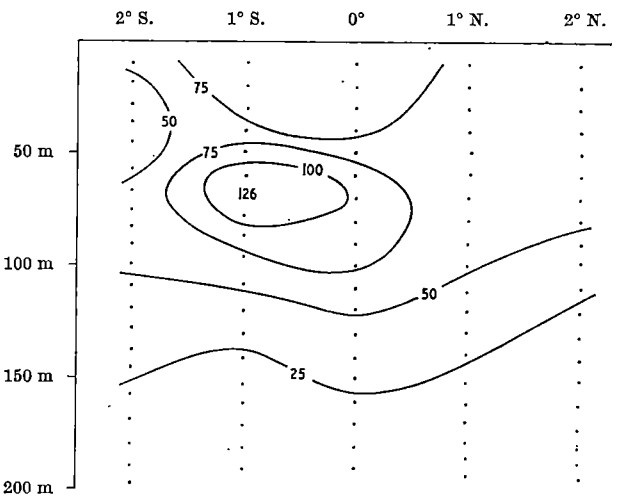


Fig. 2. Section 2, 67° 30′ E. April 26–May 1, 1964. Eastward component of velocity in cm/sec

from an observation made 2 weeks after this section was completed, by H.M.S. *Owen*[3]. At an anchor station on the equator in 55° 40′ E. on April 7, 1964, an eastward current of 90 cm/sec was found at a depth of 50 fathoms (91 m) with much weaker currents above and below.

Section 2, 67° 30′ *E., April* 26–*May* 1, 1964. On this section the surface currents were eastward, with speeds of about 90 cm/sec and meridional components converging on the equator. At the equator and 1° S. there was a significant subsurface maximum of eastward flow, reaching 126 cm/sec at 70 m depth at 1° S. The meridional components of this subsurface current were weak, with a tendency to diverge from the equator, and again there was a salinity maximum slightly deeper than the high-velocity core, decreasing in strength from north to south. It had a value of 35·42 parts per thousand at 92 m at the equator. The steepest part of the thermocline was deeper than the velocity maximum which lay between the 28° C and 29° C isotherms. There was a distinct spreading of the thermocline on this section, the separation of the 25° C and 15° C isotherms being 85 m at the equator, and only 53 m and 44 m at 2° N. and 2° S. Some indication of possible instability comes from one of the repeat observations at 2° S.; there appeared to be an eastward surge which would increase the speeds shown at that station by 30 cm/sec, exaggerating even more the asymmetry about the equator.

Section 3, 58° *E., June* 2–7, 1964. The undercurrent was still present, though weaker and shallower and displaced still farther south from the equator. The maximum eastward speed observed was 84 cm/sec at 39 m at 2° S. Surface currents had only weak zonal components and tended to diverge from the equator, being north-east at 2° N. and south-east to south at 1° S. to 3° S., 30–60 cm/sec. At the equator and 1° N. there was a weak westward surface current.

In the high-speed core, meridional components were northward, with a maximum of 49 cm/sec associated with the 84 cm/sec eastward maximum, but below that, between the 50 cm/sec and 25 cm/sec isotachs, the meridional flow was southward. The salinity maxima lay close to this lower zone, with a value of 35·30 parts per thousand at 79 m at the equator, and the steepest part of the thermocline was at about the same depth. There was no significant spreading of the thermocline, with the 25° C and 15° C isotherms varying between 75 m and 90 m separation throughout the section.

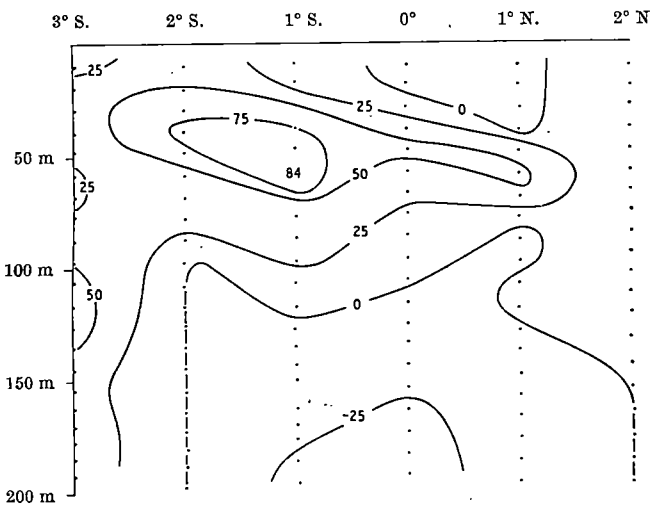


Fig. 3. Section 3, 58° E. June 2–7, 1964. Eastward component of velocity in cm/sec

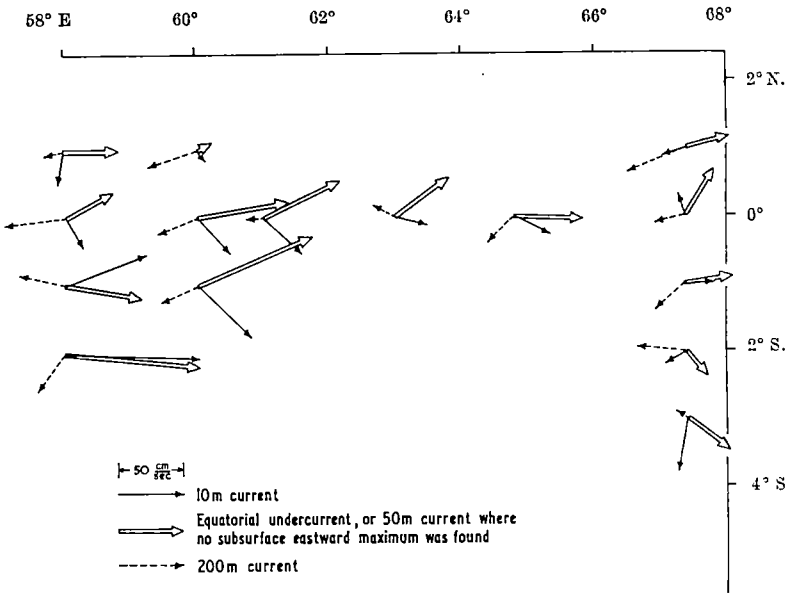


Fig. 4. Current vectors using mean of observations at 500–1,500 m depth as reference value. Lengths of arrows indicate speed. Examples shown represent 50 cm/sec. June 13–21, 1964

On our fourth visit to the equator, during June 13–21, 1964, short sections were worked in 58° E., 60° E. and 67½° E. with stations along the equator between the latter two. It seems more convenient to present these observations in plan view instead of vertical profiles. Time did not permit anchoring buoys; instead, at each station the usual current-shear measurements to 200 m were supplemented by observations at a few depths to 1,500 m using a telemetering current-meter provided by the Chr. Michelsen Institute and the Geophysical Institute, Bergen. The mean shear between 10 m and 500–1,500 m depths has been taken as representing the near-surface current, to which the upper part of the shear profile has been fitted. In Fig. 4, current vectors derived in this way are shown for each station at 10 m and 200 m depths, and in the sub-surface eastward maximum, which is generally near 50 m depth. Where no subsurface maximum was found, the 50 m value has been plotted.

Conditions at 58° E. and 60° E. were not very different from those in section 3, surveyed two weeks previously. The undercurrent appeared to slow down towards the east, but this could equally well be a time change. The salinity maxima and thermocline were again found a little deeper than the eastward velocity maxima, as in section 3, and there was little or no evidence of thermocline spreading.

Evidently, comparing these observations with those of Knauss and Taft, the equatorial undercurrent in the western Indian Ocean undergoes more complicated fluctuations than simple seasonal ones. It is hoped that it may be possible to relate these fluctuations to differences in the winds in the two years.

The work of the R.R.S. *Discovery* described here was carried out by the National Institute of Oceanography as part of the U.K. contribution to the International Indian Ocean Expedition. I thank Commander D. W. Haslam, R.N., in command of H.M.S. *Owen*, and also the Hydrographer of the Navy for permission to refer to H.M.S. *Owen's* results in advance of publication.

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SMALL-SCALE IRREGULARITIES OF THE RADIO REFRACTIVE INDEX OF THE TROPOSPHERE

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IN research on wave propagation through the troposphere at very high frequencies it is important to know the magnitude of the differences in refractive index which can occur over distances of a few centimetres or a few metres. This information is required both in investigations of propagation to points beyond the normal horizon and in studies of radar echoes observed in the absence of aircraft, clouds or precipitation (so-called 'angel' echoes). Experiments to measure these small-scale differences are in progress at the Radio Research Station, and it is the purpose of this article to summarize some of the first results obtained, using a balloon-borne, spaced-cavity, microwave refractometer. These results show much larger differences of refractive index than might have been expected on the basis of some earlier investigations of tropospheric structure carried out by other workers.

Microwave refractometers installed in aircraft have been used for several years in measurements of irregularities of the refractive index. A difficulty arises in this technique, however, because of the ambiguity resulting from the horizontal component of the motion of the aircraft, in the measurement of vertical gradients. This problem is largely avoided in soundings with captive balloons. The Radio Research Station refractometer uses three cavities in a two-channel, differential system. The cavities are connected in two pairs, and with each pair a measurement is made of the difference in the refractive index of the air in the cavities. This information, together with measurements of pressure and wind speed, can be telemetered to a ground station and recorded as the balloon ascends and descends. Possible applications of the spaced-cavity system are shown in Table 1, cavity *A* being compared with each of the others.

Table 1. Applications of Spaced-cavity Refractometer

Arrangement	Location of cavities		Quantities measured
I	o *C* o *B* o *A*	*A*, *B* and *C* all open	Vertical differences (*A*–*B*, *A*–*C*)
II	o o o *A* *B* *C*	ditto	Horizontal differences (*A*–*B*, *A*–*C*)
III	o o *A* *B* o *C*	ditto	Simultaneous vertical and horizontal differences (*A*–*B*, *A*–*C*)
IV	*A* *B* o o *C*	*A* and *C* open, *B* closed	Total mean square fluctuation, *A*–*B*, and vertical difference, *A*–*C*

Adjacent cavities can be located, in any of the four arrangements, at spacings of 0·1–1 m if required. A vertical spacing of 1 m selects those irregularities which would be important in forward scatter at, for example, a wave-length of 3 cm on a path 300 km long. The first experiments indicated the need for a high sensitivity in the refractometer and in the model now in use the residual noise-level is equivalent to fluctuations of less than 0·1 *N* unit. ($N = (n-1)10^6$, where n is the refractive index.) The investigations in progress are primarily intended to measure the small-scale differences in and near elevated isothermal and inversion layers in conjunction with vertical-incidence radar soundings[1,2]. Early theories of 'scatter' propagation did not consider the possible influence of thermal stability in the intensity and scale size of refractive-index irregularities, but more recent work[3–5] has emphasized its importance. However, no detailed measurements over distances of 1 m and less have hitherto been made. In the Radio Research Station experiments carried out so far, most soundings have been made with arrangement III of Table 1 (with spacings *A*–*B* and *A*–*C* of 1 m), for this gives a direct indication of anisotropic structure. A few trials, however, have been made with arrangements I and IV. Some selected results are summarized in the following discussion. The measurements described were all made at Cardington, Bedfordshire, at the Royal Air Force Balloon Unit.

On May 28, 1964, a sounding was made at 13.00 h G.M.T., immediately following a routine sonde ascent by a captive balloon at 12.00 h G.M.T. Conditions were clear but not anticyclonic. The sonde data showed an inversion layer in which the temperature increased by 1·5° C between heights of 0·43 and 0·50 km. Light winds (1·5–2 m/sec) prevailed throughout the soundings. Between the inversion and the ground the mean vertical gradient of temperature was about − 9° C/km (that is, the dry adiabatic value). The refractometer was equipped with three open cavities in arrangement III of Table 1. The peak and mean square values, ΔN (peak) and $\overline{\Delta N^2}$, measured at various heights are shown in Table 2, each entry being derived from 50 sample readings of the paper-chart record obtained from the telemetry receiver.

Table 2. Variation of Small-scale Differences of Refractive Index with Height through an Inversion Layer

Clear conditions: Cardington, Bedfordshire, May 28, 1964. Cavity arrangement III (Table 1)

Height (km)	Pair *A*–*B* spacing 1 m		Pair *A*–*C* spacing 1 m		Remarks
	ΔN (peak)	$\overline{\Delta N^2}$	ΔN (peak)	$\overline{\Delta N^2}$	
0·15	2·0	0·35	2·0	0·35	2-min sample: refractometer stationary
0·43–0·45	3·0	1·0	2·5	0·9	1-min sample: refractometer ascending through base of inversion
0·46	1·5	0·3	1·5	0·3	2-min sample: refractometer stationary
0·49	2·0	0·2	1·5	0·3	ditto
0·52	1·5	0·2	1·5	0·35	ditto

The largest values of ΔN (peak) and $\overline{\Delta N^2}$ were measured in the first 20 m above the base of the inversion. However, it is of interest to note that on this occasion the values of ΔN (peak), over a 1-m spacing, were almost as large in the unstable air at a height of 0·15 km as in the inversion. Values of $\overline{\Delta N^2}$, obtained from each pair of cavities over periods of the order of a few tens of seconds or more, did not indicate any marked anisotropic effects in distances of 1 m on this sounding.

Another measurement, using the same arrangement of open cavities, was made through an elevated inversion layer on the morning of May 29, 1964, at 09.30 h G.M.T. Conditions became clear during the ascent, after morning mist. Fig. 1 shows the results of this sounding, together with the coarse variation of refractive index and temperature with height obtained from the routine sonde ascents at 12.00 h G.M.T. The sonde ascent showed a maximum in the vertical gradient of refractive index in a layer between heights of 0·37 and 0·52 km. The largest differences of refractive index, over distances of 1 m in the horizontal and vertical directions, were 4–5 *N* units and were observed at a height of 0·37 km. The anemometer on the refractometer indicated a minimum value of wind speed at a height of 0·37 km.

In later experiments even larger differences in refractive index (over a 1-m spacing) were measured in elevated

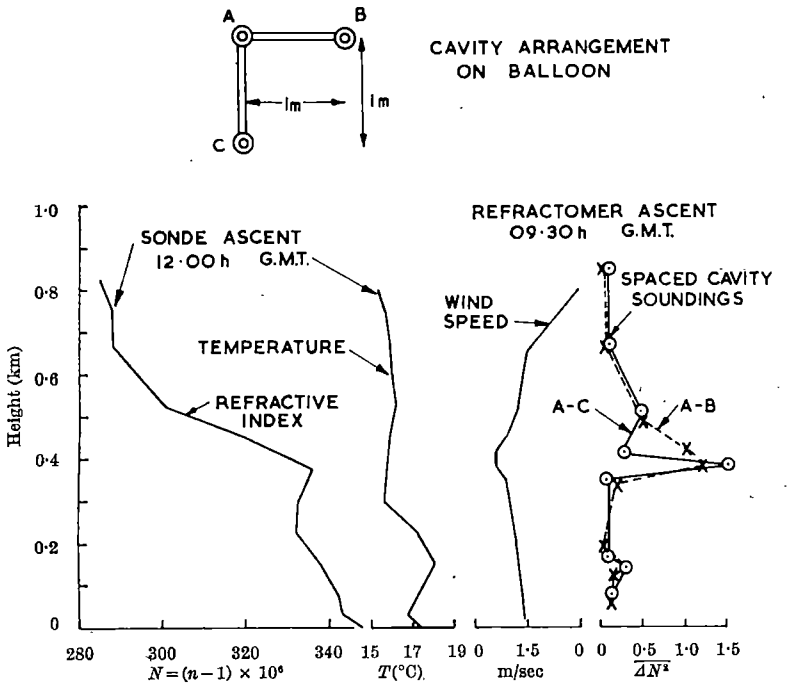


Fig. 1. Results of captive-balloon soundings; Cardington, Bedfordshire, May 29, 1964. ○ and × show the mean square variation, $\overline{\Delta N^2}$, in a 3-min sample at the heights indicated

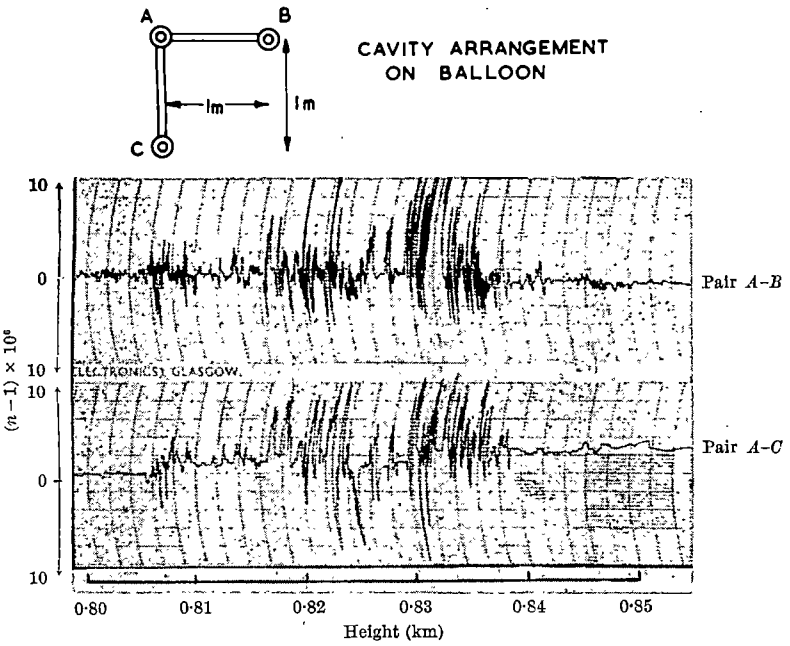


Fig. 2. Results from spaced-cavity refractometer; Cardington, Bedfordshire. 13.30 h G.M.T., July 27, 1964, height range 0·80–0·85 km

isothermal or inversion layers, in conditions which were fine but not always anticyclonic. An example of such a layer is shown in Fig. 2. Several differences of 10 N units were measured in both the horizontal and vertical directions as the refractometer ascended from 0·81 to 0·84 km. The sounding was made on July 27, 1964, at 13.30 h G.M.T., in fine conditions after morning rain, and it provides an interesting example of a well-defined layer which was not associated with anticyclonic subsidence.

One sounding, made with arrangement IV (Table 1) in anticyclonic conditions on July 1, 1964, gave results which indicated that in stable layers the scale size of the irregularities is considerably less than the value of 100 m assumed in theories of tropospheric scatter. As the refractometer moved into the layer, fluctuations of about 10 N units on the chart record obtained from the open cavities, A–C (1 m apart), were accompanied by fluctuations of 15 N units in the record from the open–closed pair, A–B. A large fraction of the intensity or total mean square fluctuation at the base of the layer was contained therefore in those irregularities extending over distances of 1 m or less.

On August 20, 1964, a first trial was made to investigate the presence of marked differences of refractive index over vertical distances less than 1 m. Two cavities were located 12 cm apart in the vertical direction and a sounding made in conditions which were fine but not anticyclonic, with patches of cloud at a height of 1·1 km. Several examples were obtained, in clear air at heights between 0·6 and 1·3 km, showing differences of 2–3 N units over the 12 cm spacing. This distance is believed to be the smallest over which a direct measurement of refractive-index difference has been made in the lower atmosphere.

Finally, it is of interest to compare the data from the spaced-cavity soundings with some previous published measurements of refractive-index irregularities. Flights made in earlier Radio Research Station work with a refractometer in an aircraft have indicated that changes of 10–20 N units can occur in a vertical distance of approximately 1 or 2 m in subsidence inversions, but precise measurements of these small-scale irregularities are difficult in soundings with aircraft for the reasons given earlier in this article. From the results of a few flights with a refractometer in an aircraft over Washington, D.C., Bussey and Birnbaum[6] deduced that, except in or near clouds, differences over horizontal distances of 0·5–5 m had amplitudes of less than 0·04 N units, that is, two orders of magnitude less than the values obtained for a 1 m spacing in some of the Radio Research Station measurements. Straiton, Deam and Walker[7] determined the mean square fluctuation, $\overline{\Delta N^2}$, in distances of 5 m and less, also from soundings using an aircraft: they obtained a maximum value of $\overline{\Delta N^2} = 5 \times 10^{-2}$ in a horizontal flight at a height of about 1 km. This value is 25–30 times smaller than the maximum measured in the sounding illustrated in Fig. 1. In measurements with a balloon-borne, capacitor-type refractometer ascending at 300 m/min, Turner and Hay[8] noted irregularities of the order of 1 N unit in a vertical distance of about 10 cm, but their soundings were limited to the first 60 m above ground.

We conclude from these initial experiments with a spaced-cavity refractometer that instantaneous differences of refractive index of a few N units over distances of 0·1–1 m can exist in summer conditions in England in the first 2 km or so above ground, even in unstable air. Larger differences (about 10 N units) have been observed in several elevated layers in which the temperature was uniform, or increased by 1°–2° C, over vertical distances of the order of 100 m. The measurements also indicate that in stable air the scale size of the irregularities is of the order of a few metres. However, further measurements are desirable in elevated layers of greater thermal

stability than those investigated in the experiments described.

The work described was carried out as part of the programme of the Radio Research Board, and this article is published by permission of the Director of Radio Research of the Department of Scientific and Industrial Research. The balloon soundings were carried out with the co-operation of the Meteorological Office, the Royal Air Force Balloon Unit, and the Ministry of Aviation. The initial experimental work was carried out by Mr. D. S. Froome. The latest model of the refractometer and the telemetry equipment were developed by Mr. C. S. Fowler. Mr. M. P. M. Hall assisted in the design of the microwave equipment.

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VERTICAL DISTRIBUTION OF RADIONUCLIDES AS MEASURED IN OCEANIC ANIMALS

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THE north-east Pacific Ocean contains two sources of artificial radionuclides: neutron-induced isotopes from the Hanford nuclear reactors on the Columbia River and fission products (fall-out) from weapons tests. Zinc-65 is one of the principal radionuclides in nearshore waters off Oregon. Although it is often a component of fall-out, most of the zinc-65 off Oregon arises from Hanford operations[1]. A number of fall-out radionuclides were also present in measurable amounts before the nuclear test ban became effective. Zirconium-95+niobium-95 and cerium-141-144 in particular have been reported in marine organisms[2,3]. This article is based on analyses of samples collected when both sources of radionuclides were present. Subsequent diminution of zirconium-95+niobium-95 through radioactive decay precludes repetition of these observations.

Animals were collected over a 24-h period on January 7–8, 1963, 50 miles off Newport, Oregon. An Isaacs–Kidd midwater trawl with a multiple plankton sampler as an opening–closing cod-end unit provided samples from three depth intervals: 0–150 m, 150–500 m, 500–1,000 m (ref. 4). Volume of water filtered was estimated from the cross-sectional area of the mouth of the trawl and distance towed at each depth interval, assuming 100 per cent filtration efficiency. A single mesh size (12 mm stretched measure) was used as a liner throughout the body of the trawl. Two tows during daylight and two after dark provided the material for radioanalysis.

Samples from each depth-interval, consisting of mixed species collections (mostly crustaceans, chætognaths and fishes), were drained to remove excess fluids (radioanalysis of this material showed that it contained only a minor fraction of the radioactivity), weighed (wet-preserved weight), ashed at 450° C, and packed into 15-c.c. plastic tubes. The samples were reweighed, and the tubes sealed. Samples were analysed in the well of a 5-in. × 5-in. Harshaw NaI(Tl) crystal used with an *ND* 130*AT* pulse-height analyser. Counting time was either 100 or 400 min, depending on sample activity. Counts were converted to picocuries per gram wet weight and per 1,000 m³ water filtered.

Zirconium-95+niobium-95 and zinc-65 activity of organisms from different depths is shown in Fig. 1. Levels of zirconium-95+niobium-95 per gram wet weight were always highest in animals from the surface layer (0–150 m), where activity averaged about nine times that in animals collected in deeper waters. Zinc-65/g was not higher in surface waters but appeared in rather uniform concentrations at all depths.

Presumably the difference in vertical distributions of the two radio-elements is related to how these elements are involved in biological processes. Zinc, a transition element, is a constituent of several enzymes. Zinc-65 is concentrated by many marine animals[3]. Zirconium and niobium apparently have relatively little biological significance in the sea. ^{95}Zr–^{95}Nb do not appear to be concentrated except by second trophic level animals, such as some copepods, euphausiids, pteropods, and salps[4]. Concentration is thought to occur through mechanical filtration of phytoplankton cells and other particulate material with which the radioactivity is probably associated. Zinc-65 and zirconium-95+niobium-95 also have different biological turn-over rates. Experiments by Chipman[5] show that, in general, fission products are poorly absorbed and are rapidly egested. Zinc-65, on the other hand, is retained over a longer period of time. This may explain why zinc-65/g is fairly constant in animals taken at all depths down to 1,000 m, whereas zirconium-95+niobium-95 are more restricted to surface waters (Fig. 1). Grazing by herbivores is normally confined to surface waters. Animals collected from greater depths, although having grazed in upper layers the previous night, could have lost most of the fission products through production of fæcal pellets[6].

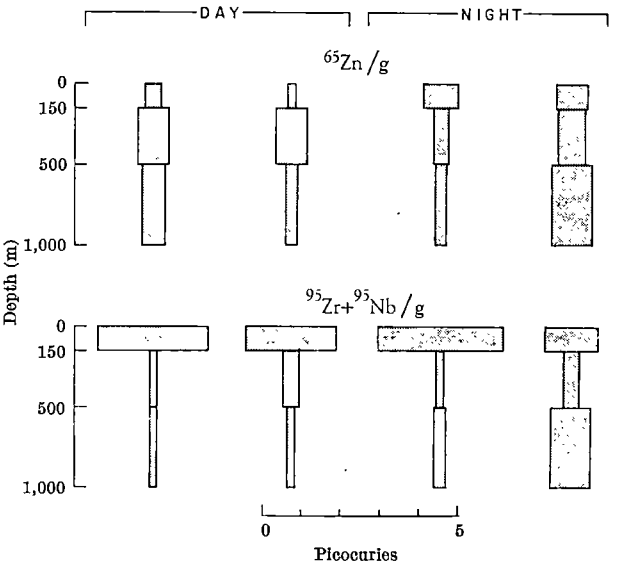


Fig. 1. Zinc-65 and zirconium-95+niobium-95/g/wet weight of pelagic animals collected from 0–150 m, 150–500 m and 500–1,000 m during day and night periods January 7–8, 1963, at 44° 40′ N., 125° 15′ W.

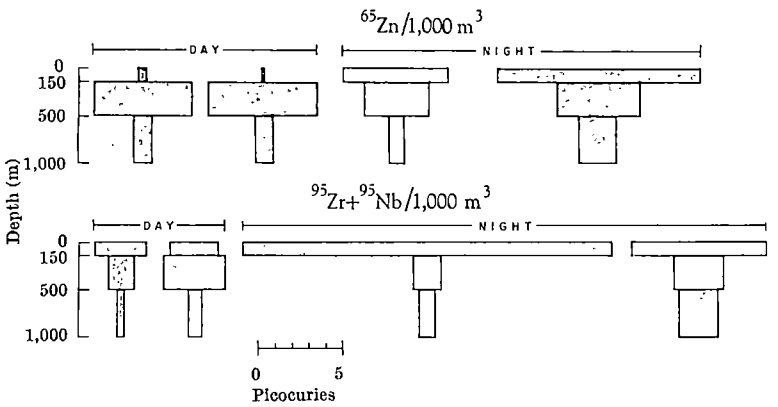


Fig. 2. Total zinc-65 and zirconium-95+niobium-95 activity in the animals filtered from 1,000 m³ of water at the three depth intervals during day and night periods

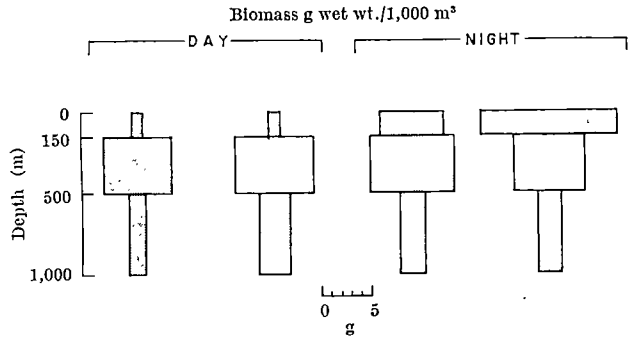


Fig. 3. Wet weight of pelagic animals per 1,000 m³ at the three depth intervals during day and night periods

Vertical distribution of zinc-65 and zirconium-95+niobium-95, as measured in animals collected at three depths, is a function of the biomass per 1,000 m³ of water and radioactivity/g (Fig. 2). Day–night differences in radioactivity occur, particularly in surface waters. Here, concentrations of both isotopes are lower during the daylight period than during darkness. After dark, activities are highest at the surface and are progressively lower at mid-depths and in deep water. During daylight, zinc-65 was highest at mid-depths and lower at the surface than at any other depths, but a similar increase of zirconium-95+niobium-95 at mid-depths during the day is not evident.

Explanations of these observed differences depend on a number of factors. Since biomass changes with time and depth, radioactivity/1,000 m³ of water might be expected to vary accordingly. A rather close correlation did exist between total zinc-65 and biomass (Figs. 2 and 3), indicating that zinc-65 was distributed quite uniformly among animals from the entire 1,000-m column. Histograms for zirconium-95+niobium-95 per 1,000 m³ (Fig. 2) are not similar to either those of biomass or radioactivity per gram. However, they must result from a combination of these two factors, since the values used in preparing Fig. 2 are products of those used in Figs. 1 and 3.

Day–night changes in the vertical distribution of radioactivity (Fig. 2) are correlated with the known diurnal vertical migrations of oceanic animals into surface waters at night. Although a night-time increase in biomass in surface waters is shown (Fig. 3), our results do not provide unequivocal evidence that vertical migrations caused these daily changes in radioactivity. Since both the total amount of radioactivity and the biomass in a column of water 1,000 m deep were larger for night collections than day collections, animals not sampled during the day may have merely avoided the net or migrated below the depth of sampling. That vertical migrations actually occur, however, is evidenced in other studies by the shift of peak catches of oceanic animals such as mesopelagic fishes from surface waters during the night to mid-depths during the day. The relatively high zinc-65 activity at mid-depths during the day and at the surface during the night can also be interpreted as evidence for such migrations (Fig. 1).

In summary, this work shows that radioactivity/g of oceanic animals may demonstrate different patterns of depth distribution for different radioisotopes. Moreover, since zinc-65 is ubiquitous in marine animals off Oregon, vertical migrations of biomass will result in a vertical flux of radiozinc. It is interesting that the largest day–night changes in zinc-65/1,000 m³ occurred across the depth of 150 m, a depth which corresponds approximately to the halocline and a rapid density change that impedes vertical mixing by physical processes in this portion of the Pacific Ocean. Further generalizations based on these limited data are obviously unwarranted, as little is known of temporal or spatial variations of these radionuclides associated with oceanic animals.

This work was supported by U.S. Atomic Energy Commission contracts *AT*(45–1)1726 and *AT*(45–1)1750. Ship operations were sponsored by grants from U.S. National Science Foundation and U.S. Office of Naval Research.

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FOAM FRACTIONATION OF CATIONIC SURFACTANTS: EFFECT OF INORGANIC ACIDS AND BASES

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FOAM fractionation has been utilized for the transfer of organic solutes which by themselves have little foaming ability, for the purification of surface-active agents, for the separation of enzymes, for the ion flotation of radioactive cations from waste streams, for the treatment of secondary sewage effluents to remove non-biodegradable organics, etc. A number of extensive reviews of the process have appeared in the literature[1–3]. Recently, several investigations have been made on the operating, systemic and geometric variables affecting the process. Grieves *et al.*[4–9] have determined the influence of surfactant, feed concentration, temperature, gas-rate, feed-rate, foam height, foam column diameter, liquid solution height, and feed position on the batch and continuous foam fractiona-

tion of anionic and cationic surfactants. Other investigations along these lines have been conducted by Brunner and Lemlich[10], by Kishimoto[11] and by Kevorkian[12].

The information available concerning the influence of strong inorganic electrolytes on the foam fractionation of surfactants from aqueous solution is very limited. Investigations[13,14] have shown that the isoelectric point is the optimum *p*H for foaming amino-acid and protein solutions. Sengupta and Pipes[15] have investigated the influence of a number of electrolytes on the continuous foam separation of alkyl benzene sulphonate, an anionic surfactant, while the influence of various salts on the foaminess of sodium lauryl sulphate solutions has been determined[16,17]. No work of this nature has been reported on cationic agents. The object of the work recorded here is the establishment of the effect of strong inorganic acids and bases on the batch foam fractionation of the ethylhexadecyldimethylammonium bromide–water system (EHDA-Br). The responses of the removal ratio and of the lesser coefficient of fractionation to varying concentrations of hydrochloric acid, sulphuric acid, sodium hydroxide and calcium hydroxide are presented together with comparison runs utilizing sodium chloride and sodium sulphate. The effects of sulphuric acid on an additional feed concentration of EHDA-Br and on a single feed concentration of hexadecylpyridinium chloride (HPy-Cl) are included.

All the experiments were carried out in a cylindrical column, 105 cm high and 10 cm diam., made of 'Lucite'. High-purity nitrogen was saturated with water, metered with a calibrated rotameter, and passed through twin, sintered glass diffusers of 50μ porosity. In each experiment, 2,000 ml. of the feed solution of EHDA-Br (together with an acid, base or salt) in distilled water were placed in the column. Nitrogen bubbles were dispersed through the solution for a period of 15 min with continuous foam removal at a port located 74·5 cm above the feed solution level. A feed concentration of 125 mg/l. EHDA-Br ($3{\cdot}30 \times 10^{-4}$ molar) was used in most of the experiments, employing a nitrogen rate of 3,700 ml./min (at 0° C and 1 atm.). Additional experiments utilized 87·5 mg/l. ($2{\cdot}31 \times 10^{-4}$ molar) feed solutions of EHDA-Br with a nitrogen rate of 4,950 ml./min and 113 mg/l. ($3{\cdot}30 \times 10^{-4}$ molar) feed solutions of HPy-Cl with a nitrogen rate of 3,700 ml./min. Prior to each run, carefully measured quantities of hydrochloric acid, sulphuric acid, sodium hydroxide, calcium hydroxide, sodium chloride or sodium sulphate were added to the surfactant feed solution, and the *p*H measured with a Beckman *p*H meter. The *p*H of the distilled water was 6·6 and the *p*H of pure 125 mg/l. EHDA-Br solutions was 6·1. This would seem to indicate that a small amount of hydrolysis of the quaternary ammonium ions occurred. Temperature was maintained at 31·5° C. At the termination of each experiment the residual solution volume was measured, and the concentration of surfactant was determined by a two-phase titration technique[18]. Random analyses of the collapsed foam were conducted for material balance verifications. The method of analysis was accurate to within ± 1·0 mg/l. The *p*H of the residual solution and the *p*H of the collapsed foam were measured.

Results of the experiments with 125 mg/l. feed solutions of EHDA-Br are presented in Figs. 1 and 2, in which the removal ratio, $V_f y_f / V_t x_t$, and the lesser coefficient of fractionation, x_r/x_t, are related to *p*H. V_f and V_t are the volumes (ml.) of the collapsed foam and feed solution, respectively, and y_f, x_r and x_t are the concentrations of EHDA-Br in the collapsed foam, residual solution, and feed solution, respectively. For maximum efficiency, it is desirable to minimize x_r/x_t while maintaining $V_f y_f / V_t x_t$ at a large value. Maximization of $V_f y_f / V_t x_t$ generally signifies an excessively large and dilute foam stream. For each experiment, the following material balances may be written:

$$V_t = V_r + V_f \qquad (1)$$

$$x_t V_t = x_r V_r + y_f V_f \qquad (2)$$

V_r is the volume (ml.) of the residual solution. V_t was held constant at 2,000 ml. For given x_t and V_t, y_f may be computed from experimental values of x_r and V_r, using equations (1) and (2).

From Fig. 1 it may be observed that the addition of sulphuric acid causes a rapid increase in the removal ratio, reaching a maximum at *p*H = 3·3 followed by a sharp decline. The behaviour with hydrochloric acid is similar except that the increase begins more slowly (as the *p*H is decreased from 6·1) and reaches a peak at *p*H = 2·7. The different behaviour of the two acids would be more pronounced if $p\mathrm{Cl}^-$ and $p\mathrm{SO}_4^{2-}$ were plotted on the abscissa instead of *p*H. In this case, the sulphuric acid curve would be shifted to 0·3 units to the *right* indicating a maximum in the removal ratio at $p\mathrm{SO}_4^{2-} = 3{\cdot}6$. The effect of the addition of strong bases on $V_f y_f / V_t x_t$ is not pronounced. For sodium hydroxide the removal ratio on the average is virtually identical to that of a pure EHDA-Br solution. For calcium hydroxide there appears to be a slight increase in the removal ratio as the *p*H is increased. The variation in the two points between *p*H = 7·0 and *p*H = 8·0 seems to be due to experimental scatter. For experiments with the *p*H of the feed solution

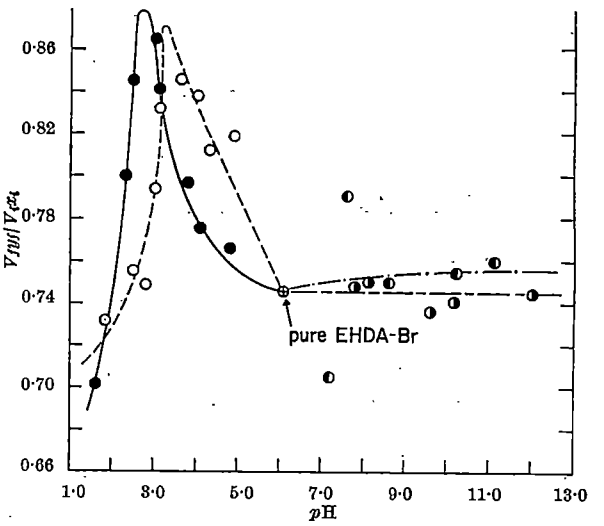


Fig. 1. Relationships between $V_f y_f / V_t x_t$ and *p*H for 125 mg/l. EHDA-Br solutions. ● –, Hydrochloric acid; ○ – – –, sulphuric acid; ◑ – – –, sodium hydroxide; ◐ – · –, calcium hydroxide

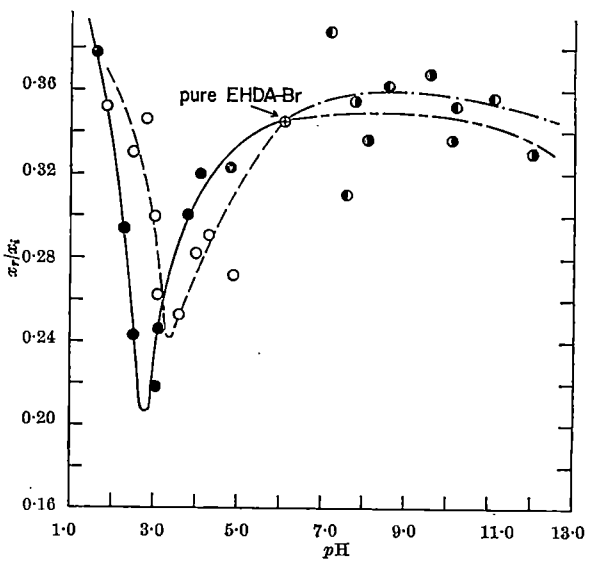


Fig. 2. Relationships between x_r/x_t and *p*H for 125 mg/l. EHDA-Br solutions. Symbols as for Fig. 1

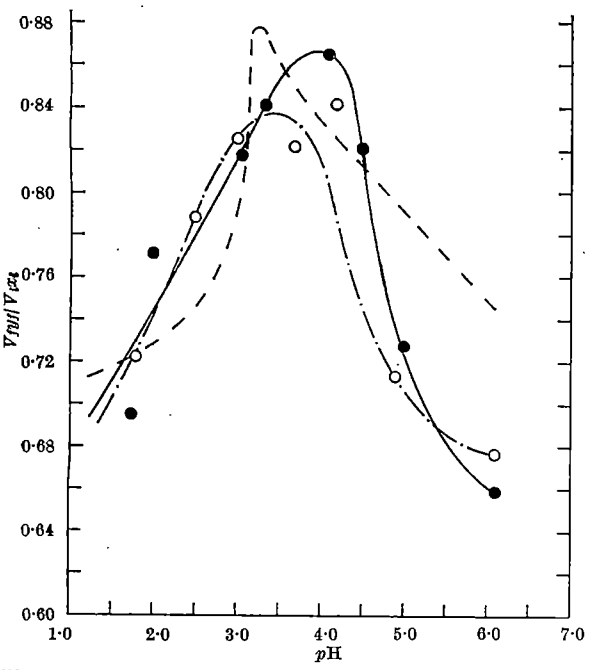


Fig. 3. Relationships between $Vfyf/ViXi$ and pH with sulphuric acid. ○—·—, HPy-Cl, 113 mg/l.; ●—, EHDA-Br, 87·5 mg/l.; ———, EHDA-Br, 125 mg/l.

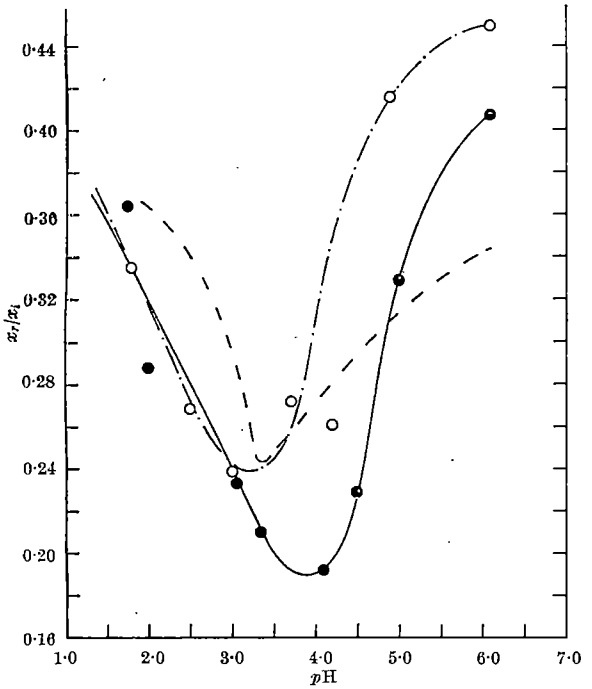


Fig. 4. Relationships between x_r/x_i and pH with sulphuric acid. Symbols as for Fig. 3

between 7·5 and 9·5, the pH of the residual solution and the pH of the collapsed foam were both lower than the feed. When the pH of the feed was between 6·1 and 7·0, the opposite tendency was observed. For all other feed pH values, no changes were noted. Part of the variation may have been due to the presence of low concentrations of carbon dioxide in the distilled water or to a slight amount of hydrolysis.

Fig. 2 indicates that the concentration of the residual solution, and thus the lesser coefficient of fractionation, behave analogously to the total removal at pH < 6·0. x_r/x_i passes through minima at virtually the same points where $V_f y_f / V_i x_i$ is maximized. Two investigations[5,7] have shown previously that when considering such operating variables as gas-rate and feed-rate, the residual concentration and removal ratio do not increase and decrease analogously. Considering values of x_r/x_i for pH > 7·0, no trend can be seen. Again the effect of pH variation on the basic side is small.

Figs. 3 and 4 present the effect of pH depression by sulphuric acid on the foam fractionation of 87·5 mg/l. EHDA-Br and of 113 mg/l. HPy-Cl solutions. The curves for 125 mg/l. EHDA-Br solutions are included for comparison. From Fig. 3 it should be noted that for the more dilute EHDA-Br solutions, a lower concentration of sulphuric acid is necessary to produce the maximum, and the effect of sulphuric acid on the removal ratio is more pronounced. The influence of sulphuric acid on HPy-Cl solutions is similar to that on EHDA-Br. The behaviour of the lesser coefficient is again analogous to that of the removal ratio. For 125 mg/l. EHDA-Br and 113 mg/l. HPy-Cl the minima are at approximately the same pH (3·2–3·3) and yield similar values of x_r/x_i at the minima (0·24–0·25). However, the shapes of the curves are quite different.

A few comparison runs were conducted with sodium chloride and sodium sulphate. Results of the experiments are presented in Table 1, together with values for hydrochloric acid and sulphuric acid (smoothed) at the same Cl^- and SO_4^{2-} concentrations. With both salts the same general trend may be observed as with the corresponding acids, with values of $V_f y_f / V_i x_i$ passing through maxima and values of x_r/x_i passing through minima. The maximum (and minimum) for sodium chloride is at a higher anion concentration than the maximum for sodium sulphate. Comparing the relative effects of H^+ and Na^+, the values of x_r/x_i for sodium sulphate and sulphuric acid are practically identical, while the removal ratio for sulphuric acid is greater at the two lower SO_4^{2-} concentrations and less at the highest concentration. The removal ratio behaves in a similar manner for sodium chloride and hydrochloric acid except that the differences are greater. In addition, there is a considerable difference in the values of x_r/x_i between the acid and the salt. A final comparison between the effects of various anions may be made by noting the experiments with sodium hydroxide listed in Table 1. Contrasted to Cl^- and SO_4^{2-}, the effect of OH^- is minimal.

Table 1. COMPARISONS BETWEEN SALTS, ACIDS, AND BASES FOR 125 MG/I. EHDA-Br SOLUTIONS

	$V_f y_f / V_i x_i$		x_r/x_i	$V_f y_f / V_i x_i$		x_r/x_i
pCl⁻		NaCl			HCl	
3·46	0·759		0·336	0·807		0·278
2·75	0·852		0·224	0·878		0·204
2·07	0·840		0·237	0·751		0·327
pSO₄²⁻		Na_2SO_4			H_2SO_4	
4·48	0·804		0·284	0·824		0·280
4·00	0·827		0·256	0·848		0·256
2·81	0·756		0·330	0·751		0·336
pOH		NaOH			H_2O	
4·40	0·736		0·368	0·745		0·344
3·90	0·741		0·336			
2·00	0·745		0·330			

The separation obtained in a foam fractionation process depends on two interrelated factors: the tendency of the surfactant molecules to be adsorbed at the gas–liquid interfaces of the bubbles as they rise through the bulk solution; the stability (persistence) and drainage of the foam phase rising above the foam-solution interface. In a batch foaming process, in addition to the single transfer stage in the bulk solution multiple stages exist in the rising column of foam, and each cross-section of foam is enriched continually by the drainage of concentrated liquid from the foam layers above the particular cross-section. The bulk solution concentration, the concentration of surfactant in the surface layers at the gas interfaces in the foam, and the concentration of entrained (draining) liquid in the foam all continually change with time. At any time, the latter two concentrations vary with height in the rising column of foam.

Most of the effects which were observed may be explained on the basis of the anions (Cl^-, SO_4^{2-}) added to the surfactant solutions rather than on the basis of *p*H. Quaternary ammonium salts are almost completely dissociated in solution and only a slight amount of hydrolysis occurs to form quaternary ammonium bases. The adsorption of surfactant ions at a gas-liquid interface involves the formation of a diffuse, electrical double layer consisting of a unilayer of surfactant ions in the surface and a Gouy region below it which is poorer in solute. The resulting potential which is set up as soon as the first surfactant ions are adsorbed acts as an activation barrier, which is reduced by an increase in the ionic strength of the solution[19]. The decrease of the surface tension of surfactant solutions on the addition of salts validates this description[16,17,20]. In the case of cationic surfactants, the nature and valency of the anions are significant factors and the initial increase in $V_f y_f / V_i x_i$ and decrease in x_r/x_i as the *p*H is lowered below 6·1 may be explained by the greater adsorption of surfactant ions and the resulting more voluminous and stable foam. In this regard, SO_4^{2-} is more effective than Cl^-, while OH^- (*p*H elevated) has little influence. From Table 1 it may be observed that there is some difference between sodium sulphate and sulphuric acid and a significant difference between sodium chloride and hydrochloric acid, indicating that changes in cations do have some effect. Similar behaviour has been noted for anionic surfactants[21] and the hydrolysis of impurities may have brought it about. As the *p*H is lowered below the values (3·3 and 2·7) corresponding to the maxima and minima with sulphuric acid and hydrochloric acid, respectively, the decrease in $V_f y_f / V_i x_i$ and increase in x_r/x_i may have been caused by the promotion of foam drainage by the electrolytes present. Foam drainage increases as the electrolyte concentration is increased due to the ability of the liquid lamellæ to thin to a greater extent as the ionic concentration is raised[22]. It is possible that this effect was shielded by the increase in the adsorption of surfactant ions at higher values of *p*H (6·1 → 3·5); then as the *p*H was further lowered the increase in adsorption levelled off and the increased foam drainage due to the electrolyte became the significant factor. Maxima in relations between foam stability and electrolyte concentration have been observed previously for anionic surfactants[17,21].

This investigation was carried out under the sponsorship of the Commission on Environmental Hygiene of the Armed Forces Epidemiological Board and was supported in part by the U.S. Army Medical Research and Development Command, Department of the Army, under research contract *DA–49–193–MD–2629*.

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A GAS-TIGHT GROWTH CHAMBER FOR INVESTIGATING GASEOUS NITROGEN CHANGES IN THE SOIL : PLANT : ATMOSPHERE SYSTEM

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LOSSES of nitrogen from the soil : plant system have never been measured directly, and gains have almost always been estimated from conventional nitrogen balance experiments. Quantitative knowledge of changes in nitrogen occurring in an enclosed atmosphere surrounding the system should not only allow satisfactory nitrogen balances to be struck, thus resolving Allison's[1] enigma, but also permit the kinetics of these changes to be studied, including the effects of such factors as temperature, soil moisture, and soil type. The use of nitrogen-15 would increase the power of such a technique; for example, by cross-labelling nitrogen fertilizer and nitrogen gas, it should be possible to identify simultaneous gains and losses instead of measuring net changes as has been invariably done in the past. This article describes a gas-tight assembly which has been successfully used in this laboratory for preliminary balance studies on normal soil : plant systems.

It is important to stress the need for a strictly closed system of small volume that will allow the prolonged growth of plants in soil under conditions as natural as possible. If the system is not strictly closed, changes due to leakage may result, especially over long periods; a large volume (relative to the size of the plants) makes the detection of small changes difficult, and abnormal or restricted conditions limit the applicability of the results obtained. Systems used by previous workers, though adequate for particular purposes, are thus defective from this point of view. For example, closed systems have been used for studies of gaseous losses of nitrogen from soil alone[2,3], but extrapolation of such results is limited. Wilson[4] and other workers[5–7] have used closed systems to study fixation, but normal conditions were not ensured and no attempt was made to follow gaseous changes. De and Mandal[8] measured the decrease in atmospheric nitrogen to estimate fixation by algae in flooded rice soils; however, their system did not enclose the whole plant. Some physiological experiments have involved closed systems[9], but these were short-term investigations lasting only a few hours. Normal plants labelled with carbon-14 have been produced in closed systems; the assembly described by Jenkinson[10] is a typical example. Here the volume was kept large to reduce the effects of the plants; small atmospheric changes would thus be undetectable. Moreover, the system was not strictly closed, and used an

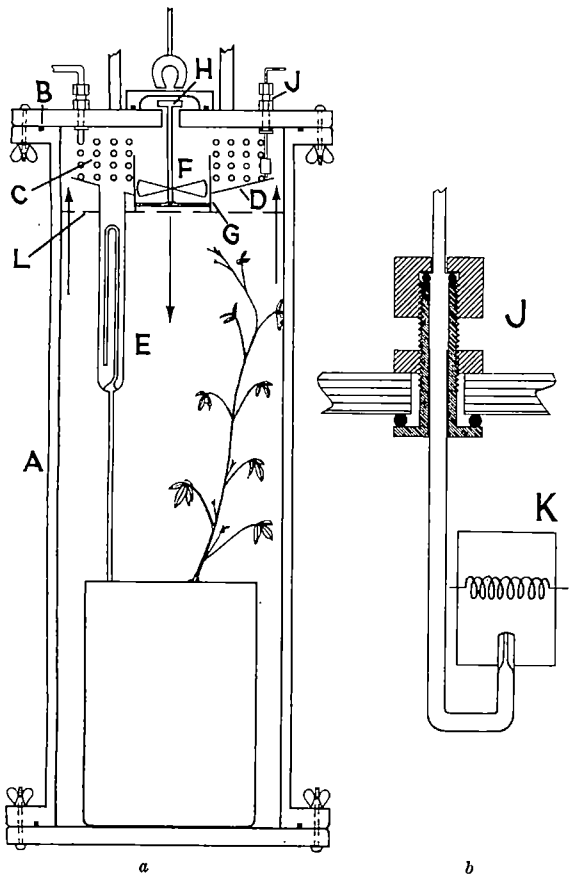


Fig. 1. *a*, Diagram of gas-tight chamber allowing prolonged growth of enclosed plants. *A*, 'Perspex' cylinder; *B*, latex gasket; *C*, cooling coils; *D*, water catcher; *E*, water siphon; *F*, fans; *G*, fan cylinder; *H*, soft iron; *J*, hydrogen microburner; *L*, metal gauze
b, Enlargement of hydrogen microburner *J*, showing nickel enclosure *K*

artificial growth medium. Thus it seems that no assembly suitable for the following gaseous nitrogen changes in a soil : plant : atmosphere system has been previously described.

An extended period of growth in a gas-tight chamber presents several difficulties. First, some form of air conditioning inside the chamber is desirable to ensure suitable temperature and humidity, and to return transpired water to the soil. Secondly, removal of oxygen produced by photosynthesis is necessary if initial gas pressure and composition are to be maintained. Expandable rubber or plastic bags would keep the total pressure at 1 atm. but would allow an increase in pO_2; furthermore, such bags permit diffusion of gases and are thus unsatisfactory. Thirdly, an adequate supply of carbon dioxide must be maintained. Finally, a sufficiently accurate and versatile method of gas analysis is necessary.

Prototype Assembly. A 'Perspex' cylinder *A* (Fig. 1*a*), 50 cm long and 15 cm internal diameter with a wall thickness of 6 mm, is fitted with a flange at either end, 2·5 cm wide and 12 mm thick. The outer surface of each flange contains a machined rectangular groove in which a gasket seal *B* is seated; this consists of a length of 3 mm dia. latex. A circular end-plate is bolted to each flange by six wing nuts.

The top end-plate supports condensing coils *C* and contains gas inlet ports, a sampling port, and electrical connexions. Water vapour condenses on the coils, which are copper tubes of 6 mm diameter, silver-plated, and cooled by water at 5°–10° C circulated continuously from a refrigerated bath. Condensed water drips on to a shallow funnel-shaped annulus *D*, also plated, and thence flows into a glass siphon *E*. Use of this siphon avoids discharge of cold condensate directly to the soil and surface roots; by altering its capacity, different cycles of wetting and drying can be imposed on the soil.

Air circulation is provided by a fan *F*, mounted centrally in the upper part of the assembly within a short cylinder *G*. The fan axis, rotating on two bearings, is connected to a short length of soft iron *H* so that magnetic coupling can be used. Air movement is sufficient to mix the atmosphere thoroughly and ensure the efficient condensation of transpired water vapour; however, if close control of temperature were desired, it would be necessary to provide more efficient circulation since air near the top of the chamber is cycled more rapidly over the coils than air around the plant. Inclusion of a simple nichrome heating wire (not shown) allows some control of temperature, and humidity can be varied by changing the temperature of the cooling water.

Excess oxygen is removed by burning pure hydrogen at the end of a capillary in a microburner *J*, a larger drawing of which is shown in Fig. 1*b*, which also illustrates gasket seals used for the various entry ports and electrical connexions. When the pressure in the system rises a nichrome ignition spiral is switched on, hydrogen admitted, and excess oxygen removed in the small flame. Heat produced is dissipated by a thin nickel enclosure *K*. This device could be automated by providing a polarographic oxygen detector[11] to operate the ignition spiral and the hydrogen inlet valve simultaneously through an appropriate control system.

Pure carbon dioxide from a cylinder is admitted through a calibrated capillary leak for a specified period each hour, injection being automatically controlled by a solenoid valve connected to a time switch. The amount injected is somewhat less than the anticipated plant requirement, and is easily adjusted if gas analysis reveals an excess. It may prove possible to control the level of carbon dioxide automatically by using a pCO_2 electrode[11] as the sensing element in a control system.

A metal gauze screen *L* is necessary to prevent plants from entering the upper part of the chamber.

Light for a single chamber is conveniently supplied by short, vertical fluorescent tubes; for a number of chambers long horizontal tubes are more convenient. The addition of incandescent bulbs improves the quality of the light. It is necessary to ensure adequate ventilation of the lighting unit to prevent overheating the chambers; light intensities of 2,000 ft.-candles can be obtained without difficulty.

Mass Spectrometric Analysis of Gaseous Changes. The most suitable method of gas analysis is mass spectrometry (for which reliable methods are available[12–14]), since this provides an accuracy, versatility and convenience that other methods cannot match.

To measure small changes mass spectrometrically it is necessary to use a comparative method in which samples are compared with a standard mixture on a double-inlet mass spectrometer. It is convenient to use as standard a sample of the initial atmosphere.

If unit pressure of component j of a mixture is admitted to the mass spectrometer, the height of the largest resulting peak is by definition the (absolute) sensitivity s_j of the component; the height of the peak at mass i is $c_{ij}s_j$, where the set of numbers c_{ij} (i ranging over the peaks of the spectrum) is by definition the cracking pattern of the component. If unit pressure of the mixture gives a peak i of height H_i, then, subject to the usual assumptions[12]:

$$\sum_j c_{ij}s_j p_j = H_i \qquad (1)$$

where p_j is the partial pressure of component j.

It is convenient to select one component of the mixture, s, as an internal reference standard. Assuming that only this component contributes to the peak r:

$$c_{rs}s_s p_s = H_r$$

Defining relative sensitivities r_j and relative partial pressures x_j by the relations:

$$r_j = s_j/s_s$$

$$x_j = p_j/p_s$$

equation (1) becomes, on division by $s_s p_s$:

$$\sum_j c_{ij} r_j x_j = c_{rs} H_i/H_r \qquad (2)$$

The unit of pressure chosen is arbitrary, and small changes do not affect the cracking patterns and relative sensitivities. Hence, if the standard mixture taken from the chamber initially is admitted to one inlet vessel of the mass spectrometer and a later sample to the other, the pressures being adjusted to give approximately equal heights at peak r, we have from equation (2):

$$\sum_j c_{ij} r_j (x'_j - x_j) = c_{rs}(H'_i/H'_r - H_i/H_r)$$

where the prime refers to the later sample. If we let:

$$\Delta H_i = H'_i - H_i$$

this becomes:

$$\sum_j c_{ij} r_j (x_j - x_j) = c_{rs}(\Delta H_i - \Delta H_r H_i/H_r)/H' \qquad (3)$$

The change in the quantity of component j in the chamber is given by:

$$\Delta Q_j = M(x'_j - x_j)$$

where M is the quantity of component s present, assumed strictly constant.

The terms c_{ij}, r_j, and H_i/H_r are constants and need be measured only once. Hence, to analyse a sample, it is only necessary to measure H'_r and ΔH_r at peak r, and ΔH_i at enough peaks to allow the ΔQ_j of interest to be found; a complete analysis need not be made, which is an important advantage if there are many samples. The method also avoids the use of specially blended gas mixtures. The sensitivity is such that changes in a component in the 8-l. chamber of 0·1–0·5 per cent, or 1–5 μmol. (whichever is larger), can be detected.

The method outlined here can also be used for measuring large changes in a component if high accuracy is not desired; if high accuracy is necessary the use of a specially blended gas mixture in place of the initial atmosphere sample will allow the calculation of x'_j from equation (3).

For the experiments on fixation presented here the 20 peak of argon was used as an internal reference. A known quantity of krypton added to the chamber (which contained 60 per cent argon) enabled M to be calculated. It was initially intended to use the krypton as internal reference, but results calculated on this basis were rather variable, probably because of effects in the mass spectrometer inlet system arising from the relatively high molecular weight of this gas. The 20 peak of argon was used in preference to the 40 peak, which was too large for convenience. The change in excess nitrogen-15 in the atmosphere, $\Delta XS^{15}N$, was calculated from the formula:

$$\Delta XS^{15}N = \Delta Q_{^{14}N^{15}N} - \Delta Q_{^{14}N_2}/135 + 2\Delta Q_{^{15}N_2}$$

with the quantities on the right expressed in millimoles and $\Delta XS^{15}N$ in milli-equivalents.

Trial Experiments. To test the chamber and associated techniques in establishing a nitrogen balance, four experiments were run. Three of these (experiments 1, 2 and 3) used pots each containing two leguminous and two grass plants, labelled nitrogen gas being added to the atmosphere, while experiment 4 used four grass plants fertilized with labelled ammonium sulphate. Excess nitrogen-15 in the atmospheres was monitored by gas analysis, and at the end of the experiments excess nitrogen-15 in plants and soils was measured by Kjeldahl and nitrogen-15 analysis.

The plants used were *Phaseolus atropurpureus* var. 'Sirato', a legume which has shown promise as a pasture plant in Queensland, and *Chloris gayana* (Rhodes grass). Each pot contained 2 kg sandy loam soil of *p*H 5·6, any nutrient deficiencies (except that of nitrogen) being corrected by addition of fertilizer. Soil temperatures and matrix suctions during the experiments were 27° C and 0·1 bar for experiment 1, and 32° C and 0·2 bar for experiments 2, 3 and 4.

After an initial period in the glasshouse one pot was taken and placed on the bottom end-plate of the chamber, legume growth being cut back. The assembly was then placed over the pot and the unit sealed by tightening the wing nuts. An artificial atmosphere was introduced by flushing the system with commercial argon, evacuating to 0·75 atm., and adding oxygen to 1 atm. from an anæsthetic bag. In experiments 1, 2 and 3 labelled nitrogen gas was added in the same way. Finally, a known quantity of krypton (approximately 4 m.mol.) was added by displacement with water. Labelled nitrogen gas was prepared by reaction of alkaline hypobromite with an enriched ammonium salt and purified by passage through a liquid nitrogen trap; no peaks at masses 31 or 45 were detected on the mass spectrometer, indicating that no oxides of nitrogen were present.

During the experiments, soil temperature was measured with a thermometer and air temperature with a shielded thermistor; humidity was followed with a thermocouple hygrometer. Pressure was measured by movement of a metal diaphragm, as mercury vapour was found to be toxic to the legume, and other manometric fluids would permit diffusion of gases. All plants appeared healthy at the end of the experiments, although some of the older legume leaves were shed soon after the plants were introduced into the chamber. The grass in experiments 1, 2 and 3 made little growth in the nitrogen deficient soil, but that in experiment 4, which had received nitrogen fertilizer, soon filled the chamber.

Experiments 1, 2 and 3 were run for 26, 23 and 13 days respectively. The atmospheres contained 18, 29 and 16 per cent nitrogen with enrichments of 4·1, 3·4 and 7·1 atom per cent excess nitrogen-15. The rates of fixation of total nitrogen, calculated from the gas analysis data for nitrogen-15, were 3·2, 1·5 and 2·2 mg/pot/day. Fixation of unlabelled nitrogen could not be followed directly because air diffused against a positive pressure into rubber tubing connecting the cylinder of carbon dioxide to the systems; it was thus admitted with the carbon dioxide. Subsequent tests have shown other sources of leakage to be negligible, at least for periods of up to a month.

The excess nitrogen-15 in the atmosphere at the end of the experiments was estimated by regression analysis of the gas analysis data, since these appeared to lie close to a straight line. Total nitrogen was determined on dried (60° C) plant material and soil using the method of Bremner and Shaw[15], the ammonia being distilled into 30 ml. of 5 per cent boric acid. An aliquot of this was taken for electrometric titration with sulphuric acid and the remainder concentrated by redistillation into hydrochloric acid for isotope ratio determination. The results are presented in Table 1.

It is seen that the recovery of nitrogen-15 is close to 100 per cent. However, the 99 per cent fiducial limits on the atmospheric decreases in nitrogen-15 indicate that, for experiments 2 and 3, these decreases are significantly different from the increases in the soil : plant system. The cause of this slight discrepancy is not known. It is possible that adsorption of gases within the soil is responsible;

Table 1. Comparison of Excess Nitrogen-15 Removed from the Atmosphere with that found in the Soil : Plant System

Exp.	Decrease of excess ^{15}N in the atmosphere (m.equiv.)	Increase of excess ^{15}N in the soil : plant system (m.equiv.)	Recovery of total excess ^{15}N in the system (%)
1	0·245 ± 0·026	0·235	99·8
2	0·084 ± 0·016	0·067	99·8
3	0·146 ± 0·021	0·121	99·6

The 99 per cent fiducial limits from regression analysis are given for the decrease of excess ^{15}N in the atmosphere. The recovery of total excess ^{15}N is based on a determination of that originally present using a synthetic gas mixture for comparison. The decreases in experiments 1, 2 and 3 were 4·9, 1·2 and 2·2 per cent respectively of the excess ^{15}N originally present.

alternatively, there may be a systematic error in the gas analysis. Errors in the recovery of nitrogen-15 from the soil : plant component are considered to be too small to cause the difference. It is clear, however, that the detection of changes of a few per cent in atmospheric nitrogen-15 is considerably more accurate than the detection of similar changes in the soil : plant component where errors in the determination of total nitrogen make the detection of small proportional changes difficult. There is no reason why a similar accuracy should not be attained for unlabelled nitrogen, provided the system is free from leaks.

The partition of nitrogen-15 fixed is shown in Table 2. These results are interesting because of their variability, which indicates that under different conditions, as in these experiments, a considerable range of partition is possible. In particular, the amount of fixed nitrogen in the grass in exp. 1 is negligible, but in exp. 2 and 3 it is of practical significance. In exp. 3, for example, the total uptake of fixed nitrogen by the grass would correspond to approximately 20 lb./acre/yr. The large proportion of fixed nitrogen-15 in the soil in exp. 2 is thought to be a real effect and not largely due to poor separation of roots from soil. Further work in this direction using controlled conditions should prove fruitful.

Table 2. THE PARTITION OF FIXED EXCESS NITROGEN-15

Exp.	Percentage of total ^{15}N fixed in		
	Legume	Grass	Soil
1	91	0·07	9
2	67	1·2	32
3	84	2·3	14

Exp. 4 was run for 34 days in an atmosphere of 7 per cent unlabelled nitrogen, 10 per cent oxygen, 82 per cent argon, and 1 per cent krypton. The grass had received 12 m.equiv. (approximately 200 lb./acre) unlabelled nitrogen as ammonium nitrate one week before the pot was placed in the chamber. A further application of 12 m.equiv. labelled nitrogen (30 atom per cent excess nitrogen-15) as ammonium sulphate was placed in the water siphon; this was automatically applied the day after the experiment began. Peaks at masses 17, 29, 30, 31, 45 and 46 (corresponding to ammonia, nitrogen and oxides of nitrogen) were monitored. No change was detected at any of these peaks. From the data it was estimated that any loss of nitrogen-15 to the atmosphere which may have occurred in these forms was less than 5 μequiv. or approximately 0·1 per cent of that applied. The peak at mass 30 indicated that there was no loss of unlabelled nitrogen as N_2O, NO, or NO_2. These results serve to establish a lower limit to the sensitivity of the technique. It must be remembered, however, that any NH_3 or NO_2 present in the atmosphere would probably be absorbed in condensed water and returned to the soil.

Excess nitrogen-15 in the soil : plant component was determined as in the other experiments, tops, roots and soil being analysed separately. The recovery of excess nitrogen-15 was 101 per cent of that applied.

Discussion. The experiments described here show that the chamber can be successfully used to strike a nitrogen balance, even where changes in nitrogen are small. It may also find application in work not directly concerned with nitrogen balance. For example, McAuliffe *et al.*[16] have applied labelled nitrogen to legumes grown with grass and used the relative enrichments resulting as an indication of nitrogen fixation by the legumes. The chamber could be used to test the validity of the assumptions underlying this technique. Again, simultaneous fixation of carbon and nitrogen could be followed from day to day under controlled conditions. The chamber may also find uses, perhaps in an adapted form, in microbiological and plant-physiological work—for example, in studying the effects of artificial atmospheres. An important advantage of the chamber is its low cost, which allows ready replication.

We thank Mr. L. Cowper for constructing the chamber, and Dr. A. W. Moore for advice. Mr. D. J. Oxenham gave further assistance, and Miss Carolyn Patterson helped with analyses.

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FLUTTERING FLIGHT MECHANISMS IN INSECTS AND BIRDS

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RECENT analysis[1–3] has demonstrated that the fluttering lift behaviour of insects and birds can be explained by a non-linear force balance accounting for superposition of the forces derived from body acceleration, aerodynamic lift, gravitation and air acceleration, such that the model takes into detailed consideration all the following factors: (1) as in Nature, the present theory requires a true flapping motion about a point hinged to the body, in contrast to the non-flapping or wing-pitching model of Holst and Küchemann[4]; (2) an instantaneous force balance is used throughout the analysis so that it is unnecessary either to neglect portions of the stroke or to take time average forces, as in the approaches of Walker[5] and Osborne[6]; (3) unlike previous models, the present analysis permits the body to oscillate freely as observed experimentally for the locust by Jensen[7] and photographed during model ornithopter flight by Holst and Küchemann[4]; (4) an aerodynamic lift force is included by a term proportional to the square of the relative body-wing velocity during flutter, a form analogous to that proposed for insects by Osborne[6] and of the square-form conventionally used in experimental aerodynamics for systems in Helmholtz flow; (5) the lift coefficient C_L in the aerodynamic lift term is permitted to have different values on the downstroke (C_L^D) and upstroke (C_L^U) as postulated by most workers in the field of insect and bird flight[4–10], the largest value of C_L observed in laboratory experiments[11] on plane aerofoils in Helmholtz flow being

2·14; (6) the present theory accounts for the experimental observation[6–10] that the wing motion (wave-form) is non-uniform, such that the ratio of the time of lowering to the time of raising, or the duration factor k, may be in the range 1·0–1·8 depending on the particular insect or bird; (7) the important air acceleration term, not present in earlier models, arises because the wings, when acting as paddles, press on the ambient air with a force that varies from one part of the cycle to the next such that the mass of displaced air, multiplied by its acceleration, will generate a non-uniform reaction force on the wings—the nature of such displaced fluid forces in the analogous case of accelerating particle-fluid systems has already been discussed[12–14].

In the present case of flapping flight the air acceleration term has been formulated, as in particle-fluid systems, by requiring the mass of displaced air to carry along with it a virtual mass which is a fraction $\varkappa$ of the displaced air, the motion of this mass of accelerated air closely following the non-uniform trajectory of the wings, with the possible exception of a difference in phase ε. It is the uneven pressure of the wings on the ambient atmosphere, arising from the periodic but non-sinusoidal wing motion, which results in the non-uniform reaction force of the displaced air on the wings, the time integral of this net force over a complete wing cycle being cumulative over a succession of wing beats, as discussed later. The fluttering-lift equation, incorporating all of the foregoing phenomena, is a complicated two-dimensional integro-differential equation with time-varying coefficients that requires a high-speed digital computer for its numerical solution—such complexity indicates why insect and bird flight has proved so intractable to simplified approaches in the past[4–6], a situation which no doubt prompted Pringle[15] to summarize the status of insect flight in 1957 as follows: "It must be stated that there is at present no simplified picture of the mechanism of insect flight which can be used to derive a mathematical theory of the way in which lift and thrust are generated by wing motion".

That most of the force induced by wing flutter is used to overcome gravity and a much smaller percentage is needed to maintain uniform horizontal flight can be demonstrated fairly generally using available experimental flight data for insects[6–10]; for example, the results of Weis-Fogh[8,9] and Jensen[7,8] on the desert locust reveal the equivalent body diameter to be about 1 cm, the average steady flight velocity as 350 cm/sec with a measured drag coefficient on the wings of less than 0·1 for observed angles of attack in the conventional aerodynamic sense of 5°–10°, while the drag coefficient for the body at Reynolds numbers of 2,500 would be[16] about 0·4, so that the total body-wing form drag in horizontal flight using conventional aerodynamic gliding concepts is estimated to be less than 50 g cm/sec^2, as compared with the gravitational force of 1,960 g cm/sec^2 on a locust weighing 2 g. Similarly, the wing lift coefficient was found[7–9] to be less than 0·8 with angles of attack of 5°–10°, so that the maximum lift that could be generated by any forward horizontal gliding in the conventional aerodynamic sense is 320 g cm/sec^2; if the flight velocity were reduced by a half to 175 cm/sec the square law would reduce the lift to one-quarter its value, or 80 g cm/sec^2. Thus the frictional drag in horizontal flight is theoretically about 2–3 per cent of the gravitational force, as also found experimentally by Weis-Fogh[9] and Jensen[7], while the vertical lift generated by any forward gliding motion is probably less than 16 per cent of the downward gravitational pull, indicating that the insect is unstable in the conventional aerodynamic sense and also that at least 84 per cent of the lift must be generated by wing flutter. Since most measurements[6–10,17] of wing frequency and stroke angle for insects are probably no more accurate than ± 25 per cent, it seems quite reasonable to neglect lift and drag produced by forward motion in comparison with the overwhelming gravitational pull. Furthermore, it is common observation that many insects, such as the bee, can rise and descend vertically in still air with scarcely any horizontal components to the body motion. Thus the applicability of conventional aerodynamic gliding principles to insect flight must be held in serious doubt since the wings must serve the dual function of forward propulsion against the body form drag as well as vertical stabilization against gravity. From the physical point of view, forward motion by wing flapping in higher-order insects could be produced by giving the wing a time-varying angle of attack and permitting it to flutter with a horizontal component as well as a vertical component—that is, in one type of wing motion the tip might follow an elliptical Lissajous figure such as one might describe with an oar when rowing a boat, while, in another type of wing motion, with wing *twisting*, the trajectory might be that of a figure of eight. In the large-winged insects, such as the locust and moth, wing folding with only slight wing twisting are the most probable causes of forward motion, while in birds the most logical mechanisms for forward propulsion are wing warping and feather twisting, particularly of the primary remiges. However, as discussed here, the force required to maintain uniform horizontal flight against body-wing form drag is less than 3 per cent of the vertical component required to balance gravity in the locust, so that the treatment of the more complicated forward motion will necessitate greater refinements in both the theoretical and experimental techniques than have hitherto been contemplated. Another aspect of insect and bird flight apparently neglected in most treatments is the influence of body contours, particularly in birds and higher-order insects, since if forward propulsion is effected by 'wing-rowing', then the body might be aerodynamically shaped to provide maximum lift with minimum drag, these being problems in common with all conventional aircraft and hydrocraft.

Thus, since gravity is by far the most overwhelming force on a fluttering wing-body system at low forward speeds, the major problem of insect and bird flight is resolved into determining the relative importance in the present theory of the anti-gravity forces generated by the aerodynamic lift term and the air acceleration term in the fluttering lift equation (1) of the first article[1], which is rewritten in the more suitable integral form:

$$u(t) = -(\rho/M)\int_0^t C_L(\tau)\int_0^L W(r)\,\{u(\tau) - r\varphi P_\tau(k, \omega\tau)\cos[\varphi P(k, \omega\tau)]\}.$$
$$|u(\tau) - r\varphi P_\tau(k, \omega\tau)\cos[\varphi P(k, \omega\tau)]|\,\mathrm{d}\tau\mathrm{d}r - gt$$
$$- (\gamma\varphi/M)\int_0^t P_{\tau\tau}(k, \omega\tau + \varepsilon)\cos[\varphi P(k, \omega\tau)]\,\mathrm{d}\tau \qquad (1)$$

The instantaneous body velocity is $u(t)$, the ambient air density ρ being small compared with the body density ρ_L such that the mass of the insect $M \approx (\rho_L + \chi\rho)V \approx (\rho_L - \rho)V$ where V is the volume of the body the coefficient of virtual mass of which is χ. The periodic function $\varphi P(k, \omega\tau)$ simulates the non-uniform wing motion (wave-form) of angular half amplitude φ and overall angular frequency $\omega = 2\pi f$ such that the notation for derivatives is $P_{\tau\tau} = \mathrm{d}P_\tau/\mathrm{d}\tau = \mathrm{d}^2P/\mathrm{d}\tau^2$. The geometry[1–3] of the axially symmetric wings is governed by the chord length $W(r)$ as a function of the radial distance r from the fulcrum, the overall wing-length being L. The lift coefficient $C_L(\tau)$ is included in the time-integral so that it can take on the periodic values of C_L^D and C_L^U on the downstroke ($P_\tau \leqslant 0$) and upstroke ($P_\tau > 0$) respectively[2]. The acceleration factor $\gamma = 8\rho(1 + \varkappa)\varphi S m_2$ is based on the volume of air (plus virtual volume) displaced by the wings, the single wing area $S = \int_0^L W(r)\mathrm{d}r$ and the second wing moment $m_2 = (1/S)\int_0^L r^2 W(r)\mathrm{d}r$ being determined by graphical integration using equivalent two-wing geometries[1–3]. Tran-

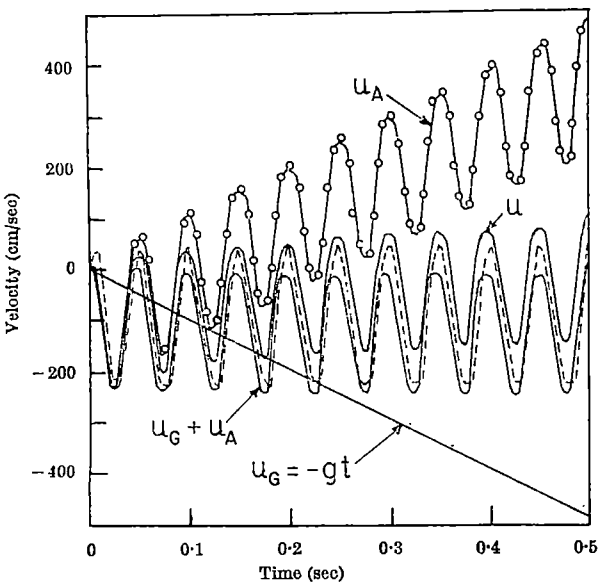


Fig. 1. Velocities u, u_A and u_G for the desert locust at $k = 1{\cdot}50$, the points (O) being U_A from equation (2) with $\mu = 0{\cdot}056$; the broken curve is u_A at $k = 1{\cdot}00$

sient velocity distribution effects, as generalized by a Basset term, have been presumed negligible for the reasons given earlier[1]. Using Stieltjes forms for the time integrals, equation (1) above has been solved numerically[1] on a high-speed digital computer (IBM 7090) starting with $u(0) = 0$ and $y(0) = 0$ to calculate $u(t)$ and the vertical distance $y(t) = \int_0^t u(\tau)\mathrm{d}\tau$. In the discussion which follows much simplification is achieved by assigning the functional symbol $u_L(t)$ to the first term on the right of equation (1) arising from aerodynamic lift generated by wing flutter, the symbol $u_G(t)$ to the gravitational term $-gt$ and $u_A(t)$ to the third term on the right arising from the integration of non-uniform air acceleration forces.

Some insight into the relative importance of the lift and acceleration terms, u_L and u_A, respectively, in relation to gravity u_G can be obtained by inspecting the vertical phugoids, $y(t)$ versus t, for the bird, locust, moth, fly and bee in the previous communication[3], it being found that the slope of the trajectory for $\gamma \equiv 0$, corresponding to no air acceleration forces, is about 15–35 per cent lower than that for free fall with no wing flutter, thereby indicating that if coupling effects are ignored the air acceleration term will contribute about 65–85 per cent of the total anti-gravity force for the case where $C_L^D = 2{\cdot}0$ and $C_L^U = 1{\cdot}5$, the wing wave-form being of the Z-type with phase lag $\varepsilon = -3\pi/4$. Higher aerodynamic lift contributions of 20–50 per cent have been found when $C_L^D = C_L^U = 2{\cdot}0$. Thus, while aerodynamic lift effects arising from wing flutter are not small, being on average 25 per cent of gravity, the major anti-gravity contribution of about 75 per cent comes from non-uniform air acceleration, thereby explaining why earlier theories of flapping flight, based solely on aerodynamic lift phenomena, were inadequate to explain insect flight.

In order to illustrate the behaviour of the non-uniform air acceleration term, Fig. 1 has been constructed based on Z-wave-form calculations for the desert locust at $\varepsilon = -3\pi/4$, $f = 20$ c/s, $\varphi = 0{\cdot}875$ rad, $k = 1{\cdot}5$, $C_L^D = 2{\cdot}0$, $C_L^U = 1{\cdot}5$ and $\gamma = 2{\cdot}80$ g cm, utilizing the equivalent two-wing geometry of Weis-Fogh and Jensen[8]. Clearly with $k = 1{\cdot}5$ the acceleration integral u_A exhibits a component oscillating in velocity around another component that increases linearly with time, this latter component being due to the non-uniformity of the wing motion since it disappears when $k = 1{\cdot}00$; it is this positive linear component that cancels the negative gravitational term $u_G = -gt$, the resultant $u_G + u_A$ being also plotted in Fig. 1 for comparison with the true body velocity u which contains the aerodynamic lift contribution u_L of equation (1) together with any coupling effects. A comparison of the velocities shows that the linear portion of u_A represents about 82 per cent of u_G, the remaining 18 per cent being accounted for by u_L and coupling effects, thus confirming the predominance of the air acceleration forces. Very similar results to those for the locust in Fig. 1 have also been obtained for the bird-, moth-, fly- and bee-types discussed previously[3]. Although not shown in Fig. 1, the magnitude of the linear contribution to u_A can be varied by changing the phase angle ε which modifies the interaction between the functions $P_{\tau\tau}(k, \omega\tau + \varepsilon)$ and $\cos[\varphi P(k, \omega\tau)]$ as demonstrated earlier[2], it being also shown that the Z-wave-form is more effective than the S-wave-form, the latter tending to produce phugoids which are relatively insensitive to the duration factor k. Since the non-uniform air acceleration integral appears to be composed of linear and oscillatory components, it seems reasonable to formulate a functional approximation U_A to u_A such that, for convenience in subsequent analyses, the two contributions are clearly separated as follows:

$$u_A(t) \approx U_A(t) = \mu(\gamma\varphi/M)(\omega_U^2 - \omega_D^2)t - (\gamma\varphi\omega^2/M)\int_0^t \cos(\omega\tau + \varepsilon)\cos(\varphi\cos\omega\tau)\mathrm{d}\tau \quad (2)$$

where ω_D and ω_U are the respective frequencies of the downstroke and upstroke segments of the Z wave-form such that the duration factor is $k = \omega_U/\omega_D$, the linear term yielding the necessary zero contribution when $k = 1{\cdot}00$; empirical coefficient μ denotes a dimensionless parameter the numerical value of which is adjusted to fit u_A as closely as possible—the fit of approximation (2) to the locust data with $\mu = 0{\cdot}056$ is shown by the points plotted in Fig. 1, similar agreement being found for the bird, moth, fly and bee when μ is 0·125, 0·099, 0·047 and 0·096 respectively, the corresponding flight parameters for these insects being tabulated in the previous article[3]. Equation (2) clearly identifies the two components present in the air acceleration term, thereby illustrating that the original non-uniform wave-form is modulated by interaction to generate a time-increasing portion and leaving a more uniform oscillatory component; it will be demonstrated in a future communication that approximation (2) serves as the basis for a general correlation of all the insect flight data of Magnan[10], Osborne[6] and Sotavalta[18], comprising 21 different insects belonging to the orders Lepidoptera, Neuroptera, Coleoptera, Diptera and Hymenoptera.

For the computing time on the IBM 7090 digital computer I thank the U.S. National Science Foundation through grant *GP*–2310, which partially supports the University of Pittsburgh Computing Center.

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INDUCTION OF IMMUNITY AND OF UNRESPONSIVENESS TO PARENTAL MARROW GRAFTS IN ADULT F_1 HYBRID MICE

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PREVIOUS work has firmly established that transplanted bone marrow cells of several sub-lines of the *C57BL* (H-$2^b/H$-2^b) strain do not grow as well in X-irradiated *C3H* × *C57BL* (H-$2^k/H$-2^b) and *A* × *C57BL* (H-$2^a/H$-2^b) F_1 hybrids as in recipient mice of either of the parental strains[1,2]. Such hybrids have been designated resistant toward grafted parental marrow merely to indicate that they are not capable of supporting optimal growth of donor hæmopoietic cells, as compared with genetically compatible recipients. The frequency of success of such grafts in segregating backcross progeny and the marrow's growth pattern in F_1 hybrids heterozygous at known H loci or at defined regions of the H-2 locus indicated that hybrid resistance (or, conversely, deficient growth of *C57BL* marrow in F_1 hybrid recipients) was controlled by a genetic determinant closely associated with the K region of the H-2 locus, in linkage group IX[2,3]. *C57BL* marrow is capable, however, of sustained and optimal growth on transplantation into irradiated mice of strain *C3H*, an allogeneic recipient[1,2]. This finding has been interpreted to mean that genetic interaction (interallelic), rather than heterozygosis for a recessive gene, may be responsible for hybrid resistance, presumably by preventing or modifying the expression of one or more isoantigens of the parental H-2^b phenotype[2,3].

In view of the known characteristics of growth of *C57BL*/10 and *C3H* strain marrow cells[2], we regard *C57BL*/10*ScSn* or *B10* as the prototype donor strain for demonstrating the phenotype 'deficient growth of parental marrow grafts in hybrids' and *C3H*/*HeSn* or *C3H* as the strain representative of the more common pattern of growth of transplanted parental marrow cells, which is indistinguishable in hybrids and in isogenic recipients.

These two strains of mice were used in the present experiments in pairs with their related congenic lines[4]. Strain *C57BL*/10-H-2^d-old or *B10·D2* is congenic with *B10* except that it carries the H-2^d allele, derived from *DBA/2*, instead of H-2^b. Strain *C3H·SW* is congenic with *C3H* except that it carries the H-2^b allele, derived from a non-inbred Swiss mouse stock, instead of H-2^k. F_1 hybrids from crosses between the congenic pair *B10* × *B10·D2* (H-$2^b/H$-2^d) were prospective recipients for marrow cells gathered from *B10* and *B10·D2* donors, while *C3H·SW* × *C3H* (H-$2^b/H$-2^k) hybrids were prospective recipients for *C3H·SW* and *C3H* marrow cells. In either case, the parental strains had, by definition, a common genetic background but different H-2 alleles. The recipient mice were females exposed to 700 or 800 r. of 300-kV (peak) whole-body X-rays at the age of 12–15 weeks and injected intravenously with graded doses of nucleated cells a few hours later. Generally, each cell dose was given to a group of 5–10 mice. Marrow was obtained from the femurs and tibias of adult female donors; the cells were suspended in Tyrode's solution and counted with a Coulter electronic particle counter after red blood cells were lysed. Five days following transplantation of the marrow, its growth was assayed in each of the recipients by measuring the uptake of 5-iodo-2′-deoxyuridine-^{131}I, a specific deoxyribonucleic acid (DNA) precursor, in the spleen. For this assay, each recipient was given intraperitoneally 0·5 μc. of ^{131}IUdR (specific activity ~100 mc./mg, Nuclear Science and Engineering Co., Pittsburgh, Pennsylvania), and 17 h later each recipient was killed, its spleen removed, and the incorporated iodine-131 radioactivity measured by means of crystal scintillation counting. The splenic uptake of ^{131}IUdR was expressed in percentage of the total radioactivity injected. Parental marrow cells from a single preparation were generally grafted into isogenic and into F_1 hybrid mice at the same time; the relationship between the mean uptake of ^{131}IUdR in spleens of isogenic recipients and the number of cells injected into the animals 5 days prior to ^{131}IUdR labelling appeared to be linear on a logarithmic plot for *B10*, *B10·D2* and for *C3H·SW* marrow (Fig. 1, curve *A*), throughout a range of cell doses from $0{\cdot}1 \times 10^6$ to $1{\cdot}6 \times 10^6$ donor cells per recipient mouse. This indicates that the competence to proliferate on transplantation into genetically susceptible hosts was not different for cells carrying the H-2^b or the H-2^d alleles; it has been reported previously[2], and it is shown in Table 1, that *C3H* cells carrying the H-2^k allele are also comparable with cells of the aforementioned types with respect to proliferative activity in isogenic recipients. The marrow cells characterized by these four phenotypes differed markedly, however, with respect to their ability to proliferate in the spleens of F_1 hybrid recipients: *B10* and *C3H·SW* cells grew deficiently in *B10* × *B10·D2* and in *C3H·SW* × *C3H* F_1 hybrids, respectively (Fig. 1, lines *C* and *B*), whereas *B10·D2* and *C3H* marrow grew without detectable impairment in hybrids of the same

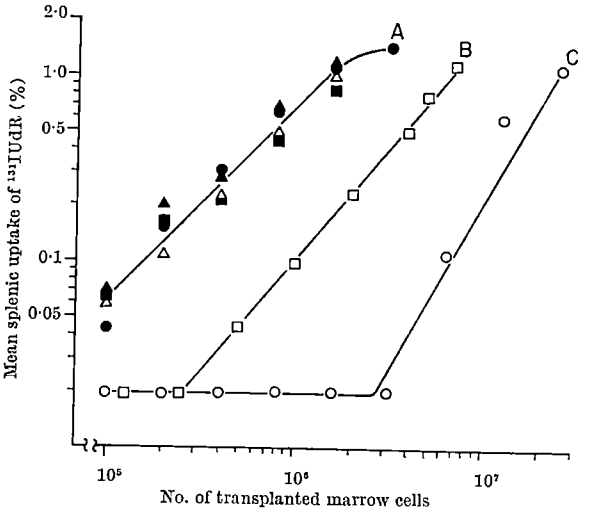


Fig. 1. Mean splenic uptake of ^{131}IUdR in relation to the number of bone marrow cells injected into X-irradiated (700 r.) recipient mice (five mice per point). Curve *A*, *B10* cells into *B10* (●); *C3H·SW* cells into *C3H·SW* (◪); *B10·D2* cells into *B10·D2* (▲); and into *B10* × *B10·D2* (△). Curve *B*, *C3H·SW* cells into *C3H.SW* × *C3H* (□). Curve *C*, *B10* cells into *B10* × *B10·D2* (○)

Table 1. Proliferation of Transplanted Parental Marrow Cells in the Spleens of F_1 Hybrid Recipients pretreated with Parental Marrow or Skin Grafts

Donor Strain	Donor No. of grafted cells	Recipient Strain	Recipient Pretreatment	Mean splenic uptake of ^{131}IUdR (per cent ± S.E.)*
B10	10^7	*B10 × B10·D2*	Tyrode's	0·58 ± 0·04 (28)
			B10 marrow	0·12 ± 0·01 (25)
			B10·D2 marrow	0·60 ± 0·03 (27)
			B10 skin graft	0·73 ± 0·08 (13)
			B10·D2 skin graft	0·89 ± 0·10 (9)
B10·D2	10^6	*B10 × B10·D2*	Tyrode's	0·69 ± 0·05 (10)
			B10 marrow	0·59 ± 0·05 (10)
C3H	10^6	*B10 × B10·D2*	Tyrode's	0·61 ± 0·09 (10)
			B10 marrow	0·70 ± 0·10 (10)
C3H·SW	8×10^6	*C3H·SW × C3H*	Tyrode's	1·02 ± 0·04 (10)
			C3H·SW marrow	0·17 ± 0·03 (10)
			C3H marrow	0·99 ± 0·07 (10)
C3H	10^6	*C3H·SW × C3H*	Tyrode's	0·61 ± 0·06 (10)
			C3H·SW marrow	0·70 ± 0·07 (10)

*Mean uptake values are given as percentage of the total ^{131}IUdR radioactivity administered ± S.E. of the means, in excess of percentage retention of radioactivity in spleens of irradiated control animals not injected with marrow. The number of recipient mice is indicated in brackets; data of several independent experiments were pooled.

parentage (Fig. 1, curve *A*, and Table 1). Thus, the simple substitution of the *H–2*^{b} allele with *H–2*^{d} conferred to the marrow cells of *B10·D2* mice the ability to grow optimally in *H–2* heterozygotes, although the genetic background remained essentially that of the *B10* strain. Conversely, substitution of the *H–2*^{k} allele with *H–2*^{b} conferred on the marrow cells of *C3H·SW* mice the trait for deficient growth in *H–2* heterozygotes, despite the *C3H* genetic background of such mice. Differences in strength of hybrid resistance were noticeable, however, on comparison of the uptake-cell dose responses in F_1 hybrids from *B10* or *C3H* parents (Fig. 1, lines *B* and *C*). This observation raises the question whether or not the strength of hybrid resistance is modified by the genetic background of the parental strains entering the F_1 cross. Alternatively, the observed differences in strength of hybrid resistance might reflect dissimilarities in the *K* region of *H–2*^{d} and *H–2*^{k} with regard to the factor(s) involved in hybrid resistance.

Since *H–2* isoantigens are thought to be of primary importance in deciding the fate of allogeneic tissue grafts[5,6], it is conceivable that the *H–2* controlled hybrid resistance may reflect differences in isoantigenic constitution between the tissues of *H–2*^{b}/*H–2*^{b} parental mice and those of their heterozygous hybrid recipients. If this were the case, one would expect that hæmopoietic cells of the strains *B10* and *C3H·SW* would be antigenic to their resistant hybrids and capable of inducing a state of sensitivity or of unresponsiveness in such hybrids towards parental marrow grafts. To test this possibility, adult hybrid mice were given one of the following treatments before irradiation and infusion with parental marrow cells: (1) intraperitoneal injection at weekly intervals of $1{\cdot}5 \times 10^7$ nucleated, viable parental marrow cells for a total of three injections; (2) intraperitoneal injection at weekly intervals of 2×10^7 nucleated, viable parental spleen cells for a total of four injections; (3) transplantation on to the mid-dorsal region of parental skin tissue taken from the donor's ear; and (4) intraperitoneal injection at weekly intervals of Tyrode's solution for a total of three or four injections. Seven days after the last of the cell injections, or 100 days after placing the parental skin grafts (accepted and in perfect condition throughout), the hybrids were irradiated and infused with a single fixed dose of parental strain or allogeneic strain marrow cells. Five days later the extent of grafted marrow proliferation was assessed by the ^{131}IUdR assay. To detect immunity, that is, an induced state in which parental marrow grafts become unacceptable to F_1 recipients which otherwise would accept them, we grafted a dose of parental marrow cells known to override the resistance of non-sensitized hybrids (Fig. 1, lines *B* and *C*, and Table 1). On the other hand, to detect unresponsiveness, an induced state in which parental marrow grafts become acceptable to recipients which otherwise would reject them we grafted a dose of parental marrow cells known not to grow detectably in non-pretreated hybrids.

The promotion of splenic incorporation of ^{131}IUdR by 10^7 transplanted *B10* marrow cells in *B10 × B10·D2*F_1 hybrids was inhibited when the hybrids were pre-injected with *B10* marrow (Table 1, upper section). However, such an effect was not observed when the hybrids were pretreated with *B10·D2* marrow or with skin grafts of either parental strain before X-irradiation and infusion with the *B10* marrow test-graft. Furthermore, the pretreatment with *B10* marrow, which resulted in reduced acceptance of the *B10* test-grafts by the F_1 hybrids, did not affect the acceptance of marrow test-grafts from parental *B10·D2* and from unrelated *C3H* donors. Similar results were obtained with *C3H·SW* marrow grafts given to a comparable series of *C3H·SW × C3H* F_1 hybrids (Table 1, lower section). Thus, the induction of enhanced hybrid resistance resembles the induction of transplantation immunity, in that it is effected by the parental cells towards which the hybrid host was known to be resistant but not by the parental marrow cells or by the parental skin grafts which were known to be histocompatible with the hybrids. Furthermore, once established, this condition was specific for cells identical with those used for sensitization.

In contrast to the previous findings, pre-injection of resistant hybrid mice with parental spleen cells instead of parental marrow cells did not enhance the hybrid resistance and led, rather, to an opposite effect, that is, to the abrogation of hybrid resistance (Table 2). This was accomplished when the hybrids were given multiple injections of spleen cells from their *H–2*^{b}/*H–2*^{b} parents over a period of 4 weeks, whereas similar treatment with spleen cells of their parent which carried an *H–2* allele different from *H–2*^{b}, or with skin grafts of either parental strain, was ineffective (Table 2). That injected spleen cells have a tendency in certain circumstances to yield specific unresponsiveness in adult allogeneic mice has been reported by several investigators[7]. In this study, a similar condition has been established in F_1 hybrid mice

Table 2. Proliferation of Transplanted Parental Marrow Cells in the Spleen of F_1 Hybrid Recipients pretreated with Parental Spleen or Skin Grafts

Donor Strain	Donor No. of grafted cells	Recipient Strain	Recipient Pretreatment	Mean splenic uptake of ^{131}IUdR (per cent ± S.E.)*
B10	10^6	*B10 × B10·D2*	Tyrode's	0·01 ± 0·001 (29)
			B10 spleen	0·55 ± 0·04 (29)
			B10·D2 spleen	0·01 ± 0·002 (16)
			B10 skin graft	0·01 ± 0·003 (18)
			B10·D2 skin graft	0·02 ± 0·005 (10)
B10·D2	10^6	*B10 × B10·D2*	Tyrode's	0·69 ± 0·05 (10)
			B10 spleen	0·60 ± 0·06 (10)
C3H	10^6	*B10 × B10·D2*	Tyrode's	0·61 ± 0·09 (10)
			B10 spleen	0·68 ± 0·07 (10)
C3H·SW	10^6	*C3H·SW × C3H*	Tyrode's	0·05 ± 0·002 (10)
			C3H·SW spleen	0·57 ± 0·06 (10)
			C3H spleen	0·07 ± 0·001 (10)
C3H	10^6	*C3H·SW × C3H*	Tyrode's	0·61 ± 0·06 (10)
			C3H·SW spleen	0·67 ± 0·05 (10)
B10	10^6	*B10*	None	0·70 ± 0·06 (10)
		C3H × B10	Tyrode's	0·02 ± 0·001 (10)
		C3H × B10†	*B10* spleen	0·64 ± 0·09 (20)
		C3H × B10†	*C3H·SW* spleen	0·32 ± 0·04 (10)
		C3H × B10†	*C3H* spleen	0·02 ± 0·001 (20)

* Mean uptake values are given as percentage of the total ^{131}IUdR radioactivity administered ± S.E. of the mean, in excess of percentage retention of radioactivity in spleens of irradiated control animals not injected with marrow. The number of recipient mice is indicated in brackets; data of several independent experiments were pooled.

† Twenty-eight and 60 days after the first of four injections of spleen cells the peripheral blood hæmoglobin of these animals was entirely of host type.

Table 3. Lack of Spleen Chimerism in F_1 Hybrid Mice after Four Injections, at Weekly Intervals, of 2×10^7 Parental Spleen Cells

Donor			Recipient*		
			Mean uptake of ^{131}IUdR (%) in recipient spleens		
Strain	Pretreatment	No. of spleen cells grafted	Strain	Unsensitized†	Sensitized†
None		None	*B10*	0·01	0·01
B10	None	2×10^5		0·07	0·08
		4×10^5		0·12	0·15
B10 and *B10* × *B10·D2*	None	2×10^5		1·54	0·09
		$2{\cdot}5 \times 10^7$‡			
B10 and *B10* × *B10·D2*	None	4×10^5		1·44	0·16
		$2{\cdot}5 \times 10^7$‡			
B10 × *B10·D2*	None	2×10^7		1·74	0·02
		4×10^7		2·76	0·02
B10 × *B10·D2*	*B10* spleen§	2×10^7		0·71–1·11–2·32	0·02–0·03–0·03
		3×10^7		1·42–1·55–1·59	0·01–0·02–0·02
		4×10^7		1·69	0·02
None		None	*C3H·SW*	0·02	0·02
C3H·SW	None	2×10^5		0·15	0·13
		4×10^5		0·39	0·33
C3H·SW and *C3H* × *B10*	None	2×10^5		1·24	0·15
		2×10^7‡			
C3H × *B10*	None	2×10^7		10·6	0·01
C3H × *B10*	*C3H·SW* spleen§	2×10^7		10·0	0·02
				1·35–1·37–1·43	0·01–0·02–0·04

* Donor and recipients were females. Recipients were exposed to 800 r. of X-rays before spleen cell infusion (4–5 mice per group).

† *B10* mice were sensitized against *B10·D2* isoantigens; *C3H·SW* mice were sensitized against *C3H* × *B10* F_1 isoantigens.

‡ The cell mixture was prepared a few minutes before the infusion into recipient mice.

§ The spleens of 11 suspected chimeras were tested 28 days after the first of four injections with *B10* or *C3H·SW* spleen cells. Eleven additional tests not reported in the table were made 32 days later with identical results.

towards parental *B10* and *C3H·SW* marrow grafts, provided that the exposure of the hybrids to the injected parental spleen cells was of sufficiently long duration. The extent of the hybrid's unresponsive state was estimated by comparing the splenic uptake of ^{131}IUdR in isogenic recipients and in pretreated hybrid recipients given test-grafts from a common pool of parental marrow cells (Table 2, lower section). Injected spleen cells of either *B10* or *C3H·SW* mice were capable of inducing unresponsiveness to *B10* marrow grafts in *C3H* × *B10* F_1 hybrids. Even though the abrogation of hybrid resistance induced by *C3H·SW* spleen cells was not as complete as that induced by *B10* spleen cells, the spleen cell factor(s) responsible for induction of unresponsiveness appeared to be similar in the two unrelated homozygous *H–2*^{b} strains, *B10* and *C3H·SW*.

It has been reported that F_1 hybrid mice infused with large doses of parental strain spleen cells sometimes become cellular chimeras[8]. Therefore, we determined the solubility and crystallographic properties of peripheral blood hæmoglobin[9] in *C3H* × *B10* F_1 hybrids, 28 and 60 days after the first of four injections with spleen cells of *B10*, *C3H·SW*, or *C3H* mice. If chimerism resulted from the injections of spleen cells, increasing amounts of donor-type hæmoglobin should become detectable as host erythrocytes age and disappear from the circulation. All the hybrids injected with *B10* or *C3H·SW* spleen cells became non-resistant to *B10* marrow grafts, as determined 7 days after the last injection (Table 2, lower section). At this time, as well as 32 days later, they did not contain detectable amounts of donor-type hæmoglobin in their blood. *C3H* × *B10* F_1 mice treated with *C3H* spleen cells also failed to become blood chimeras at similar time-intervals after the treatment. The spleens of hybrid mice which received multiple injections of parental cells were also tested for the presence of donor-type hæmopoietic spleen cells by transplantation, as described in the following. Cell suspensions were prepared from each hybrid spleen under test, and known numbers of such cells, varying from 2×10^7 to 4×10^7, were injected into irradiated *B10* or *C3H·SW* recipients, that is, into recipients isogenic with the donors of the spleen cells used to abrogate the hybrid resistance.

Prior to this cell injection the mice received intraperitoneally 50 U.S.P. units of heparin to prevent sudden death during or immediately after intravenous administration of large numbers of spleen cells. Half the *B10* or *C3H·SW* hosts were sensitized against isoantigens of the F_1 hybrid spleen donor by two intraperitoneal injections of 5×10^6 spleen cells each, given 1 and 2 weeks, respectively, before the test. This was done to prevent hybrid spleen cells from proliferating in such hosts on transplantation without impairing, however, the proliferation of eventually admixed parental-type spleen cells. Proliferation of the grafted cells was evaluated by the ^{131}IUdR method as described above, except that an intraperitoneal injection of 10^{-7} mole of 5-fluoro-2′-deoxyuridine (FUdR, gift from Hoffman LaRoche, Inc., Nutley, New Jersey) was given to the recipient mice 1 h before the ^{131}IUdR injection. FUdR is an inhibitor of the endogenous synthesis of thymidilate and reduces the competition of the latter with ^{131}IUdR for incorporation into DNA. Hence, it increases the incorporation of ^{131}IUdR in a given population of cells and therefore the sensitivity of the assay. Cells isogenic with the irradiated recipients (that is, *B10* or *C3H·SW*) were equally capable of promoting splenic incorporation of ^{131}IUdR when transplanted alone or as a mixture with F_1 hybrid cells (Table 3). The latter cells, however, were not capable of growth in presensitized recipients, their viability being established by their successful growth in unsensitized recipients (Table 3). Spleens of 22 suspected F_1 hybrid chimeras were analysed 28 days and 60 days after the first of four parental strain spleen cell injections. No spleen contained transplantable hæmopoietic cells other than those carrying the isoantigens of the hybrid donor (Table 3).

The proliferation of transplantable hæmopoietic stem cells contained in 2×10^5 nucleated parental strain spleen cells was readily measured by the ^{131}IUdR technique; since we transplanted approximately one-quarter of all the nucleated cells of a whole F_1 hybrid spleen into each of the recipients, we could, in fact, have detected the presence of one parental strain stem cell in 200–400 hybrid-type stem cells, depending on the total number of nucleated spleen cells infused (Table 3). Our findings do not exclude, however, that spleens of the unresponsive F_1 hybrids contain other parental type cells which are not hæmopoietic and do not proliferate on transplantation, as, for example, long-lived small lymphocytes.

It is suggested that induced enhancement and induced abrogation of hybrid resistance to marrow grafts from *H–2*^{b}/*H–2*^{b} donors reflect acquired immunity and unresponsiveness, respectively, to cellular isoantigen(s) of the *H–2*^{b} phenotype not present in *H–2*^{b} heterozygous F_1 hybrids. The failure of parental skin grafts from homozygous *H–2*^{b} donors to affect the response of F_1 mice to the postulated parental isoantigen(s) is in keeping with the lack of hybrid resistance to parental skin grafts[2,10] and with the lack of detectable hybrid-anti-parent humoral antibodies[5,11]. For these reasons, experiments based on rejection of skin grafts and on induction of hæmagglutinating antibodies have failed to detect this phenomenon[5,11].

This work was sponsored jointly by the U.S. Atomic Energy Commission under contract with the Union

Carbide Corporation and by research grant *CA*-01329 of the National Cancer Institute, U.S. Public Health Service.

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In vitro CYTOTOXIC EFFECT OF LYMPHOID CELLS FROM MICE IMMUNIZED WITH ALLOGENEIC TISSUE

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IT is generally agreed that lymphoid cells have a decisive role in transplantation reactions *in vivo*. Although the mechanism of their action is unknown, it is of interest in this connexion that lymphocytes from mice immunized with allogeneic transplants can cause a cytolytic destruction of cells from the donor strains *in vitro*[1–4]. This effect is immunologically specific and does not seem to be complement-dependent.

When target cells were labelled with ^{14}C-thymidine and exposed to isoantibodies *in vitro*, the release of radioactivity into the medium after trypsin treatment was found to be correlated to the cytotoxic response as determined by supravital staining[5]. A similar method was applied for measuring the effect of immune lymphoid cells on labelled donor cells *in vitro*. Observations made in parallel on the morphology of the target cells will also be described.

Mice were immunized against allogeneic tissues derived from foreign genotypes differing at the *H*-2 locus in all cases except one. Skin, tumour tissues or spleen, liver and kidney were used as indicated in Table 1. The target cells were taken from mice of the strain used as tissue donor for immunization. Cells were isolated from whole 16–17-day-old fœtuses by trypsinization, or from adult lungs and kidneys, and were cultivated in milk dilution bottles[6]. Second or third sub-cultures were made on coverslips (1 cm × 1 cm) in small Petri dishes. The culture medium in all experiments consisted of Eagle's basal medium in Earle's balanced salt solution containing 0·0172 M sodium bicarbonate and 10 per cent heat-inactivated (30 min, 56° C) calf serum. The cultures were incubated at 37° C in 5 per cent carbon dioxide in air.

For morphological observation of the cytotoxic reactions, the coverslips were transferred to Petri dishes (1 coverslip/dish) and placed under stainless-steel screens (1 cm × 1 cm) of the type introduced by Trowell for organ culture[7]. Each screen was covered by lens paper and the dishes were filled with medium up to the height of the lens paper[7,8]. Axillary and inguinal lymph nodes, spleen and thymus, or spleen only, were removed from the immunized mice, cut into small pieces, washed twice and placed on top of the lens paper. All lymphoid material removed from one mouse was placed on the screen in one culture dish. Control material was taken from mice syngeneic with target cells. The controls were either from untreated mice, or from mice inoculated with syngeneic or allogeneic tissue.

After varying times of exposure, the coverslips were fixed and stained with hæmatoxylin–eosin. In the experiments with embryonic fibroblasts, no obvious cytotoxic effect of the immune lymphoid cells was found. Numerous lymphocytes and also a few polymorphonuclear cells had fallen down from the screens. Lymphocytes gathered around the target cells, but no abundant lysis was observed up to 72 h, and the number of target cells remaining on the coverslips appeared to be similar to that in the controls, even when the seeding of the cells on the coverslips was sparse.

In contrast, adult kidney or lung fibroblasts were more susceptible to immune lymphocytes. In the immune systems, a large proportion of the cells disappeared from the coverslips within 24 h, while no apparent change in cell number was seen in the controls (Figs. 1 and 2). Furthermore, the cells remaining on the glass in the immune systems were covered by aggregated lymphocytes and had a vacuolated cytoplasm. Addition of fresh guinea-pig serum (final dilution, 1 : 20) to the incubation medium did not affect the results, which were similar to those found by other authors[1–4]. The reason for the apparent resistance of the embryonic cells is unknown, although the lesser concentration of isoantigens on their surface may be suspected to play a part[9].

For the isotope experiments only embryonic fibroblasts were used. Second or third passage cultures on coverslips in Petri dishes were incubated overnight in medium containing either 0·5 μc./ml. of ^{14}C-thymidine (Radiochemical Centre, Amersham, England; spec. act. 0·1 mc./0·83 mg).

For each experiment, coverslips from one Petri dish were washed three times with buffered saline (PBS) and were then transferred to fresh dishes under stainless-steel screens (one screen/dish) as described above. One or two screens were loaded with lymphoid material from immunized animals, or two others with lymphoid material from control mice, whereas some screens in control dishes were left empty. The volume of the incubation medium in each dish was 13 ml. After incubation for 11–45 h the coverslips were removed and washed again three times with PBS. Each coverslip was treated for 15 min with 2 ml. 0·5 per cent trypsin, and the cells were spun at 2,000 r.p.m.

Table 1. ^{14}C-THYMIDINE IN DNA OF EMBRYONIC FIBROBLASTS AFTER EXPOSURE TO IMMUNE OR NORMAL LYMPHOID TISSUE (LYMPH NODES, SPLEEN AND THYMUS) *in vitro*

Exp. No.	Target cell strain	Exposed to lymphoid tissue from*	Days after last challenge	Precultivation of lymphoid tissue	Time of exposure (h)	Complement added	c.p.m.
1	*A.SW*	*A* anti-*A.SW*	8	1 day	45	+	905
						−	866
		Normal *A*	—			+	1,002
						−	2,201
		—	—			+	656
2	*DBA*	*C57BL* anti-*DBA*	4	1 day	24	+	1,288
						−	898
		C57BL inoc.	4			+	1,259
		with *C57BL*				−	1,035
		—	—			+	991
						−	1,151
3	*DBA*	*C57BL* anti-*DBA*	7	1 day	30	+	2,376
						−	2,842
		Normal *C57BL*	—			+	2,193
						−	2,968
		—	—			+	2,755
						−	2,710
4	*DBA*	*C57BL* anti-*DBA*	9	2 days	24	−	1,786
		Normal *C57BL*	—			−	4,033
							2,350
		—				−	2,787
							2,614
5	*DBA*	*C57BL* anti-*DBA*	9	0	18	+	2,001
		C57BL inoc.	9			+	2,553
		with *C57BL*				−	2,517
		—	—			+	2,453
						−	3,098
6	*DBA*	*C57BL* anti-*DBA*	9	0	11	−	1,760
						−	2,197
		Normal *C57BL*	—			−	2,202
						−	2,262
		—	—			−	3,077
						−	2,857
7	*DBA*	*C57BL* anti-*DBA*	10	0	30	+	3,518
						−	3,479
		Normal *C57BL*	—			+	4,091
						−	4,754
		—	—			+	5,421
						−	4,149
8	*DBA*	*C57BL* anti-*DBA*	4	0	24	−	1,409
						−	1,693
		Normal *C57BL*	—			−	3,119
						−	2,461
		—	—			−	2,138
						−	2,284

* Exp. 1: Immune donors inoculated for 6 weeks twice (intraperitoneally, 10^6 cells/dose) with methylcholanthrene induced sarcoma (MSWB) and once with normal *A.SW* spleen, liver and kidney. Exps. 2–7: Immune donor inoculated for 8 weeks, 5 times (intraperitoneally, 10^7 cells/dose) with *DBA* lymphoma (AMR) and once with normal *DBA* spleen, liver and kidney. Exp. 8: Immune donors inoculated for 10 weeks, once with *DBA*-skin (by transplantation) and once with normal *DBA* spleen, liver and kidney.

for 10 min. The cell sediment was treated with ice-cold 5 per cent trichloroacetic acid (TCA) and the precipitate formed was then washed once with TCA (5 per cent) and with absolute alcohol and alcohol + ether (3 : 1). For determination of carbon-14, the precipitates were dissolved in formic acid and the radioactivity was measured at infinite thinness with a thin-windowed Geiger–Müller tube in an automatic gas flow counter after drying on plastic planchets. The values in Table 1 show the total acid precipitable radioactivity (c.p.m.) of the cells recovered from a coverslip after incubation, washing and trypsinization. In addition, the acid-soluble as well as the acid-precipitable radioactivity of the trypsin solutions, and of aliquots of the incubation media, were determined in the same way. In some experiments, unlabelled DNA (0·4 mg/sample) was added as carrier.

Table 1 shows the results of a series of eight experiments. As can be seen, the acid-precipitable radioactivity recovered from the cells exposed to immune lymphoid cells was approximately 20–40 per cent less than that found in the controls when fresh lymphoid material was used (Exps. 5–8). This effect was seen only in 2 of 4 experiments in which the lymphoid material had been precultivated for one or two days (Exps. 1–4). (The positive results of Exp. 4 are questionable because of infection found in the sister culture.) No significant loss of isotope was noted in the cultures exposed to control lymphoid cells as compared with the unexposed control cultures. A measurable loss could already be recorded after 11 h of incubation. Addition of guinea-pig serum had no effect, although its concentration under the present conditions may have been too low for complement action.

As can be seen from Table 1, the immune cells were active 4–10 days after the last challenge of the mice with antigen. These results were confirmed in a series of similar experiments, where pieces of spleen from *C3H* mice immunized with *C57BL* spleen, were added to embryonic fibroblasts of *C57BL* mice. In this series, the immune spleen cells exhibited a cytotoxic action from 2–10 days after challenge. Control spleens from *C3H* mice inoculated with syngeneic tissue or with tissue from unrelated allogeneic mice (*AKR*) were inactive.

In the previous experiments[6], the cytotoxic effect of iso-antibodies was determined by measuring the isotope released from the damaged target cell into the incubation medium after trypsin treatment. In the work recorded here, the content of isotope of the trypsin solutions, as well as that of the incubation media, was low, and no differences were found between immune systems and controls. However, since the volume of the incubation mixtures was large and since some cellular material may have been lost during washing of the coverslips after incubation, isotope may have escaped detection. Until it has been established where and in which form the missing isotope can be recovered, it cannot be decided whether the present difference between immune systems and controls was due to a release of isotope from damaged cells, or to the escape of cells from the coverslips. It should be noted, however, that the morphological observations of these embryonic cells (see foregoing) showed no striking difference in cell numbers between immune and control incubations.

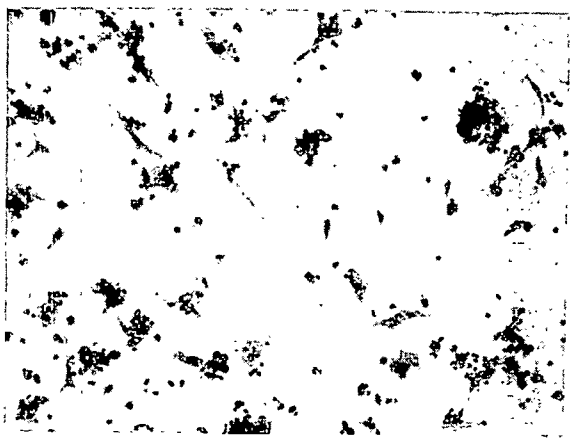


Fig. 1. Adult *A.SW* lung fibroblasts (second passage) after overnight exposure to immune lymphoid tissue from one *A* mouse immunized with *A.SW* sarcoma (MSWB) (Table 1)

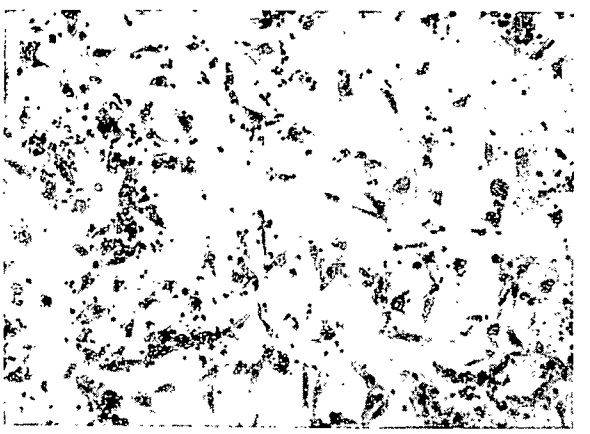


Fig. 2. Parallel control culture. Fibroblasts exposed to lymphoid tissue from one *A* mouse inoculated with syngeneic cells

In conclusion, it may be stated that even in cases where morphological observation gave no clear-cut results, the *in vitro* cytotoxic effect of immune lymphoid cells could readily be detected by the technique described here. The cytotoxic action of the lymphoid cells on the embryonic fibroblasts was immunologically specific. Although serum from the immunized mice had no cytotoxic effect under the present conditions, it cannot be excluded that the effect was mediated by antibodies of γ-globulin type, adsorbed to or synthesized by the aggregated lymphocytes[10–12].

It also remains to be established whether or not the reduced activity of the lymphoid tissue after ageing for 1–2 days in culture was significant. It is well known that isolated lymphoid tissue may retain its capacity to produce antibody during several weeks in culture[13,14].

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INTESTINAL CRYPT LESIONS IN NEONATALLY THYMECTOMIZED HAMSTERS

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THE fatal wasting syndrome following neonatal thymectomy in the golden hamster includes diarrhœa, marked loss in weight, pancytopenia[1] and immunological deficiency[2]. The basic mechanism of these changes is unknown. Work recorded here describes the characteristics and incidence of intestinal crypt lesions in hamsters thymectomized at birth, which may account for the diarrhœa and wasting of these animals. The high degree of correlation between the occurrence of enteropathology and alterations in the ratios of circulating white blood cells is noted.

A total of 274 new-born golden hamsters (obtained from Simonsen Laboratories, White Bear Lake, Minnesota) from 31 litters were divided into three groups of 143 thymectomized, 76 sham-thymectomized controls (sham) and 55 intact controls (controls). Thymectomy within 24 h of birth was carried out by the method of Sjodin *et al.*[3]. In all but two litters, litter mates of the thymectomized animals were subjected to sham operation involving exposure of the thymus without removal. The intact controls from separate litters were anæsthetized in ether and toe marked.

Blood for differential white blood cell counts was obtained following decapitation of the one- and two-week-old animals, or from the retro-orbital plexus in older animals immediately prior to death. All animals were killed by decapitation following ether anæsthesia. Twelve of the 18 thymectomized animals three weeks of age and more were killed when one or more signs of the syndrome appeared. The remainder appeared healthy but were killed to provide representatives of a particular age-group to complete the sequential study. Sham-operated and control animals were killed in each age-group. Complete gross and microscopic autopsy studies were performed on all animals used in this work. The tissues obtained were fixed immediately in 10 per cent formalin and prepared for histological examination. One-cm lengths of small bowel, usually including lymphoid aggregates, and of colon were sectioned longitudinally for more extensive evaluations of the intestinal mucosa. This report includes all animals 1–8 weeks of age, the tissues of which were adequate for complete histological investigations and the differential white blood cell counts of which were available. No attempts other than morphological observations were made to determine the presence of bacterial or viral agents.

Survey of all animals showed some differences between the experimental and control groups. The two-week survival rates of 38 per cent for the thymectomized animals versus 49 per cent for the control groups probably reflected the traumatic effects of neonatal manipulations. In general, the thymectomized hamsters showed an early gain in weight comparable to the controls, but then failed to gain, or lost, weight. The incidence of post-thymectomy wasting was about equal in the two sexes. There was no apparent correlation between the clinical conditions of the experimental animals at the time of death and the two parameters of this work, enteropathology and percentage of circulating lymphocytes.

This article will concentrate on the pathology of the intestinal crypt lesions diffusely scattered throughout the small bowel in 14 of 18 weaned, thymectomized animals. The lesions were of two types, both of which might occur in the same animal. The more distinctive (Fig. 1) appeared to comprise a disorderly proliferation of large vacuolated cells. The earliest changes in afflicted crypts included a loss of the bright red staining granules of the base cells (Paneth cells) and a slight disorganization in the orderly arrangement of the columnar lining cells. The cytoplasm of the cells became pale and swollen. The decreased intensity of nuclear staining suggested decreased nuclear chromatin content. Compression of surrounding tissue and the continued presence of mitotic figures suggested that excessive proliferation of the crypt cells had been involved in these lesions. Inflammatory response to these lesions was variable but usually minimal. No viral inclusion bodies were seen. The second type of lesion was an inflammation at the base of the crypts. There was usually flattening of the crypt lining cells, minute erosions occurred, and micro-abscesses formed (Fig. 2). The acute inflammatory reaction often involved the supporting connective tissue (lamina propria). It seemed to us that the best interpretation of these lesions was that both types were due to abnormal regeneration of crypt lining cells. In one case, the regenerative process could be considered defective, and in the other inadequate.

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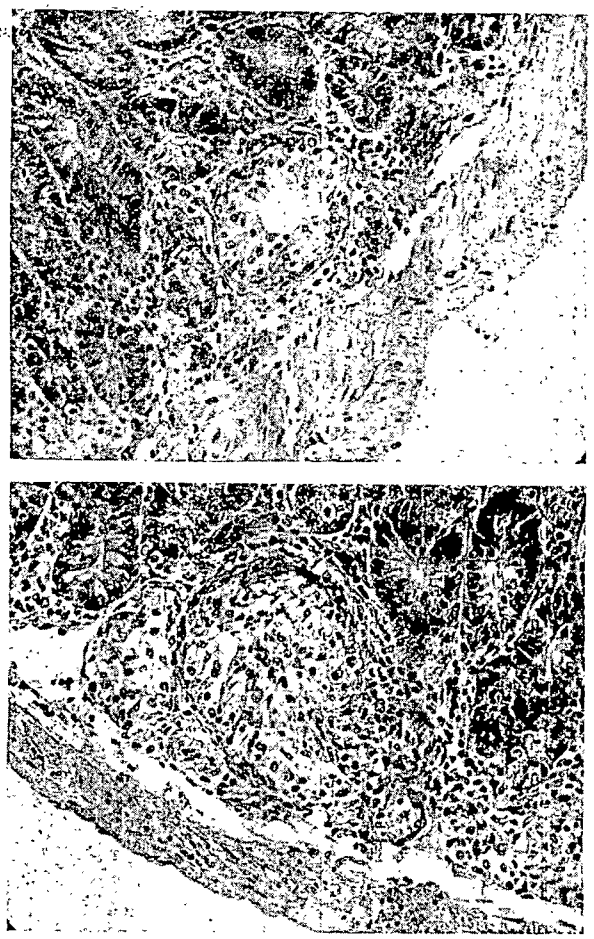


Fig. 1. Proliferative intestinal crypt lesions of neonatally thymectomized hamsters. Above, moderately advanced lesion typically located inside the muscularis with crypt architecture partially maintained by large vacuolated cells around dilated lumen; below, advanced lesion with central cell necrosis and slight acute inflammatory reaction. Note lesser involvement of two adjacent crypts (hæmatoxylin and eosin, × *c.* 133)

Histological survey of the other tissues of the thymectomized animals revealed only sporadic examples of significant tissue alterations, none of which appeared to be of infectious ætiology. Lymphocytic depletion in lymphoid organs was variable. It is of interest that the only examples of acute inflammatory lesions outside the intestinal tract which might be attributed to bacterial agents were found in three animals from the sham and control groups.

Lymphopenia has long been known to follow thymectomy. In this series, the designation of lymphopenia was an arbitrary one, representing a drop in the percentage of lymphocytes by one-quarter or more from an average level of 70 per cent. This average figure was obtained from the differential white blood cell counts of the sham and control animals found to be free of disease at autopsy. On this basis, it can be seen from columns I and III of Table 1 that lymphopenia occurred in 16 of 18 experimental

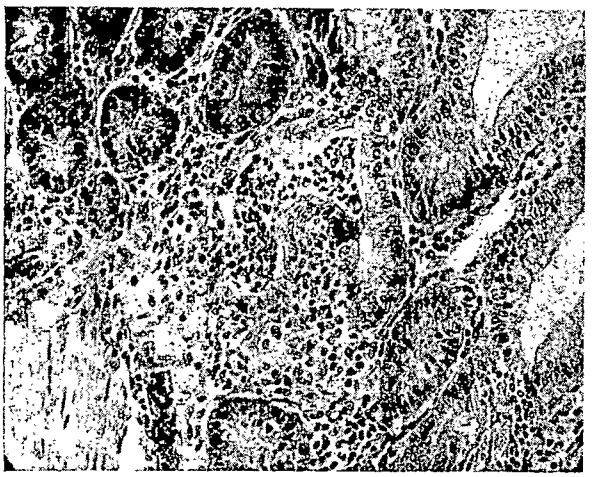


Fig. 2. Acute inflammatory intestinal crypt lesion of neonatally thymectomized hamsters (hæmatoxylin and eosin, × *c.* 133)

animals three weeks of age and over. The average proportion of lymphocytes in these 16 lymphopenic animals was 29 per cent with a range of 6–52 per cent (versus a range of 55–90 per cent in the two control groups). Although the experimental plan for this series of animals did not include total white blood cell counts to rule out a leucocytosis perhaps secondary to bowel inflammations, the hæmatological data of our pilot experiments were in agreement with the hæmatological data of Sherman *et al.*[1] and Defendi *et al.*[2], and indicated that the lymphopenia of thymectomized hamsters is both relative and absolute.

The association of intestinal crypt lesions and lymphopenia in 13 of 18 (72 per cent) neonatally thymectomized hamsters three weeks of age and older contrasted markedly with the absence of coincident lymphopenia and crypt lesions in sham-operated and control groups. The apparently normal hæmatological and enteric status of the thymectomized hamsters during the first two weeks is striking. Four additional thymectomized animals had crypt lesions, but were not included in Table 1 because differential counts were not available.

The five animals in the experimental group which did not show lymphopenia and crypt lesions were of interest. One had normal circulating lymphocyte levels and intact bowel mucosa; despite thorough search, no thymic remnant was found. Three animals had lymphopenia but no crypt lesions. The fifth animal which had crypt lesions without lymphopenia proved to be the major exception of the series. The crypt lesions noted in one sham animal (column IV of Table 1) were part of a diffuse ulceration of bowel adherent to a large liver abscess.

Microscopic thymic remnants were found in 4 of 34 animals in the thymectomized group after sectioning the thymic bed tissue at a frequency of approximately 70μ. All four of these animals showed lymphopenia and three of the four had crypt lesions. The development of one or more signs of the post-thymectomy syndrome despite the presence of minute thymic fragments in these animals suggested that a minimal amount of thymus may be

Table 1. Correlation of Intestinal Mucosal Status with Circulating Blood Lymphocyte Levels in Neonatally Thymectomized and Control Hamsters at Time of Death

Age at death (weeks)	I		II		III		IV	
	Animals with lymphopenia* and crypt lesions		Animals with normal % lymphocytes and normal mucosa		Animals with lymphopenia* only		Animals with crypt lesions only	
	Thymectomized	Sham and control	Thymectomized	Sham and control	Thymectomized	Sham and control	Thymectomized	Sham and control
7 and 8	3/5	—	—	7/10	1/5	2/10	1/5	1/10
5 and 6	7/8	—	1/8	5/5	—	—	—	—
3 and 4	3/5	—	—	2/2	2/5	—	—	—
1 and 2	—	—	16/16	16/17	—	1/17	—	—

* 52 per cent lymphocytes or less, versus 70 per cent average in sham and control animals

required for maintenance of lymphopoiesis and normal body economy.

The occurrence of these crypt lesions in thymectomized hamsters, and more recently in mice[4], raises the question of ætiology. It would be of great interest to know whether the enteric pathology is an independent occurrence or whether it is sequentially and causally related to the lymphopenia which occurs in such animals. It is possible that neonatal thymectomy removes the source of a humoral substance capable of direct action on the metabolic processes of the mucosal crypt cells. However, the high degree of association of lymphopenia with crypt lesions, and of normal lymphocyte levels with normal intestinal mucosa, suggested to us that these lesions might reflect the need of the rapidly proliferating crypt cells for components normally provided by the lymphocytes. Bryant[5] found, for example, that cells of the intestinal crypts took up radio-tagged DNA precursor from lymphocytes. These morphological and radioisotopic investigations would be in keeping with the idea that one function of the lymphocyte is to act as a nucleoprotein trephocyte[6]. The three thymectomized hamsters with lymphopenia but lacking crypt pathology, and the one thymectomized animal with normal lymphocyte count and intact mucosa, might indicate that lymphopenia preceded the intestinal lesions.

It is also possible that the enteric lesions are due to a latent virus activated in a weakened host as was recently observed with a hepatotrophic agent[7] found in neonatally thymectomized mice. Some of the characteristics of the crypt lesions would be compatible with this view. There is the additional possibility that our observations reflect the direct action of an auto-immune process as suggested by deVries *et al.*[8], and that these lesions are similar in pathogenesis to the crypt lesion observed earlier in animals undergoing homologous disease[9]. On the other hand, it is quite conceivable that the crypt lesions found in animals suffering from homologous disease are due to the lymphoid tissue deficiency known to accompany the graft-versus-host reaction[10].

This work was supported by grants from the National Foundation, U.S. Public Health Service (*HE*–02085, *AI*–00798), American Heart Association and the American Cancer Society.

We thank Dr. Dorothy Sundberg and Dr. Henry Pinkerton for their advice, and Mrs. Elizabeth Simonsen Thorp for her assistance.

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A REINTERPRETATION OF THE VISCERAL SKELETON OF *Acanthodes*

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IN a work of cardinal importance on the Acanthodii, Prof. D. M. S. Watson[1] attempted to demonstrate that these gnathostome fishes had a fully developed prehyoid (spiracular) gill-slit, and a little-modified hyoid arch skeleton which played no part in the suspension of the upper jaw. He also argued that this was true of the various groups of placoderms, and these he grouped with the acanthodians in the grade Aphetohyoidea. He believed the Aphetohyoidea to occupy an intermediate position between the Cyclostomata and Pisces with regard to the modification and incorporation in the jaw apparatus of the anterior visceral arches.

Several workers[2–5] have doubted that the aphetohyoid condition obtained in either the Acanthodii or Placodermi. Watson's arguments are overtly less strong where they concern the latter group, and Stensiö[6] has convincingly demonstrated that the hyoid arch is strongly modified in many placoderms, and that they did not have a complete spiracular gill-slit. Of particular importance are the descriptions by him and other workers of a large hyomandibula in the Rhenanida[6,7], and in at least one of the Ptyctodontida (*Rhynchodontus eximius* Jaekel)[8]. Doubt still exists about the interpretation of the visceral skeleton of the Acanthodii, and Holmgren[2] and Stensiö[3] have tried to show that these fishes are not aphetohyoid. The former's arguments are based to some extent on the assumption that acanthodians are an early branch of the selachian stock; the latter's evidence rests on the supposed course of the nerve fibres supplying what is believed to be the homologue of the actinopterygian preopercular neuromast line (see also Jarvik[5]).

It is only in the Carboniferous and Lower Permian genus *Acanthodes* that the acanthodian visceral skeleton is adequately known. However, even here discussions about its organization have been hindered by uncertainties over the structure of certain crucial regions, and by the often conflicting accounts of earlier workers[1,9,10]. I have studied a large number (about 50) of excellent, negatively prepared specimens of *Acanthodes bronni* Ag., and have been led to a partly new interpretation of the organization of the acanthodian head. This study may also throw some light on the relationships of the Acanthodii to the other classes of gnathostome fishes.

There is a series of four post-hyoidean branchial arches in *Acanthodes*, and the skeleton of the first, glossopharyngeal innervated arch, is structurally quite typical of the whole (Fig. 1). Dorsally it consists of a proportionately small epibranchial element which articulates against an infrapharyngobranchial of similar size. The latter element bears a stout, postero-dorsally directed process, against which a small suprapharyngobranchial articulates. In contrast, the hyoid arch consists, dorsally, of two elements. In all probability they were joined in life by cartilage (Fig. 1*B*), to form a large, continuous structure, which may be regarded as a hyomandibula. It lies in a broad groove on the mesial surface of the palatoquadrate (Fig. 1*A*). As the relationship of the latter to the neural endocranium is definitely fixed, through the articulation of the external otic process and basal process with the postorbital process and basipterygoid process respectively (Fig. 1 *B*, *C*), the position occupied in the head by the hyomandibula can be determined. It articulates with the neural endocranium just behind the postorbital process

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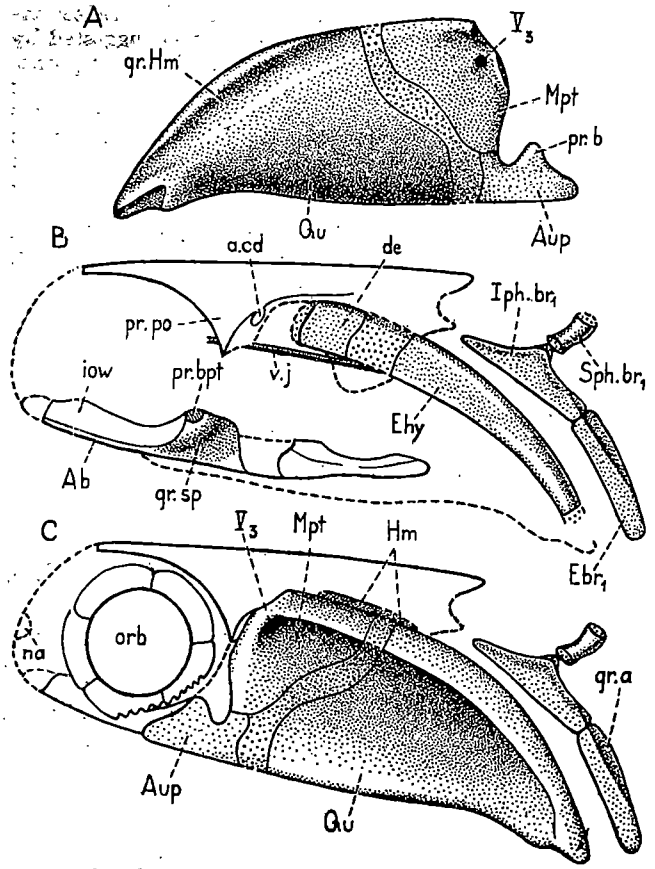


Fig. 1. *Acanthodes bronni* AG. *A*, Palatoquadrate in mesial view. *B*, Ossified regions of neural endocranium with hyomandibula and dorsal elements of first branchial arch *in situ*. *C*, As *B* but with left palatoquadrate and circumorbital plates added. After specimens from L. Permian, Lebach, Saarland (*c.* × 1). *Ab*, anterior basal element; *Aup*, autopalatine ossification; Ebr_1, epibranchial one; *Ehy*, epihyal; *Hm*, hyomandibula; $Iph.br_1$, infrapharyngobranchial one; *Mpt*, metapterygoid ossification; *Qu*, quadrate ossification; $Sph.br_1$, suprapharyngobranchial one; *a.cd*, articular condyle for fossa on metapterygoid ossification; *de*, dorsal element in hyoid arch; *gr.a*, groove for branchial nerves and vessels; *gr.Hm*, groove for hyomandibula; *gr.sp*, spiracular groove; *iow*, interorbital wall; *na*, position of nasal opening; *orb*, orbit; *pr.b*, basal process; *pr.bpt*, basipterygoid process; *pr.po*, postorbital process; *v.j.*, position of jugular vein; V_3, foramen for ramus mandibularis trigemini

and plays an important part in the suspension of the upper jaw.

The large, ventral component of the hyomandibula is undoubtedly the epihyal. The nature of the small, dorsal component is less obvious. Earlier workers[2,10,11] have regarded it as the pharyngohyal (presumably the suprapharyngohyal in modern terminology), but as the hyomandibula articulates against the endocranium dorsally and laterally to the jugular vein, as in actinopterygians, this is not a completely satisfactory explanation. Further, because of the position of the jugular vein, it is very likely that both the infrapharyngohyal and suprapharyngohyal elements are incorporated in the brain-case as in crossopterygians, actinopterygians and elasmobranchs[12,13]. In actinopterygians, the hyomandibula is composed of the fused epihyal and laterohyal elements[13], the latter forming the articular head and being derived from hyoid gill ray material. Probably the dorsal element in the *Acanthodes* hyomandibula is equivalent to the actinopterygian laterohyal, so that the hyomandibula as a whole is of the same nature in the two cases. In elasmobranchs the hyomandibula appears to be formed from the epihyal only[14]; in the Batoidei, hyoid gill ray material may form the pseudohyoid[15].

Some previous writers[6,16,17,19] have independently compared the anterior basal element of the *Acanthodes* brain-case with the parasphenoid of other gnathostomes. It is clearly not a purely dermal formation[18], however, as it includes the basipterygoid processes and the base of the interorbital wall. What is important is that in this structure there can be recognized, at each side, anterior and posterior ascending processes, and a shallow sulcus which is identifiable as the spiracular groove. There is a marked correspondence in gross morphology between the anterior basal of *Acanthodes* and the parasphenoid of early actinopterygians (Fig. 2, ref. 12): and the spatial relationships of the structure in general, and at each side, its ascending processes and spiracular groove in particular, to the hypophysis, basipterygoid process, postorbital process and articular area for the hyomandibula, are exactly the same in the two cases (compare Figs. 1 and 2).

The spiracular groove in *Acanthodes* must have contained the spiracular tube, that is, the reduced dorsal part of the spiracular gill-slit, as is the case, for example, in *Amia* and other bony fishes[12,20]. This part of the spiracular gill-slit was therefore far removed from the hyomandibula (as in bony fishes), and it is quite evident that a normally functioning gill-slit did not exist between the mandibular and hyoid arches. Further, it has been shown above that the hyoid arch skeleton is highly modified in comparison with a typical branchial arch and plays an important part in the suspension of the upper jaw. It is very probable that the ventral half of the spiracular gill-slit was closed, as I hope to discuss on another occasion. The ceratohyal and hypohyal elements lie close to Meckel's cartilage and, as assumed by Jarvik[5], it is likely that the ramus mandibularis internus VII (the post-trematic branch of the n. facialis) was situated in the normal gnathostome (pre-trematic) position on the

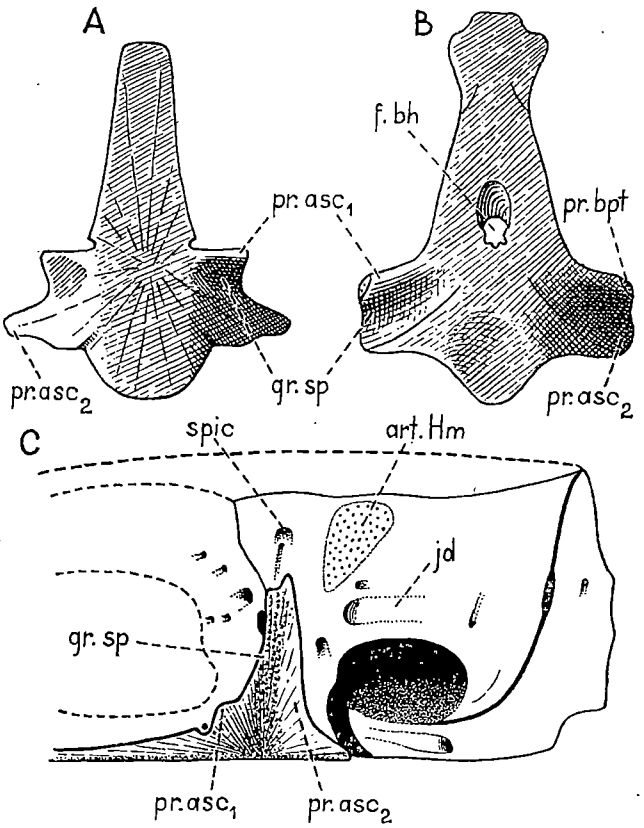


Fig. 2. *A*, *Pteronisculus magnus* (Nielsen) (Actinopterygii; Palaeonisciidae); parasphenoid in ventral view, teeth omitted; from Nielsen[20]. *B*, *Acanthodes bronni* AG.; anterior basal element in ventral view. *C*, *Pteronisculus cicatrosus* White; neural endocranium in lateral view; from Jarvik[12]. *art.Hm*, articular area for hyomandibula; *f.bh*, buccohypophysial foramen; *gr.sp*, spiracular groove; *jd*, groove for jugular vein; $pr.asc_1$, $pr.asc_2$, anterior and posterior ascending process, respectively; *pr.bpt*, basipterygoid process; *spic*, spiracular canal

mesial side of Meckel's cartilage[14]. The inescapable conclusion is that *Acanthodes* is not aphetohyoid, but is broadly the same as actinopterygians, crossopterygians and elasmobranchs in the general organization of the known regions of the head.

There remains for notation the gill-rakers that are found in *Acanthodes*. In the hyoid arch they are situated on the ceratohyal and hypohyal elements and on the epihyal part of the hyomandibula. They play an important part in Watson's arguments for the aphetohyoid condition. However, as Holmgren[2] has pointed out, gill-rakers are commonly present on the hyoid arch in present-day fishes in which the spiracular gill-slit is largely or completely closed, so their presence on this arch cannot be taken as evidence to show that the preceding gill-slit is complete and functional. It appears to me that the gill-rakers on the hyoid arch in *Acanthodes* project into the pharynx, and are associated with the presence of a large hyoidean hemibranch.

It is important to consider how far the conditions described for *Acanthodes* apply to the Acanthodii as a whole. It seems certain that *Acanthodes* is fully representative of the Acanthodiformes[21] (with the families Mesacanthidae, Cheiracanthidae and Acanthodidae) and Ischnacanthiformes, because of what is known of the palatoquadrate in the members of these groups; but there is little direct evidence about the nature of the visceral skeleton in the Climatiiformes (Climatiidae, Euthacanthidae and Diplacanthidae). Watson assumed that members of the latter group were aphetohyoid because he believed that his evidence showed that the main gill cover was carried on the mandibular arch. In the Acanthodiformes, however, it is clear from the relationship of the main, branchiostegal[5] (mandibular[1]) ray-bearing gill cover to the hyoid arch that it is hyoidean in nature, and I believe this to be true of all acanthodians (compare refs. 3–5). I find a marked lack of evidence for the view that any of the Acanthodii are aphetohyoid.

Nielsen[22] has pointed to some resemblances in the morphology of the palatoquadrate and endocranium between early actinopterygians and acanthodians (as represented by *Acanthodes*). His comparisons can be greatly extended in view of what has been outlined here, and we may also note the similar organization of the branchial arches with, for example, elongated hypobranchials, and the presence of large otoliths in acanthodians[1,23] (see also Heyler[24]). The Acanthodii seem to resemble palæoniscoid actinopterygians more closely than they do any other group of fishes, although the significance of this resemblance cannot yet be stated. Very large differences exist between the known members of these groups, particularly in the presence of body spines and the development of the shoulder-girdle. It is now clear that the Acanthodii have no important characters which serve to link them with the Elasmobranchii or Placodermi. In my opinion, these three groups are best ranked as separate classes of gnathostome vertebrates, along with the several classes of 'bony fishes'.

I thank Prof. E. Jarvik for the hospitality of the department in which this study was made, Mrs. M. Liepina for drawing the text-figures, and Dr. H. Jaeger and Prof. H. K. Erben for the loan of material. This work was supported by a NATO research fellowship.

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SYNTHESIS OF NUCLEIC ACIDS IN *Opalina ranarum* Ehrbg.

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THE opalinids are multinucleate, homocaryote Protozoa. The great majority of *Protoopalina* and *Zelleriella* are binucleate. The genera *Cepedea* and *Opalina* are multinucleate. The trophonts of *Opalina ranarum* commonly possess about 200 nuclei. The division of the nuclei is asynchronous and does not appear to be in keeping with the division of the cytoplasm. In spring, during the breeding season of amphibians, the plasmotomy proceeds quickly, resulting in the formation of small individuals or tomonts[1] which are capable of encysting. The tomonts are formed by plasmotomy which usually does not correspond to nuclear division.

The life-cycle and morphological and cytochemical peculiarities of the opalinids have been examined thoroughly[1–5]. These investigations form the basis for the present experimental work which is devoted to the examination of some regularities of DNA and RNA synthesis in the trophonts of *Opalina ranarum* during different seasons.

Experiments were carried out on the natural populations of *Opalina ranarum* isolated from the intestine of *Rana temporaria* L. After isolation the opalines were placed in small test-tubes which are generally used for *Opalina* cultures *in vitro*[4]. Ringer's solution for amphibians was used as the medium for the control cultures. Ringer's solution previously injected with radioactive precursors of DNA and RNA served as the medium for the test cultures.

Tritiated thymidine (from the Radiochemical Centre, Amersham, Buckinghamshire, England) with a specific activity of 2·4 c./mM was diluted with distilled water to 100 mc./ml. Then Ringer's solution was added to it so as to reduce the final concentration of tritiated thymidine to 20 mc./ml.

^{14}C-Adenine (from the State Office 'Isotop', Moscow, U.S.S.R.) with a specific activity of 9 mc./ml. was diluted

with Ringer's solution to a final concentration of 1 mc./ml.

The opalines were incubated in the solutions injected with tritiated thymidine and ^{14}C-adenine for 1, 3, 24 or 72 h. After incubation, the opalines were fixed in Carnoy's solution, embedded in paraffin, and the sections were cut at 5μ.

The investigation was carried out by means of autoradiography on a liquid emulsion of '*R*' type supplied by the NIKFI, Moscow, U.S.S.R. The emulsion was applied to the sections by the method described earlier[5]. The sections with the emulsion were exposed for 20 days. Prior to the application of the emulsion some of the sections were treated with trichloroacetic acid, and some of the sections were treated by ribonuclease in the experiments with ^{14}C-adenine. The rest of the sections were stained with methyl green-pyronine. The control sections to which the emulsion had not been applied were stained by Feulgen, with methyl green-pyronine and hæmatoxylin-eosin. The index (percentage) of labelled nuclei was calculated on the basis of the examination of 1,000 nuclei, and more than 50 sections.

In winter and in spring, during the breeding season of the frogs, the opalines were incubated in the medium containing tritiated thymidine and ^{14}C-adenine within 3–14 days at 4° C, and within 1–72 h at 15° C. No labelled nuclei were found in opalines after their incubation in the medium containing tritiated thymidine. On the contrary, the label was obtained in some of the nuclei and in the endoplasm of opalines incubated in the medium with ^{14}C-adenine. After 72 h of incubation 19 per cent of the nuclei proved to be labelled. The label completely disappeared after treatment of the sections with ribonuclease. Thus, the label of both the nuclei and the endoplasm depended on RNA. The nuclei on the sections stained by Feulgen did not reveal any labelling at all, and this fact confirmed the result obtained in the experiments with tritiated thymidine.

^{14}C-Adenine was used for the examination of DNA and RNA synthesis in *Opalina* trophonts during the summer. The opalines were incubated in the medium containing ^{14}C-adenine for 1–72 h at 15° C. The label was obtained in some of the nuclei and in the endoplasm of each trophont in all the cases of incubation. The percentage of the labelled nuclei depended on the time of incubation of the opalines in the radioactive medium. The number of labelled nuclei increased very quickly after 1 h incubation and achieved a maximum after 3 h incubation (52·24 per cent). After 3 h the number of labelled nuclei began increasing slowly and after 72 h incubation only about 60 per cent of the nuclei appeared to be labelled (Fig. 1). Some of these sections were treated with ribonuclease. This treatment confirmed that 42·78 per cent of the nuclei possessed the label showing the RNA synthesis (this label disappeared after the treatment). The treatment also showed that the label did not disappear in 16·72 per cent of the nuclei. It could be assumed that synthesis of DNA occurred in these nuclei (Fig. 1) and this supposition was confirmed by examination of the sections stained by Feulgen. The nuclear and endoplasmic label completely disappeared after treatment with trichloroacetic acid.

The nuclei labelled with ^{14}C-adenine as a rule possessed small nucleoli, rather dense nuclear substance, and their diameter was about 6–6·5μ. No labels were observed in the nuclei immediately after their division. These nuclei are usually situated very closely to each other and their diameter was about 3–4μ. No labels were found in nuclei with large nucleoli and vacuoles, with a diameter of about 7·5–8μ. This kind of nuclei is usually characteristic of the majority of the nuclei in opalines during winter.

When comparing the results obtained, it can be seen that the nuclei of *Opalina ranarum* do not synthesize DNA either during winter or in early spring. At the beginning

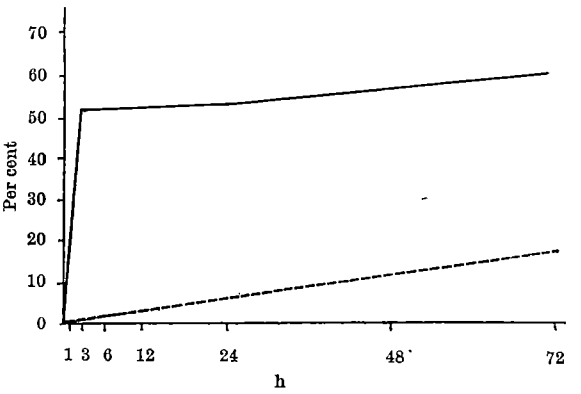


Fig. 1. The number of labelled nuclei in *Opalina ranarum* during various periods of incubation in the medium containing ^{14}C-adenine. The abscissa shows the time of incubation (h). The ordinate shows the percentage of labelled nuclei. The continuous line demonstrates the whole number of labelled nuclei; the dotted line—the percentage of labelled nuclei after the ribonuclease treatment

of spring many *Opalina* trophonts undergo a series of rapid divisions without compensatory growth. These plasmotomic processes result in the formation of pre-cysting forms and cysts containing several nuclei. Such plasmotomy which usually does not correlate with nuclear division is characteristic of opalinids and essentially differs from typical processes of palintomy[5] and syntomy. It can also be supposed that the processes of cyst formation proceed without DNA synthesis in opalines.

The results of the experiments with the incubation of the opalines in the medium containing ^{14}C-adenine show that RNA synthesis can be supposed to proceed all through the winter and spring as well. Approximately one-fifth of the nuclei of each trophont synthesize RNA during these seasons. It was important to establish that the number of the nuclei synthesizing RNA increased in summer and 42·78 per cent of the nuclei of trophonts took part in this process during summer.

Synthesis of DNA was observed in the nuclei of trophonts only in summer. This fact may suggest that *Opalina* cells synthesize the necessary storage of DNA during the summer period of the life-cycle when the Protozoa and their hosts are active. It is in summer that the intensive processes of DNA and RNA synthesis can be observed in the nuclei and in the endoplasm of the opalinids, and besides, many trophonts and their nuclei usually divide during this period of time. Thus, seasonal changes can be observed in the synthesis of DNA and RNA in *Opalina ranarum*.

The experimental results obtained also testify to asynchronism of both DNA and RNA synthesis (within the intervals investigated). The asynchronism in DNA and RNA synthesis can be confirmed by the observations of the label only in some of the nuclei of *Opalina ranarum* in summer, spring and winter as well. This observation allows us to suppose that only the labelled nuclei can synthesize DNA and RNA. Another confirmation of asynchronism of DNA and RNA synthesis in the nuclei of *Opalina ranarum* is the increase of the number of labelled nuclei during different periods of incubation of the trophonts in the radioactive medium (Fig. 1). 100 per cent labelled nuclei were not observed in any cases of incubation of the opalines, though the time of incubation was long enough for each nucleus to be labelled. Hence, the multinuclear cell of *Opalina ranarum* shows not only asynchronism in the nuclear division, but also asynchronic synthesis of DNA and RNA.

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LETTERS TO THE EDITOR

ASTRONOMY

Lunar Erosion and Brownian Motion

RECENT photographs of the lunar surface taken by the U.S. *Ranger* space craft indicate that erosion occurs on the face of the Moon. A number of speculations as to the source of this erosion have appeared. I suggest that Brownian motion provides a mechanism of lunar erosion which seems likely to account for at least part of the observed erosion.

The face of the Moon is subjected to high-velocity impact by meteorites. Optical microscopic observations of rocks (for example, calcite) which have been fractured by high-velocity projectiles (bullets) have shown that a few large chips are produced by impact and that the material along the boundaries of these chips is pulverized into very fine particles which can barely be distinguished at times 850. The typical size of these particles seems to be about $10^{-1}\mu$, and one might expect the surface of the Moon to be covered with rock fragments of similar size. In addition, satellite data indicate that the typical diameter of micrometeorites is in the micron and sub-micron range (J. Mar, private communication, 1964), which further indicates that the dust cover of the Moon might well consist of rock fragments of about $10^{-1}\mu$ diameter. These small rock fragments should have a specific gravity of about 3·3 (the mean specific gravity of the Moon) and their weight in the gravity field at the surface of the Moon (which is about 0·16 times the gravity field at the surface of the Earth) should be about $0{\cdot}53 \times 10^{-12}$ dynes. The temperature on the sunny side of the Moon is of the order of 10^3 °K, and thus the value of kT (Boltzmann's constant, $k \simeq 1{\cdot}4 \times 10^{-16}$ dyne·cm/ °K) on the sunny portion of the lunar surface is about $3/2 \times 10^{-13}$ dyne·cm. Using these values of mg and kT in the barometric formula, one finds that the typical scale of height of the dust cloud on the moon (kT/mg) is roughly 3/10 cm. The density of the dust cloud is 1 per cent of its density on the surface at a height of about four times this typical scale (for example, four 'relaxation distances'), and so the height of the dust atmosphere on the sunny portion of the lunar surface is of the order of a centimetre. As the Moon turns and each portion of the lunar surface is alternately heated by the Sun for about two weeks and then cooled to low temperatures by radiation into space, the dust cloud rises and falls with about a 28-day period. The rising and falling of the dust cloud are accompanied by a redistribution of the dust which should gradually obscure the sharp features on the Moon, and thus provide a partial explanation for the observed lunar erosion.

In addition, the sharp edge of a lunar crater will cast a shadow in which the lunar surface will be cooled rapidly by radiation into space and heated rather slowly by conduction through surrounding material. The temperature of the surface in these shadows should be quite low, causing the dust cloud to fall locally. The dust cloud on the hot sunny side of a ridge will be drawn over the ridge to condense on the shadowy portion, and thus a second mechanism of erosion obtains. Since the mean kinetic energy of the dust particles is $3/2kT$, the mean velocity of those dust particles in the sun is about 10 cm/sec; this should be about the speed at which the dust flows over the tops of ridges in passing from the hot sunny side into the cold shadows where the dust cloud is almost completely condensed. The flow of these fine abrasive fragments should wear down the sharp ridges and thus contribute to the erosion observed in the *Ranger* photographs.

I note that even if the typical diameter of fine rock dust on the Moon were as large as a micron (and this seems unlikely), the height of the dust cloud on the sunny portion of the lunar surface would be of the order 10^{-3} cm (about 10 particle diameters) and the two mechanisms of erosion given here would still operate, although at a much slower rate.

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GEOPHYSICS

Rocket Optical Observation of a Day-time Aurora

STUDIES of the day-time aurora have been restricted almost entirely to investigations by radio techniques. So far as we are aware the only published optical work is that by Noxon[1] on the red and green lines of atomic oxygen. In this communication we report the preliminary results of an investigation of the spectral region 4000–5900 Å obtained during a day-time aurora by means of a rocket probe launched from Fort Churchill, Manitoba, Canada.

The principal difficulty in an optical study of the day-time aurora is, very simply, that of knowing when an optical aurora is, in fact, present. Statistical investigations indicate some correlation between the radio and visual aurora but the correspondence is certainly not one-to-one. Furthermore, we must bear in mind that these investigations are based on night-time measurements and the situation may be different for day-time events. A second difficulty, of particular importance for rocket measurements, arises from the fact that the aurora in the auroral zone is normally quite limited in spatial extent and exhibits both rapid spatial movements and rapid intensity fluctuations. It is, consequently, fairly easy to fire a rocket at an aurora and find the aurora no longer there when the rocket reaches the altitude at which measurements begin.

Montbriand (private communication) has examined the statistical relationship between auroral absorption, as observed by the riometer, and visual auroral forms in northern latitudes. He has found that almost all the occurrences which take place (*a*) during the morning maxima between 0600 h and 0900 h, and (*b*) during which night conditions were still present, were associated with widespread diffused auroral glows. Such glows occasionally have active rays, patches and diffused surfaces within them.

As a result of the foregoing considerations the following criteria were used to decide whether the rocket should be fired: (1) the existence of moderate to strong magnetic activity; (2) the firing to be between 0600 and 0900 h; (3) riometer absorption greater than 0·5 dB; (4) increased absorption as shown by an appreciable increase in the ionosonde f_{min}; and (5) as a secondary criterion the presence of radio auroral echoes observed from the Prince Albert, Saskatchewan, radar facility. These criteria were met on the morning of July 18, 1964, and the launching took place at 0622 C.S.T. Due to technical difficulties, the firing was not at the maximum of the auroral event. Fig. 1 shows a portion of the 30 mc/s riometer record during this period. The flight times are indicated in Fig. 1. The instrument flown was a

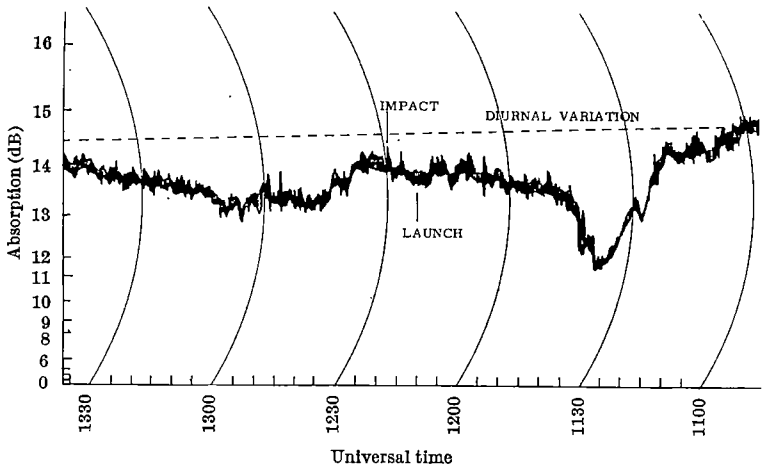


Fig. 1. Riometer absorption at Fort Churchill, Canada, from about 1100–1330 h U.T. on July 18, 1964. The estimated normal diurnal variation is indicated by a dotted line. The times of launch and impact of the rocket are indicated on the chart

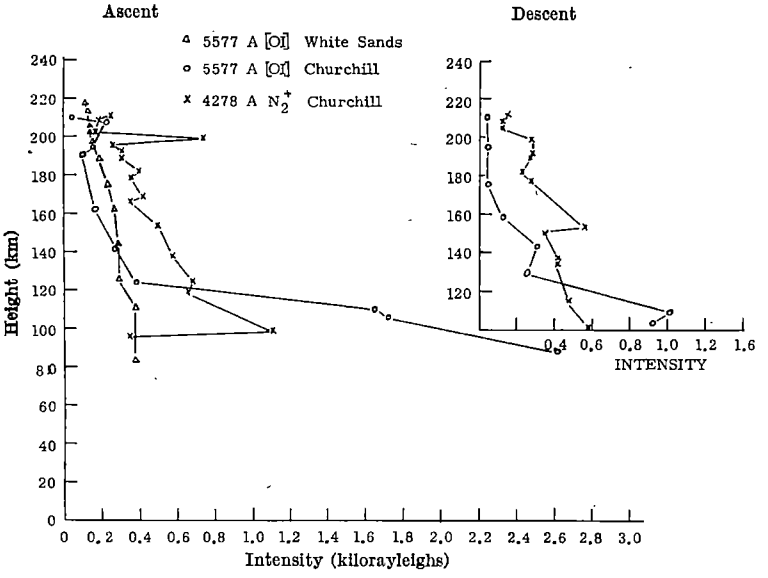


Fig. 2. Intensity variation with altitude for 4278 Å N_2^+ and 5577 Å [OI] at Fort Churchill, Canada, for July 18, 1964, compared with that for 5577 Å [OI] obtained at White Sands Missile Range, New Mexico, November 13, 1963. The intensity scale for the descent data has been displaced to avoid confusion

1-m Ebert spectrophotometer[2] with fixed slits set for a resolution of 11 Å and scanning the wave-length range 4000–5900 Å in 7 sec. The instrumental field of view was directed along the longitudinal axis of the rocket and viewed the sky through an opening exposed by the ejection of the nose cone tip. A 14-in. baffle and sun shade was mounted in front of the input slit to minimize the sunlight scattered into the instrument. The altitude of the rocket was determined by the use of a cosmic ray altimeter.

Preliminary analysis of the data leads to the following results. Because of scattered sunlight only two lines were unambiguously observed in the spectra: 5577 Å [OI] and 4278 Å N_2^+. The variation of the intensities, uncorrected for aspect, of these two lines with height is shown in Fig. 2. Also shown in Fig. 2 are the intensities of the 5577 Å line obtained from a rocket launched at 1600 M.S.T. on November 13, 1963, from White Sands, New Mexico. The same instrument, with a different grating and slit width, was used for the two flights, so that a fairly direct comparison of intensities as a function of height can be made. This comparison leads to the following conclusions: the optical day-time aurora is clearly evident in the lowest scans as a considerably greater luminosity at Churchill than at White Sands. The intensity of the 5577 Å line on the lowest altitude scan, that is, that which would be most comparable with a ground-based observation, is in the range to be expected for an I.B.C. I aurora. It is also of the same order as that reported during a part of the day-time aurora observed by Noxon[1]. We consider the height at which the luminosities are approximately equal at the two sites to represent the upper border of the aurora: Using data from both ascent and descent we determine the upper border as lying between 113 and 123 km, similar to a typical night-time aurora. The lower border cannot be determined from our data. Above 123 km the relative intensities are comparable in magnitude though they appear to fall off more rapidly with altitude at Churchill than at White Sands. This may be due to the difference in latitude, time of day, or to the presence of an auroral component even at the higher altitudes. It is difficult at present to determine which of these factors is responsible, though the descent data down to about 160 km indicate that the day-glow component at Churchill may be appreciably lower than the day-glow at White Sands. The 4278 Å N_2^+ band intensity appears to be due to particle bombardment, superimposed. The ratio of 4278 Å intensity to that at 5577 Å above 150 km is comparable to that reported by Wallace and Nidey[3] for the day-glow at White Sands. Thus we conclude that above about 120 km the optical observations refer primarily to a day-glow component and that the aurora is confined primarily to heights lower than this. Unfortunately no data on the ratio of these two lines for the normal day-glow below 150 km are available for comparison with the results of the present flight. The results reported here come from a preliminary analysis of the data.

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OCEANOGRAPHY

Scavenging of 'Dissolved' Organic Matter from Sea-water with Hydrated Metal Oxides

In an effort to recover the 'dissolved'* organic matter from large volumes of sea-water by a simple and widely applicable process, co-precipitation experiments with hydrated metal oxides were undertaken using natural sea-water.

Previously published reports on the scavenging or co-precipitation of organic matter from sea-water had indicated that such an approach might be fruitful. Recoveries of organic compounds of the order of 80–100 per cent have been claimed. Jeffrey and Hood[1] co-precipitated the organic matter from a ^{14}C-labelled, aged algal culture filtrate by increasing the *p*H to 10 to precipitate $CaCO_3$ and $Mg(OH)_2$ from the sea-water. This scavenged 79 per cent of the ^{14}C-labelled material. They recovered 95 per cent of the labelled organic matter from the aged culture

* 'Dissolved' is arbitrarily defined as that passing a 0·45μ *HA* 'Millipore' membrane filter unless otherwise noted.

Table 1. PER CENT REMOVAL OF ORGANIC CARBON FROM SEA-WATER USING HYDRATED METAL OXIDES

Sea-water volume (l.)[1]	Metal[2]	Metal ion concentration (mg/l.)	Final *p*H	Settling time (h)	Initial carbon (mgC/l.)[3]	Final carbon (mgC/l.)	ΔC	Percentage removed
18[4]	Fe	20	4·8	4	1·05	0·39	0·66	63
18[4]	Fe	20	6·1	8	1·00	0·40	0·60	60
200	Fe	20	5·0	18	2·10	1·30	0·80	38
200	Fe	20	5·3	12	1·24	0·77	0·47	38
1600	Fe	20	6·4	16	1·57	0·95	0·62	40
1·0[5]	Fe	20	5·2	4	0·48	0·36	0·12	25
0·5	Fe	100	5·0	22	1·08	0·79	0·29	27
1·0	Fe	1000	5·4	24	1·40	0·65	0·75	54
0·5	Fe	20	5·0	22	1·21	0·75	0·46	38
0·5	Fe,Mn	20,20	9·0	22	1·14	0·82	0·32	28
0·5	Mn	20	9·0	22	1·20	0·95	0·25	21
0·5	Fe	20	5·0	24[6]	1·47	0·83	0·64	43
0·5	Fe,Al	10,10	5·0	2[6]	1·27	0·79	0·48	38
0·5	Al	20	5·0	2[6]	1·50	1·25	0·25	17
0·5	Fe,Cu	10,10	5·0	2[6]	1·27	0·79	0·48	38
0·5	Cu	20	5·0	2[6]	1·33	1·11	0·22	16
0·5	Fe,Cu	20,10	8·0	24[6]	1·18	0·81	0·37	31
0·5	Fe,Al	20,10	8·0	24[6]	1·35	1·08	0·27	20

[1] All surface samples from the Scripps Institute of Oceanography pier except where indicated. The variable initial carbon is a result of sampling at different times.

[2] Metal as Fe^{3+}, Mn^{2+}, Cu^{2+} and Al^{3+}.

[3] Mean of duplicate samples. The precision of the method is ± 0·12 mg C/l. at the 95 per cent confidence limit.

[4] Surface water collected 5 miles offshore.

[5] Collected 85 miles offshore at a depth of 1,500 m.

[6] Sample centrifuged for 10 min at 2,000 r.p.m. in a clinical centrifuge after the settling time indicated.

by co-precipitation with $Fe(OH)_3$ at a *p*H of 7·6–8·21. The concentration of ferric iron used was 5·6 g Fe^{3+}/l. Tatsumoto *et al.*[2] and Park *et al.*[3] co-precipitated combined and free amino-acids from natural sea-water with $Fe(OH)_3$ (124 mg Fe^{3+}/l.) and Williams[4] recovered 0·1 mg C/l. of organic acids from deep sea-water by $Fe(OH)_3$ scavenging using 20 mg of Fe^{3+}/l. The addition of known organic compounds to sea-water in concentrations greater than those expected under natural conditions, and subsequent co-precipitation with $Fe(OH)_3$, yielded recoveries of polypeptides, organic acids, sugars and amino-acids ranging from 0 to 100 per cent (Williams[5] and Tatsumoto *et al.*[2]). In addition, Bader *et al.*[6] demonstrated scavenging of various organic compounds added to sea-water by the minerals montmorillonite and kaolinite.

In all the above cases, total organic carbon data for the sea-water were lacking. Menzel and Vaccaro[7] have recently developed a rapid and sensitive method for the determination of total organic carbon in sea-water, and this analysis has been used in the present work to assess the percentage removal of the dissolved organic carbon in sea-water samples by co-precipitation with $MnO_2.nH_2O$, $Fe_2O_3.nH_2O$, $Al_2O_3.nH_2O$, and $CuO.nH_2O$. These values are compared with the percentage adsorption of organic carbon by filter pads of activated alumina, a celite–MgO mixture and silicic acid.

The sea-water samples were collected from the Scripps Institution of Oceanography pier, from 5 miles offshore, and from a depth of 1,500 m at a station 85 miles offshore. Small samples were filtered immediately through an *HA* 'Millipore' filter, discarding the initial 200 ml. of filtrate, which contains a significant amount of organic matter leached from the filter. The 1,600- and 200-l. samples were filtered through a 35μ mesh-size net only. The samples were then acidified to *p*H 2 with organic carbon-free 6 N HCl. Iron, manganese, copper and aluminium were added as organic carbon-free solutions of $FeCl_3$, $MnSO_4$, $CuSO_4$ and $AlNH_4(SO_4)_2$. The 'Celite'–MgO mixture (1 : 1), activated alumina (neutral) and silicic acid were freed of organic matter by ignition at 450° C. The metal hydroxides were precipitated by the addition of 1 N KOH to give the desired final *p*H and allowed to settle. Organic carbon determinations were made on the initial filtered sea-water, the sea-water plus added metal at *p*H 2, the sea-water plus added metal and KOH at the final *p*H and on the supernatant liquid at time intervals thereafter, as the precipitates settled.

The organic carbon in the $Fe(OH)_3$ precipitate, after decanting the supernatant, was determined in two experiments and the carbon values checked to within 10 per cent of the values calculated from the initial and supernatant amounts. The solid adsorbers, approximately 25 g, were slurried in organic carbon-free distilled water, poured into the 'Millipore' filter assembly to form a pad 3 cm deep, through which 400 ml. of the filtered sea-water was passed; the initial 200 ml. of filtrate was discarded.

Table 1 summarizes the conditions and percentage removal of carbon by co-precipitation. Table 2 gives the carbon adsorbed by 'Celite'–MgO, alumina, and silicic acid.

The effect of the final *p*H on the percentage removal of organic matter was determined by precipitating 0·5 l. samples of sea-water (initially containing 1·26 mg C/l.) with 20 mg Fe^{3+}/l. at a final *p*H of 4, 5, 6 or 7. The percentage removal was 38, 44, 42 or 38 respectively. The percentage removal as a function of time was variable but, in general, the maximum amount was co-precipitated in 8 h and further standing did not significantly improve the yield.

Table 2. PERCENTAGE REMOVAL OF ORGANIC CARBON FROM SEA-WATER USING SOLID ADSORBANTS.

Adsorbant	Initial mg C/l.[1]	Final mg C/l.	Percentage removal
'Celite'-MgO	1·31	1·06	19
Silicic acid	1·31	1·01	23
Alumina	1·31	0·75	43

[1] The same sea-water was used in all three experiments.

The 200-l. samples were co-precipitated in a 220-l. polyethylene tank, and the 1,600-l. sample in a 1,900-l. polyvinylchloride-lined tank. All other precipitations were made in glass. There was no detectable organic carbon contamination from the reagents or from the polyvinylchloride liner. The use of the polyethylene tank gave an increase of 0·1 mg C/l.

It is evident from Table 1 that Fe^{3+} at a concentration of 20 mg/l. and at a final *p*H from 4 to 6 is the most effective scavenger of the systems studied. A five-fold increase in Fe^{3+} did not increase the yield, while a fifty-fold increase only gave an additional 12 per cent removal. This latter concentration gives cumbersome amounts of precipitate in dealing with large volumes of water. Centrifugation of the supernatant solution did not improve yields.

The amount of organic carbon scavenged from the five sea-water samples from the surface, collected at the pier, using 20 mg Fe^{3+}/l. and at a final *p*H of 5 to 6·4, ranged from 38 to 43 per cent, indicating that a relatively constant fraction of the organic matter was scavenged by $Fe(OH)_3$ from samples with variable initial levels of organic carbon. The low recovery from the 1,500-m sample was consistent with the hypothesis that deep-water organic matter has already been exposed to any indigenous clay mineral and

ferric hydroxide scavenging processes in the sea and thus contains less organic matter susceptible to further scavenging.

There may be a refractory compound or group of compounds in deep sea-water which are resistant to chemical and biochemical oxidation or utilization and which may be 'old' in the sense that the inorganic carbon in deep water is old relative to that in the surface water. This study was undertaken in the hope of isolating sufficient dissolved organic carbon from deep water to obtain an age using radiocarbon dating.

This work was sponsored by the United States Atomic Energy Commission, Contract *At*(11-1)-34, Project 108. *AEC* Ref. No. *UCSD*-34*P*-108-2.

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Uranium Distribution in Drake Passage Waters

CONSIDERING the amount of work that has been done concerning the uranium content of oceanic waters, remarkably few data can be found for the Antarctic Ocean compared with the North Pacific, the North Atlantic and the Indian Oceans[1-5]. It is possible the Antarctic waters might have a considerable interchange with the deep waters of the Atlantic and the Pacific. Information about the distribution of uranium in Antarctic waters might, therefore, contribute significantly to knowledge of oceanic circulation.

Water samples were collected during a cruise (TRIDENTE-3, February–March 1963) by R/V *Capitan Canepa* of the Argentine Navy Hydrographic Service, along the transect of Drake Passage (Fig. 1). The microquantities of uranium present in the samples were determined fluorometrically by using fused mixture with sodium fluoride[6].

To each sample of 200–500 ml., 10 ml. of mixed solution of concentrated HNO_3, aluminium nitrate solution (50 mg/ml.) and ammonium hydrogen phosphate solution (12 mg/ml.), in the ratio of 3 : 2 : 5, was added. After boiling the sea-water for 10 min to decompose the carbonate anionic complex of uranium, aluminium phosphate was precipitated at *p*H 6 by adding ammonium hydroxide. Uranium was co-precipitated with the aluminium phosphate.

The precipitate-containing uranium was ignited at 450°–500° C. The residue was dissolved in 10 ml. of HNO_3-acidified aluminium nitrate (a solution contained 900 g of $Al(NO_3)_3 \cdot 9H_2O$ in 500 ml. of 1 : 10 HNO_3) as a solvent and salting-out agent.

Uranium was extracted with 10 ml. of ethylacetate, and a 2-ml. portion of ethylacetate solution was evaporated on a platinum dish (diameter 1·5 in.) moderately heated over a water-bath. To the residue on the platinum dish, 2 g of flux (fused and pulverized mixture of K_2CO_3, Na_2CO_3 and NaF with the ratio of 45·5 : 45·5 : 9) was added and fused for 20 min at 650° C in a specially designed double-rotating electric oven. The cake obtained from rapid cooling of the fused material was stored in a desiccator. The fluorescence was measured at a wave-length of 555 mμ with a transmission-type fluorometer[6]. The uranium content of reagents used for uranium determination were checked before the analysis. The results showed that the total blank was below 0·01 μg.

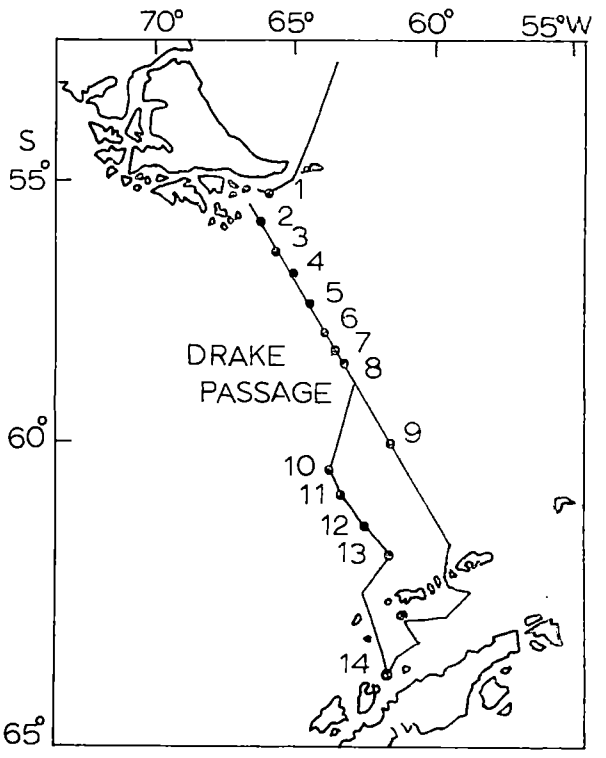


Fig. 1. Location of water sampled

The results of analyses of the surface waters are summarized in Table 1, and the vertical distribution of uranium for stations 8 and 9 is shown in Table 2. It can be seen that fairly constant values of uranium were obtained in the surface sample from elsewhere in Drake Passage. In this season, the Antarctic Convergence was located

Table 1. URANIUM IN DRAKE PASSAGE WATERS

Station	Location S.	Location W.	Temp. (°C)	Salinity (‰)	Uranium ($\times 10^{-6}$ g/l.)
1	55° 06′	66° 07′	8·9	32·58	2·8 ± 0·02
2	55° 50′	66° 05′	6·5	34·05	3·3 ± 0·03
3	56° 30′	65° 33′	5·4	34·14	3·3 ± 0·03
4	55° 52′	65° 01′	5·9	34·07	2·7 ± 0·02
5	57° 27′	64° 19′	5·0	34·14	3·0 ± 0·02
6	58° 00′	63° 50′	4·0	34·04	3·0 ± 0·02
7	58° 25′	63° 20′	3·5	33·95	3·2 ± 0·02
8	58° 28′	63° 10′	2·2	34·01	3·0 ± 0·02
9	60° 00′	61° 25′	1·2	33·88	3·0 ± 0·02
10	60° 32′	63° 34′	2·0	33·96	3·2 ± 0·02
11	60° 56′	63° 08′	1·8	33·89	3·1 ± 0·03
12	61° 28′	62° 20′	1·2	33·91	3·5 ± 0·02
13	61° 58′	61° 30′	1·1	33·79	3·1 ± 0·02
14	64° 00′	61° 41′	0·6	33·77	3·1 ± 0·02

Table 2. VERTICAL DISTRIBUTION OF URANIUM IN DRAKE PASSAGE

A. Sampling site: 58° 28′ S., 63° 10′ W. (St. 8, March 9, 1963)

Depth (m)	Temp. (° C)	Salinity (‰)	Uranium ($\times 10^{-6}$ g/l.)
0	2·20	34·01	3·0 ± 0·03
48	2·31	33·96	3·0 ± 0·03
96	1·18	34·08	3·0 ± 0·03
192	0·90	34·16	3·0 ± 0·03
384	1·85	34·42	2·9 ± 0·03
693	2·26	34·61	3·5 ± 0·03
932	2·11	34·67	3·3 ± 0·03
1,420	1·79	34·75	3·5 ± 0·03
1,903	1·42	34·78	3·4 ± 0·03

B. Sampling site: 60° 00′ S., 61° 25′ W. (St. 9, March 10, 1963)

Depth (m)	Temp. (°C)	Salinity (‰)	Uranium ($\times 10^{-6}$ g/l.)
0	1·20	33·88	3·0 ± 0·03
180	0·72	34·23	3·1 ± 0·03
360	1·95	34·58	3·0 ± 0·03
770	1·99	34·60	3·2 ± 0·03
1,820	1·18	34·64	3·3 ± 0·03

between 58° S and 59° S. However, there was no significant difference in uranium content between the samples from both sides of the convergence.

Baranov and Khristianova gave an average value of $2{\cdot}7 \times 10^{-6}$ g/l. of uranium for the southern Indian Ocean[5]. Rona *et al.* and Wilson *et al.* gave an average of $3{\cdot}3 \times 10^{-6}$ g/l. of uranium for the North Atlantic and the North Pacific waters[1,2]. An average of $3{\cdot}4 \times 10^{-6}$ g/l. of uranium was given by Miyake and Sugimura for the western North Pacific waters off Japan[4]. For the uranium content of surface waters in Drake Passage, an average value of $3{\cdot}1 \pm 0{\cdot}2 \times 10^{-6}$ g/l. is obtained, which is in the same order of magnitude as those in previous work.

In its vertical distribution, uranium tends to show a minimum concentration about the depth of 300–400 m, increasing at greater depth. A similar vertical distribution of uranium was found by Miyake and Sugimura in the area of the western North Pacific[4].

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METALLURGY

Austenite/Carbon Reaction in Cast Iron

In our experiments in collaboration with the British Cast Iron Research Association, on the gouging and machining of grey iron with a plasma jet, it came to our notice that the skin of the iron under the gouge had become white due to being heated close to or slightly above the melting point and rapidly cooled. Subsequent experiments have shown that, if we quickly passed the plasma jet over the surface of the iron so that it reached a high temperature (~1,100° C) but did not melt, a white surface layer was produced which in some cases contained graphite.

Fig. 1 shows the cementite/graphite surface layer. In the lower areas of the photomicrograph, cementite (white)



Fig. 1. Photomicrograph of the cementite/graphite surface layer (×100)



Fig. 2. Lower right-hand corner of Fig. 1 (×400)

can be seen growing out from graphite flakes. In these areas the thermal cycle has been such as to arrest the reaction. Fig. 2 is a photomicrograph of this particular area at higher magnification showing the cementite 'dendrites' in greater detail. The particular iron used contained 3·4 per cent carbon, 3·5 per cent silicon and 0·67 per cent manganese.

The mechanism of the production of this layer is probably that the rapid localized heating causes the formation of Fe_3C by reaction between the graphite and iron, and the rapid cooling retains the Fe_3C, giving it insufficient time to decompose back to iron and graphite. The surface layer comprising cementite and some graphite is hard (up to 1,000 Vickers pyramid number) and, with the added self-lubricating and oil-retaining effect of the graphite flakes, it should provide good wear resistance.

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CRYSTALLOGRAPHY

Growth of Crystals from the Melt in Glass Tubes

The rates of growth of benzophenone and salol from the melt have been investigated. Single crystals were grown freely in the melt, and the rate of advance of a single face was measured by means of a travelling microscope. In further experiments the crystal faces were allowed to grow into glass capillary tubes which were situated in the melt normal to the face. The melts were held at constant under-coolings of 4° C or less, by means of a thermostat: under these conditions it was found that on entering the capillary the face remained plane but advanced down the tube at a rapidly diminishing rate, finally practically ceasing to grow altogether. The data on an experiment with the (100) face of salol are as follows:

Undercooling 3·6° C. Capillary 1·5 mm internal diameter.
Free growing rate was 25×10^{-5} cm s^{-1}
Average rate for 1st 5½ h in capillary $0{\cdot}87 \times 10^{-5}$ cm s^{-1}
Average rate between 52 and 92 h $0{\cdot}02 \times 10^{-5}$ cm s^{-1}

When a crystal face had ceased to grow inside a capillary it was found that on touching the face inside the capillary with a glass spike the face started to advance again, but at a rapidly diminishing rate, finally coming virtually to rest once more. For example, the (001) face of a benzophenone crystal was allowed to grow into a capillary of 1·5 mm internal diameter at an undercooling of 2·0° C. The free growth rate under these conditions is about

Time (min)	Growth rate (cm s^{-1}) × 10^5
0	0·2
34	0·1
34	touched surface inside capillary with glass spike
34½	11·9
40	4·6
45	2·1
50	0·9

The above growth rates were determined from the slope of the displacement/time curve).

20×10^{-5} cm s^{-1}. Growth measurements were then begun at a time when the rate had already fallen to a very low figure.

Most of the experiments were carried out in tubes of diameter 1·5 mm or less. In one experiment, however, the (010) (the slowest growing) face of a salol crystal was allowed to grow into a much wider tube of 0·8 cm internal diameter at an under-cooling of 2·2° C: the effect was similar although the retardation was somewhat less, the results being as follows.

Time (h)	Displacement (cm)	Rate (cm s^{-1}) × 10^5
0	0	6·7
⅛	0·04	
⅝	0·087	8·2
1⅝	0·382	

The crystal which had hitherto been free growing now entered the 0·8 cm diameter tube

2½	0·480	1·6
20	1·498	
67	2·886	0·18
96	3·074	
122	3·159	0·09
146	3·198	0·045

(The rates given are the average rates over the times indicated by the brackets)

Several different types of experiment were carried out with salol which convinced us that the observed retardation is not due to accumulation of impurities in the melt in advance of the growing face. No altogether satisfactory explanation of the observation has yet been found; but tentatively two alternative lines of approach are suggested. If growth layers were normally nucleated at the edges of faces, it seems possible that when the crystal grows into, and fills, a glass tube this nucleation process would be hindered or eliminated; this would explain the retardation on entering the tube. But it would also be necessary to assume that scratching the crystal surface with a glass spike would temporarily set up defects in the surface which would enable new layers to be nucleated at those defects, until such time as the defects are healed over or grow out to the walls.

Alternatively, growth layers may always be initiated at defects in the surface (which may be screw dislocations, groups of screw dislocations, or major defects such as grain boundaries). Then it would be necessary to assume that when growing in a tube these defects either grow out to the walls or are healed over. At the same time it would be necessary to assume that fresh defects are less easily formed in a crystal that is confined in a tube than in a free-growing crystal.

When growing in capillaries at under-coolings of greater than about 6° C the advancing faces were no longer plane, the crystals were no longer transparent and appeared to be polycrystalline; under these conditions the slowing up of growth was not observed.

The results presented here suggest that the measurement of growth rates in capillary tubes, on which much reliance has been placed in the past, is no guide to the behaviour of free-growing crystals.

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CHEMISTRY

Adsorption of Krypton and Xenon on Evaporated Metal Films

Brennan *et al.*[1] quoted our earlier communication[2] in connexion with the determination of the effective cross-sectional areas of adsorbed xenon molecules, σ(Xe). However, a numerical error appears in their article. The values 13·8 and 17·2 Å² were not given in our communication and the value σ(Kr) = 19·5 Å² was not used as a reference value. Our communication indicated that we found from the measurements of xenon adsorption on evaporated nickel films at 78° K that σ(Xe) = 19·3 Å², and from measurements at 90° K that σ(Xe) = 21·8 Å². Moreover, σ(Kr) = 21 Å² was used as the reference value. The adsorbed amounts of xenon and krypton were always estimated by the same volumetric method and evaluated by means of the Brunauer–Emmett–Teller theory.

The value of the ratio σ(Xe)/σ(Kr) (xenon and krypton adsorption was measured at 78° K) was 0·92 in our communication, which is in good agreement with the value 0·95, which Brennan *et al.* calculated from their experimental data for the same system.

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Catalysed Reaction of Xenon with Fluorine

Nickel reaction vessels have been used in the past for the preparation of xenon fluorides because of the good resistance of this metal to attack by fluorine[1–3]. However, despite the apparent ease with which these compounds form, the possibility that nickel may be acting as a catalyst has apparently been overlooked. To test this possibility we have carried out the following experiments.

Xenon (99–100 per cent, balance krypton) and fluorine (at its vapour pressure at −196° C) were mixed in a fused silica vessel. A nickel filament (0·020 cm diam.) was heated electrically in the gas mixture and its temperature monitored by following the resistance. Pressure changes in the vessel were followed with an all-metal bellows manometer. The results shown in Fig. 1 were obtained with the walls of the vessel cooled to −78° C. The number of molecules that reacted was determined from the pressure changes observed when the nickel wire was heated in the range 180°–400° C. The reaction is of zero order until about half the initial material has reacted, then slows down, and has almost stopped when two-thirds has reacted. The product condenses in the form of a fine white powder on the cooled walls of the reaction vessel. The composition of the gas phase at the end of an experiment was determined by freezing the xenon in liquid nitrogen and pumping the excess fluorine away.

The ratio of fluorine to xenon (F_2/Xe) reacted was reproducibly 1·2 and was independent of the temperature of reaction over the range examined and independent of the initial F_2/Xe ratio in the range 0·8–2·0. The product, on standing at room temperature in the evacuated vessel, decomposed over about one hour to give a total pressure corresponding to the amounts of xenon and fluorine reacted.

The Arrhenius plot of the reaction rates is shown in Fig. 2. From this is derived an activation energy of 10·4 kcal $mole^{-1}$ and a pre-exponential factor of $\sim 10^{21}$ molecules sec^{-1} cm^{-2}. This last-mentioned value is comparable with the collision frequency of xenon with the nickel wire.

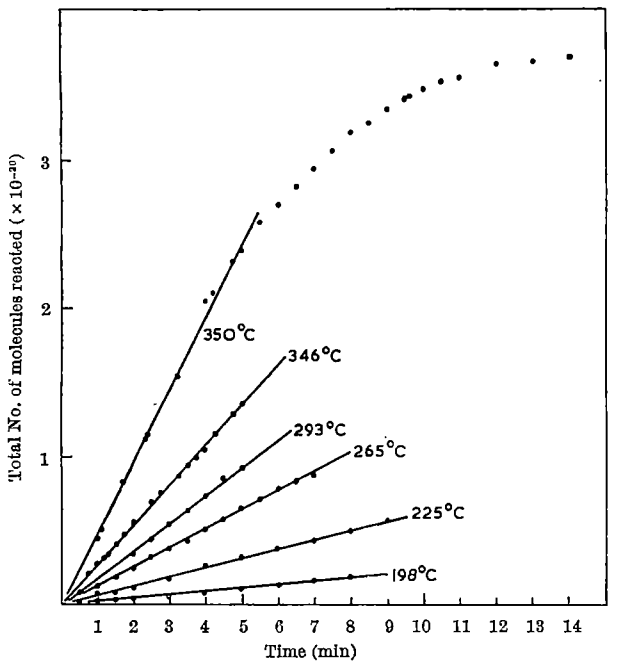


Fig. 1. Rate of reaction of xenon with fluorine at different temperatures

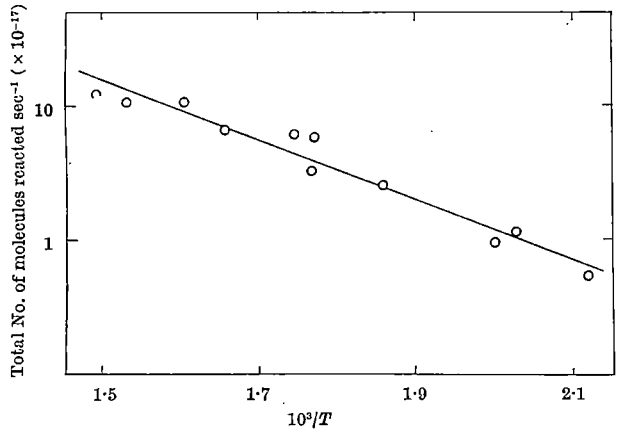


Fig. 2. Arrhenius plot for the reaction of xenon with fluorine

Reaction rates measured with the walls of the vessel at 0° C were lower and zero order conditions held over a much smaller extent of reaction (about one-quarter). This suggests that the product interferes with the reaction if it is not effectively frozen out. The product crystals, which condensed at 0° C, were larger than those condensed at −78° C, colourless, and could be readily sublimed.

Experiments in which xenon and fluorine were heated in the silica vessel in the absence of any hot metal surface and also in which fluorine alone was heated by the nickel filament showed that no reaction took place.

To compare the catalytic activity of nickel with other metals, similar experiments were carried out on copper, aluminium and platinum wires. In the temperature range examined for nickel these metals produced no measurable reaction. However, copper was effective above ~550° C with a rate corresponding to that observed at 200° C on nickel. Reaction occurred on aluminium from ~430° C up to its melting point; the rate at 450° C corresponded to the nickel rate at 200° C. In both cases a product was observed which behaved similarly to that formed in the nickel reaction. The interaction of fluorine alone with these metals under the reaction conditions was found to be small.

At about 510° C, platinum reacted vigorously to give a sharp drop in pressure. The walls of the vessel were covered with a pale yellow solid which hydrolysed on exposure to air. Addition of water to this material produced a yellow precipitate. The relative amounts of xenon, fluorine and platinum used up in the reaction were Xe : F : Pt = 2 : 12 : 1.

Electron-microscope examination of the nickel wire after reaction showed that the surface (×10,000) was roughened by polycrystalline flakes (~1μ), which were shown by electron diffraction to be composed of very small crystals of NiF_2. The surface of the copper wire was heavily encrusted with CuF and CuF_2. The aluminium surface was covered with crystals (0·2μ) of AlF_3.

The different activities observed for these metals show that the reaction between xenon and fluorine is catalysed by a nickel surface and to a lesser extent by copper and aluminium. The low activation energy measured is consistent with the ease of formation of these compounds and may be of interest when considering the energies involved in the formation of the xenon fluorides.

We thank Dr. T. A. O'Donnell of the Chemistry Department, University of Melbourne, for his advice. We also thank Mr. V. Sheludko for assistance with the experimental work.

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New High-pressure Polymorphs of Sodium Bromide and Sodium Iodide

Recently new high-pressure polymorphs were discovered for sodium chloride[1] and sodium fluoride[2]. It is reasonable to expect the other sodium halides also to show polymorphism when subjected to elevated pressures. Bridgman[3] failed to find any evidence for polymorphism in the sodium halides.

Pressures up to 50 kbars were generated in a piston-cylinder apparatus similar to that previously described[4]. Possible transitions were investigated with the volume-discontinuity method[5]. The halides were dried at 300° C pre-pressed into a cylindrical pellet about 1 in. long and 0·5 in. in diam., and wrapped in thin lead foil. Piston displacement versus pressure was plotted. Temperatures up to 200° C could be obtained by wrapping a heavy-duty heating coil around the pressure vessel.

The curves obtained for sodium bromide showed a very sluggish but reversible transition near 11·5 ± 2·5 kbars at temperatures of 175°–200° C. At lower temperatures the transition did not occur at all. The kinetics of the transition appeared to be very similar to the kinetics of the corresponding transition in sodium chloride[6] and in some alkali fluorides[7].

No transition was found in sodium iodide at temperatures ranging up to 140° C, but at 164° C a transition was easily picked up at 10·2 ± 2·5 kbars. The transition was also found at 171° C, 11 ± 2·5 kbars, and 175° C, 11·6 ± 2·5 kbars. The sodium iodide transition is much less sluggish than the sodium bromide transition, and at 194° C, where the transition pressure is 14·0 ± 2·5 kbars, the transition was still running when the piston reached the end of its travel. The change in volume is at least 8 per cent, and may be much more. A fresh set-up was made to check these values and similar results were found. The best transition pressure obtained is 14·3 ± 1·5 kbars at 190° C, being the average of the readings obtained with increasing and with decreasing pressure.

Transitions have now been found for all the sodium halides at elevated pressures. The transition in sodium chloride is one from the sodium chloride-type structure

to the cæsium chloride-type structure[1], and it is to be expected that the transitions in sodium fluoride, bromide and iodide are similar.

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Polymeric Structure of Mercury tert-Butyl Mercury Mercaptide

Investigations of metal alkoxides[1] have shown that polymeric molecules $[M(OR)_x]_n$ are generally formed unless the alkyl group (R) is bulky. Work on sulphur analogues known as metal mercaptides $M(SR)_x$ was undertaken in order to compare the structures and properties of metal alkoxides and metal mercaptides. A survey of the work on metal mercaptides showed that the lowest mercaptide, mercury methyl mercaptide $Hg(S{\cdot}CH_3)_2$, has quite a compact structure[2] having weak intermolecular Hg—S bonds. The structure is consistent with the high melting point (185°) and low solubility of this compound. The next higher mercaptide, mercury ethyl mercaptide $Hg(S{\cdot}Et)_2$, melts at a low temperature (76°) and as such has essentially a molecular structure[3]. The structures of most of the higher mercaptides are likely to be molecular since they are volatile and soluble in organic solvents. The properties of mercury *tert*-butyl mercaptide did not fit into this pattern. This compound has a low solubility and sublimes at a high temperature (135°) under low pressure. An X-ray analysis of this compound was, therefore, undertaken in order to elucidate its crystal structure.

Rotation and Weissenberg photographs (copper radiation) of the orthorhombic needles of the compound obtained by vacuum sublimation gave the following results: $a = 18{\cdot}36 \pm 0{\cdot}03$ Å, $b = 9{\cdot}18 \pm 0{\cdot}02$ Å, $c = 7{\cdot}52 \pm 0{\cdot}01$ Å; $d_{obs} = 1{\cdot}97$ g cm^{-3}, $d_{calc} = 1{\cdot}972$ g cm^{-3}; $z = 4$; space group $C222$. The $hk0$ and $h0l$ results were obtained and measured using multiple-film technique and visual estimation.

The ($hk0$) and ($h0l$) Patterson projections gave the co-ordinates for Hg_1, S_1 and S_2 and precluded the other two space-groups, namely, $Cmm2$ and $Cmmm$. Positions of the carbon atoms obtained by mercury–difference Fourier syntheses are less precise because of the presence of the heavy mercury atom in the crystal structure. The structure was refined by the successive difference Fourier syntheses and the final agreement index for all $hk0$ and $h00$ reflexions is 0·12. All calculations were made on the IBM 7040 computer using programmes written in *Fortran IV*, in this laboratory.

The results of the structure analysis of this compound are interesting. The structure of this compound is quite different from those of the other known mercaptides and consists of rows of mercury atoms (Hg—Hg = 3·76 ± 0·01 Å) parallel to the crystallographic C axis. Each mercury is surrounded by four sulphur atoms (Hg—S = 2·59 ± 0·025 Å, 2·66 ± 0·025 Å) in a highly distorted tetrahedral arrangement, angles SHgS being 87 ± 1·5°, 90 ± 1·5°, 121 ± 1·5°. A polymeric chain parallel to the C axis is formed by an infinite sharing of the opposite edges of the tetrahedra. The sulphur of the mercapto group acts as a bridge linking the two neighbouring atoms. The values of the Hg—S—Hg bridge angles are 90 ± 1·5° and 93 ± 1·5°. The dimensions of the chain are shown in Fig. 1. All chains are parallel to each other and to the C axis. The forces between the various chains are of residual type.

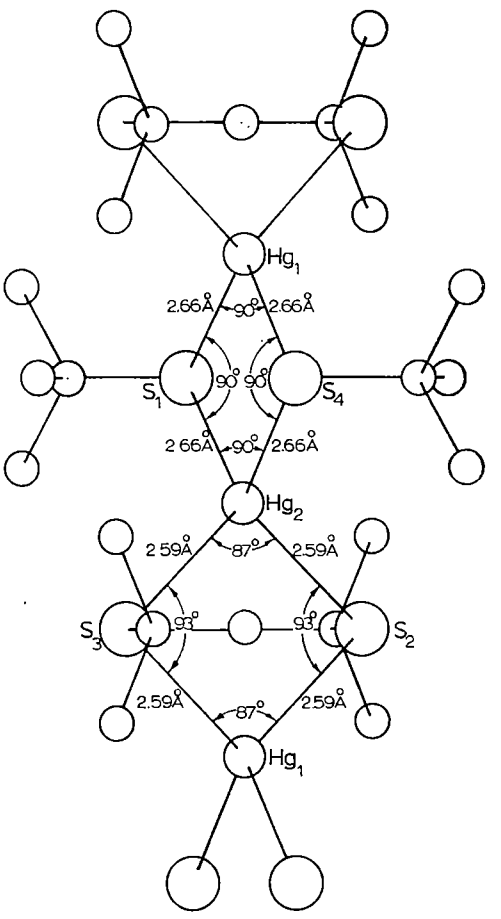


Fig. 1. Bond-lengths and bond-angles in the polymeric chain of mercury *tert*-butyl mercaptide

It is intriguing that this compound has a true polymeric structure while the lower mercaptides $Hg(S{\cdot}Met)_2$ and $Hg(S{\cdot}Et)_2$ have molecular structures. A probable explanation for this is that in all these compounds co-ordination occurs through sulphur, and in the case of sulphur, the greater the substitution, the greater is its co-ordinating power[4].

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Optical Rotatory Power of DNA and of its Complex with Acridine Orange under Streaming Conditions

This communication reports the results of experiments in which left- and right-circularly polarized light is propagated along the flow lines of a streaming solution of DNA or of the DNA-AO (acridine orange (I)) complex, and the differential molar extinction coefficient ($\varepsilon_l - \varepsilon_r$) is measured as a function of wave-length. By these means circular dichroism bands due to electronic transitions polarized parallel and perpendicular to the helix

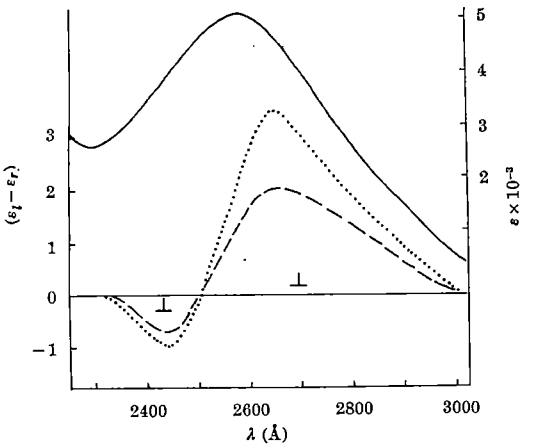


Fig. 1. The electronic absorption spectrum (———) and the circular dichroism (— — —) under stationary and (. . . . .) under flow conditions of calf-thymus DNA in aqueous solution at *p*H 6·5. ⊥, perpendicular polarization

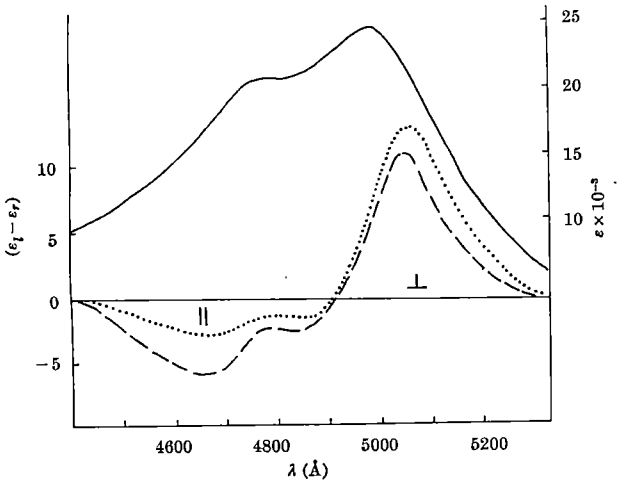


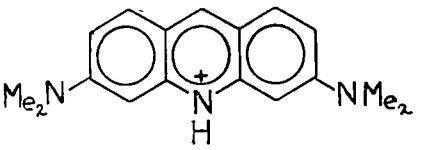
Fig. 2. The electronic absorption spectrum (———) and the circular dichroism (— — —) under stationary and (. . . .) under flow conditions of the DNA-acridine orange complex, with one dye molecule per base-pair, in aqueous solution at *p*H 6·5. ∥, parallel, and ⊥, perpendicular polarization

axis of the DNA polymer are distinguished, as light propagated along the helix axis can give rise only to transitions with perpendicular polarization. With the small flow rates employed (3 c.c./sec through a cylindrical cell 4 mm in diameter) the degree of orientation of the rod-like polymer molecules along the flow-lines is small, but it is sufficient to produce circular dichroism changes (Figs. 1 and 2) which allow the polarization directions of the bands to be identified.

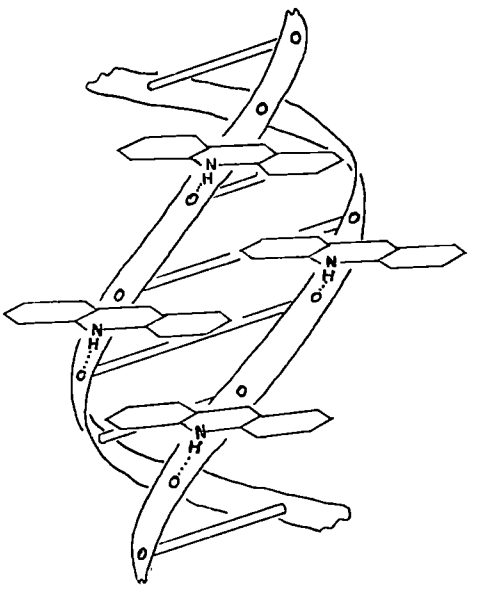
The area of a circular dichroism band measures the rotational strength of the electronic transition responsible for the absorption. Current theories[1,2] of the optical rotatory power of helical polymers distinguish two types of rotational strength which are dependent on the pitch and on the radius of the helix, respectively. The rotational strengths of each type derived from a given monomer excitation sum to zero in principle, and in each case the observed circular dichroism should consist of two bands, or groups of bands, with equal areas and opposed sign. The two types differ, however, in that the pitch-dependent rotational strengths are associated only with perpendicularly-polarized transitions, whereas the radius-dependent rotational strengths derive from transitions which are polarized parallel and perpendicular to the helix axis of the polymer.

The results obtained for DNA show (Fig. 1) that both the circular dichroism[3] bands associated with the 2600 Å absorption increase in area under the flow conditions adopted, and that their rotational strengths are of the pitch-dependent type derived from perpendicularly polarized transitions. Accordingly the monomer excitations responsible for the measured optical rotatory power of DNA are the lowest-energy $\pi \rightarrow \pi^*$ transitions of the purine and pyrimidine base-pairs and not the $n \rightarrow \pi^*$ transitions[4], which have electric moments directed parallel to the helix axis in DNA. The magnetic moment of the $n \rightarrow \pi^*$ transition, directed in the molecular plane of the purine or pyrimidine base, may contribute to the rotatory power of DNA, however, as a non-zero rotational strength requires an electric and a magnetic transition moment which are either parallel or antiparallel.

Structures proposed for the DNA-AO complex are the intercalation model[5], in which each dye molecule (I) is sandwiched between two adjacent base-pairs, and the tangential stacking model[6], in which each dye molecule is held by an ionic bond between a dimethylamino group of (I) and a phosphate residue of DNA. In both models the molecular plane of the dye (I) is orientated perpendicular to the helix axis of the DNA, so that in the DNA-AO complex all $\pi \rightarrow \pi^*$ transitions of the dye should have a perpendicular polarization, and should give pitch-dependent rotational strengths only. However, the present flow experiments show (Fig. 2) that, of the two circular dichroism bands of the DNA-AO complex associated with the 5000 Å acridine orange (I) absorption, one is polarized parallel and the other perpendicular to the helix axis of the DNA, the band areas decreasing and increasing, respectively, on streaming. Accordingly these circular dichroism bands are due to radius-dependent rotational strengths, which require that the molecular plane of the dye (I) is orientated neither parallel nor perpendicular to the helix axis of DNA in the complex and that the super-helix of bound dye molecules has a non-zero radius. As the parallel-polarized circular dichroism band of the complex at 4670 Å has a negative rotational strength, the super-helix of bound dye molecules is left-handed, indicating that the dye molecules



(I)



(II)

are accommodated in neither the large nor the small groove of the right-handed sugar-phosphate helix of the DNA molecule. The perpendicular (5050 Å) and the parallel polarized (4670 Å) circular dichroism bands of the DNA-AO complex lie at wave-lengths (Fig. 2) where the unpolarized absorption is relatively strong and weak, respectively, suggesting that the long-axis of the dye molecules, which is the polarization direction of the 5000 Å $\pi \rightarrow \pi^*$ transition of the free dye (I), is orientated at an angle $90° > \alpha > 45°$ to the DNA helix axis in the complex.

The conclusions of the work recorded here suggest the structure (II) for the DNA-AO complex. The primary basic centre of acridine orange (I) is the nuclear nitrogen atom, which is bound in the complex (II) by an ionic or a hydrogen-bond to a phosphate residue of the DNA in such a manner that the long-axis of the dye molecule is inclined at an angle to both the helix axis and the sugar-phosphate strands of the DNA. Dispersion forces between dye molecules bound at adjacent sites maintain the molecular planes parallel in the dye super-helix, but the degree of ordering is probably not large since the observed circular dichroism of the DNA-AO complex (Fig. 2) is an order of magnitude smaller than that[7] of the helical polymer formed by pseudo-isocyanine.

This work was aided by contract *N*62558–4135 between the Office of Naval Research, Department of the U.S. Navy, and the University of East Anglia.

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BIOCHEMISTRY

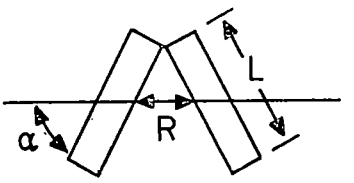
Geometrical Rearrangement of Aggregated Chlorophyll *in vivo* during the Greening Process

It has been previously reported[1] that the emission maximum from aggregated chlorophyll, in *Euglena gracilis* strain *Z*, shifts toward longer wave-lengths as the concentration of chlorophyll increases in the cell. In an earlier paper[2] it was assumed that the shift toward longer wave-lengths arises from the formation of a distribution of molecular aggregates of different sizes. In this treatment, the geometry of the aggregates was presumed to undergo no change during the greening process. A calculation of the aggregate sizes and their absorption maxima corresponding to the experimentally observed emission bands was made.

Alternatively, the observed shift could arise from a single-sized aggregate which undergoes a change in the angle and distance between the molecules forming the aggregate. An expression given by McRae and Kasha[3]:

$$\Delta\tilde{\nu}_A = \frac{4m^2}{hcR^3}\left(\frac{N-1}{N}\right)(1+\cos^2\alpha) \qquad (1)$$

describes quantitatively the absorption properties of dye aggregates, where $\Delta\tilde{\nu}_A$ is the separation, in wave numbers, between the two aggregate absorption bands, m is the transition moment of the monomer, N is the number of monomer units forming the aggregate, R is the distance between charge centres, and α is the angle between the plane of the molecules and the axis of the aggregate. These parameters are indicated in the schematic representation of the structure of the chlorophyll dimer, shown here.



As seen from the geometry, $L \cos\alpha = R$. We can eliminate the cos α term from equation (1) to give:

$$\Delta\tilde{\nu}_A = \frac{4m^2}{hcL^2}\left(\frac{L^2+R^2}{R^3}\right)\left(\frac{N-1}{N}\right) \qquad (2)$$

This equation can be rewritten in terms of emission as described previously[2], where $\tilde{\nu}_F' - \tilde{\nu}_F''$ is the separation, in wave numbers, between the monomer and aggregate emission maxima.

$$\tilde{\nu}_F' - \tilde{\nu}_F'' = \frac{2m^2}{hcL^2}\left(\frac{L^2+R^2}{R^3}\right)\left(\frac{N-1}{N}\right) = \delta \qquad (3)$$

Rearranging terms yields the cubic equation:

$$\delta R^3 - BR^2 - BL^2 = 0 \qquad (4)$$

where $B = \frac{2m^2}{hcL^2}\left(\frac{N-1}{N}\right)$.

It is assumed that the observed shift in emission maximum arises from a change in the distance between charge centres, R, in an aggregate of size, N, and that the centre of charge for chlorophyll is concentrated in the centre of the molecule. A calculation of the aggregate emission maximum, $\tilde{\nu}_F''$, in wave numbers, as a function R, can be made from equation (4). In these calculations the wave number for the monomer emission maximum, $\tilde{\nu}_F'$, is taken as 14,556 cm^{-1} (687 mμ) (ref. 2). The value of B is calculated to be $0{\cdot}714 \times 10^{-5}$ E.S.U./erg cm^3 when the aggregate is a dimer (that is, $N = 2$) and $m^2 = 3{\cdot}26 \times 10^{-35}$ E.S.U. (ref. 4) and $L = 15$ Å (this corrected value supersedes that given in ref. 5). From the relationship $R = L \cos\alpha$, the angle α is also calculated. These values and the analogous ones for larger-sized aggregates (that is, $N = 3, 4, 6$ and ∞) are given in Table 1. The results are also plotted in Fig. 1.

Table 1. Geometrical Values for an Aggregate Calculated from Emission Data

N	Long wave-length emission maximum (observed) mμ	δ cm^{-1} (observed)	R (Å)	α
2	717	609	6·8	63°
	720	668	6·6	64°
	728	820	6·1	66°
	734	933	5·8	67°
3	717	609	7·6	60°
	721	687	7·3	61°
	728	820	6·8	63°
	734	933	6·5	64°
4	717	609	8·0	58°
	721	687	7·6	60°
	728	820	7·1	62°
	734	933	6·8	63°
6	717	609	8·3	56°
	726	782	7·6	60°
	734	933	7·0	62°
∞	717	609	8·9	54°
	721	687	8·2	57°
	728	820	7·9	58°
	734	933	7·6	60°

The first aggregated form observed in *Euglena* (light, 7 h) has an emission maximum at 717 mμ (ref. 1). If only dimers are present, then $R = 6{\cdot}8$ Å and $\alpha = 63°$. After *Euglena* has been in the light for 80 h, the emission maximum is shifted to 734 mμ. If this form is exclusively

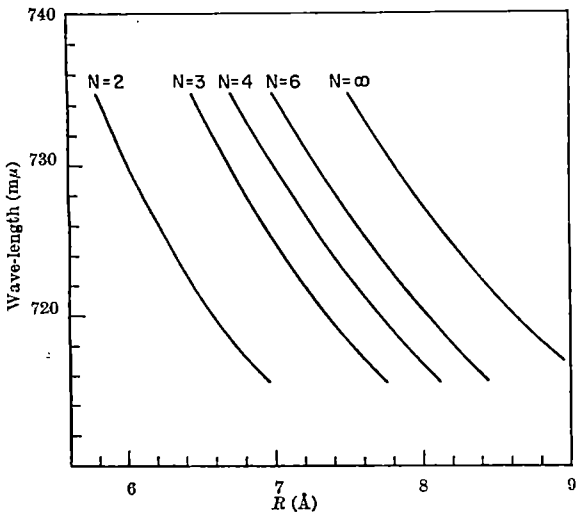


Fig. 1. Relationship between the long wave-length emission maximum from *Euglena* and the distance between charge centres, R, of the molecules forming the aggregate. The relationship is shown for aggregates of different sizes; N being the number of monomer units forming the aggregate. Emission is measured at 77° K and sensitized with blue light (436 mμ)

dimeric, R is 5·8 Å and α is 67°. If the aggregate size *in vivo* is larger than dimeric, then, for a given emission maximum, the value of R is increased considerably with increasing N (Fig. 1) and α decreases.

Up to this point it has not been possible to determine if the observed spectral shift of fluorescence *in vivo* arises from an increase in aggregate size or from a change in the geometry of the dimer, or a combination of these factors. Resolution of the *in vivo* absorption spectra into monomeric and aggregate components should enable one to decide between these possibilities, as shown here.

A relationship between α and the extinctions of the two aggregate absorption bands can be obtained[4] from the work of McRae and Kasha[3]:

$$\frac{\varepsilon''_s}{\varepsilon''_L} = \tan\alpha \qquad (5)$$

where $\frac{\varepsilon''_s}{\varepsilon''_L}$ is the ratio of the extinctions of the short and long wave-length absorptions of the aggregate. If, in the aggregate absorption spectrum, this ratio remains constant during greening (while the emission maximum shifts to longer wave-lengths), then α, and hence R, also do not change. Then the shift in emission maximum to longer wave-lengths can be ascribed solely to an increase in aggregate size. In that event the aggregate size, N, can be determined uniquely from the absorption or emission data. Substituting equation (5) in equation (1) we obtain:

$$\delta = 2\left(\frac{N-1}{N}\right)\frac{m^2}{hcL^3}\left[2+\left(\frac{\varepsilon''_s}{\varepsilon''_L}\right)\right]\sqrt{1+\left(\frac{\varepsilon''_s}{\varepsilon''_L}\right)^2} \qquad (6)$$

when $\frac{\varepsilon''_s}{\varepsilon''_L}$ is constant the wave number separation between the aggregate absorption bands *in vivo* should also vary during the greening process according to equation (1) reflecting increased aggregation. If $\frac{\varepsilon''_s}{\varepsilon''_L}$ varies the shift in long-wave-length emission may be resolved into contributions due to a change in geometrical arrangement of the aggregate and to an increase in size of the aggregate. For the case where the experimentally determined value of δ varies linearly with $\left[2+\left(\frac{\varepsilon''_s}{\varepsilon''_L}\right)^2\right]\cdot\left[1+\left(\frac{\varepsilon''_s}{\varepsilon''_L}\right)^2\right]^{\frac{1}{2}}$, then the shift in long wave-length emission is due solely to geometrical rearrangements. Furthermore, for this case N may be determined uniquely with the aid of equation (6). For the case where δ is not a simple function of the ratio $\frac{\varepsilon''_s}{\varepsilon''_L}$, then both the aggregate size and geometry are changing; with the aid of equation (6) a value of N can be estimated.

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Synthesis and Metabolism of Halothane-1-^{14}C

Evidence that the volatile anæsthetics are not biologically inert has been developed recently in reports from this laboratory. We have measured the extent of production of carbon dioxide from ^{14}C-diethyl ether, ^{14}C-chloroform and ^{14}C-methoxyflurane ($^{14}CH_3OCF_2CCl_2H$)[1]. Carbon-14-labelled halothane ($CF_3CBrClH$) has not been available to us until recently when it was prepared by one of us (E. R. L.).

Halothane-1-^{14}C was synthesized starting with chloroacetic-1-^{14}C acid obtained from New England Nuclear Corporation. The amount used was 93 mg with a total isotopic activity of 1 mc. This was mixed with 30 g of cold chloroacetic acid. This mixture plus 170 g sulphur tetrafluoride and 50 μl. water were placed in a 300 ml. stainless steel pressure vessel and heated with shaking to 198° C (2,100 psig). These conditions were maintained for 63 h. In this manner, 25 g of trifluoroethyl chloride-1-^{14}C was prepared and afterwards brominated by means of a vapour phase thermal bromination in the apparatus shown in Fig. 1.

Trifluoroethyl chloride-1-^{14}C (25 g) was dissolved in liquid bromine (33 g) at 0° C and placed in the jacketed dropping funnel. The temperature of the solution was maintained at about 0° C by circulating ice water. The solution was added dropwise over a period of 30 min and heated to 540° C. The effluent from the reaction was condensed in the dry-ice-acetone trap.

The condensate in the trap was allowed to warm to about 0° C and the condensed hydrogen bromide flashed off. The excess bromine was destroyed by adding aqueous sodium bisulphate to the trap. The water-insoluble layer was transferred directly to the post of a small distillation column. The distillation yielded 8·5 gm of unreacted trifluoroethyl chloride-1-^{14}C and 8·4 g of crude halothane-1-^{14}C. The unreacted trifluoroethyl chloride-1-^{14}C was dissolved in 12 g of bromine and recycled through the reactor, yielding an additional 4·6 g of halothane-1-^{14}C.

The crude halothane-1-^{14}C (13 g) was purified by gas-liquid chromatography using a 5/8 in. × 10 ft. *bis*-(2-ethylhexyl) sebacate on 'Chromosorb' column with a temperature of 100° C. After three passes through the column, 6·25 g of halothane-1-^{14}C was obtained having a purity greater than 99·9 per cent. This material did not contain any 1,1,1-4,4,4-hexafluoro-2,3-dichlorobutene-2.

The halothane-1-^{14}C was then used to examine the possibility that this material is converted to carbon dioxide. This was carried out as described previously[1]. The labelled material was injected intraperitoneally in 0·1-ml. quantities which contained $5{\cdot}57 \times 10^6$ DPM. Following the injection, the animal was placed in a metabolism cage which allowed the complete recovery of expired gases including the carbon dioxide. The urine was also collected.

The extent of conversion of the number one carbon of halothane to carbon dioxide is very limited since only

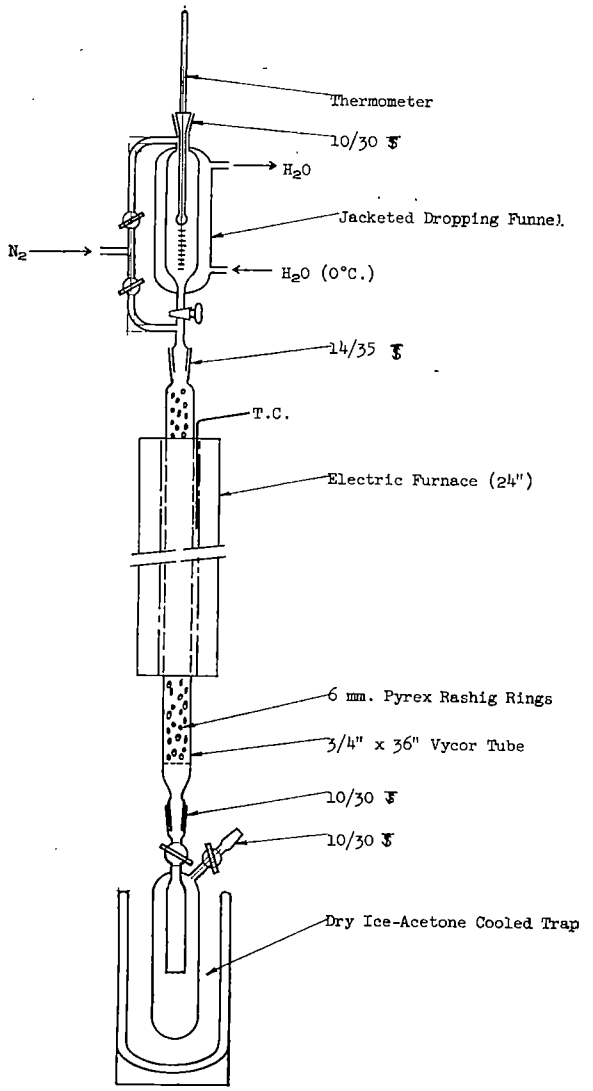


Fig. 1. Apparatus for bromination of trifluoroethyl chloride-^{14}C

0·84 per cent (0·41–1·23 per cent from nine animals) of the injected dose appeared as carbon dioxide in 24 h. This amount of conversion may be due to one of two causes. (1) This may represent the extent of defluorination of the number one carbon and thus the complete breakdown of the molecule. (2) This may represent an easily metabolized impurity, although the purity data do not indicate this.

The extent of labelled metabolites appearing in the urine was also determined. In a 24-h period, an average of 1·56 per cent (0·95–2·68 per cent from nine animals) of the injected dose was found in the urine as non-volatile, unidentified products. Presumably, however, they are dechlorinated and/or debrominated halothane residues conjugated or in some other manner made non-volatile. We have shown that dechlorination does occur[1,2].

The total recovery of all isotopic activity in a 24-h period was 95–103 per cent.

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Differential Action of Neuraminidase on Human Serum Alkaline Phosphatases

Boyer[1] noted that blood serum from normal adults produced in starch-gel zymograms one or two major zones of phosphatase activity between the transferrin and the haptoglobin 1–1 regions. Occasionally there was also a faint band near the origin. Similar patterns were later reported by other investigators[2–5].

Arfors *et al.*[5] described a phosphatase polymorphism based on the number of bands in the major zones. Type-1 phosphatase is characterized by a single band in the leading zone, and type-2 phosphatase by one or two additional bands in the slower major zone. A relationship was found between the phosphatase type and the Lewis blood group. Among individuals with type O red blood cells, serum samples of all 16 who were Le (a+ b-) contained type-1 phosphatase, and the sera of 30 of 41 individuals who were Le (a-b+) contained type-2 phosphatase. The authors suggested that one of the components in the slower zone may be complexed with a blood group substance (Le^b or H), since its enzymatic activity was decreased after incubation with anti-Le^b serum. A correlation between serum phosphatase type and red blood cell group has also been demonstrated recently in cattle[6] and in sheep[7].

This report concerns a fundamental difference in the structure of the human serum alkaline phosphatase isozymes. Incubation of serum with neuraminidase, prior to electrophoresis, decreased the anodal migration of three of the four isozymes observed in starch-gel zymograms. The results indicate that terminal neuraminic acid occurs in three of the isozymes, but is absent from one of them. The neuraminidase-resistant enzyme was inhibited by L-phenylalanine, an organ-specific inhibitor for intestinal alkaline phosphatase[8].

Serial dilutions of *Vibrio cholerae* neuraminidase (Behringwerke AG., Marburg, Germany) were prepared in 0·05 M sodium acetate, 0·06 M NaCl, 0·004 M $CaCl_2$, *p*H 5·6. The greatest dilution which, when added to an equal volume of serum, decreased the electrophoretic migration of type-1 phosphatase was considered, arbitrarily, to contain 1 unit per 0·05 ml. To each 0·05 ml. portion of serum was added an equal volume of neuraminidase; the mixtures were incubated for 20 h at 37° C and horizontal starch-gel electrophoresis[9,10] of the incubation mixtures was performed. The methods of Boyer[1] and of Arfors *et al.*[5] were used in the preparation of the zymograms. We find that the two procedures give comparable results. The method of Boyer produces less background staining, but it is not so sensitive as the method of Arfors *et al.*

Fig. 1, a photograph of the zymograms, shows that incubation with neuraminidase decreased the rate of anodal migration of type-1 phosphatase and of the similar fast component of type 2. The slow component of type 2 was unaffected, even when the concentration of neuraminidase was 10,000 times that required to produce detectable changes in the fast component. The activity of the neuraminidase-resistant component was almost completely abolished by staining in substrate mixture[5] containing 0·01 M L-phenylalanine (Fig. 2).

We examined five type-2 sera which showed two bands in the slow major zone. In all cases, only the faster of the two components was neuraminidase-resistant. After incubation with neuraminidase, the sensitive component could not be located on the gel. Possible explanations are that it did not enter the anodal side of the gel, that it migrated to the same position as the neuraminidase-treated fast component, or that the removal of neuraminic acid inactivated the enzyme. The migration of the faint band near the origin was also decreased by the action of neuraminidase. This band, not discernible in Fig. 1, was detected in each of 30 sera when the staining process was continued for 24 h.

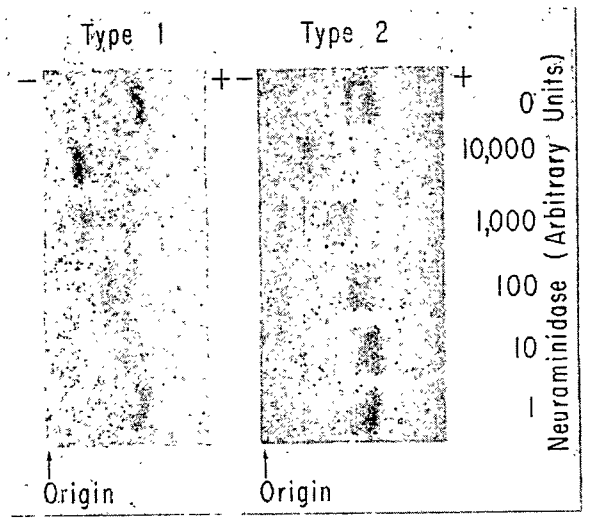


Fig. 1. Starch-gel zymograms of serum alkaline phosphatases, showing the effect of neuraminidase. Six portions of serum, each treated with the indicated quantity of neuraminidase, were inserted in each gel. After electrophoresis the gels were stained according to the method of Boyer[1]. The ends of each of the stained bands have been marked on the photograph, using the original gel as a guide

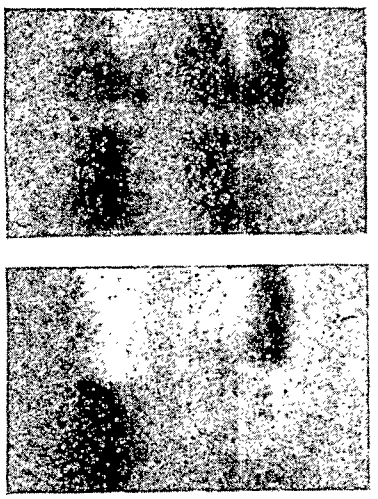


Fig. 2. Starch-gel zymograms, showing specific inhibition by L-phenylalanine of the slow component of type-2 phosphatase. The preparation of the two gels differed only in the inclusion of L-phenylalanine in the substrate mixture for the lower one. The upper insertion in each gel was serum plus buffer; the lower, serum plus neuraminidase. The origin was at the extreme left; the anode was to the right

The decreased anodal migration is caused by the removal of neuraminic acid (or a derivative thereof), which has a free carboxyl group. This technique has been used to demonstrate the occurrence of neuraminic acid in several plasma proteins. It has been particularly useful because the loss of neuraminic acid did not interfere with the specific tests used to detect the proteins. For example, incubation with neuraminidase does not abolish the binding of iron by transferrin, the interaction of haptoglobin with hæmoglobin to form a peroxidase complex, or the binding of thyroxin by thyroxin-binding globulin[11]. Also, we have observed decreased mobility, but unaltered enzymatic activity, of ceruloplasmin and pseudocholinesterase after incubation of serum with neuraminidase. In the case of pseudocholinesterase, Svensmark and Kristensen[12] have clearly shown that the decrease in mobility is due to a change in the isoelectric point of the enzyme from 2·9–3·0 to 6·7–7·0 as a result of treatment with neuraminidase.

Immunological evidence[13,14] indicates that there are at least three antigenic classes of human non-specific alkaline phosphatases. The apparently closely related enzymes of liver, bone, spleen and a major kidney component form one class; intestinal phosphatase and placental phosphatase, which are partially cross-reactive with each other and with a major kidney component, represent the other two classes[13]. The zymograms of Hodson and associates are labelled to show the liver enzyme in the fast major zone, and intestinal and bone phosphatase in the slow major zone. The inhibition investigations of Fishman and Kreisher[4], using L-phenylalanine, support the view that the intestinal enzyme is localized in the second zone. Our results demonstrate that it is this phenylalanine-sensitive enzyme which is resistant to the action of neuraminidase. A minor component near the origin can be seen in zymograms of various tissue extracts prepared by Boyer[13]. The tissue-of-origin of the similar serum component, therefore, cannot be specified at present.

Heretofore, the genetically determined protein variants have been found to differ either by amino-acid substitutions in polypeptide chains[15] or by the association of fundamental units in various combinations, as, for example, in the lactate dehydrogenases[16]. The present work suggests an additional way in which isozymes may differ, namely, in their carbohydrate components. It is possible that in detailed structural analysis of the glycoproteins all three modes will be encountered simultaneously.

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A Specific Elastolysate Test

Since the discovery of the splitting effect of pancreatic elastase on elastin[1,2], many authors have described methods for estimating elastolytic activity. In this laboratory we have attempted to develop a salt turbidity test as a more convenient method, by using an alkaline salt, Na_2CO_3, or a neutral salt, NaCl, or an acidic one, $(NH_4)_2SO_4$.

Elastolysates produced by acting of elastase on elastin[2–5] become turbid when treated with saturated solutions of Na_2CO_3, NaCl or $(NH_4)_2SO_4$, and the maximal turbidity values in elastolysates with each of these first two saturated salt solutions are in a ratio of 1 : 2, and 1 : 1 with that of $(NH_4)_2SO_4$ (v/v).

The intensities of the turbidities in these reactions depend first on the kind of saturated salt solution and indicate the following sequence: NaCl < Na_2CO_3 < $(NH_4)_2SO_4$. Under these conditions, saturated solutions of Na_2CO_3 or NaCl do not give any turbidity reaction with some other native proteins or proteins digested by elastase, while $(NH_4)_2SO_4$ has a general protein-precipitating effect.

Each of these saturated salt solutions yields a different value for the same elastolysate specimen at a given time,

and finally determines its different curve of behaviour (Table 1).

Table 1

Time of the elastolysate (min)	5	10	15	20	25	30	60	120	240
Turbidities (colorimetric divisions)									
with sat. Na_2CO_3 :	14	41	59	66	72	79	95	97	88
with sat. NaCl :	0	15	23	36	44	47	—	88	78
with sat. $(NH_4)_2SO_4$:	22	97	128	142	148	149	—	170	180

All these turbidities and precipitates are of different stabilities and thus most of the turbidity values (photocolorimetric divisions), especially in the experiments with Na_2CO_3, are not completely in accordance with those of the corresponding precipitates estimated by kjeldahlometry or gravimetry. Nevertheless, the comparison of these results with those of other general methods shows that this Na_2CO_3 turbidity test is useful for following the activity of elastase on elastin, and it is concluded that these turbidity reactions can be taken as specific reactions for elastin and elastolysates. They have some other advantages over other methods in that (*a*) the reagent is easy to prepare and is stable and consistent, and (*b*) the reactions take only 10 min. The results can be graded objectively, but only semi-quantitatively.

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Interactions of Peroxidases and Catalases between *Phaseolus vulgaris* and *Pseudomonas phaseolicola* (Halo Blight of Bean)

The increased activity of oxidative enzymes in plants caused by fungi, viruses or other organisms has been known for many decades[1]. It has also been suggested that the resistance of plants to micro-organisms was due to compounds, probably phenolics, which were formed after the invasion of the pathogen[2]. Since then in many cases correlations between higher activities of phenol oxidases or peroxidases and resistance have been reported[3]. In the case of halo blight of beans, it was suggested that peroxidases play a part in resistance[4]. In fungus-infected beans the appearance of a 'phytoalexin' which induced resistance has been reported[5], but recent investigations showed that it was difficult to explain all resistant reactions in bean plants with only the phytoalexin concept[6]. It is not known if a specific compound induces resistance against the halo blight organism or if a general enhancement of oxidative processes (hypersensitive reaction) is the primary cause of resistance.

Our investigations showed that increased activities of peroxidases caused by the infection could be detected earlier in the resistant variety and that the activity remained higher during the first few days after infection as compared with the susceptible variety. This would suggest that in the case of the susceptible combination the bacteria did not trigger, or were able to delay, this defence mechanism of the bean plant. Attempts to study interactions of host and parasite were often complicated by the need to differentiate between the contributions of the two organisms. Therefore we hoped to obtain a better understanding of the biochemistry of this disease by analysing the proteins of healthy and infected bean leaves and of the bacteria. The first experiments showed clearly that the infection does change the protein composition of the bean leaf and that this change is different in susceptible and resistant varieties[7]. By application of a new technique, polyacrylamide disk electrophoresis, better characterization of the protein changes was attained.

The conditions for growing the bean plants, for infecting the leaves, and for extracting the proteins were the same as described earlier[7]. The disk-electrophoresis method of Ornstein and Davis[8] was used with some minor technical modifications[9]. Bands of enzymatic activity were located on the gels by standard histochemical methods[10].

Staining with amido-black did not reveal marked differences between protein bands of healthy and infected leaves. However, zymograms for several enzymes showed changes due to infection. The details will be published later. In general, increased activities of the host enzymes were observed for oxidases and dehydrogenases, whereas some hydrolytic enzymes (phosphatases, esterases) of the host showed lower activity after infection. In most cases, when new zones were seen in infected leaves, they appeared to be of bacterial origin (for example, acid phosphatase, esterase, malate dehydrogenase, catalase).

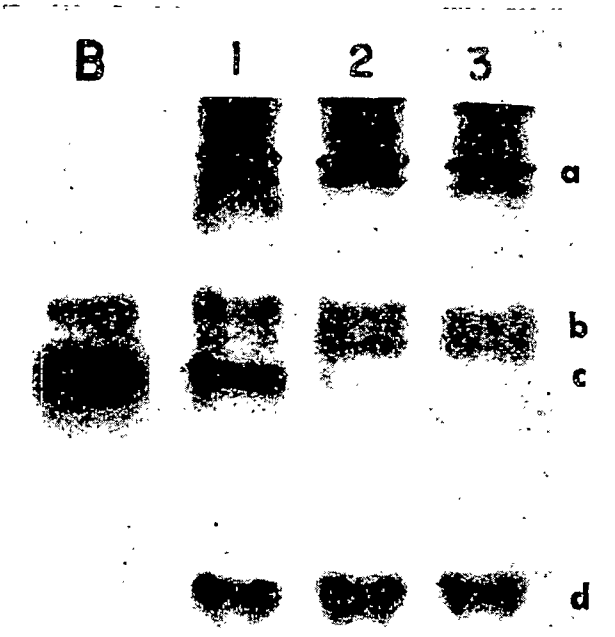


Fig. 1. Peroxidases and catalases in bean leaf and bacterial extracts after disk-electrophoresis. Bands *a-d* were developed with 2×10^{-2} M catechol and 0·3 per cent hydrogen peroxide.
B, bacterial extract; 1, infection centre; 2, surrounding yellow halo; 3, surrounding green tissue

One of the most striking changes was the appearance of bacterial catalase in the infected leaves, as shown in Fig. 1 (band *c*), when catechol (2×10^{-2} M) and hydrogen peroxide (0·3 per cent) were used for development. It was established that band *c* was a catalase because it showed a positive reaction with the starch–iodide method[11], and it did not possess detectable peroxidatic activity with guaiacol as a substrate. Two commercial catalase preparations showed the same characteristics and also oxidized catechol. In contrast the main plant, band *b*, had peroxidatic activity with guaiacol as well as catalytic activity and all upper bands (*a*) showed only peroxidatic activity. Band *d* is probably a phenol oxidase. As shown in Fig. 1, sonic extracts of bacteria grown on peptone–dextrose–beef-extract agar showed a strong band at the same position with the same biochemical characteristics as those described for band *c*. Other evidence for the

bacterial origin of band *c* may be derived from the fact that extracts from the 'greasy' infection centres contained a large amount of this catalase whereas only low activity was found in the surrounding yellow halos and no activity could be detected in the surrounding green tissue (see Fig. 1, 1–3). In contrast, systematically chlorotic leaves contained only low amounts of band *c*, which is in accordance with the report that these leaves contain only a few bacteria[12]. A faint positive reaction was obtained from infected resistant leaves, but this faint zone tended to fade in later infection stages. It has been reported repeatedly[13,14] that in resistant leaves low populations of inoculated bacteria are able to survive for some time. In susceptible leaves band *c* was detectable as early as one day after infection and became very strong during the latter stages of infection. Whether the enzyme is actively excreted by the bacteria or appeared only because of bacterial autolysis is not known. However, its high activity in infected tissues suggested that this enzyme influenced the host metabolism.

One explanation for the influence of catalase on the host metabolism would be an inhibition of host peroxidases by competition for hydrogen peroxide. *In vitro* tests with guaiacol showed that bean peroxidases are markedly inhibited by bacterial extracts. This inhibition could be reduced by adding higher concentrations of hydrogen peroxide and no inhibition occurred with boiled bacterial extracts. Curves for the inhibition of bean peroxidase by commercial catalase and for the reversal of the inhibition by hydrogen peroxide were very similar to those for bean peroxidase and bacterial extracts. When guaiacol was oxidized by a plant extract and hydrogen peroxide the dark brown coloured solution could be decolorized by addition of catalases as well as bacterial extracts.

The peroxidatic activity of catalase is often considered physiologically more important than catalytic activity[15]. We also found *in vitro*, under relatively high concentrations of hydrogen peroxide, catechol was oxidized by the bacterial catalase but guaiacol was not. This indicates that peroxidases oxidize compounds which are not affected by catalases under our conditions. Similar differences were reported for the coupled oxidation of phenolic hydrogen donors by peroxidases and catalases[16]. We do not know which peroxidatic pathways are involved in the hypersensitive reactions in the bean leaf and if these reactions could also be mediated by catalases.

In order to examine the effect of catalases *in vivo* we fed bean shoots with commercial catalase. This treatment did not enhance oxidation but markedly inhibited ageing reactions which normally progress rapidly in cuttings. That is, the yellowing as well as the increase in peroxidase activity were delayed by treatment with catalase. In addition an electrophoretically detectable peroxidase band, which normally increased during ageing, was very low. The same effect can be seen in Fig. 1 where the main plant peroxidase band *b* shows a lower activity in extracts from the infection centres, as compared with the surrounding tissue (compare Nos. 1–3).

We also compared two equally fast-growing bacteria. *PP* 8 (ref. 17) was very virulent and showed high catalase activity. *B*-845 (ref. 18) showed low virulence (based on symptom expression) and low catalase activity. During the first days after infection, the increase in peroxidase was higher in the bean leaves infected with *B*-845 compared with *PP* 8 infected leaves. However, another very slow growing strain[19] showed very little catalase activity but was nevertheless virulent and the increase in host peroxidase was relatively low. Another mechanism of virulence seems to be operating in this case. This might be the degree of toxin production, the chemical nature of which as well as its mode of action are still unknown. It has been suggested recently for *Pseudomonas tabaci* that the toxin production is high in slow-growing strains, and negative correlations with catalase activity were reported[20]. This would be in accordance with our own observation that a non-toxin producing but virulent strain of *Pseudomonas phaseolicola*[17] showed a very high catalase activity.

Catalases are very common among bacteria and fungi; however, not much is known about their biochemical or plant pathological importance. The latter may be partially due to the fact that until recently it was difficult to estimate to any degree the participation of host and parasite in biochemical reactions of the host–parasite relationship. The demonstration of a strong bacterial catalase in infected plants and other observations led us to the suggestion that with certain combinations bacterial catalases may be one factor for virulence by depressing the activity of peroxidases involved in the defence reactions of the host.

This work was supported in part by a research grant from the Hermann Frasch Foundation.

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Presence of Several Phenolic Components in Fruit Proanthocyanidins

Forsyth and Roberts[1] reported that chromatographically homogeneous cacao proanthocyanidin dimer yielded epicathechin and a new leucocyanidin when heated with 0·1 N hydrochloric acid, and cyanidin when heated with stronger acid. Similar dimeric proanthocyanidins were isolated from *Crataegus oxyacantha* and *Gleditschia triacanthos* by Freudenberg and Weinges[2–5]. The astringent proanthocyanidins isolated from various fruit tissues, unlike the above dimers, were found to be highly polymerized and not chromatographically mobile. This was true of the originally isolated persimmon proanthocyanidin[6,7] and of the more recently isolated persimmon[8] and apple[9] preparations. These purified preparations, which were not mobile on paper in any of the solvent systems tested, were, however, found to yield mobile phenolics without any accompanying anthocyanidins when heated with dilute hydrochloric acid. Under these conditions several chromatographically identified phenolics and a non-mobile but apparently less highly polymerized fraction were obtained. The original preparation, and the residue after dilute acid hydrolysis, which was insoluble in ethyl acetate, were found to yield identifiable anthocyanidins on heating with more concentrated hydrochloric acid. The results of these observations, which continued investigations previously reported on the mechanism of acid conversion of proanthocyanidins into their corresponding anthocyanidins[10], are reported here.

Portions of 50–80 mg of purified apple, carob, grape and persimmon proanthocyanidins were dissolved in 25 ml. of 0·1 N hydrochloric acid and heated in boiling water for 20 min. The apple preparation was obtained from 'Gravenstein' apple flesh as the *n*-butanol-soluble, hexane-precipitated fraction[9]. The persimmon preparations were compared; one was the methanol-soluble, acetic acid-insoluble preparation from Japanese astringent persimmons ('Yokono' variety), dissolved in ethanol and precipitated with ether[6,7], the second was obtained from California-grown astringent persimmons ('Niu Nai') by methanol extraction, precipitation with lead acetate, deleading, solution in ethanol and precipitation with hexane[8]. Both of the persimmon preparations gave similar results. The grape preparation was from 'Colombard' grape pomace, as butanol-soluble and ether-precipitable fractions[10]. The carob preparation was obtained from a methanol extract of green carob pods after lead precipitation and deleading, as the butanol-soluble hexane precipitable fractions. In all cases solution in butanol or in ethanol and precipitation with ether or hexane were repeated to purify the preparation further.

The dilute acid hydrolysate, free of visible anthocyanidin, was extracted with 40 ml. of ethyl acetate. The ethyl acetate extract after dehydration with sodium sulphate was concentrated in a rotary vacuum evaporator at below 40° C to 2 ml. The concentrate so obtained gave a strong vanillin-positive reaction and formed a pigment with diazotized *p*-nitroaniline which was characteristic of flavonols. Two-dimensional ascending solvent front chromatograms were prepared from the ethyl acetate extract using *n*-butanol–acetic acid–water (6 : 1 : 2) as the first solvent and 6 per cent acetic acid as the second solvent[11]. Two chromatograms were prepared, one developed by spraying with diazotized *p*-nitroaniline and the other with vanillin-hydrochloric acid. The mobile phenolics that were found were identified by colour with the above reagents, by fluorescence in ultra-violet light, by reported R_F values in the literature, and checked by comparison with authentic phenolics which were chromatographed under similar conditions or co-chromatographed.

The apple-flesh preparation yielded *d*-catechin and *l*-epicatechin. Both the persimmon preparations yielded gallic acid, *l*-gallocatechin and *l*-gallocatechin gallate. The grape preparation yielded *d*-catechin, *l*-epicatechin, *l*-epicatechin gallate, protocatechuic acid, phloroglucinol, kæmpferol, quercetin and an unknown phenolic. The carob pod preparation yielded gallic acid, *d*-catechin, *l*-epicatechin, *l*-gallocatechin, *l*-epicatechin gallate, phloroglucinol, ferulic acid, quercetin and myricetin.

It is interesting that, except in the case of the persimmon, *d*-catechin and *l*-epicatechin occurred in the dilute acid hydrolysate of the astringent proanthocyanidin. In the proanthocyanidins, which were predominantly leucodelphinidin, persimmon and carob, gallic acid was found. The occurrence of quercetin and phloroglucinol in both the grape and carob preparations was also of interest. The occurrence of quercetin in the grape was paralleled by myricetin in the carob as corresponding flavonols.

On strong acid hydrolysis[9] the apple-flesh preparation yielded two anthocyanidins, predominantly cyanidin and much smaller concentrations of pelargonidin. Both the persimmon preparations yielded largely delphinidin with smaller amounts of cyanidin. Ito and Oshima[6] had previously reported the presence of leucodelphinidin-3-glucoside in persimmon proanthocyanidin. The grape preparation yielded only cyanidin and the carob preparation yielded mainly delphinidin with smaller concentrations of cyanidin. This preparation, however, was made from the methanol extract of whole green carob pods including the seeds. Goldstein, however, had observed (unpublished) that the carob pod tissue free of seeds yielded only delphinidin while the carob seed testa yielded only cyanidin.

The occurrence of a number of chromatographically identifiable phenolics in the products of dilute acid hydrolysis, varying from two for the apple tissue preparation to eight or more for the carob and grape tissue preparation, and the presence of more than one anthocyanidin in the products of concentrated acid hydrolysis indicate either that the proanthocyanidins are quite complex or that the preparations contained mixtures of leucocyanidins, leucodelphinidins and various phenolic compounds. Additional purification by continuous liquid–liquid extraction and fractional elution is being carried out in an attempt to obtain more highly purified preparations for chemical identification.

We thank Dr. T. Nakabayashi of the University of Shizuoka, and Dr. A. Holmes of the Indian Tea Association, for samples of authentic polyphenols.

This work was supported in part by U.S. Public Health grant *EF*–00080–02.

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Sub-cellular Localization of Rat Kidney Alkaline Phosphatase

In mammalian cell cultures alkaline phosphatase is regulated by both inductive and repressive mechanisms[1]. In preparation for further investigations of the mechanisms of alkaline phosphatase control, the sub-cellular localization of this enzyme was re-investigated, using newer techniques[2,3]. Previous investigations, using the kidney and intestine of several different mammalian species, have shown that the highest alkaline phosphatase activity resides on the microsomes[4–6].

Three separate fractionations and analyses were performed using different rats. A representative experiment is described.

An adult rat was killed by decapitation. The kidneys were removed and the capsules stripped. The combined weight of both kidneys was 3 g. Further manipulations were carried out at 4° C. The kidneys were minced, washed thoroughly on a nylon filter with 0·25 M sucrose and homogenized in 27 ml. of 0·25 M sucrose with a motor-driven 'Teflon' homogenizer (*TRI-R* Instruments No. *S*-23 pestle and *S*-37 tube). Ten-ml. portions of homogenate were carefully layered over equal volumes of 0·34 M sucrose and fractionation proceeded as outlined in Fig. 1. Nuclei were isolated by the method of Hoogeboom[2], except that calcium chloride was omitted from the solution since calcium ions in the suggested concentration (0·18 mM)[3] inhibit alkaline phosphatase activity. Following centrifugation at 600 r.p.m. (International refrigerated centrifuge, yoke No. 253) for 20 min, the top layer was separated from the bottom layer which contained the sedimented nuclei. The top layer was fractionated into a mitochondria–lysosome fraction and a microsome fraction by the method of Ernster *et al.*[3]. The fraction that sedimented at 12,500 r.p.m. in Spinco rotor No. 40 (Beckman model *L* ultracentrifuge) was called the mitochondria–lysosome fraction. We focused our further purification efforts on the supernatant containing the microsomal sub-structures. These were sedimented at

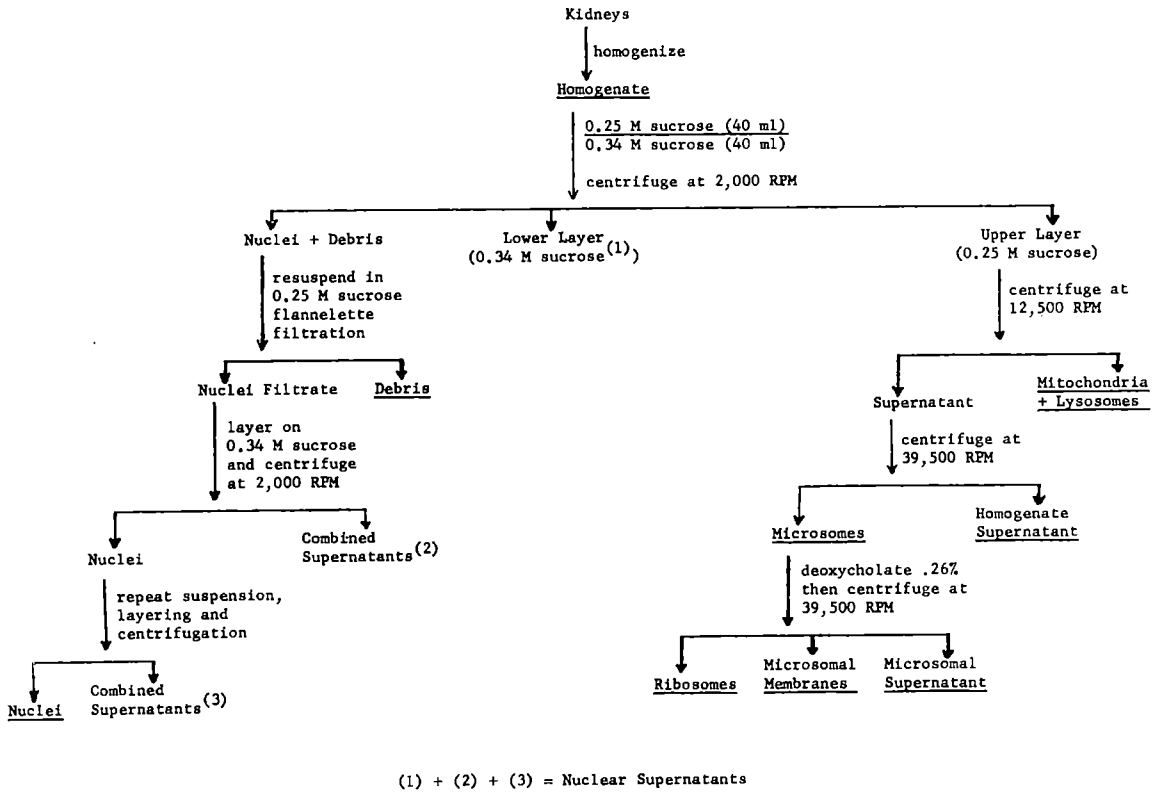


Fig. 1. Fractionation of rat kidney sub-cellular particles. The underlined fractions were assayed

39,000 r.p.m. in Spinco rotor No. 40 for 2 h. The pellet, containing microsomes, was re-suspended in 0·25 M sucrose and re-sedimented as indicated here. This now washed microsomal pellet was re-suspended in 0·25 M sucrose. Three-quarters of the microsome fraction was taken and further fractionated into ribonucleoprotein particles (ribosomes)[7], microsomal membranes and a microsomal supernatant by treatment with 0·26 per cent deoxycholate at *p*H 7·4 as shown in Fig. 1 (ref. 3). Ernster *et al.* have demonstrated that the tightly packed bottom layer of the two-layered precipitate is predominantly ribosomes, whereas the loosely aggregated upper layer of the precipitate consists of partially disrupted microsomal membranes. These two layers were easily separated by washing the membranes away from the ribosomes. The latter pellet could only be re-suspended by homogenization.

The nuclear sediment in the bottom layer of the initial centrifugation supernatant was re-suspended in 20 ml. of 0·25 M sucrose. This fraction was filtered through a cloth flannelette and re-sedimented twice from 0·25 M sucrose through 0·34 M sucrose[8]. Nuclei prepared by this procedure appeared reasonably free of other visible cell particles using Wright stain and light microscopy[8].

The activities of enzymes which are concentrated in certain cell particles have been used as criteria for cellular fraction homogeneity[8]. The β-glucuronidase and acid phosphatase were assayed as enzymes localized in lysosomes. Acid phosphatase can be found in certain nuclear fractions, but the significance of this finding is uncertain[8]. The specific activity of the alkaline phosphatase[9], acid phosphatase[9] and β-glucuronidase[10] of all fractions were compared (Table 1). The particulate fractions were diluted in a standard volume of 0·25 M sucrose and thoroughly mixed with an equal volume of 1 per cent deoxycholate, *p*H 8·4 (ref. 1). The resulting mixture is a micellar suspension allowing maximum expression of enzymatic activities. Protein was determined by the method of Lowry *et al.*[11].

Certain tentative conclusions can be drawn from Table 1. In agreement with Morton's findings[4], almost all (98 per cent) of the alkaline phosphatase is sedimented

Table 1

Fraction	Alkaline phosphatase Units (10^3)	*S.A.*	Acid phosphatase Units (10^3)	*S.A.*	β-glucuronidase Units	*S.A.*	Alkaline phosphatase Total units
Whole homogenate	134	1·9	24	0·34	1·2	0·17	16,800
Nuclear debris*	110	1·6	26	0·38	1·4	0·21	4,600
Nuclear supernatants†	160	2·6	19	0·31	0·9	0·06	6,400
Nuclear fraction	137	1·8	25	0·33	1·2	0·16	690
Mitochondria lysosome	295	1·9	103	0·67	3·3	0·22	5,900
Supernatant of original homogenate	11	0·1	2	0·02	0·4	0·05	500
Microsomes	205	3·2	26	0·40	0·6	0·09	1,025‡
Microsomal membranes	54	1·7	6	0·19	0·3	0·06	270
Ribosomes	32	1·6§	9	0·17	0·4	0·12	160
Microsomal supernatant	223	4·0	25	0·45	0·8	0·16	2,240
Light membranes from microsomal supernatant	76	5·4	4¶	0·13	0·0¶	0·00	—
							21,785 = 130% recovery

* Filtrate from flannelette filtration of nuclei.
† Combined supernatants of 0·34 M sucrose and all washings from nuclear preparation.
‡ Total units for an aliquot of 1/4 of the microsomal fraction.
§ In two other experiments, the alkaline phosphatase specific activity was about 1/4 that of the microsomal membranes.
¶ Not measured in this experiment due to low yield. Results obtained from another experiment.

by centrifugation at 105,000*g*, indicating its close association with cell particles.

The microsome fraction has high specific activity when compared to nuclei, mitochondria–lysosomes or homogenate supernatant. On further fractionating the microsomes, it was found that microsomal membrane, as well as the ribosomes, have high alkaline phosphatase specific activity, while exhibiting minimum contamination by other fractions. The microsomal supernatant also contains alkaline phosphatase. However, on centrifugation at 130,000*g* for 3 h, this enzyme activity is almost entirely sedimented. The sediment consists of light microsomal membranes containing the highest alkaline phosphatase specific activity of any cell particle. These membranes have no measurable β-glucuronidase, as shown by Table 1.

Although the nuclear fraction appeared homogeneous by light microscopy, it is contaminated with other particles, as shown by the high specific activity of both acid phosphatase and β-glucuronidase. The lysosome-containing fraction has the highest acid phosphatase and β-glucuronidase specific activities, as expected. The high alkaline phosphatase specific activity of both the nuclear and mitochondria–lysosome fraction may be due in part to contamination by cell particles with high alkaline phosphatase content. However, the alkaline phosphatase activity is so high that it is probable that both these fractions contain intrinsic alkaline phosphatase. For all the contamination to be due to microsomes it would require 50 per cent of the protein of these fractions to be microsomal protein.

When the fractionation described here was performed using 0·18 mM calcium chloride, the results were qualitatively similar, although only 68 per cent of the alkaline phosphatase activity was recovered, presumably due to inhibition of enzyme activity by calcium ions.

The findings indicate that microsomal membranes contain most of the alkaline phosphatase associated with the microsome fraction. Although the nuclear and mitochondria–lysosome fractions were contaminated by other cell particles, their high alkaline phosphatase specific activity implies intrinsic localization within them.

We thank Dr. Salah Al-Askari and Dr. Felix Rapaport for their advice on fractionation techniques.

This work was supported by genetics training grant 2 *T*1 *HE*–5307 of the U.S. Public Health Service and research contract *U*–1296 of the Health Research Council of the City of New York.

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Qualitative Estimation of Erythrocyte Catalase by Starch-gel Electrophoresis

Erythrocyte catalase can be assayed in the usual quantitative way as well as by qualitative starch-gel and starch-block electrophoresis[1–3]. The electrophoretic method permits a more subtle characterization of its normal and abnormal properties. In comparative examinations of catalase from cord blood and blood samples of abnormal hæmoglobin carriers, no abnormal electrophoretic behaviour could be observed[3]. An atypical catalase was identified recently by a somewhat slower electrophoretic mobility in some members of a Caucasian family[4].

The principle of known electrophoretic assays consists of the negative iodine–starch reaction at the catalase location, thus forming white spots within the dark environment. The method given here utilizes hæmoglobin added in small concentrations to the starch gel as indicator of catalase. The presence of catalase prevents the peroxidative transformation of colourless *o*-dianisidine into the coloured product by action of hæmoglobin. Thus in one step the hæmoglobin fractions as well as catalase can be observed on a brown background.

For preparation of starch gel, 20 g of potato starch and 48 g of soluble starch are dispersed together with 90 ml. of *tris*-EDTA-borate-buffer, *p*H 9·0, and are added during constant stirring to 360 ml. of the same buffer boiling in a steam. When the starch becomes gel-like, 0·7 ml of a solution containing 5 g of hæmoglobin in 100 ml. are added, mixing it well with the hot gel. Following this the gel has to be evacuated by a water pump until no more air bubbles arise which could disturb electrophoresis. This gel is poured into the proper form, which can be cooled in a refrigerator after 0·25 h.

Hæmolysates are prepared in the usual way from erythrocytes washed three times in 0·9 per cent sodium chloride and shaken with carbon tetrachloride. For electrophoresis Whatman No. 3 *MM* paper cuttings 12 mm by 7 mm are soaked in the hæmolysate, blotted well between filter paper and placed into the proper gel position. In order to prevent drying of the gel liquefied paraffin (melting point 42°–44° C) is poured directly over it. Running time is 4–6 h at 60 m.amp, 400–500 V +12° C. Thereafter the gel is cut into layers 3 mm thick and is subjected to hæmoglobin staining (100 mg *o*-dianisidine in 200 ml. 96 per cent ethanol + 100 ml. McIlvaine buffer, *p*H 4·7+0·5 ml. 30 wt. per cent hydrogen peroxide).

The separated hæmoglobin fractions are indicated by a strong brown colouring (Fig. 1), while the hæmoglobin



Fig. 1. Demonstration of hæmoglobin and catalase in starch-gel electrophoresis, *p*H 9·0. Left, normal adult blood: middle, blood of a 4-week-old infant: right, purified liver catalase of the cow

added during gel preparation produces an even and slightly brownish to violet background. The locations of catalase are indicated by white areas. The mobility of catalase is determined by *p*H: at *p*H 9·0 it is somewhat higher than HbA_2; raising the *p*H produces a movement towards the cathode, lowering the *p*H a movement towards the anode. The width of the catalase fraction depends on its concentration: a reasonably bordered separation is obtained from concentrations found in 0·8–1·0 g per cent hæmoglobin solutions. The sensitivity of the assay, though of little meaning for a qualitative consideration, is limited by 100-fold dilution of a 15 g per cent hæmolysate.

The advantage of this method lies in the simple procedure as well as the simultaneous demonstration of hæmoglobin and catalase. On one hand, a qualitative judgment is obtained on both hæm proteins; on the other hand, the relative mobilities can be used for characterization of catalase.

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PHYSIOLOGY

Drinking caused by Constriction of the Inferior Vena Cava in the Rat

Injection of osmotically effective solutes in hypertonic solution causes nephrectomized rats to drink enough water to dilute the injected solute to isotonicity, so long as the initial increase in osmotic pressure does not exceed about 15 per cent[1]. With intact kidneys, however, less water is drunk because the solute can be eliminated in hypertonic urine, though the degree of hypertonicity depends on the rate of urine flow[2]. If drinking water is withheld excretion is even more rapid.

Isotonic depletion of body fluid by bleeding or by peritoneal dialysis is also an effective stimulus of drinking[3,4]. After depletion by peritoneal dialysis large amounts of water may be drunk; there is also extreme antidiuresis and a fall in the amounts and concentrations of sodium and potassium in the urine. In the acute stages of depletion the body fluids may become profoundly hypotonic as a result of the water intake, but later this hypotonicity is rectified by the development of a marked preference for saline[5].

In this communication some experiments in which the inferior vena cava was ligated are described. Caval ligation results in a sharp diminution in venous return to the heart and a fall in cardiac output and might be expected to have similar effects on fluid metabolism as bleeding and peritoneal dialysis. Complete and sudden occlusion of the inferior vena cava above the renal veins and below the hepatic veins does not cause loss of consciousness in unanæsthetized animals, though mean arterial pressure is immediately halved. This fall does not persist, and the pressure gradually returns to normal over the next few days. Ascites does not occur, the procedure is well tolerated and such effects as there are appear transient.

Male albino rats weighing between 150 and 300 g were anæsthetized with ether. The inferior vena cava was approached through a small muscle splitting incision in the right loin and was completely ligated above the entry of the renal veins and below the hepatic and right adrenal veins. The animals were weighed and then placed in individual metabolism cages with water available but no food. Drinking was recorded continuously for 6 h, and at the end of this period the urine excreted was collected for analysis and the animals again weighed. Serum freezing points were measured in some instances to confirm that caval ligation alone had no effect on osmotic pressure. The control animals were subjected to a sham operation. In another group of experiments ligated and sham-operated animals were given various doses of 2 M sodium chloride intraperitoneally at the time of operation. Special care was taken to seal the wounds to prevent leakage of saline.

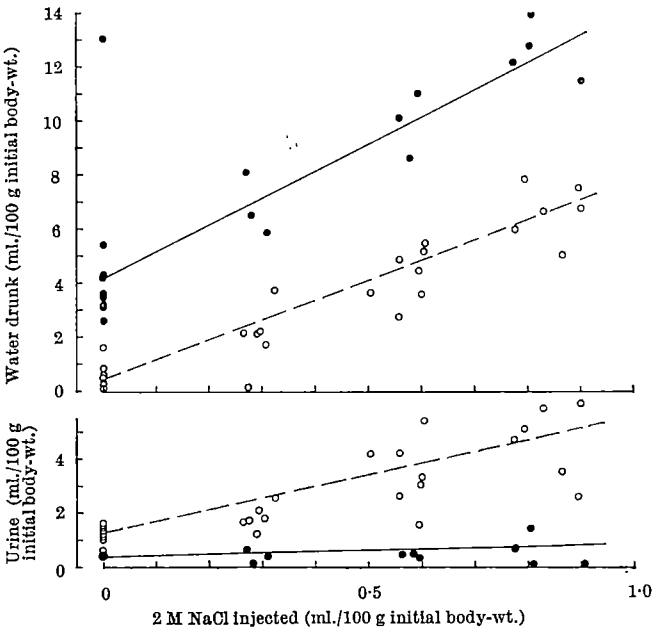


Fig. 1. Amounts of water drunk and urine excreted in 6 h by rats allowed to drink after injection of 2 M sodium chloride. ○, sham-operated; ●, caval ligation. The sloping lines were fitted by eye. Each point is one observation, except the urine of the uninjected ligated group which is a mean value

In the 6 h after operation the ligated rats drank more water and excreted less urine than the corresponding sham-operated rats (Fig. 1). Ligated animals generally started to drink within 30 min of operation, and even after the largest doses of saline, drinking was mainly completed in the first 3 h. The rate of drinking tended to decline exponentially. The failure of osmotic diuresis in the ligated group after hypertonic saline, and the very low urinary sodium and potassium, are shown in Table 1.

Table 1. Urine volume and amounts of sodium and potassium excreted in 6 h after injection of ligated and sham-operated animals with 2 M sodium chloride. Mean values of animals in Fig. 1 with the number of observations in parentheses

Sham-operated				Ligated			
2 M NaCl ml./100 g	Volume ml./100 g	Na μM/100 g	K μM/100 g	2 M NaCl ml./100 g	Volume ml./100 g	Na μM/100 g	K μM/100 g
(9) 0	1·16	75	171	(9) 0	0·4	26	30
(6) 0·292	1·82	474	231	(3) 0·288	0·39	18	18
(7) 0·575	3·48	863	234	(3) 0·582	0·43	23	27
(6) 0·845	4·7	1,225	309	(4) 0·823	0·59	47	66

The amounts of water drunk by the ligated rats injected with saline are comparable with the amounts drunk by nephrectomized rats after the same dose of saline, and since urine production was minimal, the water retained, as measured by the change in weight, was sufficient to dilute the saline to isotonicity.

If the effects of caval ligation on drinking are to be attributed to the failure to excrete the injected salt it might be predicted that caval ligation in nephrectomized rats would be without effect. This was tested by repeating the aforementioned experiments on bilaterally nephrectomized rats, the nephrectomy being performed at the same operation as the other procedures. There is certainly no significant difference between the changes in weight

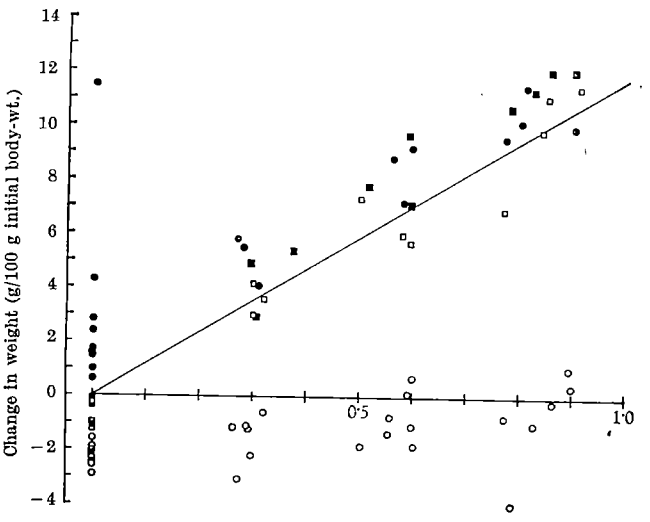


Fig. 2. Changes in weight in 6 h of rats allowed to drink after injection of 2 M sodium chloride. ○, sham operated; ●, caval ligation; □, nephrectomized; ■, caval ligation and nephrectomy. The sloping line delineates the changes in weight required to dilute the saline to isotonicity, assuming no excretion

of the nephrectomized group and the nephrectomized ligated group (Fig. 2), nor is there any obvious difference between both nephrectomized groups and the ligated groups with intact kidneys after the larger saline loads. Turning now to the groups which were not given saline, there is a significant ($P \simeq 0{\cdot}02$) difference between the changes in weight of the ligated group with intact kidneys and both nephrectomized groups, and a highly significant ($P < 0{\cdot}001$) difference between the ligated and sham-operated groups with intact kidneys.

The interpretation of these results is that caval ligation is an effective stimulus of drinking in rats with intact kidneys, but that when hypertonic saline is also given this effect is masked by the large amount of water drunk to neutralize the osmotic stimulus. Secondly, since caval ligation seems ineffective in nephrectomized rats, the kidneys play a part in the response, possibly by release of renin or some other renal factor such as that described by Asscher and Anson[6].

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Nitrogen Balance Experiments: Some Theoretical Considerations

The method[1] for the determination of nitrogen balance indices (K) by measuring the slope of the line relating either absorbed or intake N to N balance has been well tried. The interpretation of the results depends on treating the experimental points as falling on a straight line in the region below nitrogen equilibrium, and values for K may be shown to be theoretically equal to values for either biological or net protein utilization (NPU) depending on whether data for absorbed or intake nitrogen are used. For example:

$$\text{N balance} = \text{endogenous excretion} + K \times \text{N intake} \quad (1)$$

$$\therefore K = \frac{\text{N balance} - \text{endogenous excretion}}{\text{N intake}} = \text{NPU} \quad (2)$$

However, it has recently been shown[2] that NPU varies with the concentration of protein in the diet (P) according to the equation:

$$\text{NPU} = b(1 - kP) \quad (3)$$

where b and k are constants. In view of the work of Allison and Anderson[1], we[2,3] took the view that below maintenance NPU was constant, but re-examination of the literature suggests that the efficiency of nitrogen utilization falls over the whole range of protein concentration and that the relationship plotted by the American workers should be slightly curvilinear. This does not invalidate nitrogen balance index as a method of assaying protein value since the slopes of the lines would be similar, but raises some points of theoretical interest. For example, it is possible to explain why some values for K and indeed some biological values would indicate efficiencies of retention that are greater than 100 per cent. Several such values appear in the literature, and one might suspect that others have been found but rejected as apparently impossible.

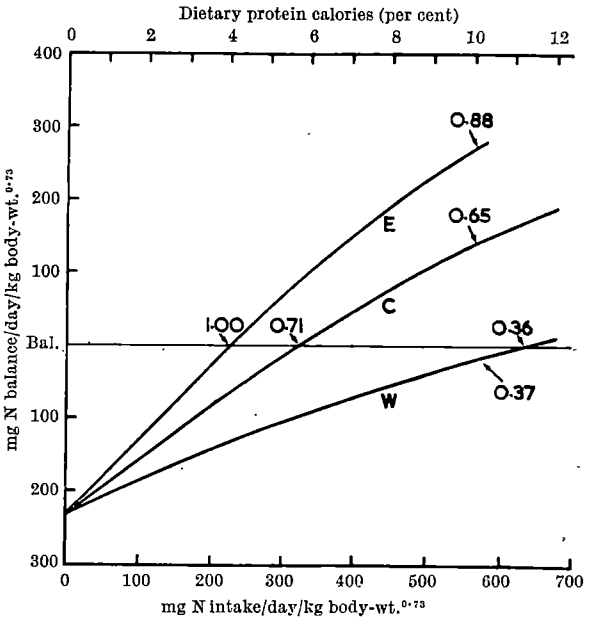


Fig. 1. Relationship between nitrogen balance and nitrogen intake for three proteins (egg, E; casein, C; wheat gluten, W), to be expected when caloric intake is twice basal calories/day. The numerals indicate the slopes of the curves at the points indicated

Fig. 1 shows for three proteins the relationship between nitrogen balance and nitrogen intake expected on the basis of equations 1, 2 and 3*. It is assumed that the animals were fed a number of calories per day equivalent to twice the basal metabolism, and the percentage of protein in the diets is shown; these conditions are similar to those chosen by Allison and Anderson[1]. It will be seen that the lines depart from linearity to an extent less than would be detected experimentally. The upper curve represents egg protein which has been shown[5] to have a biological value of 100 when the diet contains 4 per cent protein. In order to obtain a slope of unity at this percentage, it is necessary to assume retentions greater than 100 per cent from diets containing less than 4 per cent egg protein. Such values have been reported; for example, a mean of 115 from 10 determinations for egg white[6] and values of 128 for an amino-acid mixture[7]. Further evidence is supplied by actual demonstrations[4] of a curvilinear relationship between nitrogen balance and nitrogen absorbed for egg protein with a maximum slope of 1·23. These high values

* By inserting values for NPU given by equation (3) for K in equation (1), the relationship between nitrogen balance and nitrogen intake is seen to be of the form: N balance $= AP - kAP^2 -$ endogenous N, where A is a constant ($b \times$ caloric intake/25). This equation is of the same form as that presented by Forbes, Vaughan and Yohe (ref. 4).

are in agreement with protein scores as usually defined but admitting values greater than 100. According to food tables[8], egg has an excess of all the essential amino-acids over the reference pattern given by the Food and Agriculture Organization[9] and would score 114 on its content of tryptophan.

The physical meaning of nitrogen retentions of more than 100 per cent needs explanation. In determining the nutritive value of proteins we are concerned with the instantaneous effect of the diet on the animal, and observe the fate of the nitrogen it consumes; losses of urinary or fæcal nitrogen of endogenous origins are allowed for in the calculations made (equations 1 and 2). It is generally assumed that these losses are constant irrespective of the diet consumed, but it is now known that they may be reduced in certain cases. For example, body protein is spared by diets containing low levels of egg protein[10] when rats excrete less nitrogen than on a non-protein diet. Similar data have been obtained with dogs[11]. Non-protein diets supplemented with methionine also reduced nitrogen loss[10,11]. Reductions of endogenous loss result in calculated efficiencies greater than 100 per cent. Since such diets promote the re-cycling of amino-acids derived from the tissues, it is reasonable that they are rated accordingly. Absorbed amino-acids enter the metabolic pool derived from the catabolism of tissue proteins and it is the amino-acid balance of the resultant mixture which determines the rate of anabolism. Since the tissue proteins are limited by the sulphur amino-acids, at low levels of protein intake the pool may be effectively supplemented with methionine. Similarly, as the tissue proteins have a relatively high proportion of lysine they will supplement diets low in this amino-acid: this would account for the positive NPU values obtained with diets totally deficient in lysine[12,13].

The foregoing considerations show that treating the results of Allison and Anderson[1] as a linear or curvilinear relationship makes little difference to the nitrogen balance indices obtained. It is also clear that NPU values rise as the protein concentration is lowered below that required for maintenance, but this fact does not alter the validity of the equations[2,3,14] derived for the prediction of protein values. The evidence shows that these equations now apply below maintenance as well as above. However, the increased values of NPU below maintenance make little difference to net dietary protein values which are low and of small practical interest.

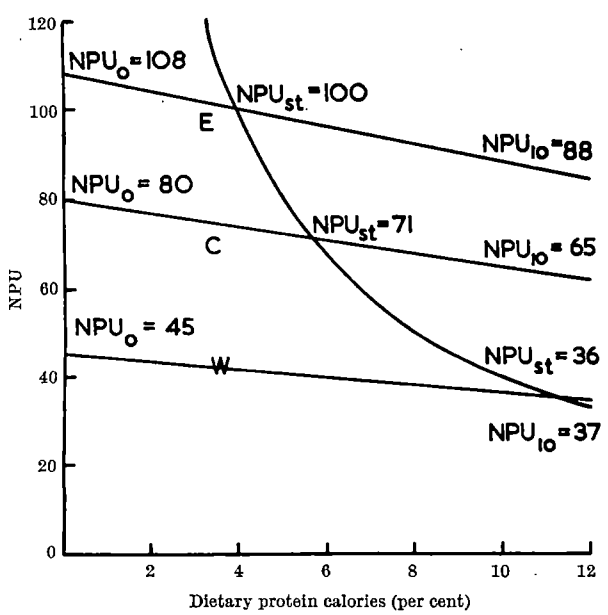


Fig. 2. Relationship between net protein utilization and protein concentration for three proteins: egg (*E*), casein (*C*) and wheat gluten (*W*). The numerals indicate the values for NPU a specified protein concentrations

The foregoing observations are of practical importance, as in order to predict the protein values of diets from chemical data the relationship between protein score and NPU must be evaluated. Thus, it is usual to determine diets containing 10 per cent protein (NPU_{10}) and to calculate the value of NPU at maintenance (NPU_{st}): an alternative parameter is the intercept of equation (3) on the NPU axis (NPU_0). The relationship between these values is illustrated in Fig. 2 using data obtained for egg, casein and wheat gluten. It is clearly necessary to specify in all cases which of the values for NPU is being used. It may well be that NPU_{st} and NPU_0 correlate with protein scores better than NPU_{10}, which was chosen by Block and Mitchell[15]. Certainly the design of an 'ideal' amino-acid pattern for calculation of protein scores should take the differences between the values into account.

It will be noted that the experiments of Allison and Anderson[1] differ from those of Mitchell and Carman[5] and from those of Munro[16]. In the work of Allison and Anderson[1] the caloric intake was fixed and thus the protein intake and concentration varied. Mitchell and Carman[5] fixed the concentration of protein and the caloric and protein intake varied. On the other hand, in the work described by Munro[16] the protein intake was fixed and the caloric intake and protein concentration varied. Apparent discrepancies between the results of these experiments are resolved by our theory of protein metabolism[17], which takes into account the interrelationship between the various dietary factors that influence nitrogen balance[18].

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Temperature Discrimination in the Skin

Thermoreceptors reported in the skin of the cat, rat and dog[1–3] had properties similar to the thermoreceptors found in the tongue[4]. The afferent fibres, however, had low conduction velocities characteristic of non-myelinated axons. The receptors fell into two categories: those excited by a rise and those excited by a fall in cutaneous temperature, above or below the neutral temperature of the skin. At certain temperatures each of the fibres was firing steadily and when tested would continue to respond at a nearly constant rate of discharge for 30 min or longer. The very low conduction velocities of all the afferent fibres, less than 1·5 m/sec, was difficult to fit with results based on psycho-physical and nerve-block techniques in man, which indicated that most of the afferent fibres mediating cutaneous thermal sensations

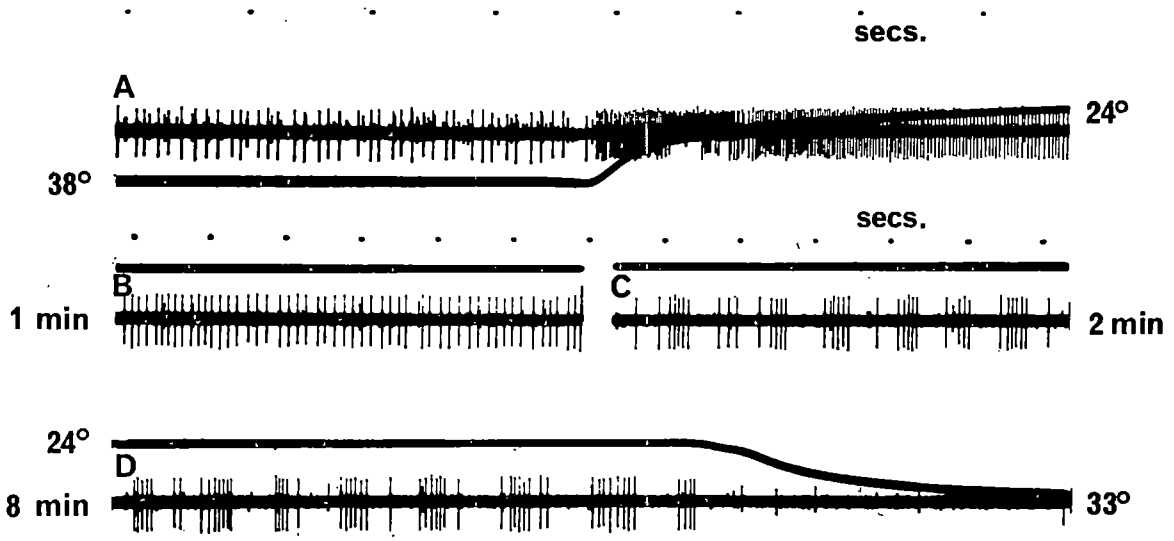


Fig. 1. The discharge of impulses in a cold receptor in response to lowering the temperature of the skin. In record *A*, the upper trace shows the impulses in the fibre and the lower trace shows the temperature of the skin. Initially the skin had been held at 38° C and just before the start of the upper record was allowed to cool slightly. This produced the regular discharge of impulses. The water flowing through the thermode was then suddenly switched to 24° C. As the cutaneous temperature was falling there was an acceleration of the discharge which was maximal (peak 120/sec) before the skin had reached the new steady temperature. *B*, 1 min after the change the skin temperature was still falling very slowly and the discharge was still regular. *C*, 2 min after the change the temperature was steady and the grouped discharge had made its appearance. *D*, 8 min later the grouped discharge was still present and disappeared when the temperature was raised. The time marks are at 1-sec intervals

had conduction velocities faster than 4 m/sec[5], although Landau and Bishop[6] have more recently brought forward evidence that in man, too, some thermal sensations can be mediated by non-myelinated fibres in peripheral nerves.

Monkeys were used in the experiments reported here and the thermoreceptors found had several differences in properties from the thermoreceptors in the cat, rat and dog. The monkeys were anæsthetized with chloralose and 'Nembutal' and the afferent fibres were dissected from either the median nerve or the musculocutaneous nerve in the upper arm. The cutaneous temperatures were controlled by circulating water, controlled to $\pm 0{\cdot}02°$ C by a thermostat, through a closed metal cylinder, 10 mm diameter (the 'thermode'), which rested on the skin. The temperature was registered by a thermistor bead mounted on the surface of the thermode in contact with the skin.

The endings of the afferent fibres were either in the hairy skin of the radial side of the forearm or in the glabrous skin of the palm, thumb, first or second finger. The receptive fields, measured with a thermal stimulator which had a tip diameter less than 75μ, were always small, ranging from 0·25 to 0·6 mm^2. Each fibre ended in only one temperature-sensitive spot in the skin. These small areas of maximal thermal sensitivity may correspond to the cold spots in human skin first reported by Blix[7].

The sizes of the afferent fibres were assessed by measuring the conduction velocity in individual fibres from a pair of electrodes placed distally, either on the nerve trunk or the fibre-ending in the skin, to the nerve filament on the proximally placed recording electrodes. Measurement from the skin invariably gave a lower value for the velocity than for stimulation of the nerve trunk, as reported previously for the cat[1], probably due to tapering of the axon as it approaches its endings in the skin. The conduction velocities of the 14 fibres measured were, with two exceptions, in the range 3·6–15 m/sec so that the fibres were myelinated, with diameters ranging from about 1–3μ. The exceptional fibres had conduction velocities below 1·5 m/sec and were probably non-myelinated, with diameters below 1μ. The monkeys thus differs markedly in this respect from the cat, rat and dog in which all the thermoreceptors examined in detail have had afferent fibres with conduction velocities below 2·5 m/sec, that is, were non-myelinated fibres, less than 1μ diameter.

All 19 thermoreceptors examined as single units in the present experiments were excited by lowering and inhibited by raising the cutaneous temperature. No receptors excited by small increases in skin temperature were found although in multi-fibre preparations not examined in detail there were some fibres comparable to the heat receptors of the cat[1]. A possible reason for the failure to find sensitive 'warm' receptors is that they are all innervated by non-myelinated axons. The peak frequency of discharge on suddenly cooling the skin, by lowering the temperature of the thermode resting on the surface from 40° C to 20° C, was about 150 impulses/sec. A striking and characteristic property of these cold-sensitive fibres was the grouping of impulses into bursts separated by intervals of silence (Fig. 1). This pattern of discharge was most conspicuous when the cutaneous temperature was steady, but was also recorded when the temperature was changing. The number of impulses in a burst varied at different times between 2 and 8, but at any particular temperature was usually constant or fluctuated by only one or two impulses. The bursts of impulses lasted from 1/10th to about 1 sec and the interval between bursts had similar durations. Whenever the temperature either rose or fell, even if the rate of change was less than 0·05° C/sec, the grouped discharge was altered and eventually disappeared to be replaced either by a more steady stream of impulses or by silence (Fig. 1*A* and *B*). The grouped discharge returned when the temperature was once again steady (Fig. 1*C*). The frequency of the adapted discharge depended on the temperature of the skin and was maximal, not usually greater than 120/sec for each fibre at some temperature in the range 20°–35° C. Many of the fibres had a regular grouped discharge of impulses at cutaneous temperatures above 35° C but not above 40° C, so that they were active at temperatures above the normal temperature of the skin. On two occasions there was a discharge above 46° C which corresponds to the paradoxical discharge reported by Dodt and Zotterman[8]. Although a fibre might be silent at the higher temperatures it would nevertheless fire when the skin was cooled to a temperature above that at which there was an adapted discharge, and in these conditions the fibre would become silent again after a few seconds.

Exact measurements of the thermal sensitivity were not made, but all the fibres could be excited by temperature changes of less than 0·5° C and the grouped

discharge could be altered by much smaller changes than this, less than 0·05° C/sec. A comparison of the thermal sensitivity with the threshold of cold spots in human skin revealed a comparable sensitivity at threshold.

None of the receptors could be excited by innocuous mechanical stimulation such as moving hairs, stroking or pressing on the skin. Some units were excited by pressing a small probe firmly on the skin using a force (5 g wt.) about 2,000 times the threshold of the most sensitive cutaneous mechanoreceptors. The thermal and mechanical receptive fields were coincident so it is likely that the mechanical stimulus was acting on the temperature-sensitive elements.

The sizes of the receptive fields, the thermal sensitivity and persistence of discharge at constant temperature taken together with size of the afferent fibres suggest that if, as seems likely, man and monkey have similar cutaneous receptors, then the receptors described here have a crucial role in thermal sensation. They are probably the cutaneous cold receptors, specifically signalling thermal conditions in the skin and thus the environment. Hensel and Boman[9] have recorded from one similar afferent unit in human experiments. Whether warm receptors also exist, that is, afferent nerve-endings specifically excited by small rises in skin temperature, cannot be decided on the present results. They have been described in other animals and the failure to find any may result either from the actual paucity of warm receptors, and indeed warm spots are distributed much less thickly in human skin than are the cold spots, or because the receptors are innervated by non-myelinated axons.

This work was done during the tenure of a Locke research fellowship of the Royal Society.

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Increase of Muscle Permeability to Aldolase in Several Experimental Atrophies

After denervation the whole sarcolemma becomes as sensitive to acetylcholine as the neuromuscular junction of the normal fibre[1]. An increase of the rate of exchange of ^{24}Na and ^{42}K between interior and exterior of the denervated muscle has been observed[2] and this increase can be attributed to an increase in sarcolemma permeability. From this it could be supposed that denervation would also affect other properties of the sarcolemma. It was, therefore, decided to determine whether the sarcolemma of the denervated muscle showed an increase in permeability to protein molecules, because it is known, mainly from the data of Zierler[3–7], that aldolase, and probably other glycolytic enzymes, moves out of the fibres during incubation of normal skeletal muscle of rat *in vitro*, probably by diffusion. This work has been extended to other conditions leading to atrophy of the muscle, namely, tenotomy, partial ischæmia and caloric deprivation.

Extensor digitorum longus muscle from young albino rats (body-wt. between 50 and 80 g) was used throughout. Denervation was performed by excising 1 cm of the sciatic nerve at the level of the hip joint. For tenotomy, the proximal tendon of the muscle was severed. Partial ischæmia was caused by ligature of the femoral artery in the proximal region of the thigh. Caloric deprivation was obtained by giving to the rats one-third, by weight, of the amount of food consumed by controls of the same initial weight, fed ad libitum. The contralateral normal muscle was used as a control in the experiments involving denervation, tenotomy, or ligature.

Table 1. Mean Differences between Normal and Atrophic Muscle in Aldolase Efflux and Total Aldolase Content

Time after beginning of experiment		Aldolase efflux as percentage of total aldolase denervation		Total aldolase content (A.U./mg)
12 h	(6)	−0·06 ± 0·12	(6)	−0·97 ± 0·55
1 day	(6)	−0·40 ± 0·59	(6)	−0·58 ± 0·25
2 days	(5)	−1·95 ± 0·44*	(5)	−0·42 ± 0·99
3 ,,	(11)	−2·14 ± 0·48†	(11)	+0·36 ± 0·59
4–5 ,,	(5)	−1·99 ± 1·03	(5)	+2·62 ± 0·91*
6 ,,	(6)	−1·59 ± 0·39†	(6)	+4·30 ± 0·75†
12 ,,	(6)	−3·87 ± 1·31*	(6)	+7·06 ± 0·96†
18 ,,	(8)	−2·17 ± 0·89*	(8)	+5·50 ± 1·22†
		Tenotomy		
6 h	(6)	−0·67 ± 0·27	(6)	−0·51 ± 0·27
12 ,,	(5)	−1·22 ± 0·32*	(6)	−0·73 ± 0·35
1 day	(5)	−1·14 ± 0·29*	(5)	+0·32 ± 0·45
2 days	(4)	−1·24 ± 0·20†	(4)	+1·27 ± 0·73
4–5 ,,	(7)	−4·87 ± 2·01	(7)	+0·48 ± 2·44*
8 ,,	(6)	−3·01 ± 0·76*	(6)	+1·98 ± 1·97
18 ,,	(2)	−0·62	(2)	+1·20
		Ischæmia		
5 days	(4)	−0·31 ± 0·50	(4)	−0·05 ± 0·45
10 ,,	(7)	−0·41 ± 0·30	(7)	−0·32 ± 0·89
20 ,,	(9)	−2·28 ± 0·77*	(7)	−1·58 ± 0·91
		Deficient diet		
10 days	(7)	+1·23 ± 0·44*	(7)	−0·51 ± 0·22
20 ,,	(10)	+2·62 ± 0·49†	(10)	+0·97 ± 0·73
40 ,,	(6)	+0·74 ± 0·22*	(6)	−2·17 ± 1·16

Values represent the mean result ± *S.E.M.*; number in parenthesis
*Difference significant at the $P < 0·05$ level.
†Difference significant at the $P < 0·01$ level.

In nearly all the experiments the muscle was incubated for 1 h at 38° C, in standard Warburg bottles, in 5 ml. of a modified Krebs–Ringer glucose-phosphate solution, *p*H 7·4 (ref. 4). At the end of the incubation period aliquots of the fluid and of the homogenate of muscle (prepared in a Potter–Elvehjem apparatus) were immediately assayed in duplicate for aldolase according to Sibley and Lehninger[8]. Effluent aldolase activity is expressed as percentage of total aldolase activity (residue in the muscle + effluent).

The results (Table 1) show that a significant increase in aldolase efflux *in vitro* takes place after denervation as well as after tenotomy and ligature of the femoral artery, whereas in the fourth type of atrophy, that due to caloric deprivation, the opposite effect is observed, namely, a decrease of the efflux. The increase in permeability is roughly similar in the three types of atrophy, but differs notably in the time of onset. In the case of tenotomy it is already evident after 12 h, while after denervation it is apparent at 2 days. In both conditions it precedes the decrease in weight of the affected muscle, whereas in the case of ischæmia, the increase in efflux is delayed and follows the decrease in weight of the muscle.

On the basis of the relation found by Zierler[4] between weight of the muscle and aldolase efflux, it could be supposed that, at least in the more advanced stages of atrophy, the increased efflux could be attributed to the decrease in weight of atrophic muscle. However, calculation of the correlation coefficient between weight and efflux did not show any significant correlation in the first three types of atrophy. These results are, therefore, in agreement with the hypothesis that, in muscular atrophy due to denervation, tenotomy and ischæmia, the sarcolemma is altered and that this alteration causes an increased efflux of aldolase.

The difference in aldolase efflux from denervated muscle in comparison with controls was not altered by lack of glucose, anoxia, a high potassium medium or insulin, although increased efflux was observed in the atrophic and the normal muscle. This suggests that the increase in permeability observed in atrophy is secondary to modifications of the metabolism of muscle. In fact, it could be expected that, if the membrane alteration were primitive,

the reaction of the sarcolemma of the pathological muscle would be different from that of the control, as suggested by Zierler[9].

It is clear from our results that there is no relation between acetylcholine hypersensitivity and increased efflux of aldolase in the denervated muscle. The increase in permeability is already present two days after denervation, when there is only a beginning of the extension of the area of sensitivity to acetylcholine. Its full development takes at least a week[1]. Furthermore, the increase in the efflux of aldolase is also evident after tenotomy, and in the ischæmic muscle. It is known that in the atrophies of disuse there is no clear increase in sensitivity to acetylcholine[10].

A further difference between the various types of atrophy investigated is evident in the total aldolase activity, which is lowered in the denervated muscle much more than the fresh weight, whereas in the other types of atrophy its decrease is parallel to that of the weight.

An increased permeability of the muscle fibre to aldolase has been observed directly[9] in the case of the hereditary muscular dystrophy of mice. The recent data of Dowben and Zuckerman[11] point to an increased permeability of muscle after local X-ray irradiation. Our data provide further evidence of an increase in permeability of muscle cells as a general response to noxious stimuli and metabolic alterations.

This work was assisted by a grant from Muscular Dystrophy Associations of America, Inc.

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PHARMACOLOGY

Influence of Hypothermia on the Radioprotective Effects of β-Mercaptoethylamine

In 1951 Bacq *et al.*[1] showed that β-mercaptoethylamine (MEA) could protect mice against ionizing radiation. Essentially, three mechanisms for the protective action of this compound have been considered. They are pictured as scavenging free radicals[2,3], lowering the oxygen tension in tissues[4,5], or forming complexes with sulphur-containing proteins by means of —S—S— bonds[6,7]. In previous work we showed that the protective action of MEA is increased by surface-active substances and by hyaluronidase[8].

The radioprotective action of MEA seemed also to be enhanced by hypothermia. It is well known that hypothermia has a radioprotective effect by inducing hypoxia[9]. It is the purpose of this communication to establish whether the effect of MEA under the conditions of hypothermia is greater than of MEA alone and to investigate the mechanism of the MEA action.

One hundred and twenty *A/Jax* male mice weighing 22–25 g and aged 60–70 days were used throughout. Sixty animals were used for controls with physiological NaCl-solution and MEA. Another 60 animals were given an injection of a mixture of 'Hydergin' (2 mg/kg body-wt.), 'Propaphenin' (5 mg/kg body-wt.) and 'Dolcontral' (4 mg/kg body-wt.), and then cooled down to 20°–25° C by placing them in a room with normal temperature (+20° C) for 60 min and then in a cold room (+2° C) for 30 min. A further 30 animals were irradiated immediately. Another 30 received, 15 min before irradiation, an intraperitoneal injection of MEA (3 mg/1 ml. physiological NaCl solution). The mice were irradiated with 800 r. (170 kVp., 22 m.amp, 0·5 mm Cu- and 1·0 mm Al-filter, 85 r./min). The statistical analysis was done by using the χ^2 test with the correction of Yates[10].

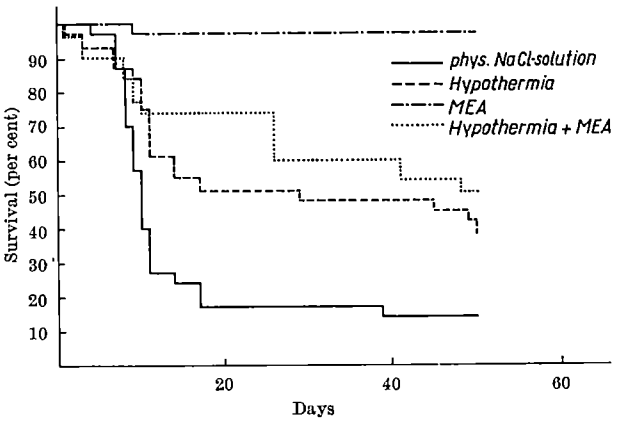


Fig. 1. The effect of hypothermia on the radioprotective action of MEA in male *A/Jax* mice

Fig. 1 shows the survival curves obtained for mice injected with physiological NaCl solution, MEA, or under hypothermia alone, or in the combination of hypothermia and injection of MEA.

It can be observed that the protection afforded by hypothermia alone is lower than the protection of MEA alone. The combination of hypothermia and MEA shows no potentiating or additive effect. On the contrary, Table 1 shows that the action of MEA under the conditions of hypothermia is lowered in such a way that no significant difference between hypothermia alone and hypothermia in combination with MEA is demonstrable.

Table 1

	Control (NaCl)	Hypothermia alone	MEA alone	Hypothermia +MEA
Control (NaCl)	5	++	+++	++
Hypothermia alone	14		+++	0
MEA alone	29			++
Hypothermia + MEA	18			

0, Difference not significant; +, difference significant ($P < 0.05$); ++, difference significant ($P < 0.01$); +++, difference significant ($P < 0.001$).

These results suggest that in the hypothermic conditioned hypoxic mice the radioprotective effect of MEA is not evident. The failure to demonstrate a significant additive protective action when MEA and hypoxia were combined is taken to indicate that MEA and hypoxia act at some stage through a common pathway for radio-protection. This view was substantiated by Patt, Blackford and Straube[11], who found that hypoxia and cysteine had no additive effect in diminishing the radiosensitivity of rabbit thymocytes, and by Weiss[12], who showed that cysteamine did not afford a significant radioprotection to the hæmopoietic system of severely hypoxic mice.

To explain the fact that MEA is not effective in protecting mice when the oxygen tension is lowered it can be assumed that MEA takes part in the oxygen-dependent production of free radicals. Ormerod and Alexander[13] showed by electron-spin resonance investigations of irradiated salmon sperm head DNA that the radioprotective effects of cysteamine were brought about by the transfer of a hydrogen atom from the SH-group of cysteamine to the radicals produced in DNA.

We may therefore assume that MEA reacts with radiation-induced radicals $R\cdot$ of biologically important molecules in competition with oxygen in such a way that the peroxidation of the radicals is prevented.

$$RH \text{---}\sim\rightarrow R\cdot + HS{\cdot}CH_2{\cdot}CH_2{\cdot}NH_2 \rightarrow RH + NH_2{\cdot}CH_2{\cdot}CH_2{\cdot}S\cdot$$

$$RH \text{---}\sim\rightarrow R\cdot + O_2 \rightarrow RO_2$$

This hypothesis will be tested further by investigating the quantitative relations between lowered oxygen tension and the concentration of MEA in the tissues on the radiation sensitivity of hypothermic mice.

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Semi-automatic Transfer of Volatile Liquids to Paper Chromatograms

THE recent publication of a device for applying liquids to paper chromatograms[1] prompts this report of a simple arrangement for the reproducible transfer of small volumes which is in routine use in this laboratory for the application of steroid standards to paper chromatograms. It consists of a lambda pipette clamped in the vertical position with the pipette tip firmly against the surface of the chromatography paper which is supported on a perforated metal disk. A stream of compressed air for drying passes through holes in the metal disk against the lower surface of the paper (Fig. 1). In practice the pipette is filled with solvent and positioned while keeping a finger over the upper orifice to prevent leakage. Gradual transfer of solvent to paper begins when the finger is released. A Medtec Spotting Device was employed, but a simpler device for support of the paper and delivery of a gas stream would serve the purpose.

Experiments were performed with a variety of solvents (100–500 μl. volume) containing Sudan III as indicator, together with Whatman No. 2 chromatography paper and compressed air stream at room temperature. The spots which occurred using chloroform, benzene, ethyl acetate, or dichloromethane had diameters of 7 mm or less, while those with methanol were less satisfactory (8 mm diameter). Unsatisfactory results were obtained with water, due to tearing of the paper or spread of the solvent over a large area despite attempts to hasten drying by use of an infra-red lamp.

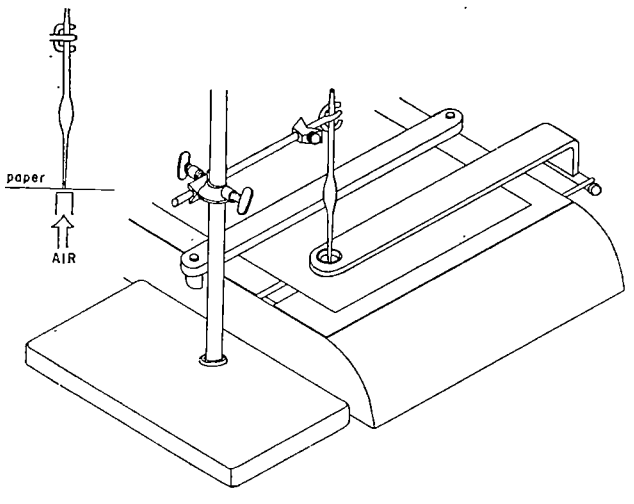


Fig. 1. Device for transfer of liquids to paper chromatograms

The time required to deliver 500 μl. chloroform was 7·5 min, benzene 14 min, and ethyl acetate 23 min. Transfer of solute to the metal disk is negligible provided the air stream is moderately vigorous and drying is effective. This device is applicable to wide chromatography paper, and semi-automatic delivery allows several chromatograms to be spotted in parallel if desired. The chief limitation appears to be the inability to handle large solvent volumes. Our spots appear to be slightly larger than those reported by Clarke and Sowter[1], perhaps because we used an air stream at ambient temperature, a choice which was dictated by the thermolabile nature of the solutes.

This investigation was supported by U.S. Public Health Service research grant *AM*–06607 and training grant 2*A*–5230, National Institute of Arthritis and Metabolic Diseases, and by the Oregon Heart Association.

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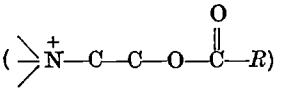
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Inhibition of Synthesis of Acetylcholine by Some Esters of Trimethoxybenzoic Acid

HEMICHOLINIUM (HC-3) has been shown to possess a strong inhibitory effect on acetylcholine (ACh) synthesis in nervous tissue which can be effectively antagonized by choline[1–6]. Similar activity, though to a lesser degree, has been demonstrated in tetraethylammonium (TEA)[7,8]. Replacement of the ethyl group of TEA by methyl, propyl or butyl results in a significant reduction of the inhibitory activity. Recently, we have demonstrated that troxypyrrolidinium (ethyl-2(3,4,5-trimethoxybenzoyloxy) ethyl pyrrolidinium iodide) possesses hemicholinium-like activity but is less toxic[9]. In the work recorded here, a series of trimethoxybenzoic acid esters are compared to determine the structure/activity relationship.

As shown in Table 1, the common feature in all compounds is the 'aminoethanol' moiety:



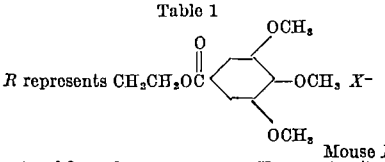
where the nitrogen is quaternized and the oxygen is linked to carbon which is in the carbonyl form. These compounds produce the characteristic toxic features of HC-3 (muscular weakness and respiratory depression at sublethal doses and respiratory paralysis followed by asphyxial convulsions at lethal doses). The LD_{50} (intraperitoneal) for mice, estimated by the method of probits[10], is shown in Table 1. Like HC-3, the toxic effects of all the compounds, with the exception of trimethylammonium (I), the morpholino derivatives with propyl (V) and butyl (VI) ester linkage, could be effectively antagonized by choline. The optimal protective dose of choline (100 mg/kg) raised the LD_{50} value 2–3 times. The compounds produced failure of transmission in the cat's superior cervical ganglion stimulated presynaptically at high frequency (20 shocks/sec). The ganglia lost 60–70 per cent of the preformed stores of ACh when stimulated for 30 min in the presence of triethylammonium (troxonium-

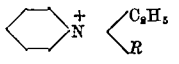
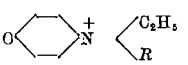
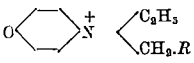
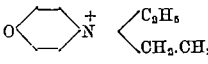
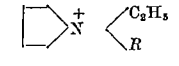
II) or pyrrolidinium derivative (troxypyrrolidinium-VII) at a dose of 200 μg/kg/min (intravenous). Loss of the ganglionic ACh could be prevented by simultaneous administration of choline. The compounds also possess some post-synaptic component in their action as indicated by reduction in the ganglionic stimulating effect of ACh. The morpholino derivative with the butyl ester linkage (VI) was the most potent in this respect.

Relative ability to inhibit ACh synthesis was investigated in minced brain preparation of mice incubated at 37° for 1 h in eserinized-bicarbonate Locke solution (*p*H 7·4: 95 per cent oxygen : 5 per cent carbon dioxide) as described by Bhatnagar and MacIntosh[8]. The test substance dissolved in the medium was added to the incubate to make a final concentration of 0·01, 0·1 or 1 mM. Two samples without the drug served as control, while two samples similarly prepared but not incubated were used for estimating ACh at zero time. Free and bound ACh (cf. ref. 8) was estimated by the cat's blood pressure method[11]. The 'total' ACh synthesized, which represented the net increase in both free and bound forms of ACh during the period of incubation, was determined and the ED_{50} for each compound was calculated (Table 1). Under our experimental conditions the amount of total ACh synthesized in absence of test substance was 12·3 ± 0·8 μg/g of wet brain tissue in 1 h, of which about one-third was in the free form.

All compounds except the trimethylammonium derivative (I) were found to be potent inhibitors of ACh synthesis. Replacement of the trimethylammonium group with triethylammonium, pyridinium (III) or morpholinium (IV) led to about 30-fold increase in the inhibitory activity. This effect became even more pronounced when the trimethylammonium group was replaced by the pyrrolidinium group (VII). The latter compound was 6–8 times as potent as the triethylammonium, pyridinium or morpholinium derivatives and possessed one-half to one-third the activity of HC-3. In addition to the quaternary *N* group, length of the ester link considerably influenced potency of the compounds. For optimal activity ethyl ester link was found to be essential (IV), as replacement of it with propyl (V) or butyl (VI) led to a progressive decline in activity. The anionic part of the molecule was of little significance.

Table 1



No.	Structural formula	X^-	Mouse LD_{50}* (mg/kg, i.p.)	ED_{50} (μM)†
I	$(CH_3)_3.\overset{+}{N}.R$	Tosylate	400 (376–426)	2,000
II	$(C_2H_5)_3.\overset{+}{N}.R$	Tosylate	23 (20–27)	60
III		I-	24 (23–25)	85
IV		I-	33 (30–37)	60
V		Tosylate	35 (31–40)	300
VI		Tosylate	63 (58–69)	600
VII		I-	32 (28–36)	10
	HC-3		0·18 (0·17–0·2)	4

* Sixty animals used for each compound. Ninety-five per cent confidence limits in parentheses.
† Concentration producing 50 per cent inhibition of ACh synthesis in mouse minced brain. Each figure represents an average of two experiments.

Release of ACh from the tissue to the medium was not significantly affected except by troxypyrrolidinium (VII) and the morpholino derivative with the ethyl ester linkage (IV), where at the highest concentration (1 mM) the ratio of free to bound ACh was reduced from a control value of 0·39 to 0·06.

The inhibitory effect could be reversed by addition of choline to the incubation medium. Investigations with troxonium (II) and troxypyrrolidinium (VII) showed that the concentration of choline required to reverse completely the inhibitory effect was about ten times the molar concentration of the compound. Activity of choline acetylase, the enzyme which catalyses the transfer of acetyl radical from acetyl-coenzyme *A* to choline, was not significantly affected.

These investigations show that among the compounds investigated pyrrolidinium derivative (VII) was the most potent inhibitor of ACh synthesis. For optimal activity the ethyl ester linkage was found to be essential while the anionic part of the molecule was of little significance. They appeared to act in a manner identical to that of HC-3, that is, by competing with choline for the intracellular sites of acetylation or with ACh for the storage sites.

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PATHOLOGY

Production of *Trypanosoma cruzi* Cysts *in vitro*

Trypanosoma cruzi, the causative agent of American trypanosomiasis or Chaga's disease, has been found to produce cysts readily in culture if the medium is treated with a urease preparation. These forms resemble closely the description of cysts of some trypanosomes in their insect hosts[1–3].

Routine cultures of either the Brazilian or Corpus Christi strains of *T. cruzi* were made in screw-capped tubes containing a liquid medium. Each tube consisted of 5 ml. of 'Trypticase Soy Broth' (BBL) which had been enriched with 0·05 ml. of citrated human blood (outdated blood bank material). To start a fresh culture, 0·5 ml. of culture, between ten and fifteen days old, was added to this medium. The cultures were kept at room temperature. The usual forms of *T. cruzi* in culture were regularly obtained in this medium. A slightly modified medium, using serum and hæmolysed material made cell-free by centrifugation, was also used.

Two hundred mg of a crude urease powder (jack bean urease, 2 × National Formulary (U.S.A.), from Mann Research Laboratories, Inc., New York 6) was suspended in 10 ml. of twice-distilled water and the insoluble portion was centrifuged. The clear supernatant was sterilized by passage through a Selas filter of 03 grade porosity. Volumes ranging from 0·01 ml. to 0·1 ml. of this preparation added to fresh cultures of *T. cruzi*, as described above, and kept

at room temperature, produced cysts in about five days. As little as 10 μg of the urease powder per ml. of culture appeared to be sufficient, while 1 μg per ml. did not have this effect. Cyst formation was obtained in concentrations up to 500 μg of urease per ml., but with 1 mg/ml. it did not occur. Incubation at 37° C was unsatisfactory. Heating the urease preparation for half an hour in a 99° C water bath destroyed its cyst-inducing property.

The first effect of the urease preparation on the flagellates was to slow them down. The cells gradually increased in size and became highly granular. In a few days a structure resembling a vacuole, situated centrally or eccentrically, became very prominent in many of the organisms. Some of these organisms, now mostly spherical in shape, were found to contain a number of flagellated daughter cells within the vacuolar space. These were seen "writhing and twisting over each other within the enveloping periplast like a bunch of eels in a sack", to use the phraseology of Minchin and Thomson[1]. The area between the outer limits of the organism and the vacuole was clearly visible and appeared as a cyst wall of considerable thickness in the living state. Single cysts were observed; but the tendency was to form clusters in this medium. They were found to be at different stages of development and in different sizes. The cysts measured 10–25μ in diameter. Fig. 1*a* shows a cluster of cysts at the top; and below, a single cyst at the right, and a pair at the left. Half a dozen flagellates were in motion inside the upper cyst of the pair (arrow) at the time this picture was taken. Some of the amorphous structures are debris from the culture medium, in and out of focus. Fig. 1*b* was obtained by pressing down a group of cysts. Two organisms clearly show the thick, granular cyst wall around the vacuoles. These were all from a culture two weeks old.

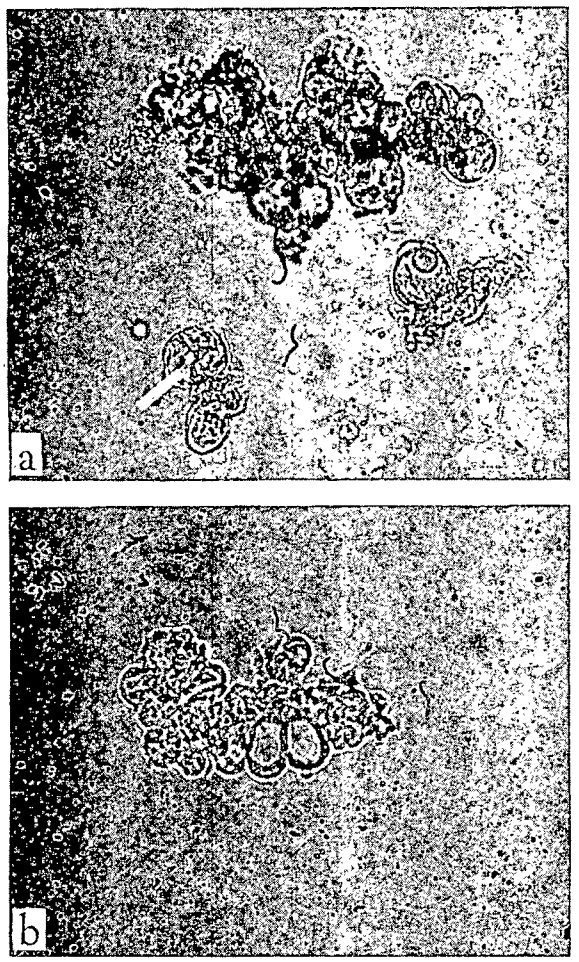


Fig. 1. Cyst formation in *Trypanosoma cruzi*. (*a*) Grouped and isolated cysts. About six flagellates were in motion inside the cyst, indicated by the arrow. (*b*) Gentle compression of cysts shows the thick granular cyst wall in optical section

Further work is in progress to determine whether this effect is specifically due to urease or to some other factor present in the crude preparation. Steinert[4] found urea to be the factor responsible for the transformation of the crithidia of *Trypanosoma mega* into its trypanosomal form.

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IMMUNOLOGY

Effect of Actinomycin C on Immune Responses *in vivo*

SEVERAL workers have reported that the actinomycins do not suppress immune responses *in vivo*[1–4]. Wust *et al.*[5] found that actinomycin *D* injected into rats at the same time as sheep red cell antigen caused a short delay in appearance of the immune response, but did not affect the maximum antibody level reached. Butler[6] reported a slight depression of the secondary response to diphtheria toxoid on administration of actinomycin *D*. Nathan *et al.*[3] report actinomycin *D*, but not actinomycin *C*, active in their screening test. Actinomycin *D* has been found an effective inhibitor of antibody synthesis *in vitro*[7,8].

According to Reich[9], the actinomycins inhibit DNA-directed RNA synthesis at low concentrations, but have little effect on DNA synthesis except at high concentrations. Inhibition of RNA synthesis is dependent on the binding of actinomycin to the guanine moiety of DNA. The experiment described here was performed in an attempt to locate the period of messenger RNA production in the synthesis of antibodies to sheep red cell antigen.

On day 0, fifteen groups of male *A2G* mice (15–22 g body-wt.) and a control group were given 0·2 ml. of a 5 per cent sheep red cell suspension in saline intravenously. On each of days −7 to +7 one group of mice was injected intravenously with 0·75 mg/kg actinomycin *C* in saline solution (LD_{10}). Mice in the day 0 group were given actinomycin within 1 h after injection of sheep red cells. All the mice were bled by decapitation on day +9, sera being collected individually and titrated as previously reported[10]. Results are shown in Fig. 1.

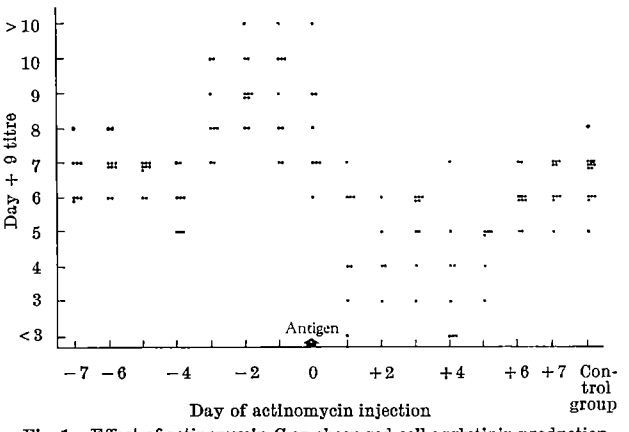


Fig. 1. Effect of actinomycin *C* on sheep red cell agglutinin production in *A2G* mice. Mice were injected with SRC antigen on day 0 and with actinomycin, 0·75 mg/kg, before or after injection of antigen. Sera were taken on day +9 and titrated individually. Titres are shown as reciprocals of the end-point dilutions, expressed as powers of the base 2

It is clear that actinomycin *C* has an action on antibody synthesis similar to that of antimetabolites and most alkylating agents, as it reduces the amount of antibody produced if given one to four days after the antigen[2,11]. It can be inferred that the period of maximal messenger RNA production is within these limits. Mice given actinomycin at a later stage of the primary response do not show significant suppression of antibody levels compared with untreated mice. Whether the observed reduction in titre on day +9 is temporary, that is, due to a lengthening of the induction period (cf. Wust *et al.*[5]), or permanent is being investigated.

Actinomycin given as a single dose in the 72-h period before injection of antigen enhances the antibody-level in mice bled on day +9. Similar effects have been observed with, for example, colchicine[12,14], colcemid[13] and 5-fluoro-2-deoxyuridine[15]. Rabbits given colchicine on days −1, 0 or +2, and sheep red cell antigen on day 0, had enhanced hæmolysin titres when bled during the next 3–4 weeks[12]. White[13] reported enhanced titres of antitoxin in guinea-pigs given colcemid 6 h before 5 *Lf* diphtheria toxoid. It has been suggested that colchicine may act by producing general cell damage, leading to release of metabolic products that aid in inducing the immune response and stimulate proliferation of antibody-producing cells[12,13]. Actinomycin, because of its action on messenger RNA, may divert a greater number of cells to the production of antibody against the antigen in question. The mechanism of this action cannot be really understood until the number of cells producing antibody are known.

Similar results have been obtained with another particulate antigen, T.A.B. vaccine, titrating the levels of *Salmonella typhi* H agglutinins in the serum. Experiments are in progress to compare soluble with particulate antigens.

The observed effects of actinomycin described here may be dependent on the dose-level used. Hirata and Redlich[14] found that hamsters given colchicine immediately after rabbit red-cell antigen had enhanced titres of antibody on day + 7 if non-toxic doses were used, but depressed titres with near lethal doses. This point needs further investigation.

I thank Farben Fabriken Bayer AG, Wuppertal-Elberfeld, for a gift of actinomycin *C*, and Dr. M. C. Berenbaum for his advice.

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HISTOCHEMISTRY

Formazan Reaction as a Confirmatory Test for Aldehyde Groups in Histochemistry

One of us has predicted elsewhere[1] that not all neutral mucopolysaccharides can be demonstrated in tissue sections by the periodic acid–Schiff technique because the 'dialdehydes' which are formed by the periodate oxidation of their *vic*-glycol groups may be transformed into hemiacetals or hemialdals which are known to be unreactive towards Schiff's reagent *in vitro*[2]. On the other hand, all periodate-engendered 'dialdehydes' appear to react with aromatic hydrazines[2,3] including salicyloyl hydrazide and 2-hydroxy-3-naphthoic acid hydrazide[1]. Therefore, in tissues which have been treated with periodic acid, those mucin sites which take up hydrazine reagents but not Schiff's solution probably contain 'dialdehydes' in the hemialdal and/or hemiacetal forms. This hypothesis has now been confirmed by showing that the formazan reaction is negative in these sites.

Only aldehyde primary arylhydrazones (such as aldose phenylhydrazones) having a replaceable (acidic) α-hydrogen in their imino bond can be converted to coloured formazans by coupling with diazotized aromatic amines in an alcoholic or aqueous pyridine medium[4]. The phenylhydrazones of ketones[5] and 'dialdehydes' in the cyclic hemialdal form[2,6] cannot undergo this reaction.

The methods described in the literature[7–9] for carrying out the formazan reaction on tissue sections containing 'dialdehydes' engendered from neutral mucopolysaccharides or glycogen by periodic acid never worked in our hands. Pearse[8] has reported similar negative results with the 'mono' aldehydes produced in tissues by the acid hydrolysis of nuclear DNA, the hydrolysis of plasmal acetals, or the auto-oxidation of unsaturated lipids. We believe the failures are due to the extensive degradation of the aldehyde phenylhydrazones soon after their formation by the strong, hot solutions of phenylhydrazine acetate used in these methods. Such degradations have been established *in vitro*[10,11].

We have found the following technique to be reliable for differentiating aldehyde groups *in situ* from ketones and suspected hemialdals or hemiacetals. (1) Bring deparaffinized sections to water. (2) Immerse in a fresh 5 per cent solution of phenylhydrazine in 10 per cent acetic acid at room temperature for 3–5 min. (3) Wash briefly in 10 and 5 per cent solutions of acetic acid, followed by a final rinse in water. Blot the slide dry, and place on ice until the temperature of the section is approximately 0°–5°. (4) Put one drop of cold pyridine and one drop of a freshly prepared solution of diazotized aniline on top of the section, and leave for 3–5 min. (Diazotized aniline is prepared as follows: Dissolve 0·13 ml. aniline in 0·5 ml. water and 0·5 ml. hydrochloric acid, and cool to 0°. Add 0·1 g sodium nitrite dissolved in 1 ml. water slowly to the

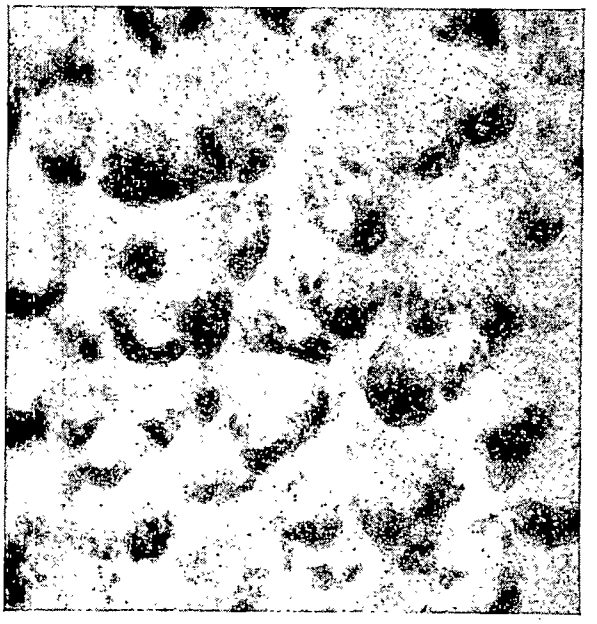


Fig. 1. Guinea-pig liver. 1 per cent periodic acid (*p*H 4·5) 8 min; uranium–formazan reaction (× 180). The 'glycogen flight' is evident

aniline mixture, keeping the temperature below 5°.) (5) Rinse in water. (6) Examine as a water mount. A light blue filter can be placed in the illuminating light path if the light yellow-brown formazans are difficult to see clearly. Photograph if required. (7) Remove the cover-slip and take the section through the alcohols to absolute alcohol. (8) Immerse in a warm (37°) 10 per cent solution of uranyl acetate in absolute ethanol for 10 min. (9) Take the sections down to water, wash successively in dilute (5 per cent) acetic acid and water, dehydrate to absolute alcohol, clear in xylene, and mount in 'Permount'.

Yellow-brown formazans can be observed in sites containing aldehydes after stage 6. Unfortunately, their contrast in sections mounted in 'Permount' is very poor. The orange-yellow uranium–formazan complexes formed after stage 9 are easily discernible.

The formazans formed from the 'dialdehydes' of periodate-oxidized glycogen in guinea-pig liver (fixed in ethylene glycol monomethyl ether acetate[12]) and of the neutral mucopolysaccharides in guinea-pig gut are shown in Figs. 1 and 2 respectively. In sites marked with an arrow in Fig. 2, formazans are absent. These sites in serial sections give strong reactions with hydrazide reagents but no reaction with Schiff's solution after oxidation in periodic acid. These sites probably contain 'dialdehydes' in the hemialdal form.



Fig. 2. Guinea-pig gut. 1 per cent periodic acid (*p*H 4·5) 8 min; uranium-formazan reaction (× 240). The formazan reaction is positive in the superficial goblet cells, whereas in the deep crypts of Lieberkühn it is negligible or absent

The amount of formazan ultimately formed from periodate-oxidized polysaccharides is maximal when the sections are immersed in the phenylhydrazine solution for 3–5 min. After immersion for about 7–8 min, the capacity for formazan formation decreases and is zero after 15–20 min. Why this should happen is still undetermined. Possibly the 'dialdehyde' phenylhydrazones are transformed to hemiacetal or hemialdal derivatives, or alternatively[13] to benzeneazoalkane derivatives.

Neither 'stabilized' diazonium salts nor freshly diazotized amines other than aniline, such as sulphanilic acid[14], *p*-amino-*N*,*N*-dimethylaniline, or morphilino-2 : 5-diethoxyaniline, would couple with aldehyde phenylhydrazones *in situ*, contrary to their ability to do so *in vitro*.

The ability of formazans to form highly coloured complexes with metals[15] has been utilized to improve the contrast of formazans *in situ*. The appearance of these complexes in the electron microscope is now being examined.

One of us (P. J. S.) thanks the Department of Scientific and Industrial Research for financial support.

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RADIOBIOLOGY

Distribution and Excretion of Dentifrice Fluoride

The inclusion of 1 mg of fluoride per g of paste in a stannous fluoride dentifrice has led to the suggestion that chronic fluoride poisoning could arise during the prolonged use of such a preparation. Although it can be calculated that systemic fluorosis will not follow even the most abnormal use of a stannous fluoride paste this conclusion is, as yet, supported by only scanty experimental evidence.

To estimate the amount of fluoride retained in the body after the use of a sodium fluoride dentifrice[1] and various simplified fluoride dentifrices[2], the fluoride recovered from the toothbrush and rinsing water has been determined chemically[1] and by the use of radioactive fluorine[3]. In addition, urine excretion of fluoride has been estimated in children[3,4] and in adults[5] who were using a commercially available fluoride dentifrice, but data on the ingestion and excretion of fluoride after using a single brushful of a proprietary stannous fluoride dentifrice are lacking. This information has been obtained in the following experiment.

Immediately after emptying the bladder, ten male subjects, ranging in age from 23 to 33 years, brushed their teeth with a weighed quantity of a stannous fluoride dentifrice ('Crest', Procter and Gamble Co.) labelled with 1–2 μc. ^{18}F at the time of use. After brushing for 1 min the mouth was rinsed with 25 ml. tap water. The unwashed brush and the rinsings were retained for radioactivity counting. Urine samples were collected at hourly intervals over a 4-h period. The subjects ate a sandwich lunch immediately after collection of the first urine sample. Their fluid intake was limited to avoid excessive dilution of ^{18}F in the urine which might have led to counting inaccuracies. The residual paste was washed from the used toothbrushes, and duplicate 5-ml. samples of the suspended washings were counted in a well-type scintillation counter. Aliquots of the expectorated rinsings, and hourly urine samples, were similarly counted. Standards were prepared from weighed quantities of ^{18}F-labelled dentifrice.

The results (Table 1) show that a mean of 25 per cent of the fluoride, measured as radioactive fluoride, was recovered from the 10 unwashed brushes after each had

been used for 1 min. 51 per cent of the fluoride was found in the water used for rinsing the mouth. Thus the mean total recovery of fluoride from 10 subjects was 76 per cent—a percentage recovery similar to that reported for a sodium fluoride dentifrice[1]. If slight losses on the lips, which could not be measured, are ignored, 24 per cent of the fluoride must have remained in the mouth or have been swallowed. Some of this retained fluoride was absorbed because 1·3 per cent of the ^{18}F was excreted in the urine in the 4 h following brushing. The short half-life of ^{18}F made it impracticable to follow the excretion longer.

Table 1. Percentage Distribution of ^{18}F after Toothbrushing (10 Subjects)

	Range	Mean	S.D.
Recovered on brush	12·1–45·4	24·8	±10·9
Recovered in mouthwash	33·6–66·0	50·9	±14·1
Total recovered	51·7–86·4	75·7	± 9·5
Retained	13·6–48·3	24·3	±10·0
Excreted in urine	0·12– 5·3	1·3	± 1·4

Before drawing conclusions from excretion of ^{18}F in urine collected for only 4 h it must be stated that its rapid absorption and urinary excretion have been demonstrated in human subjects[6]. In these experiments with toothpaste more fluoride would have been excreted in a longer time, but because the rate of excretion of ^{18}F was falling the percentage increment would have been small. Therefore, this 4-h examination underestimates the excretion of a single dose of dentifrice fluoride, but nevertheless provides a reasonably accurate measurement.

The distribution within the body of the 24 per cent fluorine-18 which was not recovered from the brush or the mouthwash is incompletely known. From data on the *in vivo* uptake of ^{18}F from labelled dentifrice[7], it can be calculated that the enamel of a full complement of teeth will retain less than 1 per cent of the fluoride, leaving at least 23 per cent to be swallowed. Direct measurement of the absorption of ^{18}F, and fæcal estimation of unabsorbed fluoride, are impracticable with this radioisotope, but it has been shown that when additional fluoride is given to subjects for short periods half the fluoride absorbed is excreted in the urine[8]. Therefore, it seems that the subjects in this toothbrushing experiment absorbed about 3 per cent of the fluoride originally present on the toothbrush. Between 0·5 and 1·0 g of paste, containing, according to the manufacturer, 1·0 mg of fluoride per g, was used. Therefore, 15–30 μg of fluoride was absorbed by the subjects but half of it had been excreted 4 h after brushing the teeth. This amount is only 5–10 per cent of the daily physiological variation in urinary fluoride excretion, so that it is not surprising that earlier workers[3–5] failed to detect an increased fluoride excretion in subjects using a stannous fluoride dentifrice. The toothpaste would have had to be used very many times a day to produce a significant increase in the fluoride excreted in the urine.

The very small contribution to fluoride storage made by this stannous fluoride dentifrice indicates that under the conditions described here this preparation will not cause chronic fluoride poisoning.

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Zinc-65 in Human Hair

In 1963, we reported that the hair of rats injected with strontium-90 retained a significant amount of the radionuclide, and suggested that measurement of the radioactivity of the hair might furnish a non-destructive method of estimating the bone content, or body burden, of the radioisotope[1]. This concept has been confirmed recently by Della Rosa *et al.*[2] in work on the strontium-90 content of beagle hair during and following the chronic ingestion of the radioelement.

We are now collecting information on the accumulation of radionuclides in human hair and have begun with investigations on zinc-65. This is a logical progression, since it has been known for some time that radiozinc accumulates in rat hair[3–5], chicken feathers[6], and fish scales[7]. Zinc-65 is a potential health hazard through its formation in fall-out and nuclear reactions as proved by its occurrence in public waters[8,9]; in meat[10], milk[11], seafood[7,12], and vegetables[9]; in cyclotron[13] and reactor[14] workers; and its increasing level in the body burden of the Rongelap Islanders[15].

The radiozinc determinations were carried out as part of an investigation of the zinc content of head hair obtained from male Egyptian dwarfs suffering from zinc deficiency and presenting with the syndrome of iron deficiency anæmia, hepatosplenomegaly and hypogonadism[16,17]. Hair from the nape of the neck was obtained from each of six dwarfs who had been injected intravenously with 36–80 μc. of zinc-65 chloride some months prior to the sampling and were receiving oral medication with zinc sulphate. One-gram samples of hair were washed with a detergent, dried, and counted in a well counter with an efficiency of about 28 per cent. The data showing time in months after injection, dose of zinc-65, and hair radioactivity are given in Table 1. As is evident, the radioactivity of the hair was low in samples taken after 19 months but relatively high in the single sample of hair taken after six months from dwarf 6. There does not seem to be a direct relationship between the radiozinc dose and hair-level in dwarfs 1–5. This variation may be due in part to differences in level of medication with zinc sulphate over the 19–22 months of observation. The radioactivity of the hair from the dwarfs does not compare with the high values measured in rat hair, where this appendage may become the most radioactive part of the body with continued ingestion of zinc-65.

Table 1. Radioactivity of Hair from Egyptian Dwarfs injected with Zinc-65

Dwarf	Months after injection	Zinc-65 dose (μc.)	Hair radioactivity (c.p.m./g)
1	22	80	62
2	21	50	85
3	21	60	0
4	20	36	72
5	19	60	0
6	7	65	678

The determination of the level of radioactivity in the hair, together with the identification of the radionuclide present, may become a very useful tool in radioecology whenever long-lived radioisotopes are being investigated. Measurements already published show that iodine-131 (ref. 18), selenium-75 (ref. 19), sulphur-35 (ref. 20), and vanadium-48 (ref. 21), as well as strontium-90 (ref. 22) and zinc-65, accumulate in the hair of laboratory animals. We also have unpublished observations which show that calcium-47, chromium-51, cobalt-58, and iron-59 are retained by rat hair. Further observations on man are needed to establish that these observations on animals apply equally well to human beings.

We thank Dr. A. R. Schulert, NAMRU-3, Cairo, Egypt, for the hair specimens from the dwarfs.

This work was supported in part by grants *CA* 03952, U.S. National Institutes of Health, and *RH* 00042,

Division of Radiological Health, Bureau of State Services, U.S. Public Health Service; and by a grant from the Aero Space Medical Division under approval of the Surgeon General, U.S. Air Force.

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BIOLOGY

Sex Ratios in Marsupial Pouch Young

ALTHOUGH sex ratios favouring males at birth are well known in eutherian mammals[1,2], we do not know of any published data showing a disparity between males and females in marsupial pouch young beyond the 0·25 level of probability.

A sample of 420 pouch young of the macropod *Macropus canguru* and 410 of *Macropus rufus* was collected in south-west Queensland, Australia. Pouch young with pes measuring less than 8 mm were excluded from both samples because the scrotum is not clearly defined in smaller animals. Pouch-young sex ratios of the phalanger *Trichosurus vulpecula* were recorded in New Zealand between 1953 and 1961, the same criteria being used for sexing and animals weighing less than 1·7 g being rejected. A standard χ^2 test ($\chi^2 = 6{\cdot}06$) indicates that the assumption of a constant sex ratio for the nine yearly samples can be accepted; the samples were therefore pooled, giving a total of 626 pouch young.

None of these three samples provides evidence of differential mortality during life in the pouch. Sex ratios of the macropod pouch young with pes measuring more than 140 mm do not differ significantly from those of young with smaller pes ($\chi^2 = 0{\cdot}05$ for *M. canguru*; 1·36 for *M. rufus*). This also applies to the pouch young of *Trichosurus vulpecula* below and above 53 g in weight ($\chi^2 = 0{\cdot}47$).

The sample of *M. canguru* pouch young comprised 242 males and 178 females, a ratio, with 95 per cent confidence limits, of $(136 \pm 26)\ M/100F$. Approximate confidence limits were assigned by the formula[3]:

$$\frac{100\,M}{F} \pm \left(100\sqrt{\frac{(M+F)\,M}{F^3}}\right)1{\cdot}96$$

There is less than one chance in 500 that the disparity is due to sampling variation. As there is no evidence of differential mortality we assume that there is a disparity at birth.

Table 1. SEX RATIOS IN SAMPLES OF MARSUPIAL POUCH YOUNG

Species and reference	Males	Females	Total	χ^2*	P†
Didelphidae					
Didelphis virginiana					
Ref. 4	34	29	63	0·40	0·5
,, 5	21	27	48	0·75	0·4
,, 6	107	109	216	0·02	0·9
,, 7	470	441	911	0·86	0·4
	632	606	1,238	0·55	0·5
Phalangeridae					
Trichosurus vulpecula					
Ref. 8	69	59	128	0·78	0·4
,, 9	11	6	17	1·47	0·25
,, 10	71	66	137	0·36	0·5
	332	294	626	2·32	0·1
	483	425	908	3·71	0·1–0·05
Peramelidae					
Perameles nasuta Ref. 11	57	55	112	0·04	0·8
Macropodidae					
Setonix brachyurus Ref. 12	279	322	601	3·08	0·1–0·05
Macropus robustus Ref. 13	281	315	596	1·94	0·2
Macropus rufus	202	208	410	0·01	0·9
Macropus canguru	242	178	420	9·76	0·005–0·001

* Figures in this column are χ^2 values for a disparity between males and females.

† P = probability of drawing a more extreme sample from a population with a sex ratio of 1 : 1.

The published figures for *Trichosurus vulpecula* and those presented here can be assumed to come from populations with a common sex ratio ($\chi^2 = 1{\cdot}10$; d.f. = 3), and all were therefore pooled, giving $(114 \pm 15)M/100F$. calculated from a total of 908 pouch young. The disparity is probable at the 0·1–0·05 level. Each of the four sex ratios favours males (Table 1). This suggests that the disparity may be real despite the non-significant χ^2 value, but about 750 more determinations would be needed for a 0·01 probability if the ratio were, in fact, $114M/100F$.

Pouch-young sex ratios of other marsupial species are listed in Table 1. In no case do they clearly indicate a dominance of males in the populations from which they were drawn. Only *Macropus canguru* shows a disparity at birth, but further data might possibly establish an excess of males in *Trichosurus vulpecula*.

The figures for the two species of *Macropus* were collected while the first author was at the School of Biological Sciences, University of Sydney. We thank Mr. C. L. Batcheler and Dr. W. G. Warren for advice.

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Molluscicidal Activity of Surface-active Agents on the Snail *Biomphalaria sudanica*; a Vector of Bilharzia

PREVIOUS work on a naturally occurring substance which inhibits growth in *Biomphalaria sudanica*[1] indicated that its toxicity was partly due to its surface activity. It was therefore decided to test various commercially produced surface-active agents, at various concentrations, on the survival of this species of snail.

As the result of requests to British manufacturers of surface-active agents for samples of their products, we obtained more than 100 compounds, which were dissolved at 5 different concentrations, ranging from 0·1 to 1,000 p.p.m., in an artificial medium containing the major inorganic ions found in Lake Victoria, in the concentrations in which they occur in the lake.

The surface tension of these solutions was determined at 30° C by means of the drop-weight method of Harkins and Brown[2], and the apparatus used was virtually identical with that described by Harkins and Alexander[3].

For the 101 detergents tested it was found that 5 were non-toxic to the snails, even at concentrations of 1,000 p.p.m., that 22 were toxic at 1,000 p.p.m., 39 at 100 p.p.m., 23 at 10 p.p.m., 10 at 1 p.p.m., and, finally, 2 were found to kill the snails at a concentration as low as 0·1 p.p.m. Death of the snail was sometimes obviously due to hæmolysis, but this effect did not occur each time, and the amount of hæmolysis was certainly unrelated to the toxicity of the surface-active components.

The substances which killed the snails at the lowest concentration so far investigated (0·1 p.p.m.) were the tertiary amines 'Empigen *AE*3' and 'Empigen *AN*' (the surface tensions measured at this concentration were respectively 71 and 65 dynes cm^{-1}).

No direct correlation could be detected between the surface activity of the detergents and their toxicity on *Biomphalaria*. 'Ethylan *PC*', for example, had a surface tension of approximately 37 dynes cm^{-1} at a concentration of 1,000 p.p.m., but this proved, at this concentration, to be non-toxic to the snail. On the other hand, the most surface-active substance tested so far, 'Deciquam 222' (which at a concentration of 1,000 p.p.m. gave a surface tension of approximately 13 dynes cm^{-1}), killed the snail at a concentration of 1 p.p.m. and a surface tension of 69 dynes cm^{-1}.

It was found that the cationic detergents were, on the whole, more toxic than the anionic or non-ionic ones.

Mixtures of anionic and cationic agents were found, on the whole, to be less toxic then the surface-active agents separately. Thus, a mixture containing 100 p.p.m. 'Perlankrol *TL*' and 100 p.p.m. 'Cibaphasol *C*' did not kill snails even after 89 h whereas the separate components, at the same concentration, killed them after 20 and 5 h respectively. The addition of 'Silicone *MS* antifoam *A*' (Hopkin and Williams) to the surface-active agents lowered the toxic concentration in most cases by a factor of 10–100. The influence of the silicone was particularly evident in the case of the anionic and cationic agents. From these experiments it can be concluded that the toxic effect of most surface-active agents on *Biomphalaria* has both a chemical and a physical origin.

It was found that the eggs of *Biomphalaria sudanica* were more resistant to the surface-active compounds than was the snail itself. While the snails were killed within 1 h in certain detergents at a concentration of 1,000 p.p.m., most of the embryos in the eggs survived this concentration for more than 24 h. Concentrations of 10 p.p.m. and less of any of the surface-active agents investigated had no detectable harmful effect on the embryos.

These findings may be useful in the selection of surface-active agents which, apart from their own molluscicidal activity, can also be used for the dispersion of almost insoluble molluscicides.

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Some Biological and Biochemical Properties of the Polarizing Factor in *Hydra*

TRIPP[1] proposed a growth pattern for *Hydra* which was later confirmed by Brien[2] and Burnett and Garofalo[3]. Beneath the hypostome of *Hydra* a growth region exists where cell proliferations constantly occur. Cell divisions in this area force adjacent cells distally to the tentacle tips and proximally to the basal disk. When cells reach the extremity of the body column they die and are sloughed off. Thus, the animal is capable of continual growth without a change in form.

Burnett[4] postulated the existence of a growth-stimulating principle which controls cell division and ultimately head formation along the length of the body column. According to this hypothesis, the growth-stimulating material is concentrated in the hypostome. It diffuses proximally from this location in a gradient until it reaches the basal disk. The reason that heads fail to form along the entire column is because of the presence of a postulated inhibitor, produced in the sub-hypostomal growth region which also diffuses proximally. This inhibitor specifically blocks the action of the stimulator and the ratio of stimulator to inhibitor in any body region determines the form of that region. For example, in the budding region the inhibitor is dilute and the residual stimulator in this region induces cell divisions necessary for the elaboration of a new polyp. If the growth pattern in *Hydra* is chemically controlled as hypothesized, it should be possible to define quantitatively the mode of action of these agents and to purify them.

This communication reports preliminary investigation into the chemical and biological nature of the growth stimulatory material only. We have isolated a substance from *Hydra* capable of inverting polarity. The material is obtained from homogenates of *Hydra* boiled 10 min to stimulate nematocyst discharge. (Nematocyst toxin is lethal to the *Hydra* after its release from the capsule.) The active factor is located in the supernatant of a fraction sedimented after centrifuging the homogenate at 25,000*g* for 50 min. Annuli of tissue excised from the gastric-budding region junction of *Hydra pirardi* when exposed to these extracts for 4 h exhibit subsequent excessive tentacle or head growth during regeneration. Inverted polarity develops in more than 20 per cent (0·222) of the surviving animals as opposed to spontaneous production of supernumerary tentacles in less than 2 per cent (0·016) of 387 control animals tested (that is, annuli allowed to regenerate in culture solution without prior incubation in an extract containing growth-stimulating principle). No control animal ever developed an excessive number of tentacles, only their placement along the body column was abnormal, and here never more than two tentacles were located outside the oral ring.

Growth-stimulating material is concentrated in the distal portion of normal animals (Table 1). The activity of this area approximates that of whole animal extracts. Although the mechanism of action of the extracted material is unknown, it probably enters the assay animal by diffusion during the incubation period. Evidence for this conjecture is, first, the material can be collected in agar blocks and can be introduced afterwards from the

Table 1. Biological and Biochemical Properties of Hydra Growth-stimulatory Material

Biological properties

Type of extract	No. of surviving animals	No. animals with inverted polarity	Proportion $\frac{p_1}{n_1}$
(1) Normal animals	234	52	0·222
No extract (controls)	387	6	0·016
(2) Distal halves (hypostome)	48	11	0·229
Proximal halves (peduncle, basal disk)	54	8	0·148
(3) Sexual animals			
4 weeks at 9° C	37	5	0·135
8 weeks at 9° C	13	0	0·000
(4) Normal animals, 4–8 h exposure	64	8	0·122
(5) Depressed animals	40	2	0·050
(6) Mustardized animals	29	2	0·068
(7) Nervous system destroyed	127	15	0·118

Biochemical properties

Treatment of extract	No. surviving animals	No. animals with inverted polarity	Difference in proportion	P
(1) Boiled 10 min	70	8	0·108	< 0·05
(2) Dialysis	100	12	0·102	< 0·05
(3) Extractions				
Ether	79	12	0·069	> 0·15
Acetone-ether	41	6	0·076	> 0·25
(4) Digestions				
RNase	45	5	0·111	> 0·05
Trypsin	39	3	0·145	< 0·05
Pepsin	29	2	0·154	*
Effects of chemical solutions				
(1) KCN (10^{-6} M)	45	3	0·155	< 0·05
(2) LiCl (10^{-6} M)	24	1		
(10^{-5} M)	25	2	0·161	< 0·01
(3) NaF (10^{-6} M)	13	1		
(10^{-5} M)	21	2	0·134	*
(4) ACC (10^{-3} M)	65	6	0·130	< 0·05
(5) Noradrenaline (5×10^{-5} M)	19	1	0·170	*

*, Additional experiments in progress to secure a sample size comparable to others prior to final analysis.

agar into a test animal, and, secondly, exposure of annuli to fresh extract between 4 and 8 h of regeneration results in 50 per cent less abnormal growth than if exposure to the extract occurs during the first 4 h after cutting. Wound healing is effectively completed after 6 h and an epidermal mucous film, resistant to the penetration of most exogenous free materials, is intact.

Preliminary examination of the extract with the electron microscope indicates the presence of a particulate material (900 Å). This material is not species specific, for it has been isolated from *H. viridis* as well as *H. pirardi*.

Animals in physiological depression (tentacles clubbed, inability to feed) possess only minute quantities of the active material (Table 1). *Hydra* exposed to nitrogen mustard, which specifically eliminated interstitial cells from the animals, also show only trace amounts of the material (Table 1). Also, it has been demonstrated that during sexuality growth in *H. pirardi* ceases[4]. It will be seen in Table 1 that after 4 weeks at reduced temperatures (the stimulus-inducing sexuality in this species) activity falls to nearly one-half that of normal animals. After 8 weeks at reduced temperatures activity is lacking entirely. Extracts of animals treated with leucomethylene blue to eliminate the nervous system result in a 50 per cent decrease in the number of animals developing inverted polarity.

Initial biochemical characterization of the growth-stimulating principle (Table 1) reveals that the material is relatively heat stable, not extractable with lipid solvents, digested by proteolytic enzymes, and slowly dialysable. Its action is not significantly mimicked by inhibitors of carbohydrate metabolism, for example, sodium fluoride, oxidative inhibitors such as potassium cyanide and lithium chloride, or by neurogenic compounds such as acetylcholine or noradrenaline. Preliminary purification with *G25* grade 'Sephadex' shows the active material to be retained on the column for an extended period.

It should be stressed that the ability of growth material isolated from the peduncle and basal disk to produce excessive head development is only half that of material extracted from the hypostomal region (Table 1). This further substantiates the idea that the stimulator extends in a gradient along the body column.

We suggest that the growth-stimulating principle is responsible for polarity in the *Hydra*. Whenever a portion of the body column is transected and an annulus removed, a head always forms on the original distal cut surface and a base on the proximal. If the growth factor extends in a gradient from the head to the base, then growth activities would be maximal at the distal cut surface. Cell divisions here would then liberate an inhibitor suppressing growth activities at the proximal surface.

At present, the chemical nature of the stimulator is not known. The present biochemical analysis suggests that the active factor may be a peptide.

This work was supported by grants from the U.S. National Science Foundation and the U.S. National Institutes of Health.

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Rotting of Apples of the Variety 'Bramley's Seedling' by *Nectria galligena*, Bres.

The first records of fruit rotting by *N. galligena* were made by Salmon and Wormald in Kent[1] and Dillon-Weston in the Wisbech area[2], where it occurred on the variety 'Worcester Pearmain'; it has since been reported as troublesome on a number of dessert varieties in various European countries[3–6]. Typical infection occurs at the blossom end of the fruit, either through the open calyx[7] or lenticels[8], and the disease became known as eye rot[1]. The rot frequently develops while the fruit is still on the tree[2], and the cream-coloured conidial masses of the imperfect stage, *Cylindrocarpon mali* (Allesch.) Wr., appear on the surface[1,2]. Such fruit, if left on the tree, become mummified, and perithecia are formed during the winter following infection[2].

Hitherto, fruit rotting by this organism of the variety 'Bramley's Seedling' has not been reported, although the wood-canker phase is widespread. *N. galligena* was identified as the causal organism of a number of rotted apples of 'Bramley's Seedling' held in gas and barn store in Co. Armagh during 1961–62 and 1962–63. Approximately 20 per cent of the fruit from one particular orchard, held in gas storage until May 1963, was found to have rotted, and more than half this loss was attributable to *N. galligena*. Wood cankers were particularly abundant in this heavily manured orchard.

During the storage period of 1963–64 samples of rotted apples, taken from various stores in Northern Ireland, were examined and the causal organism, if any, isolated. A total of 395 isolations were made and the percentages of this total attributable to the following principal organisms found were: *N. galligena*, 30 per cent; *Penicillium expansum*, 21 per cent; *Gloeosporium* spp., 13 per cent; *Phomopsis mali*, 10 per cent. *N. galligena* was by far the most serious cause of loss, particularly in the late storage period.

Most of the rots produced by *N. galligena* on 'Bramley's Seedling' fruit developed at the stalk end. The rotted area had a somewhat sunken appearance, was dark brown in colour with a distinct margin between the healthy and rotted areas (Fig. 1). Conidial masses were sometimes found on the surface. Internally the rotted tissue was soft and moist, light brown in colour, and frequently had a somewhat grained appearance, the striations running parallel with the line of radial advance. Microscopic



Fig. 1. Natural rot of Bramley's Seedling apple caused by *Nectria galligena*, viewed from the stalk end

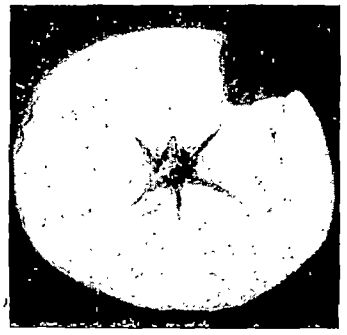


Fig. 2. Section through an unripe apple artificially inoculated with *Nectria galligena*, showing the arrested development of the rot

examination showed that the hyphæ advanced intercellularly and that eventually the host cells were completely disintegrated. Naturally infected fruit, partially buried in moist peat, and left exposed outside during the winter, developed perithecia with mature asci in about three months.

Artificial inoculation using the cork borer method[9] indicated that the fruit at harvest time showed an unexpected form of resistance to invasion by the fungus. During the first 20 days of incubation at 20° C the rot in all fruit reached an external diameter of 15–20 mm; but its further development was arrested and a distinct ridge developed at the edge as the rotted tissue shrank (Fig. 2). Internally the rot was firm and relatively dry; it was bordered by a layer six to seven cells thick with dense brown granular contents (Fig. 2). In many cases it recommenced in an erratic manner after incubation for 35–40 days; but in approximately a quarter of the fruit no further rotting took place and attempts to re-isolate the fungus from them failed, presumably because the hyphæ were dead. It can only be assumed that during the development of the rot in unripe fruit some chemical substance is elaborated which is highly toxic to the fungus. Similar inoculation of riper fruit did not show this phenomenon and the rot developed unhindered; it was similar in appearance to that described for naturally infected apples and the fungus was readily re-isolated.

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Fine Structure of Tyloses

Microchemical methods have shown that in several species, tylosis walls consist of more than one layer[1]. The outer layer is covered by an amorphous granular material while the inner surface has a crossed microfibrillar structure[2]. In the present investigation of *Eucalyptus obliqua* L'Herit. and *E. miniata* A. Cunn. ex Schau., it was found that at least a part of the granular material is derived from the denatured contents of the vessel, and that on extraction in 2 per cent sodium hydroxide the surface could be seen to have randomly arranged microfibrils, as in a primary wall. Ultra-thin sections of budding tyloses showed that, even in the early stages of development, their walls consisted of two layers. There was an outer layer, which appeared amorphous in sections, and an inner multi-lamellate layer with, at least in the more mature tyloses, very regular alternation of the direction of the microfibrils in adjacent lamellæ. This was particularly obvious in the sclerosed tyloses of *E. miniata* where the multi-lamellate layer contributed largely to the thickened wall. The structure of the inner layer, therefore, was quite unlike that of the secondary wall of the ray cell from which it arose, and resembled more that of some green algae such as *Valonia*[3].

In *E. obliqua* the walls of the fully developed tyloses were of the same thickness as those in the initial stages of growth, so that wall deposition must accompany wall extension. In some cases, even where the tylosis had not yet bulged out into the lumen of the vessel, its wall was almost as thick as the secondary wall of the ray cell.

Particular interest, however, attaches to the origin of the tylosis wall. In sections through the base of tyloses in *E. obliqua* (Fig. 1) it could be seen that the tylosis wall is continuous with neither the primary nor the secondary walls of the ray cell, but with a distinct layer (arrowed Fig. 1) lining the inner surface of the ray cell secondary wall. This layer was particularly well developed on the wall adjacent to the vessel and was quite different in texture from the secondary walls of either the ray cell or the vessel. In the initial stages of development, the tylosis wall formed a separate layer lining the pit membrane, but, as the tyloses developed, the pit membranes

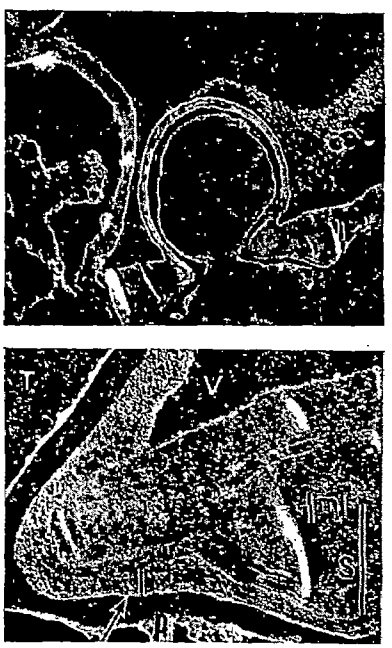


Fig. 1. Above, electron micrograph of ultra-thin section of budding tyloses of *E. obliqua*. (× *c.* 1,330). Below, detail of tylosis base (× *c.* 5,660). *ml*, Middle lamella + primary walls; *S*, secondary wall of the ray cell; *V* lumen of vessel; *P*, lumen of ray cell; *T*, lumen of tylosis

ost their separate identity, except perhaps at the bases of the tyloses. It would appear that the tylosis-forming ayer originated beneath the pits, as it was commonly hickest in these regions, and spread over the adjacent ayers of the secondary wall, in the same way that the ndividual layers of the secondary wall extend progressively from the central regions to the tips of the fibres[4].

The organization and origin of the tylosis wall indicate that this is in some ways a unique structure. The outer layer has the organization of a primary wall, and in view of the fact that the multi-lamellate layer is well developed even in immature tyloses, the tylosis wall is perhaps better regarded as a thick primary wall rather than a secondary wall. This means that the ray cell, having synthesized a secondary wall at the time of the initial differentiation of the cell, reverts to synthesizing a primary structure during tylosis production. Such reversion from a crossed fibrillar structure to a more random arrangement of the microfibrils has also been observed in some green algae following plasmolysis of the cell[5].

Investigations are continuing into the cytoplasmic organization of tyloses with particular reference to the behaviour of the nucleus.

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Factors affecting the Emergence of Primary Tillers from the True Leaves of the Main Shoot of a Rice Plant

Katayama[1], in his investigation of tillering in some cereals, reported that in the main culm and its tillers, the initiation of tillers and the development of leaves proceeded successively, maintaining a definite regularity and interval.

I have found (unpublished observations) that the first and second primary tiller of a swamp rice plant (variety *BG* 79) together contribute more than 50 per cent of the total shoot number of a plant, regardless of the fertility of the soil and plant population density.

Various factors affect the production of shoots in rice plants. It has been shown, for example, that tillering is affected by soil moisture[2–4] and by soil fertility[5–8].

The first relationship investigated was that between the leaf stage of the seedlings at the time of transplanting, and the emergence of the primary tillers from the first true leaves. This is affected by the moisture of the soil to which the seedlings are transplanted. Seedlings were transplanted to soil in pots at their first-, second- and third-leaf stages. The soil in half of the pots for each leaf-stage group was flooded (3 cm standing water) and the soil in the others was maintained at 80 per cent saturation. Tiller number was counted at the sixth-leaf stage (Table 1).

Flooding depressed the appearance of the primary tiller and the number produced fell when the seedlings were transplanted at a later leaf-stage. There was a significant difference between the effect of flooded and 'dry' soil when the seedlings were transplanted at the first- and second-leaf stages. This is shown by the analysis of the interaction.

The second factor investigated was the effect of time of commencement of flooding on the emergence of the primary tiller from the first true leaves.

The seedlings were raised in plastic buckets each containing six plants. Seedlings in half the buckets were uprooted and then replanted at the first-leaf stage. The soil in one-third of the buckets containing replanted and undisturbed seedlings was flooded at the first-, second- and third-leaf stages. Tillers were counted at the sixth-leaf stage of the main shoot and the results are shown in Table 2.

Table 1. Effect of Leaf Stage and Soil Moisture at the Time of Transplanting on the Emergence of the Primary Tiller from the First True Leaves

	'Dry' soil	Flooded soil	Leaf stages First	Second	Third
Total tiller per treatment	30*	5	20*	13*	2
F value		15·9*		6·4*	
L.S.D. ($P = 0·05$)		4·97		4·07	

Interaction (leaf stage × soil moisture saturation)

	Leaf stages First	Second	Third
'Dry' soil (*D*)	15	13	2
Flooded soil (*F*)	5	0	0
(*D*–*F*)	+10†	+13†	+2

L.S.D. ($P = 0·05$) = 4·07

* Significant ($P = 0·01$).
† Significant ($P = 0·05$).

Table 2. Effect of Replanting and of Leaf Stage at the Time of Flooding on the Appearance of the Primary Tiller from the First True Leaves

	Replanted seedlings	Non-replanted seedlings	*L.S.D.* $P = 0·05$	Leaf stages First	Second	Third	*L.S.D.* $P = 0·05$
Total tiller per bucket	2·25	5·0*	1·5	4·50*	2·25	4·13*	1·8
F value			16·0*		4·2*		

Interactions (replanting × leaf stage at time of flooding)

	Leaf stages First	Second	Third
Replanted (*R*)	3·25	1·25	2·25
Non-replanted (*NR*)	5·75	3·25	6·00
(*NR*—*R*)	+2·5†	+2·0	+3·75†

L.S.D. ($P = 0·05$) = 2·5

* Significant ($P = 0·01$).
† Significant ($P = 0·05$).

Seedlings that were not replanted produced significantly more tillers than the replanted ones. Seedlings growing on soil flooded as from the first- and third-leaf stages produced more tillers than those growing on soil flooded as from the second-leaf stage, irrespective of whether the seedlings were replanted or not.

It appeared that the seedlings received a setback at the time of transplanting and that it was less severe when they were transplanted at the first- or second-leaf stages than at the third. The effect was more strongly adverse when the seedlings were transplanted to anaerobic than to aerobic soil ('dry' soil). The roots of the rice seedlings were probably not adapted to anaerobic conditions, especially when they were raised on aerobic soil in the nursery. The sudden transfer to anaerobic soil probably prevented the growth of the primary tiller from the first true leaves. Unpublished results have shown that this tiller emerges at the fourth-leaf stage of the main shoot. It probably started its growth at the second-leaf stage of the main shoot, so that a change in the soil environment at this stage would have the most adverse effect on its emergence.

The third factor investigated was the effect of the presence of subtending leaf lamina and sheath on the emergence of primary tillers from the first and second true leaves. The seedlings were raised in pots each containing six plants. The treatments applied to the first and second leaves of the seedlings were (*a*) leaf lamina not removed, (*b*) half-leaf lamina removed, (*c*) whole leaf lamina removed, (*d*) leaf lamina and sheath removed (the organs were removed with scissors). The appearance of the tillers was observed at the seventh-leaf stage. The total numbers of tillers produced for treatments (*a*), (*b*), (*c*) and (*d*) were 23, 27, 10 and 22 respectively. The least significant difference ($P = 0·05$) was 8.

The removal of the leaf sheath probably exposed the bud in its axil to light and this probably stimulated its growth. When the leaf lamina was intact it appeared to have received and conveyed the light stimulus to its axillary bud, and this was then stimulated to grow. This influence of leaf lamina on bud growth may account for the usual

lack of tiller development from the axils of the coleoptiles of a rice plant.

Observation made on plants grown in pots showed that the leaf stage of the main shoot is also a factor governing tiller emergence from the main shoot. Table 3 shows the leaf stage of the main shoot at the time of emergence of each primary tiller.

Table 3. Leaf Stage of the Main Shoot at the Time of Appearance of each Primary Tiller

Primary tiller	1	2	3	4	5	6
Leaf stages	½ fourth	fifth	½ sixth	½ seventh	½ eighth	½ ninth
Primary tiller	7	8	9	10	11	
Leaf stages	½ tenth	½ eleventh	½ twelfth	½ thirteenth	fourteenth	

Fractions indicate the unfolded portion of the leaf blade.

It was also observed that before the appearance of a new tiller on the main shoot the preceding one was always in its second-leaf stage. The leaves developing early on the main shoot were usually smaller than those which developed later. The latter, therefore, took longer to unfold. Also, the second leaf of each primary tiller increased in size as the tillering process progressed upwards along the axis of the main shoot. The time taken for the second leaf to unfold was, therefore, progressively prolonged as the tillering process progressed upwards along the main stem. The differences in the unfolding period of the leaves, it is suggested, might be partly responsible for the increased interval between the appearance of a new primary tiller and a preceding one as shown in Fig. 1.

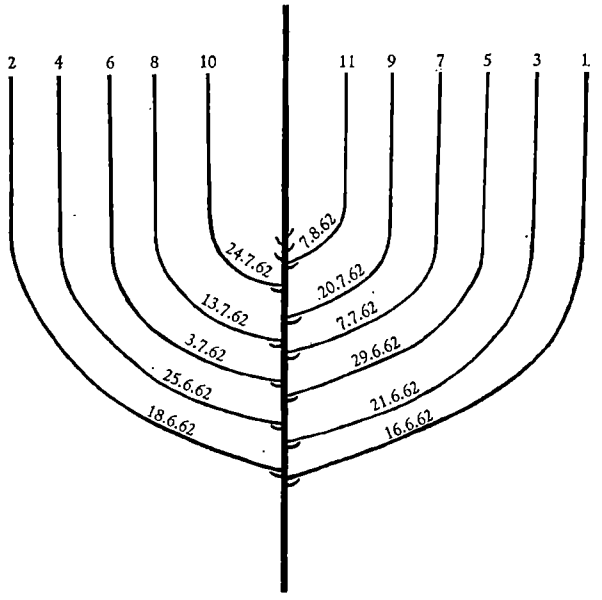


Fig. 1. Diagrammatic representation of a rice plant showing the mode of primary tiller development and the dates of emergence from the leaf sheaths of the subtending leaves on the main shoot

Where light and plant nutrients are not lacking it is possible to identify fairly accurately, from the leaf stage of the main shoot, the last tiller that has emerged from the main shoot during the tillering period. However, at low light intensity and in poor soil conditions, the development of primary tillers on the upper nodes of the main stem will be inhibited and under these conditions one cannot identify the last-formed tiller merely by knowing the leaf stage of the main stem. In a nursery where rice seedlings are overcrowded the competition for light and nutrients may inhibit the development of the primary tillers from the first-formed leaves of the main stem. According to the data shown in Table 1, if such seedlings are transplanted to the field at the sixth-leaf stage, only the primary tillers from the fourth leaf upwards are very likely to be produced. This will result in a reduction in the yield of the crop, especially if the plants are widely spaced, for I have, as stated, data showing that the primary tillers from the first and second true leaves together contribute more than 50 per cent of the yield of a rice plant.

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Leaf Spiral and Yield in Coconuts

This communication refers to the recent article by Satyabalan, Ninan and Krishna Marar[1], who perhaps unconsciously give more publicity to my findings relating to foliar asymmetry in the coconut palm[2–4]. The very first line of their statement, referring to the work of Petch[5], is misleading. Petch did not deal with leaf-spirals of coconut in his paper and, in fact, there is no mention of leaf or foliar-spiral, or the arrangement of leaves along five spirals in a clockwise or anti-clockwise direction as reported. Presumably Petch's article was not available to the above authors. Petch explained the structure of the fibres in the coconut stem. Neglecting the outer rind, he found in a stem the vascular bundles sloping to the left in the outer 3·5 cm, followed by an abrupt reversal of direction for the next 3·5 cm. This was again followed by another reversal to the original left in the remaining inner core of the stem. In another stem, the condition was reversed; the same three regions were present, but in the outer the bundles sloped up to the right, in the intermediate to the left, and in the inner to the right again. These two types of palms were regarded by Petch as right- and left-handed coconut trees.

Quotations from, and reference to, my work cover about a third of Satyabalan and others' communication. But if they had given due importance to the following sentence of my paper given within brackets ("My yield data relate to a small locality in Kerala and I do not claim that this will be the situation elsewhere."), much of their criticism could have been spared. My figures on the nut-production were from Kayangulam, a small town in central Kerala. In one part of their communication, the foregoing authors isolate this locality as "a place affected by a devastating virus disease of coconuts", but later on claim that Kayangulam and Kasaragod (extreme north-west of Kerala, about 500 km away from Kayangulam) have the same variety of palms "growing under almost identical climatic and soil conditions".

I am trying to obtain from India and abroad yield data on the two types of coconuts as well as other plants which show asymmetry of the stem, leaves or flowers. Through the kindness of Dr. M. N. Sproat, director of agriculture, Trust Territory of the Pacific Islands, kernel yields from 100 left-handed and an equal number of right-handed coconut palms on Ponape Island for the year 1963 were weighed. The left–right difference of kernel yield is quite high, being 13·48 per cent (that is, 31·24–27·53 lb. of kernel/tree/year) and this fact substantiates my findings. The repeat experiments of Reber's[6,7] reversed beans confirmed the production of a greater weight of beans and an increase in weight ratio of beans to shucks. The flowers of Malvaceae and Bombacaceae are asymmetric[8,9] and the capsules developed from the left-handed *Bombax ceiba* flowers produced a slight excess of developed seeds. Of the 200 fruits gathered from known flowers of *Hibiscus esculentus*, those from left-handed flowers bore, on an

average, 5·27 per cent more seeds, although the difference is not statistically significant.

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In vitro Production of Embryos from Anthers of *Datura*

Observations of natural phenomena such as adventive embryony and production of plantlets from the leaf margins of *Bryophyllum* support the view that although cells may differentiate and appear to behave differently, their genetic potentialities remain the same. As a corollary to this view, differentiation operates not through a segregation mechanism but due to selective and programmed suppression or stimulation of genic activity.

In recent years this view has been greatly strengthened by the use of *in vitro* culture technique, and the regeneration of perfectly normal plantlets from mature tissues of the stem and root has been demonstrated even in cases where such a phenomenon has not been observed in Nature. Thus buds differentiate under suitable treatment from tobacco pith callus[1], from stem callus of potato[2] and horseradish (*Armoracia*)[2,3], from root callus of *Isatis*[4], *Convolvulus*[5] and carrot[6,7], from bud scales of *Lilium*[8] and from embryonal callus of *Cuscuta*[9] and other plants. In several other instances embryo-like microscopic structures have been reported from callus masses, although their further development into plantlets has not been observed[10,11]. To the best of our knowledge, however, thus far there has been no report of the origin of embryos from anthers. The observation which we report here was made during attempts to grow callus from *Datura* anthers for an examination of cell multiplication in liquid suspensions.

Anthers of *Datura innoxia* Mill. growing wild in the university campus were sterilized with chlorine–water and planted in various nutrient media. The basic nutrient medium comprised Nitsch's or White's minerals and 2 per cent sucrose. This was supplemented with various growth substances such as indolyl-3-acetic acid (IAA, 10^{-6} M), kinetin (10^{-6} M), casein hydrolysate (1,000 p.p.m.), yeast extract (1,000 p.p.m.) or fruit juices like coconut milk (15 per cent), cherry juice (10 per cent), grape juice (10 per cent), litchi juice (10 per cent), pineapple juice (10 per cent) and plum juice (10 per cent) singly or in combination.

The inoculated anthers varied in maturity from the archesporial stage to mature pollen grains. However, the younger anthers did not grow in any of the media and eventually dried up. Those inoculated at the pollen grain stage showed vigorous proliferation on media containing 10^{-6} M indolyl-3-acetic acid and 10^{-6} M kinetin. The proliferations seen in Fig. 1 consisted of warty outgrowths, about 1 or 2 mm in diameter, sometimes arising all around the anther. In media containing both yeast extract and casein hydrolysate, proliferation occurred to a much lesser extent. All the proliferated masses grew satisfactorily on sub-culturing and have now been maintained for about 12 weeks.

Curious behaviour was observed in anthers implanted in media containing (1) casein hydrolysate (1,000 p.p.m. + 10^{-6} M IAA + 10^{-6} M kinetin, (2) coconut milk, (3) grape juice and (4) plum juice. For a long time these anthers showed no change at all and after 6–7 weeks the cultures were about to be discarded. Then, suddenly, numerous embryo-like structures projected out of the anther from all sides (Fig. 2). The embryos possessed root-shoot axes and cotyledons. Moreover, they were green and looked like perfectly normal embryos, with the only difference that most of them were polycotyledonous with as many as 10 cotyledons. Dicotyledonous embryos were also present, but in much smaller numbers. Many of these embryos have developed into normal seedlings. The effectiveness of different media in producing embryos varies. On media containing coconut milk and plum juice almost all the anthers showed the production of embryos. In the remaining two media only about 50 per cent of the anthers did so. In the basic medium, without added growth substances or extracts, no embryos were formed. Most of the embryos appear to be located inside the pollen sacs and are free-floating. They may have arisen from the pollen grains or from the connective tissue of the anther. Further work is in progress to ascertain this point.

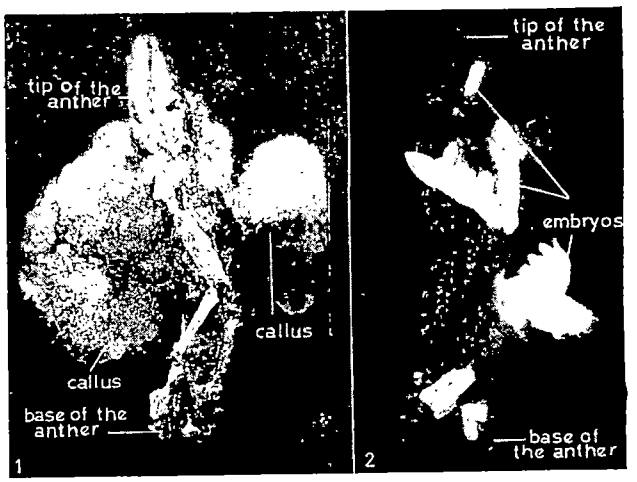


Fig. 1. Anther showing proliferation on basic medium containing IAA 10^{-6} M and kinetin 10^{-6} M
Fig. 2. Anther showing embryos from all sides on basic medium containing 15 per cent coconut milk

We thank Prof. P. Maheshwari for his advice.

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Agar-diffusion Technique for estimating Gibberellin Production by Plant Organs

So far, all published results relating to the gibberellin content of plants, or plant organs, have been obtained by various gibberellin-extraction techniques. This procedure is useful in the determination of the gibberellin content at any point in time, but it is often desirable to be able to estimate the rate of gibberellin production by various plant organs. In the course of our work on the gibberellin relationships of organs in the sunflower plant (*Helianthus*

annuus), we have adopted a modification of the classical technique of agar-diffusion. The method is simple and flexible and, as will be shown, it is reasonably sensitive and quantitative.

The plant organs are stood on, or 'plugged into', 1·5 per cent washed agar-gel blocks and left for a period of time to allow materials to diffuse from the cut surfaces into the agar blocks. The actual number of organs used, the way in which they are placed on the agar, and the size of the agar blocks can be varied to suit the particular tissues under study. The length of the diffusion period may be critical, and it is advisable to establish the most suitable time for the particular material under test. The diffusion can be carried out under a variety of environmental conditions, although in our work we have mainly used a régime of continuous light from fluorescent tubes at 22° C, under high humidity. A simple example of the method is shown in Fig. 1, where an apical bud of a young sunflower plant is plugged into a small agar cylinder (8 mm diam. × 5 mm high) standing on a 1-in. diam. aluminium planchet under an inverted glass vial.

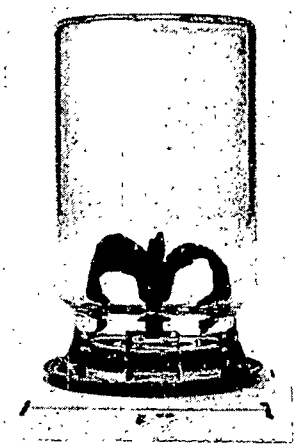


Fig. 1. Typical arrangement for agar-diffusion of excised plant organs

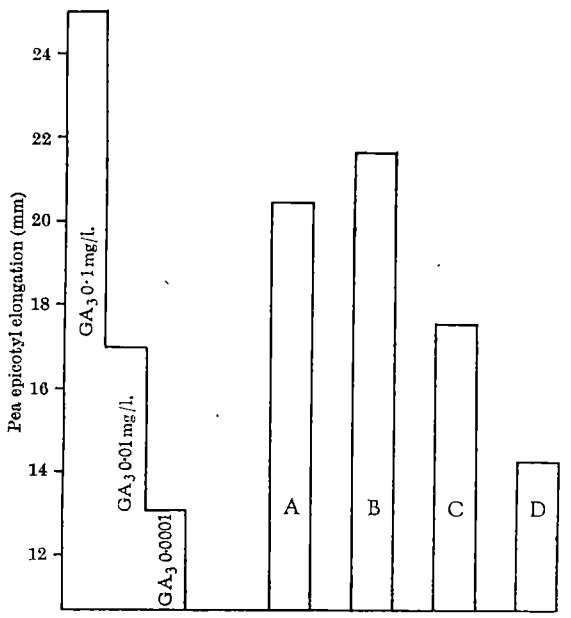


Fig. 2. Dwarf-pea epicotyl elongation assay of gibberellin-like materials obtained from agar blocks which had been in contact with fifty sunflower apical buds for varying periods of time. (*A*, 12 h; *B*, 24 h; *C*, 40 h; *D*, 12 h, followed by a period of 28 h without the apical buds present.) Elongation of epicotyls in the presence of known concentrations of gibberellic acid (GA_3) is also shown

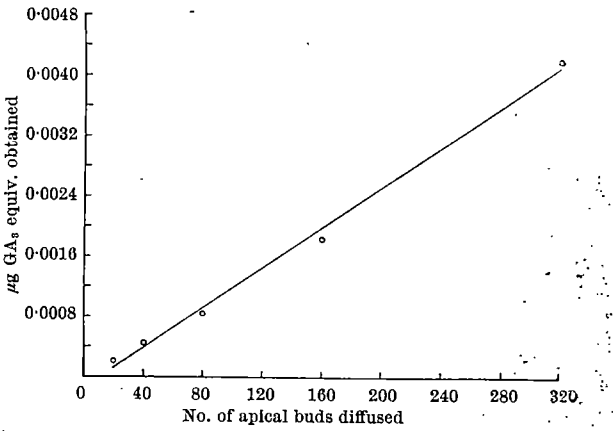


Fig. 3. Linear relationship between number of sunflower apical buds and amount of gibberellin-like material obtained from them by agar diffusion. Results expressed in μg GA_3-equivalents

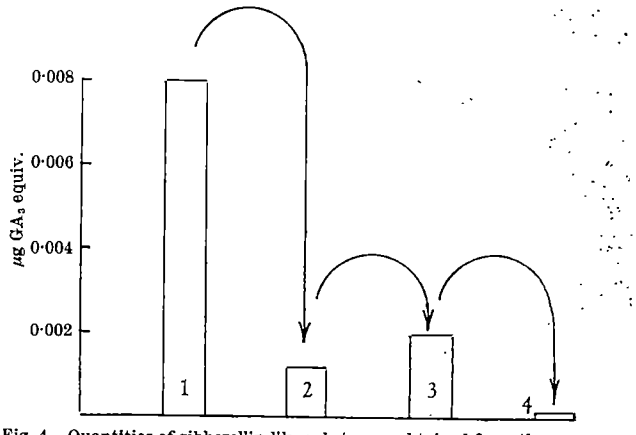


Fig. 4. Quantities of gibberellin-like substances obtained from the same sample of fifty sunflower apical buds during consecutive 12-h diffusion periods. The initial diffusion period was commenced immediately after excision of the buds

At the end of the diffusion period the agar blocks are frozen at − 15° C, the water phase of the agar gel, together with dissolved substances, freezing out. The frozen agar blocks are then extracted with absolute methyl alcohol at room temperature. The methanol extract is then drawn through a Buchner funnel and the remaining dehydrated agar is washed with more methanol. The methanol extract plus washings is reduced to an aqueous phase under vacuum at 35° C, and the aqueous residue acidified to *p*H 2·5 and extracted with ethyl acetate. The ethyl acetate fraction is then either reduced to a small convenient volume for paper or thin-layer chromatography, or reduced to dryness for gibberellin assay without chromatography. In the latter event, the dried extract can be taken up in water or other desired solvent.

With sunflower tissues, we have standardized the diffusion period at 18 h in the light at 22° C. Diffusion periods longer than 24 h have been found to result in a reduction of the amount of gibberellin-like materials in the agar blocks. This is seen in Fig. 2, where a comparison is made of the amounts of gibberellin-like substances obtainable from identical samples of 50 sunflower apical buds left to diffuse for 12, 24 and 40 h, and from agar blocks which had been in contact with 50 apices for 12 h and then left to stand out of contact with any plant tissue for a further period of 28 h. The assay used to determine gibberellin-like activity in this and other experiments reported here was a dwarf pea epicotyl-elongation test described previously[1]. It can be seen that most activity was recovered from the agar following a 12- or 24-h diffusion period, less being recovered after 40 h. The fall between 24 and 40 h would appear to be due to inactivation of gibberellin-like

material within the agar blocks, since there was a marked reduction in recoverable gibberellin-like activity when the agar was left to stand for a period of 28 h following a 12-h diffusion period. Fig. 3 shows the linear relationship between the number of sunflower apical buds diffused and the amount of gibberellin-like substances obtained, expressed in terms of gibberellic acid (GA_3) equivalents. It is likely that the amount of gibberellin diffusing out from an excised organ changes with time even under constant environmental conditions. That this occurs with excised sunflower apical buds is demonstrated in Fig. 4, which contains data obtained in an experiment where the same sample of 50 apical buds was transferred to fresh agar blocks at 12-h intervals. At the end of the first 12-h diffusion period activity equivalent to 0·008 μg GA_3 was obtainable from the agar, whereas at the end of the fourth 12-h period a negligible amount was present. Thus, the initial rate of gibberellin diffusion from the buds into the agar was not maintained. The reason for the decline is obscure; lack of respiratory substrate or of gibberellin precursor are possible factors.

The efficiency of this method for extracting the agar blocks has been evaluated by incorporating known amounts of GA_3 into 1·5 per cent agar blocks of the type used in these experiments, and estimating recovery of GA_3 by means of the dwarf pea epicotyl assay. Earlier experiments were conducted using a technique involving extraction of macerated agar blocks in absolute methanol, but without prior freezing of the agar. When this was done recovery rates of the order of 40 per cent or less were obtained; freezing the water out of the agar blocks before methanol extraction has raised the average recovery to 88 per cent in 30 experiments with a range of 72–100 per cent.

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ENTOMOLOGY

Distribution of Alkaline Phosphatase in the Malpighian Tubules of the Desert Locust

Small quantities of alkaline phosphatase have been found in the malpighian tubules of several insect species. Histological investigations suggest that it is not uniformly distributed in the cells of the tubules but that some regions contain the enzyme while others do not[1,2]. This communication summarizes some observations on alkaline phosphatase activity in the malpighian tubules of the desert locust, *Schistocerca gregaria* Forsk, at different stages of development.

Three groups of locusts were chosen to represent immature, mature and aged stages in the life-history of the adult. The alimentary canal of each locust was carefully removed and histological sections were prepared of the malpighian tubules taken from the region of the cæca and posterior mid gut. Alkaline phosphatase was identified by the Gomori–Takamatsu technique[3] and controls stained simultaneously[4]. In each series a minimum of two hundred tubules were examined.

In sections from the region of the cæca there was a positive alkaline phosphatase reaction in 21 per cent of the tubules in immature locusts, 52 per cent in mature locusts and 62 per cent in aged adults. The corresponding values from sections prepared from the mid gut region were 22, 20 and 28 per cent. When present, the phosphatase tended to be localized at the inner border of the cells adjoining the lumen of the tubule, but in a few observations additional concentrations were observed at the base of the cells. Intracellular phosphate was identified in some of the cells which had shown a negative enzyme test.

These observations confirm that small quantities of alkaline phosphatase occur in the malpighian tubules of the desert locust but suggest that it is differently distributed in the proximal and distal portions of the tubules. Such variations may be due to the different functions of the two parts of the tubule[5]. The slight increase in phosphatase concentrations in the older locusts agrees with observations published on the ageing of vertebrate tissues[6], but the wide range of variation between the individuals within this group suggests that further investigations of their immediate physiological state are necessary before comparisons can usefully be made.

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Multimodal Interneurones of Locust Optic Lobe

The auditory interneurones which ascend the ventral cord of the migratory locust have been traced forward only as far as the neck connectives[1,2]. Fibres which give a similar response can occasionally be found in the lateral parts of the protocerebrum or in the optic lobes by probing with indium-filled platinum-plated microelectrodes. They have not been found, although sought, in the corpora pedunculata. However, by probing in the optic lobe, higher-order neurones which give a different set of responses to auditory stimuli are commonly encountered. These also respond to vibration or touch stimuli of all kinds, to a wide range of visual stimuli via either compound eye, but only to high-intensity stimulation of the ocelli. The most easily found example is in the outer layers of the medulla in the optic lobe. Typically they consist of units which are presumably large because they give large spikes, with a background spike discharge ranging from 1 impulse per 10 sec to 10 per sec. Sensitivities to all modalities are low, compared with other units which respond typically to only one of the modalities. No evidence of association between inputs has been found, although sought. When the stimulus is repeated regularly at intervals of up to 10 sec, the response almost always declines in number of impulses, occasionally down to no response with irregular return of the response to some of the stimuli. The upper limit of repetition frequency varies greatly between units, or between what appear to be the corresponding units in different animals. Also, the rate of decline of the response varies greatly as between units for a standard frequency of stimulus repetition. Responses to different modalities adapt at different rates. Adaptation to a stimulus of one modality does not usually influence the response to a different modality of stimulus newly introduced, demonstrating an independence of inputs, and separate adaptation to each.

The same regional separation of adaptation is always encountered when a stimulus is introduced into a new part of the visual field when a different part of either eye has been adapted out by repetition. Effective stimuli are intensity changes, such as those caused by moving shadows rather than small movements at constant illumination; but the distinction between these types of stimulus is not clear for units with a wide field, within which separate small areas adapt separately. Some units respond at 'on', others at both 'on' and 'off'. These units, therefore, have some properties required of 'novelty units' for visual, as well as for other, stimuli. They have binocular wide-field coverage, and there is evidence, from

the adaptation investigations, that each part of the visual field (down to areas subtending a few degrees) has separate inputs. An anatomical correlation with large widely-ramifying horizontal fibres in the other layers of the medulla is probable. The units can be either efferent or afferent, depending on the stimulus. With auditory responses, no clear-cut discrimination of stimuli coming from the two sides of the animal can be found, although recordings have been made on scores of occasions from either optic lobe. Other characteristics of these fibres include: (*a*) unaccountable bursts of activity not preceded by any noticeable stimulus but sometimes associated with movement; (*b*) the persistence of the individuality of the parameters of the response over many hours, sometimes in units held for 3 days; although (*c*) after long periods of quietness in the dark the thresholds drop and activity is more persistent and more readily aroused; (*d*) after a pause of several minutes the first response is frequently very much more intense and sustained than when repetitions of a few seconds are used.

The function of these units is difficult to understand. Clearly they respond to any stimulus which would 'startle' the locust, and because they are multimodal they cannot be much use for specific responses. It may well be that all their normal responses, with repeated stimuli, are incidental, and only the large responses after a long pause may be 'effective', whatever that implies. In the absence of correlative behavioural response, the increased spike frequency is the only sign of a 'start' and, if it does represent an arousal, what then is aroused? From these and related investigations on insect neurones, it is apparent that there is a population of non-specific interneurones which can have many diverse inputs, all of which must be investigated before the unit is fully characterized. With reference to the part played by hearing in swarming and sexual behaviour it must be remembered that in these units the nature of the stimulus is not recoverable from the response of the unit alone, so purely auditory interneurones must lie elsewhere. The existence of these units, with widespread arborizations, implies that a response which appears at first to be of central sensory interneurone might on further examination be found to be modified by this non-specific system.

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Some Aspects of Weevil Behaviour affecting Population Establishment

It has been recognized for some time that it is necessary to assess the significance of low-density pest populations in stored grain. These may be survivors from a treatment (probably pre-adult stages) or migrants entering hitherto uninfested material; either may give rise to larger populations. On the other hand, 'tolerable levels' of infestation may exist, below which a population would fail to establish itself.

To estimate the probability of a female in a sparse population being fertilized, 20 kg of English wheat, of 14 per cent moisture content, was stored in plastic bags into which single pairs of one-week-old virgin grain weevils (*Sitophilus granarius* L.) were released. Five replicate bags were set up for each of 4, 8, 12, 16 and 20 weeks at 25° C. After storage for these periods, the females were sieved off, dissected and examined for sperm. Two of the females examined after four weeks had been fertilized and four after eight weeks. All those kept longer than this had been fertilized.

It was apparent from this experiment that at 25° C a single pair could initiate a population. Furthermore, under field conditions, such initial densities would most likely escape detection. Two lines of investigation were therefore commenced: first, demonstration of sexual attraction in this species and, secondly, a long-term analysis of population dispersion in grain and in particular those factors influencing aggregation. Olfactometer experiments failed to give any evidence of intersex attraction, however, and attention was directed to discovering some feature of behaviour which might facilitate contact between adults of differing sex. Presence on the surface of the grain was compared for individual virgin and mated males and females of the same age. Ten one-week-old individuals of each category were taken from the same culture and isolated in 250 g of grain (14 per cent moisture content) at 25° C. The number found on the surface of the grain each day for ten consecutive days was recorded and the results are summarized in Table 1.

Table 1. Mean Number of Individuals per Day on the Surface of the Grain

	Virgin	Mated
Male	3·0	2·7
Female	2·6	1·0

The main conclusions are: (1) The mean number of females on the surface of the grain was significantly higher ($P < 0.05$) for the virgin than for the mated ones. Other investigations on this species have shown that the mated female spends a large proportion of her time below the surface boring in grain[1]. (2) The mean numbers of mated and unmated males on the surface of the grain were not significantly different. Among mated males, however, only in four cases was the same individual seen at the surface on two consecutive days, whereas in unmated males this occurred ten times, and in two cases the same individual was seen on the surface for up to five consecutive days. Other investigations on this species have shown that mated males are constantly changing their position in the grain, apparently at random.

Observation of individual behaviour therefore indicated that some mechanism does exist which facilitates contact on the surface of the grain between unmated adults of different sex.

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Selection of some Strains of *Anopheles atroparvus* with Different Behavioural Responses to Contacts with DDT

Changes in the house-haunting habits of *Anopheles*, observed during campaigns of residual spraying with DDT, may be of various kinds. Moreover, they may interfere in a complicated way with malaria transmission[1]. Much attention has been directed in recent years to the appearance of strains showing a hyper-irritability towards the insecticide, leading to decreased insecticide contact and increased survival[2–5].

Although it is most probable that such strains originate by selection from a genetically mixed population, analysis is difficult in the absence of any information on the genetical mechanism involved and on the situation before spraying. Using the capacity to escape from an enclosed space as a criterion of irritation, it has proved possible in the laboratory to select two strains differing widely in this aspect, starting from a single Dutch strain of *Anopheles atroparvus*, isolated from the field and kept for two years in the laboratory. Both strains, at the end of the selection experiment, appeared to differ from the original colony (Fig. 1). The criteria utilized in selection were: (*a*) Escaping during 15 min from a horizontally-placed tube, lined internally with paper impregnated with DDT

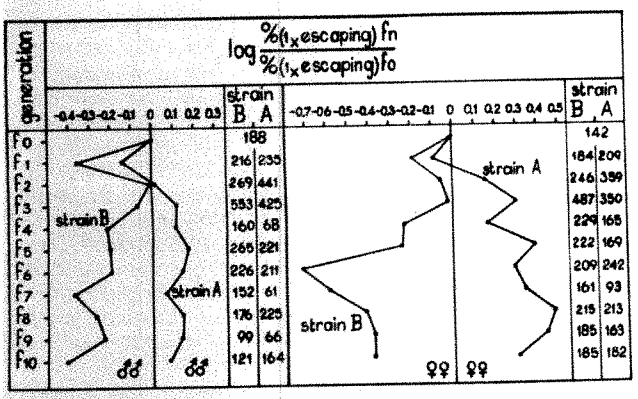


Fig. 1. Log $\frac{\text{per cent } (1\times\text{escaping})\ f_n}{\text{per cent } (1\times\text{escaping})\ f_o}$ of strain *A* and strain *B*.

Strain *A*, selected for escaping three times; strain *B*, selected for not escaping three times. Numbers of mosquitoes tested are given in the two columns to the right of each graph

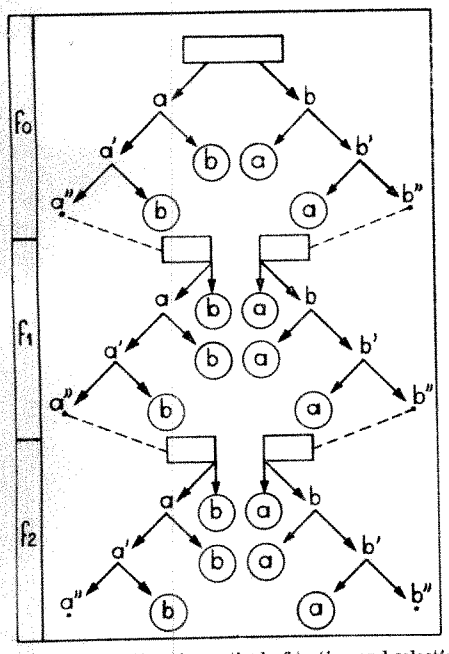


Fig. 2. Scheme illustrating the method of testing and selection, based on repeated testing and successive elimination. *a*, mosquitoes escaping; *b*, mosquitoes not escaping; rectangle, sample of mosquitoes; circle, mosquitoes to be eliminated

dissolved in 'Risella' oil. (*b*) Not escaping during the same time from the same tube. (*c*) Reacting identically in three successive tests with 24 h interval (Fig. 2).

Random reactions are largely excluded by the application of criterion *c* (Tables 1 and 2). This method of selection was first described in a paper by Beard[6]. The results of the selection are given for generations 1–10 inclusive (Fig. 1). It was realized that escape might be influenced as much by the natural activity of the mosquitoes as by the test substances, namely, DDT and its solvent, 'Risella' oil.

On testing these possibilities separately, it was found that selection for natural activity indeed affected the escape-rates (control tests, Tables 3 and 4). A definite irritation effect was found (Table 3), due to: (*A*) 'Risella' oil; (*B*) DDT dissolved in 'Risella' oil; (*C*) DDT crystallized on dry Whatman No. 1 filter paper.

The strains obtained show an identical difference in escaping in a vertical direction in tests done during 15 min from a cage measuring 50 × 50 × 50 cm, lined with paper impregnated with DDT dissolved in 'Risella' oil (Table 4).

Table 1. Percentage of Females escaping (Reaction *a*) in Generations *f4–f9* Inclusive, in three successive Tests done in Contact with 4 per cent DDT-impregnated Paper with Strain *A* (selected for Escaping Three Times; Numbers tested in Parentheses)

Test 1	Test 2	Test 3
50·5 (1,049)	59·5 (507)	62·7 (303)

Difference between Test 1 and Test 3: $\chi^2 = 13{\cdot}998$ $P = < 0{\cdot}001$

Table 2. Percentage of Females not escaping (Reaction *b*) in Generations *f4–f9* Inclusive in Three successive Tests done in Contact with 4 per cent DDT-impregnated Paper with Strain *B* (selected for not Escaping Three Times) (Numbers tested in Parentheses)

Test 1	Test 2	Test 3
90·4 (1,221)	93·6 (854)	95·6 (702)

Difference between Test 1 and Test 3: $\chi^2 = 16{\cdot}747$ $P < 0{\cdot}001$

Table 3. Percentage of Mosquitoes of the Original Strain and of the Strain selected for Escaping Three Times (*f8–f10*), escaping after Contact with Different Papers (Numbers tested in Parentheses)

Material tested	Percentages of mosquitoes escaping: Males		Females	
	Original strain	Strain *A*	Original strain	Strain *A*
Whatman filter paper impregnated with 'Risella' oil	14·0 (86)	60·5(151)	13·2(114)	51·9(106)
Crystallized DDT on dry Whatman filter paper	—	50·6 (87)	—	22·8(158)
Whatman filter paper impregnated with DDT dissolved in 'Risella' oil	54·8(188	65·2(164)	21·8(142)	43·4(182)
Dry Whatman filter paper	5·1 (99)	10·9 (46)	2·7 (73)	4·3 (47)

Table 4. Percentage of Engorged Females of Generations *f5–f9* Inclusive escaping within 15 min from a Cage with Dimensions 50 × 50 × 50 cm (Numbers tested in Parentheses)

	Paper impregnated with 4 per cent DDT in *Risella* oil	Dry Whatman paper
Strain *A*	65·3 (632)	19·7 (386)
Strain *B*	27·4 (995)	13·9 (540)

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MICROBIOLOGY

Pimelic Acid as a By-product of Azelaic Acid Degradation by *Pseudomonas* sp.

In 1955 Wright *et al.*[1] demonstrated that pimelic and azelaic acids stimulate the biosynthesis of biotin in *Aspergillus niger*. Since then, three groups[2–4] of workers have established that pimelic acid is a precursor in the biosynthesis of biotin. It remained to be tested whether or not pimelic acid is a by-product of the microbial degradation of azelaic acid.

In our experiments we have used *Pseudomonas* sp., strain *W*, which utilizes azelaic acid as the sole carbon source. The organism was isolated from garden soil and grown in a medium containing mineral salts and azelaic acid. Whole cultures were acidified and continuously extracted with ether. From cultures in the exponential phase of growth, both azelaic acid and pimelic acid have been isolated in crystalline form. Pimelic acid was characterized on the basis of chromatographic properties, melting point, mixed melting point with authentic material and infra-red spectrum. Continuous ether extraction of the culture solution near the end of growth gave a mixture of acids in very small amounts and larger amounts of an unidentified end-product which behaves chromatographically like an acid, is brown in colour and has the consistency of an oil. In the light of the work previously

referred to, our investigation explains the role of azelaic acid in the biosynthesis of biotin.

This investigation was supported by U.S. Public Health Service research grant *HE*–04138–06 from the National Heart Institute.

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Metallic Sheen in *Pseudomonas aeruginosa*

Hadley[1] noticed what he termed silvery patches either as haloes or crescent-shaped areas, around some corroded colonies in cultures of *Pseudomonas aeruginosa*. Several later workers[2–5] showed that these patches of iridescent metallic sheen were distinct from bacteriophage plaques. Warner[6] expressed the opinion that sheen was a sign of bacterial variation, and that variant colonies within the surface culture produced an oily or waxy metabolite which lysed the parent strain. Sierra and Zagt[7] attributed the phenomenon to lipolytic activity.

The production of metallic sheen is dependent on the composition of the solid medium on which the culture is grown. On a suitable medium, such as Oxoid nutrient agar No. 2 or blood agar, about half a large collection of *Ps. aeruginosa* cultures, but no other species of *Pseudomonas*, produced a sheen. Although it was generally possible to select a sheen-negative strain from sheen-producing cultures by picking single colonies, the production of sheen appeared to be an inherent property of strains of *Ps. aeruginosa* under natural conditions. The presence or absence of sheen has been shown to be a constant feature of cultures belonging to the same serological and pyocine-type that had been isolated from epidemiologically associated infections[8]; sometimes the production of sheen proved a useful 'marker' for the separation of two distinct strains which belonged to the same type.

Another observation with a possible application in the recognition of strains of *Ps. aeruginosa* was that certain cultures had the ability to inhibit sheen-production by some but not by all cultures of *Ps. aeruginosa*. Mayr-Harting (personal communication) also observed the presence of sheen-inhibiting as well as sheen-stimulating substances. In the course of an investigation of the production of pyocines[9] it was noticed that, when two different strains of *Ps. aeruginosa* were grown close to each other as streaks on a solid medium, the production of sheen by one of them was sometimes inhibited, although the growth was unaffected in other respects. It was thus possible to design a simple technique in which eight different 'indicator strains' were superimposed as streaks or drops on a lawn of a sheen-producing culture (Fig. 1). Several different patterns of inhibition of sheen production were observed. Such a test might be used, in combination with other typing systems, for the further sub-division of common types.

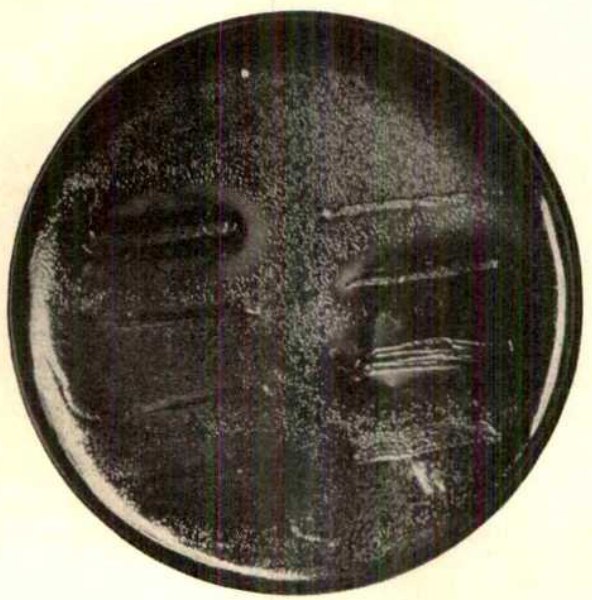


Fig. 1. Four 'indicator' strains showing the phenomenon of sheen-inhibition while four others do not show it

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GENETICS

Chromosomes of the Ring Seal

This communication reports the chromosome analysis of the ring seal. The only report concerning the karyotype of an aquatic mammal prior to this was by Makino, who reported in 1948 the chromosome number of Dall's porpoise, *Phocoenoides dallii* True, to be 44 (ref. 1).

Through the courtesy of Dr. Carleton Ray of the Aquarium of the New York Zoological Society, fresh kidney tissue was obtained at the time of autopsy from a male ring seal, *Phoca hispida krascheninikovi* Naumov and Smirnov, an animal which inhabits the Bering Sea. The kidney tissue was removed, using sterile precautions, and was taken directly to the laboratory, where the surface was seared in a flame, the capsule removed, and the tissue prepared for culture by the technique reported in detail by Rappaport[2].

After three successive transfers, the cells were treated with colchicine for 5 h, expanded with hypotonic saline for 20 min, and fixed with acetic alcohol. The acetic alcohol cell suspension was air-dried on slides, and stained with acetic orcein. The slides were scanned, and representative metaphase plates which appeared to be unbroken and to contain little or no chromosome overlap were photographed.

Twelve suitable metaphase plates were examined. Each contained 32 chromosomes. A representative metaphase plate with its karyotype is shown in Fig. 1. The chromosomes were classified into seven groups. The first contains a single large chromosome pair with pronounced satellites. The second group, somewhat smaller than the first, contains 2 pairs with sub-median centromeres. Group 3 contains 6 pairs with median centromeres; group 4, 2 pairs with sub-median centromeres; group 5, 1 pair with acrocentric centromeres; and group 6, 3

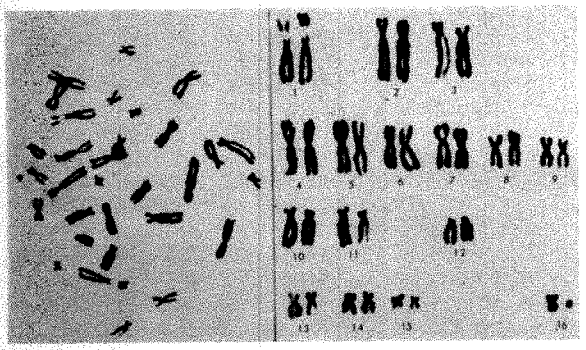


Fig. 1. Typical metaphase plate and karyotype of *Phoca hispida kraschenínikovi* Naumov and Smirnov

pairs with metacentric centromeres. Group 7, representing the sex chromosomes, contains metacentric chromosomes; one is similar in size and morphology to those in Group 6; the other is smaller than any other in the complement.

The small number of chromosomes, most of them metacentric, is consistent with Robertson's law which states that the more specialized animals contain chromosomes with an increased incidence of metacentricity, possibly due to centric fusion[3]. It is also possible that centric fusion may be related to the low chromosome number noted in many specialized species. Further karyological examination of aquatic mammals will aid in the study of the relationship between chromosome morphology and evolution.

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AGRICULTURE

Lateral Drag of Chemicals in Soil by a Plough and a Rotavator

INFORMATION was needed on the extent to which a plough and a rotavator would drag soil-applied chemicals if ploughing or rotavating was done at right-angles to band treatments. A green fluorescent insoluble powder, of particle sizes between 5 and 10μ, was used as a tracer. This method had previously given good results in tracing the distribution of insecticides in soils[1]. In this instance the tracer was thoroughly mixed with powdered talc (not fluorescent) to simulate an aldrin dust. Strips of bare soil, in good tilth, 6 ft. long and 1 ft. wide, were marked out; the talc containing the tracer was evenly spread over these surfaces and lightly mixed into the top inch to prevent blowing. Three random core samples were taken from each strip to the depth to which the implements were set. The plough was started into the ground well before the strip so that it passed over at normal working speed and then on to a distance of 30 ft. It circled and went over the strip again in the same direction, continuing until all the treated area had been turned in. A similar procedure took place on the second strip with the rotavator. Ploughing was 8 in. deep, rotavation 6 in.

Three random samples were then taken 1 ft. from the edge of each strip up to 10 ft. and again at 15, 20 and 30 ft. As it seemed, from observation, that the rotavator might be throwing soil rearwards, samples were also taken 1 ft. from the back edge of that strip.

Samples were dried in the laboratory and put through a 1-mm sieve and then very thoroughly mixed. Soil from each position was evenly spread out in a Petri dish and the number of fluorescent particles observed in 100 random microscope fields was counted under ultra-violet light. The diameter of the field of the microscope was 2 mm. The three samples taken on each strip before ploughing or rotavating were examined separately to check on evenness of spread. The three samples taken after working from each foot distance away were bulked.

Table 1. COUNTS OF FLUORESCENT PARTICLES IN 100 RANDOM MICROSCOPE FIELDS FROM SAMPLES AT VARIOUS DISTANCES

Sample	Plough	Rotavator
Before ploughing	96, 98, 91	69, 72, 79
1 ft. back	—	57
1 ,, forward	55	8
2 ,, ,,	18	7
3 ,, ,,	14	4
4 ,, ,,	11	1
5 ,, ,,	7	—
6 ,, ,,	4	—
7 ,, ,,	3	—
8–9 ,, ,,	2	—
>9 ,, ,,	—	—

Clearly a considerable amount (of the order of 50 per cent) of the tracer was dragged by the plough for a foot, and up to about 10 per cent for 4 ft. After that only small amounts were found, and none after 9 ft. This does not imply that none of the tracer was carried more than 9 ft., but that the concentration beyond that was too low to be detected easily.

The results with the rotavator show that the main shift of material was a throwing to the rear of more than 75 per cent when the machine first went over the treated strip. An appreciable amount was taken forward up to 3 ft., but the total was only of the order of 25 per cent.

In the only other piece of work on soil drag of which I am aware, Njøs and Steenburg[2] used radioactive ammonium phosphate. At a distance of 25 cm from their plots the rotary cultivator carried with it less than 10 per cent of the tracer and this was also found for the plough at a distance of 100 cm. They did not test rearward throw. They also found that at 800 cm the carry-over was small for both implements, of the order of only 0·1 per cent. The results obtained by fluorescent tracers agree well with those from the use of radioactive material and they do not require highly specialized knowledge and equipment.

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Effects of Low-temperature Storage on the Oxygen Uptake of Soil

A VALUABLE indication of the biological activity of soil is given by its uptake of oxygen or evolution of carbon dioxide. Large numbers of samples have frequently to be collected at the same time in survey work or in detailed field experiments, and immediate examination of the soils is not possible. Air drying has been widely used for storing soils, but on rewetting them a marked increase in respiratory activity is usually found[1–3].

Few experiments have been made with soils stored at low temperatures. Gooding and McCalla[4] found no difference in carbon dioxide production between previously frozen and unfrozen soils over several days. Although Antoniani *et al.*[5] and Soulides and Allison[2] found increased activity in previously frozen soils over short incubation periods the changes were smaller than with air-dried soils[2]. Freezing would appear to offer possibilities for maintaining respiratory activity in soil, and the effects of storage of four soils derived from different parent materials are described here.

Opouriao loam was collected in a tobacco field, and the other soils under grass–clover pastures, at a depth of

0·5–3 in. Some of their properties are given in Table 1. The soils were sieved through a 2-mm sieve, mixed thoroughly and stored in portions of about 350 g in polythene bags at −20° C. The soils were thawed at room temperature and allowed to stand for 24 h. Oxygen uptake was determined in Warburg flasks, the flask constants being calculated by the method of Webley[6]. The flasks were allowed 90 min to reach equilibrium, after which readings were made at 20-min intervals for 2 h. Any increase in activity which may have occurred on thawing the soils had subsided by the time the determinations were made, and constant rates of oxygen uptake were found with all soils.

From the results presented in Table 2 it is apparent that freezing had not changed the activity of these soils in any consistent way. Increased uptake of oxygen was found with two of the previously frozen soils, but values in general were close to those given by fresh samples. The measurements were not greatly affected by the period of storage.

It is conceivable that freezing may have lowered the numbers of viable micro-organisms[2] in these soils while rendering organic substrates more available for respiration, thus producing an apparent similarity between frozen and fresh samples. It would appear more likely, however, that their numbers remained essentially unchanged or that any decrease was counterbalanced by increased activity of the remaining organisms, for when excess glucose solution was added to the soils marked increases in oxygen uptake were obtained in both previously frozen and fresh samples.

These results suggest that storage at low temperatures may be suitable for maintaining the respiratory activity of other soils. Freezing has also been reported to maintain other biochemical activities such as nitrification in soil[7].

Table 1. Properties of Soils

Soil	Parent material	pH	Moisture (%)	Organic C (%)	Total N (%)	C/N
Waikakahi silt loam	Greywacke loess over marl	6·6	14	2·9	0·29	10
Judgeford silt loam	Greywacke loess	5·6	46	6·7	0·46	15
Kairanga silt loam	Alluvium from mixed sedimentary rocks	5·6	19	3·9	0·40	10
Opouriao loam	Alluvium from volcanic ash	6·0	13	2·3	0·21	11

Table 2. Oxygen Uptake of Fresh and Frozen Soils

Soil	Period of storage at −20° C (weeks)	Oxygen uptake, μl./g dry soil/h: Soil	Soil + glucose
Waikakahi	Fresh	7·4 ± 0·6	14·3 ± 0·5
	4	6·5 (89)	13·7 (96)
	9	6·8 (92)	14·5 (101)
	12	7·2 (99)	14·0 (98)
	16	6·4 (86)	14·8 (103)
	24	6·3 (85)	15·4 (108)
	Mean of frozen samples	6·6 ± 0·1 (89)*	14·5 ± 0·7 (101) *N.S.*
Judgeford	Fresh	8·8 ± 1·0	31·2 ± 1·0
	4	8·0 (91)	27·4 (88)
	8	7·1 (81)	28·3 (91)
	15	8·1 (92)	28·4 (91)
	24	8·0 (91)	29·3 (94)
	27	8·4 (95)	27·3 (88)
	32	10·0 (114)	25·6 (82)
	42	8·3 (94)	27·3 (88)
	Mean of frozen samples	8·3 ± 0·9 (94) *N.S.*	27·7 ± 1·1 (89)†
Kairanga	Fresh	7·0 ± 0·2	13·8 ± 1·2
	4	7·6 (109)	12·1 (88)
	12	7·8 (111)	12·5 (91)
	23	8·3 (119)	14·9 (108)
	Mean of frozen samples	7·9 ± 0·4 (113)‡	13·2 ± 1·5 (96) *N.S.*
Opouriao	Fresh	3·4 ± 0·2	9·1 ± 1·1
	4	4·7 (138)	9·9 (109)
	14	3·8 (112)	8·6 (95)
	Mean of frozen samples	4·3 ± 0·6 (126)*	9·3 ± 0·9 (102) *N.S.*

Soil (3·0 g) and water (3·0 ml.) or an aqueous solution of glucose (1·5 per cent; 3·0 ml.) were shaken continuously in Warburg flasks, with KOH solution (10 per cent; 0·2 ml.) in centre well, at 24° C. Four replicates were used for fresh and three for frozen soils at each period.

Results of fresh and mean frozen soils are means ± standard deviations. Figures in parentheses are values as percentages of fresh soils. Significance of differences between fresh and frozen soils, per cent: *N.S.*, $P > 5$.

*, $P < 5$; †, $P < 0{\cdot}1$; ‡, $P < 1$.

On the other hand, experiments with the four soils in this laboratory, to be reported elsewhere, have shown that mineralization of nitrogen was greater in most cases with previously frozen than with fresh samples[8] and that the activity of soil enzymes in hydrolysing starch or sucrose was lower in previously frozen samples. It would appear that the effects of freezing must be determined separately for each biochemical activity to assess its value for storing soils.

I thank Mr. I. R. Gibson for chemical analyses and Mr. D. C. McDonald for determining the specific gravities of the soils.

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PSYCHOLOGY

Confirmation of Apparent Genetic Assimilation of Behaviour in *Drosophila melanogaster*

In a recent report[1] from this laboratory an apparent modification of food preference in *Drosophila melanogaster* was described, under the combined and opposing effects of natural and artificial selection. A deficiency in the design of that experiment was that the method used to measure food preference behaviour was confounded with a measure of the activity of flies. We have repeated this experiment with a new method of testing.

The general description of our methods can be found in the earlier paper. The new test method was as follows: A Y-tube was used into which the flies were introduced. One arm of the tube ended in a chamber in which peppermint-adulterated oatmeal-and-molasses agar was provided, while normal uncontaminated agar was placed in the chamber at the other end of the Y-tube. All flies were tested at the same time of day, and after fasting for a standard number of hours. The numbers of flies choosing each food were counted.

The results were identical with those in the earlier experiment. The most important results may be summarized thus. Flies selected for liking peppermint in each generation, and reared on it, produced a population which became strongly inclined to choose the peppermint food in the choice situation. Flies selected for aversion to peppermint, but reared on it, likewise produced a population which was strongly attracted to it. When the latter population was put back on normal food after eight generations they continued to show a preference for peppermint-scented food. Since the graphs of the data are so similar to those of the original experiment, we do not reproduce them here.

It seems plausible to say that since the experiments were begun from a new population of wild-type flies in each case, it is extremely unlikely that the effect is due to the trapping of a rare gene, and that it seems rather to be a genuine case of 'genetic assimilation'[2].

We thank the Medical Research Council for financial support for this work.

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, November 2

BRITISH SOCIETY FOR THE PHILOSOPHY OF SCIENCE (in the Joint Staff Common Room, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. E. R. Leach: "What are Social Anthropologists Trying To Do?"*

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (joint meeting with the Royal Aeronautical Society, at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. J. I. Kanter: "N.A.S.A. Research and Technology Programmes in Space Navigation".

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (joint meeting with the Science and General Division, at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. A. J. Ellison: "Modern Introduction to the General Theory of Electrical Machines".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 6 p.m.—"The Lost Sahara" (Colour films with commentary and introduction by Mr. A. R. Hanbury-Tenison and Mr. James Wellard).

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Mr. A. Hart: "Research Project Evaluation".

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (joint meeting with the Woolwich Polytechnic Chemical Society, at the Woolwich Polytechnic, Thomas Street, London, S.E.18), at 7.30 p.m.—Dr. J. Leggett: "The Photochemical Apparatus of the Chloroplast".

SOCIETY FOR VISITING SCIENTISTS (at the English-Speaking Union, Dartmouth House, 37 Charles Street, London, W.1), at 7.30 p.m.—Discussion Meeting on "Computers in Publishing". Chairman: Mr. Richard Clay. Speakers: Mr. C. J. Duncan, Dr. T. A. Margarison and Mr. Fred Simmons.

Tuesday, November 3

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p.m.—Dr. J. H. Bird: "Australia: Coast, Mountain, and Back Country".*

INSTITUTE OF FUEL (at the Institution of Mechanical Engineers, 1 Birdcage Walk, London, S.W.1), at 5.30 p.m.—Mr. J. T. S. Gundry, Mr. B. Lees, Mr. L. K. Rendle and Mr. E. J. Wicks: "The Use of Ammonia for Reducing Air-Heating Corrosion at Bankside Generating Station, C.E.G.B."*

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, London, S.W.1), at 5.30 p.m.—Mr. R. M. Wynne-Edwards: Presidential Address.

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. W. Wharton and Mr. B. J. Rogers: "The Problem of Vestigial-Sideband Transmission and Reception".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 5.30 p.m.—Prof. R. E. D. Bishop: "Stability and Instability". (Afternoon lectures for Sixth Form Boys and Girls from Schools in London and the Home Counties. Further lectures on November 4, 10 and 11.)

ROYAL MICROSCOPICAL SOCIETY (in the Lecture Theatre, Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 5.30 p.m.—Prof. Dr. Ernst Ruska: "Very High Resolution in the Electron Microscope".

UNIVERSITY OF LONDON (at the Imperial College of Science and Technology, London, S.W.7), at 5.30 p.m.—Prof. S. Eilon: "Reflections on Management Science".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. R. Finlayson: "Comparative Pathology of Arterial Disease". (Fifth of fourteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)*

PLASTICS INSTITUTE (at the Wellcome Building, Euston Road, London, N.W.1), at 6.30 p.m.—Prof. C. E. H. Bawn, F.R.S.: "Recent Developments in Ionic Polymerisation".

Wednesday, November 4

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Du Cane Road, London, W.12), at 2 p.m.—Dr. L. C. Payne: "Clinical Computing".*

SOCIETY FOR ANALYTICAL CHEMISTRY (at the Wellcome Building, Euston Road, London, N.W.1), at 3 p.m.—Meeting on "Toxic Substances in Air".

COLOUR GROUP (Great Britain) (in the Physics Department, Imperial College, Prince Consort Road, London, S.W.7), at 3 p.m.—Mr. K. Hacking: "The Relative Visibilities of Spatial Variations in Luminance and Chromaticity"; Mr. A. R. Robertson: "High Accuracy Colorimetry".

INSTITUTION OF MECHANICAL ENGINEERS, EDUCATION AND TRAINING GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 4.30 p.m.—Mr. S. V. Haves and Mr. S. A. Tobias: "The Project Method of Teaching Creative Mechanical Engineering".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Prof. E. O. Willoughby: "A Push-Pull Parallel Aerial System for Navigation and Application to Communication Problems with Simple Methods of Side-Lope Cancellation".

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (joint meeting with the Science and General Division, at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "The Training of the Industrial Engineer" opened by Mr. C. J. Foster.

ROYAL METEOROLOGICAL SOCIETY (at 49 Cromwell Road, London, S.W.7), at 5.30 p.m.—Dr. F. Pasquill: "Turbulence and Mixing in the Atmosphere".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. A. G. Brodie (Illinois): "Human Jaw Growth and Tooth Eruption".*

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, TELEVISION GROUP (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Mr. B. W. Osborne: "U.H.F. Television Reception over Long Distance Paths".

SOCIETY OF CHEMICAL INDUSTRY, AGRICULTURE GROUP (joint meeting with the Oils and Fats Group, at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Mr. R. B. Wright: "The Use of Antioxidants in Fishery Products".

SOCIETY OF ENVIRONMENTAL ENGINEERS, PACKAGING GROUP (in the Mechanical Engineering Department, Imperial College of Science and Technology, London, S.W.7), at 6 p.m.—Mr. D. C. Allen: "The Package Environment in Relation to Design Requirement".

UNIVERSITY OF LONDON (at the Institute of Neurology, National Hospital, Queen Square, London, W.C.1), at 6 p.m.—Mr. N. Veall: "Some Radioisotope Techniques for Cerebral Circulation Studies".*

Thursday, November 5

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 10.30 a.m.—Discussion Meeting on "Symmetry Properties of Sub-Nuclear Particles" organized jointly by Prof. C. F. Powell, F.R.S., Prof. R. H. Salitz, F.R.S., Prof. P. T. Matthews, F.R.S., and Prof. A. Salam, F.R.S.

SOCIETY OF CHEMICAL INDUSTRY, MICROBIOLOGY GROUP (at the School of Pharmacy, 29 Brunswick Square, London, W.C.1), at 2.30 p.m.—Meeting on "Practical Problems in Detergency and Disinfection".

INSTITUTE OF PETROLEUM (at 61 New Cavendish Street, London, W.1), at 4 p.m.—Prof. M. R. Fenske (Pennsylvania State University): "New Chemicals and Fuels by Oxidation".

MINERALOGICAL SOCIETY (at the Geological Society of London, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Scientific Papers.

INSTITUTION OF CIVIL ENGINEERS, TRANSPORTATION ENGINEERING GROUP (at Great George Street, London, S.W.1), at 5.30 p.m.—Informal Discussion on "Devices for Pricing the Use of Congested Roads" introduced by Mr. G. J. Roth.

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. Uno Lamm: "Mercury Arc Valves for H.V., D.C. Transmission".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. I. C. Roddie: "Nervous Control of Limb Blood Flow". (Sixth of fourteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation".)*

INSTITUTE OF REFRIGERATION (at the National College for Heating, Ventilating, Refrigeration and Fan Engineering, Southwark Bridge Road, London, S.E.1), at 6 p.m.—Dr. D. R. Scott and Dr. R. S. Azad: "Air Flow Measurement in Refrigerating and Air-Conditioning Systems".

INSTITUTION OF MECHANICAL ENGINEERS, APPLIED MECHANICS GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "How do we Design Against Brittle Fracture?"

UNIVERSITY OF LONDON (at the School of Pharmacy, Brunswick Square, London, W.C.1), at 6.30 p.m.—"Latin America: Past and Present. Indian Civilization: Peru and the Incas".*

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (joint meeting with the Brunel College Chemical Society, at Brunel College, Woodlands Avenue, Acton, London, W.3), at 7 p.m.—Prof. B. C. L. Weedon: "The Synthesis of Polyenes".

Friday, November 6

CLAY MINERALS GROUP OF THE MINERALOGICAL SOCIETY (at the Geological Society of London, Burlington House, Piccadilly, London, W.1), at 11 a.m.—Scientific Papers. 4.45 p.m.—Annual General Meeting.

UNIVERSITY OF LONDON (at Birkbeck College, Malet Street, London, W.C.1), at 6 p.m.—Prof. G. Ekman (Stockholm): "Psychological Scaling. I: Direct and Indirect Scaling Methods".*

SOCIETY OF CHEMICAL INDUSTRY, FINE CHEMICALS GROUP (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Mr. R. B. Wright: "The Practical Application of Antioxidants in Food, Pharmaceuticals and Polymers".

TELEVISION SOCIETY (in the Conference Suite, I.T.A., 70 Brompton Road, London, S.W.3), at 7 p.m.—Dr. R. W. G. Hunt: "Factors Affecting the Acceptability of Colour Reproduction".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Dr. R. D. Keynes: "The Electric Eel".

Saturday, November 7

ASSOCIATION OF CLINICAL BIOCHEMISTS, SOUTHERN REGION (at St. James Hospital, Sarsfeld Road, Balham, London, S.W.12), at 2 p.m.—Scientific Papers and Demonstration.

LONDON COUNTY COUNCIL (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Dr. I. W. Cornwall: "British Association Experiment of the Weathering and Preservation of Archæological Monuments".*

Monday, November 9

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Submarine Telephone Cables—Protection, Damage and Repair" opened by Mr. R. J. Halsey and Mr. N. E. Holmblad.

UNIVERSITY OF LONDON (at Birkbeck College, Malet Street, London, W.C.1), at 6 p.m.—Prof. G. Ekman (Stockholm): "Psychological Scaling. II: On the Psychological Function and Related Problems".*

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.15 p.m.—Capt. J. M. Loyd: "Across the Wahiba Sands".

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Lecturer in Inorganic and Physical Chemistry in the Science Department, Birkenhead Technical College—The Director of Education, Education Offices, 63 Hamilton Square, Birkenhead (November 9).

University Lecturer (with a special interest in genetics, including microbial genetics) in Botany—The Administrator, Department of Botany, The University, South Parks Road, Oxford (November 10).

Lecturer and an Assistant Lecturer in the Department of Applied Mathematics and Theoretical Physics—The Secretary of the Appointments Committee of the Faculty of Mathematics, The University, Arts School, Bene't Street, Cambridge (November 11).

University Demonstrator in the Department of Animal Pathology, to assist with teaching and research in virology or morbid anatomy—Dr. T. D. Kellaway, Department of Pathology, The University, Tennis Court Road, Cambridge (November 11).

Lecturer or Senior Lecturer (with a science degree specializing in geophysics or related discipline and some research experience) in Geophysics in the Department of Geology and Mineralogy, University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Brisbane and London, November 13).

Lecturer/Senior Lecturer in Applied Mathematics at the University of Sydney, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 20).

Demonstrator (with a degree in science, agricultural science, veterinary science or rural science) in Physiology at the University of New England, Armidale, New South Wales, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 27).

Chair of Geography at the University College, Dar es Salaam, University of East Africa—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (November 30).

Lecturer in Mathematics and a Lecturer in Statistics at the University of Otago, Dunedin, New Zealand—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, November 30).

Lecturer (preferably with research interest in geophysics) in Physics at the University College, Dar es Salaam, University of East Africa—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (November 30).

Lecturer or Assistant Lecturer in Biochemistry at the University of Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (November 30).

Senior Lecturer and a Lecturer in the Department of Mathematics University of the Witwatersrand, Johannesburg, South Africa—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, November 30).

Senior Lecturer or Lecturer (inorganic chemist) in the Department of Chemistry, University of the Witwatersrand, Johannesburg, South Africa—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, November 30).

Lecturer (agricultural scientist with a sound knowledge of the Australian environment and with postgraduate training and research experience in ecology and plant physiology) in Agronomy at the University of Sydney, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, December 4).

Lecturer in Pharmacology at the University of Sydney, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, December 4).

Lecturers (3) in Physics at the University of Sydney, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, December 4).

Reader in Chemical Pathology at Westminster Medical School—The Academic Registrar, University of London, Senate House, London, W.C.1 (December 7).

Chair of Psychology—The Secretary, University of St. Andrews, College Gate, St. Andrews, Fife, Scotland (December 31).

Assistant Director (with an honours degree in botany, a sound knowledge of two or more modern languages, the ability to write good English, and preferably some editorial experience) of the Commonwealth Bureau of Plant Breeding and Genetics—The Director, Commonwealth Bureau of Plant Breeding and Genetics, School of Agriculture, Downing Street, Cambridge (January 15).

Agricultural Officers (with a degree in agriculture with, if possible, experience in agricultural extension work overseas, or a degree in agriculture including agricultural economics with, if possible, experience in economic investigations into peasant farming) in Bechuanaland, for agricultural extension work in African and European farming areas or economic investigations into peasant farming—The Appointments Officer, Room 301, Department of Technical Co-operation, Eland House, Stag Place, London, S.W.1, quoting Ref. RC. 213/19/01.

Biochemist or Chemist (with a good honours degree and preferably two or three years' research experience) for research on the nutritional value, in animal feed, of wheat and wheat products—The Director, The Research Association of British Flour Millers, Cereals Research Station, Old London Road, St. Albans, Hertfordshire.

Graduate Research Assistant (with an interest and preferably experience in steroid work) to assist a team investigating endocrinology of human growth (Dr. J. M. Tanner)—The Secretary, Institute of Child Health, Great Ormond Street, London, W.C.1.

Physics Technician (with G.C.E. "A" levels in mathematics or physics) or Radiographer for work on the clinical application of isotope techniques—The Assistant Secretary, University College Hospital, Gower Street, London, W.C.1.

Research Fellow (graduate with experience in the field of steroid biochemistry and preferably a knowledge of gas chromatography) in Steroid Biochemistry in the Department of Zoology, St. Bartholomew's Hospital Medical College, to participate in research on the male reproductive system and the effects of various agents such as exogenous hormones and ionizing radiation—The Clerk to the Governors, St. Bartholomew's Hospital, London, E.C.1, quoting Ref. AC/979.

Scientific Officer (with a good honours degree in physics or engineering) to study dynamics of air and droplets in and around foliage—The Secretary, National Institute of Agricultural Engineering, Wrest Park, Silsoe, Bedford, quoting Ref. N/64/ECD/25/6.

Senior Lecturer or Lecturer in Numerical Analysis—The Academic Registrar, Hatfield College of Technology, Hatfield, Hertfordshire.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Department of Scientific and Industrial Research. Visualizing the Facts. Pp. 16. (London: Library, Department of Scientific and Industrial Research, 1964.) [89

Tin-Alloyed Grey Iron Cylinder Blocks. By A. J. Tache and R. M. Cage. (Publication No. 342.) Pp. 16. (Perivale, Greenford: Tin Research Institute, 1964.) [89

British Medical Bulletin, Vol. 20, No. 3 (September 1964): Symposium on Fibrinolysis. Pp. viii+173–250. (London: The British Council, 1964.) 30*s.* [89

Ministry of Labour; Ministry of Labour and National Insurance, Northern Ireland. Code of Practice for the Protection of Persons Exposed to Ionising Radiations in Research and Teaching. Pp. iv+63. (London: H.M. Stationery Office, 1964.) 4*s.* 6*d.* net. [89

National Institute of Industrial Psychology. Report No. 16: Statistical Records about People at Work. By R. B. Buzzard and J. L. Radforth. Pp. i+22. (London: National Institute of Industrial Psychology, 1964.) [89

The Association of the British Pharmaceutical Industry. Attitudes towards the Pharmaceutical Industry amongst Family Doctors and the General Public, 1962 and 1964. Pp. 12. Attitudes towards the National Health Service amongst Family Doctors and the General Public, 1962 and 1964. Pp. 8. (London: The Association of the British Pharmaceutical Industry, 1964.) [89

An Exploratory Study of the Perception of Causality. By Dr. Lambros Houssiadas. (*The British Journal of Psychology*, Monograph Supplements, No. 36.) Pp. vi+88. (Cambridge: At the University Press, 1964.) 22*s.* 6*d.* net. [89

The Journal of Applied Ecology, Vol. 1, No. 1 (May 1964). Edited by A. H. Bunting and V. C. Wynne-Edwards. (Journal of the British Ecological Society.) Pp. vi+1–218. Published twice a year 70*s.* net. Annual subscription, post free, 120*s.*; U.S. and Canada 20 dollars. (Oxford: Blackwell Scientific Publications, 1964.) [89

Incorporated Association of Headmasters. Honours Courses in Mathematics in the Universities of Great Britain. By A. P. Rollett. Pp. ii+61. 3*s.* 6*d.* Honours Courses in Mathematics in the Universities and Colleges of Advanced Technology in Great Britain, Supplement, No. 1. Pp. 28. 1*s.* 6*d.* (London: Incorporated Association of Headmasters, 1963 and 1964.) [89

Other Countries

University of California Publications in Geological Sciences. Vol. 47: Cenozoic Chronology of the Sierra Nevada, California. By G. Brent Dalrymple. Pp. 41. (Berkeley and Los Angeles: University of California Press; London: Cambridge University Press, 1964.) 1.25 dollars. [89

Biosynthesis of DNA. By Prof. Arthur Kornberg. (Notes on the Thirty-eighth Annual Priestley Lectures. Edited and Sponsored by Mu Chapter, Phi Lambda Upsilon and Associated Departments.) Pp. x+134. (University Park, Pennsylvania: The Pennsylvania State University, 1964). 3.25 dollars. [89

Annual Report of the Surgeon General, United States Army, Fiscal Year 1963. Pp. x+165. (Washington, D.C.: Office of the Surgeon General, Department of the Army, 1964.) [89

Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Geological Maps. Map 1143A: McQuesten, Yukon Territory. Map 1144A: Ymir (Nelson, East Half), British Columbia. (Ottawa: Director, Geological Survey of Canada, 1964.) [89

Northern Rhodesia: Ministry of Natural Resources. Annual Report of the Department of Water Affairs for the year 1963. Pp. 9. (Lusaka: Government Printer, 1964.) 2*s.* 6*d.* [89

Reactor Centrum Nederland. Summary of Activities, July 1963—July 1964. Pp. 15. (The Hague: Reactor Centrum Nederland, 1964.) [89

Smithsonian Miscellaneous Collections. Vol. 146, No. 6: A Revision of the American Vultures of the Genus *Cathartes*. By Alexander Wetmore. Pp. 18. (Publication No. 4539.) (Washington, D.C.: Smithsonian Institution, 1964.) [89

Tanganyika: Department of Geological Survey. Geological Maps. Quarter Degree Sheet 90 and 90M: Mnazi (Kalimawe). Quarter Degree Sheet 143: Meia Meia. Quarter Degree Sheet 234: Chombe. (Dodoma: Department of Geological Survey, 1963.) [89

Metropolitan Life Insurance Company. Statistical Bulletin, Vol. 45, (July, 1964): Large Accident Toll Among Men at the Working Ages. Mortality Trends for Major Types of Heart Disease. Slight Decrease in Longevity of Policyholders in 1963. Rise in Frequency of Suicide. Pp. 12. (New York: Metropolitan Life Insurance Company, 1964.) [89

Arctic Institute of North America. Technical Paper No. 14: Eskimo Administration: II—Canada. By D. Jenness. Pp. 186. (Montreal, Washington, D.C., and New York: Arctic Institute of North America, 1964.) [89

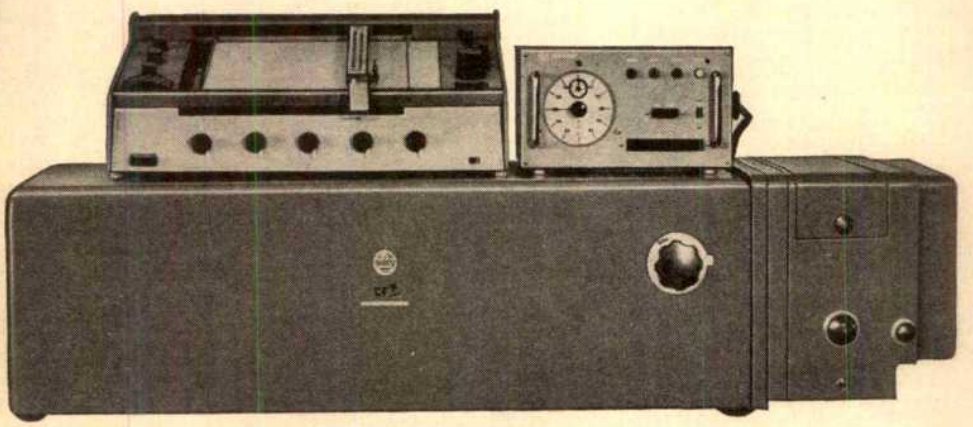
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Inquiries should be addressed to Dr. L. Krause, Head, Department of Physics.

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Further particulars can be obtained from the Registrar, University College of Swansea, Singleton Park, Swansea, to whom applications should be sent by November 30, 1964.

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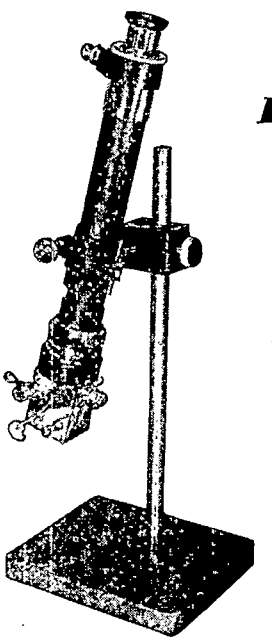


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THE SCIENTIST'S CHANGING ROLE IN WORLD POLITICS

AN address, entitled "The Changing Role of Scientists in International Affairs", was delivered by Byron L. Johnson to the Sigma Xi Chapter of Colorado State University, and is published in the *American Scientist* (June, 52, 138*A*; 1964). Mr. Johnson discussed the varied functions which the scientist has come to assume in society, more particularly when, associated with the government in some capacity, he has some responsibility in the relations between one country and another. He mentioned the Pugwash meetings, but only to stress the need of such discussions if we are to restore the essential minimum of international confidence and trust without which successful negotiation is impossible. Apart from indicating the various parts the scientist can now play in international affairs, however, he was content to insist that the scientist must continue to pursue truth with singleness of purpose, as well as being a full participant in human affairs, understanding and supporting the humanities and the social sciences as well, and taking a completely human view of human beings. While he admitted the existence of a division, in government and in the university, between science and the humanities, with imperfect communication between them, he regarded the idea of 'two cultures' as an exaggerated dichotomy and believed that ultimately the two will blend.

Mr. Johnson indicated some of the tensions which the nationalist pressures of politics have put on the scientist and emphasized that the role of the scientist has been largely dictated by the conception of the national interest, but he did not in this address do more than hint that the scientist might render much more constructive and far-reaching contributions to human welfare if he were able himself to formulate the questions and suggest priorities for the allocation of resources. Mr. Johnson gave a further reminder that the solution lies, not in the provision of unlimited resources for research, but in the wise selection of objectives. If scientists are to play the part of advisers over a wide range of topics, and assist in the formation of public policy, their advice should be disinterested as well as authoritative. However, when he suggested that some scientists might serve in elective office, since public policy requires informed judgment in scientific matters, he did not recognize that in doing so the scientist becomes primarily a politician, responsible for decisions that are none the less political because they are determined partly on scientific considerations.

Dr. Caryl Haskins's Elihu Root Lectures are of a different order*. They attempt to review the direction and nature of some of the trends of scientific and technological change as they affect aspects of the foreign policy of the United States to-day, and as they may be expected to influence it in the future. Much of its thought is to be found in his reports as president of the Carnegie Institution of Washington for 1960–61 and 1961–62 and, as with those reports, his insight and wisdom are as refreshing to the reader in Britain as in the United States. The book should, once and for all, dispose of the idea that science and foreign policy are things apart, and passages in it bear closely on some points in the recent Plowden Report on representational services overseas (*Nature*, 202, 1039; 1964).

This relation between science and technology and foreign policy is well outlined in the introductory chapter to the book, and throughout the book the emphasis is on the forces of science and technology in relation to the internal strength and development of peoples, on their binding force among peoples and on the significance of technology and science for the emerging nations of the world. He then considers science and technology in relation to somewhat more advanced 'intermediate' nations in the technical, economic or political sense, such as Pakistan and Indonesia, before finally considering some scientific and technical aspects of international solidarity in Western Europe, and the nature and position of science and technology in the U.S.S.R. and China.

From the outset Dr. Haskins makes clear two personal biases which determine his whole approach. The first, which will be familiar to readers of his presidential addresses, is that the scientific revolution of the West, encompassing the years from Galileo and Copernicus to the years following Newton, has a meaning for the present day and for the new nations which is far more than historical. He believes that it is of great contemporary interest for three compelling reasons. First, it brought to the West, and can now be expected to bring to others in the future, a world view so radically new that it may well transform the outlook of those who experience it. Secondly, it brought for the first time, and may well bring again elsewhere, the real conviction that not only can significant aspects of the laws of Nature be understood by man, but also that they can be effectively used for man's practical gain. Thirdly, and most fundamental, our society, so dominated by science and technology, sets an overriding value on the search for truth, defined not only in the context of viable and intellectually satisfying propositions, but ultimately as the faithful description of our natural and swiftly changing social worlds as we can best delineate them.

This commitment at a very deep level to the search for truth as an overriding value involves social consequences as well as a powerful commitment to the scientific way. So long as a society considers truth in this light, and so long as the search for truth has a high value, that society harbours powerful incentives to remain dynamic and flexible. It is compelled for that very reason to set a high value on, and to protect, the independent and original mind, for it is on such minds that the effective search for truth depends. This notion of truth and the valuation of the search for truth, both linked so closely to the scientific concept, do much to ensure that a society remains flexible and preserves, in contrast to the traditional society, the power to evolve.

Dr. Haskins's second bias is that, closely woven and closely interdependent as are science and technology in our civilization to-day, and much as we take their intimate relation for granted in all we do, they are significantly different in origin and nature. This idea also Dr. Haskins has developed in his presidential addresses, and in this book he further shows how important the difference may be for the new nations, especially in respect of technical assistance. Indeed, probably the major importance of the

* *The Scientific Revolution and World Politics.* By Caryl P. Haskins. (The Elihu Root Lectures, 1961–62.) Pp. ix + 115. (Published for the Council on Foreign Relations by Harper and Row, New York and Evanston.) 3.50 dollars.

book lies in its clarification of some of the wider issues to be considered in giving technical aid to the new nations. Dr. Haskins would not claim that much that he says is original, but the issues that arise in the impact of technology on the emerging nations and their implications for technical aid have rarely, if ever, been presented more brilliantly, more lucidly, nor, be it added, more urgently.

The chapter in which he discusses the implications of the scientific revolution for the new nations is no less brilliantly presented. He makes essentially all the points which Dr. S. Dedijer made last year in his article on underdeveloped science in under-developed countries (*Nature*, 201, 1153; 203, 1313; 1964), but the chapter is equally a valuable contribution to the debate on scientific change which has continued since Dr. A. C. Crombie asked his searching questions at the Symposium on the History of Science at Oxford in July 1961. Dr. Haskins's deep understanding of the needs and problems of the developing nations is matched by his appreciation of what is at stake, the urgency of the situation, and the bearing of technical aid on scientific and technical advance generally.

Consideration of the relations of science and technology to world affairs even in the context of the developing nations is imperative, not merely from the point of view of humanity itself, but of world stability and world order Besides possibly helping the emerging countries at the most practically significant level, it could contribute to a clearer comprehension of the operation of social forces centring about scientific and technical change which are at present little understood, but which in their consequences could affect dramatically the whole configuration of world power in the years ahead. Even in Britain the relation of social forces to scientific and technical change is as yet little understood, and Dr. Haskins can well write that never in world history can such understanding have been more important than it now is and will continue to be in the age before us—an age of which an inevitable symbol will be the comprehension of scientific and technical phenomena on a far larger scale than ever before, and a new order in their control.

In the three chapters comprising the second part of the book, "Beyond the Scientific Revolution", Dr. Haskins does not touch quite the same heights, but he is no less stimulating, and what emerges most clearly from his analysis of the needs of such nations as Pakistan and Indonesia is the demand which effective technical aid makes on clear and critical thinking. Technical aid is no field for facile generalizations or political catchwords: each problem demands treatment with means appropriate to the purpose to be served, not with formula devised to deal with other situations or even with a general problem. This is irksome to the politician or administrator but it is vital for success, and while it may demand much of the scientist or technologist it is in line with his whole training and outlook. Some of Dr. Dedijer's observations, addressed to the leaders of the emergent countries, could not be better underlined.

Of Western science and the growing international co-operation in research and education, Dr. Haskins observes that international institutions must be firmly based on national institutions, and that the level of education and the formation of coherent and effective national policies for the support of science, together with the development of appropriate professional organizations, are essential factors. He looks briefly at all that this implies for the United States before considering, in conclusion, the problems presented by Soviet Russia and by China. Here, he admits that he is on less certain ground, but his conjectures are reasoned and shrewd, optimistic rather than pessimistic. Not everyone will agree with his judgement, nor will his questioning of the magnitude of the effort being devoted in the United States to the field of space research and a lunar landing be everywhere applauded. None, however, can lightly dismiss his searching question whether, in the long run or in the critical view of the world, spectacular space achievements command more enduring respect than outstanding success in bringing assistance at an effective level to those countries which need it most acutely. The context in which the question is poised epitomizes the theme of the whole book.

TRAFFIC PROBLEMS AND THE COUNTRYSIDE

THE pamphlet entitled *Traffic in the Lake District** recently issued by the Friends of the Lake District is a timely contribution to considerations of a problem which has much wider implications than the future of the Lake District as we now know it. The pamphlet was inspired by the Buchanan Report on *Traffic in Towns* (*Nature*, 201, 1059; 202, 219; 1964) and, while it is concerned essentially with one particular aspect of the problems created by the rapid expansion of motor transport—which is estimated to rise from about 10·5 million vehicles early in 1963 to 18 million by 1970 and 27 million by 1980—it directs attention to a problem which threatens the countryside in general and could destroy alike the whole purpose of national parks and nature reserves. Indeed, the problem is one that calls for forethought and consideration in all regional planning represented by such plans as have been outlined for Scotland, for the North-East and the South-East, and perhaps especially in such projects as the Channel tunnel.

* Friends of the Lake District. *Traffic in the Lake District*. Pp. 11. (Ulverston: Friends of the Lake District, 1964.)

The Friends of the Lake District have long foreseen the danger, and as far back as 1938 pointed out that, unless the development of motor transport and roads was checked, the Lake District would cease to be a place of quiet and refreshment. Again in 1960 they re-stated the problem and its dangers in a full analysis, showing that widening and straightening roads only attracted more cars, so that the process proved self-defeating. The most casual observer in any part of Britain is, indeed, likely to be aware of country roads which have been greatly improved to allow for the traffic they carry, but which have become more and not less dangerous because of the temptations to excessive speeds.

The case for much greater intelligence in road policy in country districts and for a livelier appreciation of the purposes to be served scarcely needs to be argued, though undoubtedly there is a considerable task of education to be undertaken, both among the general public and among highway and local authorities. So far as the Lake District is concerned, however, the present pamphlet once more puts forward convincingly the argument that the Lake

District should be subject to treatment quite different from that which might well be appropriate in more densely populated areas. The Friends of the Lake District believe—and with some justification—that popular opinion now inclines towards a sane policy. Moreover, they are prepared to accept that to a large extent the existing road system in the Lake District is adequate on most days of the year and that some delay and congestion must be tolerated by the motorist at week-ends and holidays as part of the price to be paid for the preservation of the traditional character and essential value of the Lake District.

The pamphlet begins by quoting the concern expressed by the Minister of Transport as to the possible effect on the Lake District of a direct link between the M6 motorway and Manchester. It also includes the observation by the Minister of Housing and Local Government, made at a National Parks Conference, that the more congested Britain becomes the greater will be the need for oases of peace and quiet, such as are provided by the parks. The danger is that this relatively small-scale and often fragile beauty may be destroyed by the crowds who will want to enjoy it. In the first review of the development plan of the National Parks Board the Lake District Planning Officers expressed the definite view that this was just what was happening. In the Lake District National Park this problem dwarfs all others. The pamphlet quotes the warning given in the Buchanan Report of the menace to our civilization that the motor-car in its collective aspect represents, and insists that, unless the greatest care is exercised, the countryside of Britain will be ruined by the end of the century.

By an accident of geography the Lake District escaped the full effects of the Industrial Revolution a hundred and fifty years ago: its isolation has now been ended and only definite and planned intervention is likely to avert its ruin. To reshape its roads in an attempt to allow all imaginable holiday traffic to pass freely and quickly would destroy the essential character of the Lake District valleys, but, if highway authorities are to cease to think only of road widening as a means of coping with increasing traffic, national policy seems imperative. The proposals for widening the road over Dunmail Raise, between Kendal and Ambleside, and up the Langdale valley, to which the pamphlet refers, are appalling in their implications, yet at present the highway authorities have a virtually uncontradicted say in all that concerns roads in the Lake District. The folly of failing to provide the national park authorities with effective powers could scarcely be better illustrated. Moreover, in laying down standards for the width and form of construction for roads feeding into or debouching from motorways, the Minister of Transport has not faced the implications of his own standards.

Here again, the Lake District roads need critical consideration in their special context rather than in the national plans for roads in general, and this could apply elsewhere in areas of special scientific interest or outstanding natural beauty, in other national parks, in nature reserves, etc. The keynote of the pamphlet, however, is not simply the urgency of the situation: it is above all a plea for thought—for the kind of critical forethought of which Lord Brain was thinking when he reminded us in his presidential address to the British Association at Southampton (*Nature*, 203, 903; 1964) that we have still no population policy. Ultimately this problem is a derivative of population, as well as other changes, in Britain, and the Friends of the Lake District do well to call for a pause in order to think quietly about what is being done and where we are going. In particular, they urge the Minister of Housing and Local Government, who carries the major share of effective responsibility in the matters concerned (including grants to the highway authorities), to commission something on the lines of a Buchanan Report on traffic problems in national parks. Certainly the body responsible for such a report should offer recommendations for easing traffic difficulties while retaining unimpaired the heritage of natural beauty which the parks were created to preserve.

Support for this view and its implications is not hard to find. Although Prof. C. Buchanan was concerned in an inaugural address, entitled "Transport and the Community" (now published†), with transport in towns, his concept of 'environmental capacity'—that any urban area has a finite capacity for motor traffic according to the environmental standard selected—is applicable to national parks, nature reserves, the coast or areas of plain countryside if certain environmental objectives are to be secured. This obviously requires further consideration, quite apart from decisions about the objectives and the price which can reasonably be paid to achieve them, but it is interesting to note how Prof. Buchanan relates the problem to that of the population explosion also referred to by Lord Brain.

Prof. Buchanan directs attention, not simply to the expected rise in population of at least 20 million in 40 years, to the quadrupling of the number of motor vehicles, the intensification of the demand for industrial productivity, but also to the extensive demands for new or enlarged facilities for recreation—all of which may cause severe pressures within the life-time of many young people now at college. He suggests that we might begin now by making a few basic global calculations as to how the population should best be distributed if broadly defined standards of living are to be secured in the various localities. Even in dealing specifically with problems in towns, his suggestions are addressed to individuals as well as to local and transport authorities, and some have their rural connotations. This is especially true of what he has to say about professional collaboration, which could well be a decisive factor. Obviously it provides the basis of such schemes as the Solway Barrage proposal of Dr. R. L. Drew, or the Morecambe Bay Barrage proposal now under investigation: balanced plans are only possible as a result of collaboration between those with such skills as the architect, engineer, planner, surveyor, sociologist and economist. However, as Prof. Buchanan also notes, collaboration would be required in quite a new spirit between numerous local authorities and public bodies, both in the preparation of plans and in their continuing implementation.

Nor is this all. Such co-operation is very necessary at ministerial level, between such Ministries as those of Transport, Housing and Local Government, Power, Industry, Trade and Regional Development, and Education and Science. Meanwhile, the danger persists that the position may be hopelessly prejudiced by sporadic and independent action or decisions. While such effective collaboration may lie in the future, certain Ministers, like those of Transport and of Housing and Local Government, do possess sufficient powers to restrain encroachments such as 'improvement' in the Lake District threatens. It would be intolerable if those powers were not used with wisdom, boldness and energy. Action taken to provide

† *Transport and the Community.* (Inaugural Lecture, February 18, 1964.) By Prof. Colin Buchanan. Pp. 175–193. (London: Imperial College of Science and Technology, 1964.) 2*s*. 6*d*.

the county councils with the powers suggested by Prof. Buchanan could be beneficial in rural areas. His lecture follows the lines of his earlier larger report, *Traffic in Towns*, and is a further stimulus to the critical and imaginative thinking that is urgently needed if the needs of a civilized community are to be met in the Britain of to-morrow. The study conference last November, *The Countryside in 1970*, like the preliminary enquiry made for the Royal Society of Arts into the *Impact of Industry on Amenities in the Countryside*, is an indication of the awakening to which the Friends of the Lake District refer. Prof. Buchanan's lecture and the pamphlet *Traffic in the Lake District* underline the closing words of the report on the enquiry *Industry and the Countryside*: "the frightening thing about the present day is the rate of change". Whether from the point of view of amenity or of natural resources, effective action on at least regional if not national lines is imperative and should be based not on sectional views but on the interests of the community as a whole.

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Geographers and the Tropics

Liverpool Essays. Edited by Robert W. Steel and R. Mansell Prothero. Pp. vii+375. (London: Longmans, Green and Co., Ltd., 1964.) 47*s*. 6*d*. net.

THE twentieth International Geographical Congress, held in London during July 20–28, 1964, and attended by some 2,200 delegates, was preceded and followed by a number of specialist symposia at universities throughout the British Isles, at which small groups gathered for exchange of views. Under the leadership of Prof. R. W. Steel, whose name is rightly associated with geographical research in tropical Africa, the meeting at the University of Liverpool was devoted to the problems of tropical geography. This collection of fourteen essays was published to mark the occasion: the authors have all been associated in some way or other with the Department of Geography at Liverpool. Apart from the fact that the City and Port of Liverpool have always had close associations with many and varied tropical countries, this is the only unifying factor. Indeed, the collection illustrates the quite remarkable diversity of interest of geographers working on tropical problems.

In his introductory essay Prof. Steel reviews geographical work with special reference to tropical Africa and gives a valuable selective check list of 160 works published over the past thirty years. Physical geography is represented by P. H. Temple (Makerere University College) on change in the level of Lake Victoria; E. M. Driscoll on landforms in Northern Australia; Dr. S. Gregory deals with rainfall in Mozambique, and Joan M. Kenworthy with water resources in East Africa. Prof. Paul Wheatley, now at the University of California, Berkeley, devoted his period in the University of Malaya at Singapore to acquiring a proficiency in reading Chinese both modern and medieval. A rich field of original material has thereby been opened to the Western World, and he writes on Chinese knowledge of East Africa prior to A.D. 1500 in a scholarly and fully documented essay—the longest in the collection.

While the significance of the mobility of labour in sparsely populated tropical Africa is well known, Dr. R. M. Prothero has set out to study systematically the continuity and change in African population mobility, while I. F. Masser, now a consultant planner, looks from another angle at changing patterns of African employment in Southern Rhodesia. Settlement is the subject of Dr. G. Kay's study of the Ushi of Northern Rhodesia [as it was then], and F. Lees deals in detail with an example of Chinese settlement in Malaya over the past 130 years. Dr. Alice Dyson has made a brief summary of part of her lengthy doctoral thesis on land use in Trinidad.

Applied geography is represented by L. S. Bhat's essay on regional planning in India, and Prof. A. T. A. Learmonth's "Retrospect on a Project in Applied Geography in Mysore State" draws on his year of research there. It is left to the final essay by R. Lawton on "Liverpool and the Tropics" to bring Liverpool into the picture. He deals with the changing and expanding trade of the port from the early nineteenth century to the present day.

The volume is well produced; many of the essays are illustrated by clear, well-executed maps and diagrams. Diversity of interest should secure a place for this book in every serious library, even if its classification will stretch the ingenuity of librarians.

L. Dudley Stamp

MOUNTAIN PEOPLE

The Sherpas of Nepal

Buddhist Highlanders. By Prof. Christoph von Fürer-Haimendorf. Pp. xix+298+55 photographs and 2 maps. (London: John Murray, 1964.) 35*s*. net.

MANY anthropologists must have envied Prof. Haimendorf his chance. The Sherpa valleys of northern Nepal, closed to Europeans down the years, were opened to a few Western travellers and climbers from the start of the 1950's; the first reports of the Sherpas at home were of a friendly, welcoming, laughing people, with some special gift of cheerfulness—what better task than to put on record their exceptional way of life and to analyse its secret of success? By 1953 Prof. Haimendorf was in the field, and in just under ten years he spent a total of twenty-five months living and working with the Sherpas. Already a Himalayan anthropologist of international standing, he could use his rich chances to the full. He has already published shorter examinations of various problems, and now he gives a long and detailed account of the Sherpa way of life.

In the main it bears out the travellers' tales of spontaneous and rather simple gaiety, but some things indeed are new: there is, for example, far more division in society than had been recognized at first, with the semi-untouchable *khamendeu*, and with the original Sherpa families holding pride of place in the community over other waves of immigrants from Tibet. The structure of society is far more complex than the first impression of the Sherpas' open gregariousness had led one to expect, and yet it functions amicably. This may be traced to the ready acceptance of social obligations and responsibilities, largely on the basis of a rota, a system which is carefully and thoroughly described. There is an easy code of sexual morals. Then there is Buddhism, largely benign, which interests Prof. Haimendorf particularly, and on which he writes at length. His account of this is full and sympathetic: he is quite at home, for example, in dealing with such eastern specialities as re-incarnated lamas, offering us, if not outright belief, at least an apparent suspension of disbelief. Sherpa religion came from Tibet, and, as in pre-Communist Tibet, there are here many monks engaged in a wide variety of religious practices, but it would be wrong to see these as in any sense imposed on an indifferent laity who groan under the economic burden—Prof. Haimendorf shows that the great expansion in the Sherpas' religious foundations in fact came after the introduction of the potato had brought increased prosperity, and the practice of religion is the way in which the Sherpas choose to spend their surplus wealth. Yet, however cheerful the great religious festivals, however much at one are the monks and those who support them, it is hard for anyone who is not a Buddhist highlander to be really happy that so much energy and man-power,

good and learned man-power, should go to waste in the smoke of funeral rites.

It is so good that a scholar of Prof. Haimendorf's calibre could make this record of a traditional society before it evolves or disappears. For, as the Sherpas' isolation is being eroded, so their way of life must change, and even as one reads this book a host of unanswered questions spring to mind. Prof. Haimendorf refers to the trading rupture with Tibet. But what is the effect of the big expeditions, of the many visitors (of anthropologists, even) into the long-closed land? Moreover, what about the influx of refugees, the United Nations agricultural experts, the school at Khumjung? The Sherpas' society, so rare and valuable, must now adapt to a hurricane of change; and once one has known them and laughed with them and cared about them, one's main concern cannot be the past but the hazards lying ahead.

JENNIFER BOURDILLON

INORGANIC AND ANALYTICAL CHEMISTRY

Supplement to Mellor's Comprehensive Treatise on Inorganic and Theoretical Chemistry

Vol. 8, Supplement 1: Nitrogen (Part 1). Prepared under the direction of an Editorial Board. Pp. lxviii+619. (London: Longmans, Green and Co., Ltd., 1964.) 280*s*. net.

Boron Hydrides

By Prof. William N. Lipscomb. (The Physical Inorganic Chemistry Series.) Pp. ix+275. (New York and Amsterdam: W. A. Benjamin, Inc., 1963.) 15.40 dollars.

Boron-Nitrogen Chemistry

An International Symposium sponsored by U.S. Army Research Office—Durham at Duke University, Durham, N.C., April 23–25, 1963. (Advances in Chemistry Series, 42.) Pp. x+330. (Washington, D.C.: American Chemical Society, 1964.) 7.50 dollars.

Concise Inorganic Chemistry

By J. D. Lee. Pp. xii+248. (London, New York, Toronto and Princeton, N.J.: D. Van Nostrand Co., Ltd., 1964.) Paper-bound, 27*s*. 6*d*.; Clothbound, 50*s*.

Vapour Pressure of the Chemical Elements

By A. N. Nesmeyanov. Edited by Robert Gary. Pp. xiii+462. (Amsterdam, London, New York: Elsevier Publishing Co., 1963.) 105*s*.

Analytical Chemistry of Thorium

By D. I. Yabchikov and E. K. Gol'braikh. Translated by A. Aladjem. (Analytical Chemistry of Elements Series.) Pp. vi+250. (Jerusalem: Israel Program for Scientific Translations; London: Oldbourne Press, 1963.) 80*s*.

Analytical Chemistry of Niobium and Tantalum

By Ross W. Moshier. (International Series of Monographs on Analytical Chemistry, Vol. 16.) Pp. v+278. (London and New York: Pergamon Press, 1964.) 90*s*.

Analytical Chemistry of Ruthenium

By T. D. Avtokratova. Translated by IPST Staff. (Analytical Chemistry of Elements Series.) Pp. 224. (Jerusalem: Israel Program for Scientific Translations; London: Oldbourne Press, 1963.) 88*s*.

THE volume of Mellor follows the style of the supplements so far published. The collection of numerical data, often tabulated, is a most valuable feature. The table on p. 30, for example, gives recent values for the heat of dissociation of the nitrogen molecule varying from 115·3 to 272 kcal mole^{-1}. The theory is always concisely presented, as in the discussion of multiple bonded nitrogen. There is an excellent section on nitrides, occupying 90 pages. In this a reference to a paper giving details of the preparation of the important magnesium nitride has been overlooked. The section on ammonia is very complete and informative, although it does not seem to deal with deuterammonia. The account of the industrial synthesis of ammonia is very good; from a very large patent literature a critical and significant selection is presented. The oxidation of ammonia is dealt with only briefly; presumably this will appear in a later part concerning nitric acid. The section on active nitrogen does not include more recent work which has largely cleared up the nature of active nitrogen; the editors should have taken steps to have had the article brought up to date. The book is one which is indispensable in all chemical libraries.

The chemistry of boron is expanding at such a fast rate that summaries will be welcome. This applies particularly to the hydrides of boron. A book by one of the leading authorities must, therefore, raise hopes that chemists will now have before them a well-digested survey of the whole subject. The book by Lipscomb does not seem to me to meet this need in a wholly satisfactory way. The chemistry is dealt with only very briefly and the bulk of the text is concerned with structure, which, needless to say, is covered in great detail. The style, unfortunately, is not always clear and gives the impression of haste. The discussion of the three-centre bond is far from clear and specialized concepts such as equivalent orbitals are introduced without explanation. The book will appeal more to mathematical physicists than to chemists.

A section of boron chemistry of particular interest is concerned with compounds containing boron and nitrogen, and the book on this subject is very informative and interesting. It is true that it is largely concerned with the organic chemistry of boron, but this is so closely linked with the inorganic chemistry that it seems permissible to include it here. The text consists of a series of papers presented to a symposium, but the continuity is more evident than is common in books of this kind. Theory finds a place, but preparative chemistry and descriptions of reactions occupy a position of honour, and the volume as a whole is one which chemists with different interests should find attractive and valuable.

The concise survey of inorganic chemistry by Lee is a book which students at all stages should find useful and interesting. The author points out that a knowledge of valency theory is no substitute for a knowledge of chemical facts, and he attempts to combine these two aspects of the science; he hopes that his book is long enough to cover the essentials and short enough to be interesting. I think that he has been very successful in achieving what he set out to do, and also that there is a need for something of the sort to be done with organic chemistry, which is tending to become too stifling for students.

The book on the vapour pressure of the elements gives a good account of the methods of measurement and a critical discussion of the results. There is also a useful table of the physical properties of the elements. In modern technology a knowledge of the properties of metals at high temperatures is very important and a critical review of the vapour pressures is valuable. Recent recorded values of high-temperature melting and boiling points often differ by hundreds of degrees.

The three books on the analytical chemistry of thorium, niobium and tantalum, and ruthenium include more than the titles might suggest. They give, particularly that on ruthenium, valuable surveys of the general chemistry of the elements, and the physical properties. It is mentioned that the X-ray result that thorium has a face-centred cubic lattice has recently been corrected, that values for the melting point vary from 1,450° to 1,850° and for the boiling point from 3,000° to 5,200°. Niobium and tantalum are important elements, and a good survey of their analytical chemistry is welcome; it includes

X-ray and radiochemical methods. Ruthenium was discovered in Russia, and a book on it by a Russian author continues the tradition. The original is one of a series intended to comprise about fifty volumes and to be completed in about five years, issued by the Institute of Geochemistry and Analytical Chemistry of the U.S.S.R. Academy of Sciences. The translation leaves much to be desired; incorrect chemical names are used and sometimes impossible chemical reactions are given. Only an expert chemist could find a way through the mistakes. All three books have good bibliographies. J. R. Partington

SHOCK WAVES AND CHEMISTRY

Chemical Reactions in Shock Waves

By Edward F. Greene and J. Peter Toennies. Pp. xvi + 352. (London: Edward Arnold (Publishers), Ltd., 1964.) 65*s*. net.

In the past fifteen years, shock waves have become an important experimental tool for the physical chemist. In the shock tube, gas temperatures of thousands of degrees are attained within a few molecular collisions—instantaneously, in fact, compared with the rate of vibrational relaxation and chemical reaction. The gas is only heated for a millisecond, but the development of photoelectric and other optical devices with a rapid response makes it possible to measure both relaxation rates and equilibrium properties within this time.

The first two-thirds of this book is a revised version of a monograph first published in 1959 in *Fortschritte de Physikalischen Chemie*, which describes the hydrodynamic theory and experimental techniques used. It opens with a chapter comparing the shock tube to other methods, and listing its advantages and limitations. Among the latter might well have been included the difficulty in varying pressure and temperature independently over a wide range, and the complications that arise in making rate measurements in an adiabatic system. Exothermic reactions, in particular, are difficult because the flow is potentially unstable behind the front.

The next two chapters derive the conservation equations needed to calculate the density and temperature of the hot gas, and show how they can be solved for real gases. The complicated procedures involved in coupling these hydrodynamic equations to chemical equilibrium are carefully explained. After a brief account of shock-front structure, the analysis of experimental records of vibrational and chemical relaxation is given: it is a pity this section does not include a discussion of the effects of shock attenuation on rate measurements.

The chapter on the production of shock waves rightly concentrates on gas-driven shock tubes, and includes a useful account of deviations from one-dimensional flow due to boundary layer formation: these often limit the time of observation available. After this the methods, mainly optical, used to measure the velocity of the shock and the properties of the hot gas are described. The final chapter gives a detailed account of a few outstanding experiments chosen to illustrate the range of possible measurements.

The remaining third of the book is occupied by tables summarizing shock-tube work up to the middle of 1963. Each entry gives all the essential features of the investigation, object, method, results and comments. They are classified first by the type of investigation: equilibrium properties and equation of state of gases and condensed phases, spectra, transport properties, shock front structure, vibrational relaxation and reaction kinetics; and secondly by the substance investigated. The diversity of high-temperature topics investigated is matched by the variety of journals and reports in which the work appears. The authors are to be congratulated both on the completeness of these tables and on the skilful way the entries have been prepared and arranged.

The cover suggests that the book is intended for chemists and physicists who wish to apply shock waves to their own problems: they will find it a useful guide if they are not dissuaded from the technique by the complications that become evident in the early chapters. But the more specialized topics will be appreciated by those already working in this field, and both active and potential workers will find the tables of the greatest value. The volume is well produced, and the price is not unreasonable.

B. P. Levitt

PREPARATION AND REACTIVITY OF ORGANOSILICON COMPOUNDS

Synthesis of Organosilicon Monomers

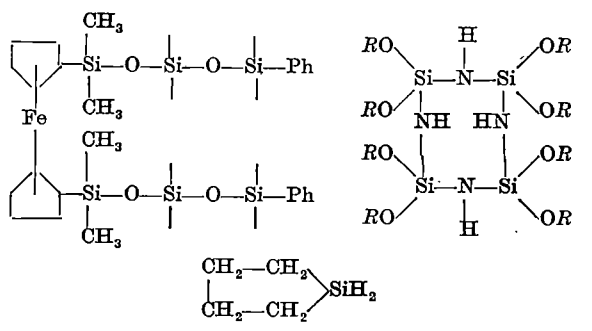
By A. D. Petrov, B. F. Mironov, V. A. Ponomarenko and E. A. Chernyshev. Authorized translation from the Russian by C. Nigel Turton and Tatiana I. Turton. Pp. 492. (New York: Consultants Bureau, 1964.) 22.50 dollars.

Chemists concerned with silicon polymers will be aware of the Russian publications on the relevant monomers. To have this essential information presented in English not only affords a means of checking items and impressions, but also greatly expedites the handing-on of knowledge to new postgraduates and to recruits into an industrial organization. It is a comfort to have this account of the experiences and views of the Russian chemists.

The use of the term 'monomers' is specific, and labels the compounds as precursors to polymers; but the chemistry described means much more than this, and fortunately the authors have assigned little space to polymerization. Rather may the book be considered to be a source of information for chemists, college or industrial alike, whose interests may not be in silicon polymers. Of more general significance are the reactive centres in the monomers, located by no means exclusively at the silicon atom. The influence of the silicon atom on the reactivity of atoms or groups, immediately or more remotely located, steric effects, and the transmission of electronic effects through the silicon atom, afford truly exciting properties for investigation. Therefore this ready access to vital information on the preparation of a series of organosilicon compounds having a stepwise range of reactivity is a windfall to many chemists who are not specifically committed to silicon polymers.

There are clear indications of the availability of the compounds, either by the usual laboratory facilities, or from commercial sources, and the three long tables dealing with unsaturated organosilicon compounds, aromatic organosilicon monomers, and physical properties of silicon hydrides, provide a list of the compounds prepared which can easily be scanned.

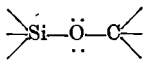
Details are given of the formation of the Si—C link by organometallic (for example, *R*Mg*X*), catalytic (Cu—Si 'direct' synthesis), and pyrolytic procedures. By subsequent processes, other atoms or groups are substituted for the reactive ones in the first stage product. Thus are constructed compounds with linkages depicted as follows:



Special features are, first, the silicohydrocarbons having alkenyl, $R_2Si(CH_2.CMe{=}CH_2)_2$, or alkynyl groups, and Si—H and Si—Si bonds; and secondly, the organosilicon hydrides, including compounds such as $RSiHCl_2$. In addition to the lithium aluminium hydride method, other procedures for forming the Si—H bond are described. Addition to a double bond is merely one example of the fascinating reactivity of the Si—H bond:

$$\rangle Si{-}H + CH_2{=}CHX \rightarrow \rangle SiCH_2CH_2X$$

The relative reactivity of the C—C and C—Si bonds has been much considered; but the variation of the relative reactivity of the Si—O and O—C bonds in:



and the basic function of oxygen in such compounds, according to the groups attached to Si and C, are matters in need of much further attention; p_π—d_π bonding to silicon from both the lone pairs on oxygen has been suggested as a probability in certain cases.

Points of comparison among the analogous compounds of Ge, Sn and Pb provide a fitting ending to *Synthesis of Organosilicon Monomers*, which deserves a place in every chemical laboratory whatever the bias.

W. Gerrard

ELECTRICAL CONDUCTIVITY OF GERMANIUM

Progress in Semiconductors
Vol. 8. Edited by Prof. Alan F. Gibson and Prof. R. E. Burgess. (A Heywood Book.) Pp. vi+244. (London: Temple Press Books Ltd., 1964.) 70*s*. net.

THE latest volume of *Progress in Semiconductors* departs from the usual practice in this series and contains only one article, a substantial contribution, by Dr. E. G. S. Paige, to the literature on germanium, devoted exclusively to electrical conductivity. The sheer bulk of the present data on the transport properties of semiconductors, and of germanium in particular, poses its own problems for the planner of review articles. In the present instance the editors have evidently considered that a closely related group of topics in a well-defined field could be treated advantageously, in a unified manner, by a single author. This inevitably involves a rather wasteful duplication of material—the present article overlaps several others which have appeared in earlier volumes. Moreover, in assessing the value of a work of text-book dimensions, the element of topicality plays little part in view of the lengthy period (two years in the present case) required for publication.

What Dr. Paige has provided is a workmanlike and authoritative monograph dealing in detail with the theory of electrical (bulk) conduction in germanium and its application to experimental results. The object has not been to shed new light on the foundations of the theory, or to examine controversial aspects, but rather to present a comprehensive account within a firmly established framework.

Of the five main sections the central, and longest, contains an analysis of experimental data on carrier mobilities, Hall effect and magneto-resistance effect, in terms of the relevant scattering mechanisms and the structure of the conduction and valence bands. High-frequency phenomena, including microwave cyclotron resonance, and impurity conduction are also dealt with, but in far less detail. The theoretical foundation is laid in the first two sections, which introduce the solutions of the Boltzmann equation, specific to the germanium band structure (and non-degenerate statistics), and the calculation of relaxation times from a detailed investigation of scattering mechanisms. The last two sections deal respectively with the effects of elastic deformations and 'hot electron' problems.

In a review article of this nature, where many results are quoted and few are derived, no concessions are made to the beginner—nor, indeed, would they be expected. This is essentially a work for specialists who already possess an adequate grasp of the subject. The book (for as such it must be regarded) suffers from one minor but irritating disadvantage: if it is opened at random, there is no clue, either from the page heading or from the labelling of the equations, which indicates the exact place. The list of contents at the beginning does not help since page numbers are not given. An adequate index is, however, provided. As a source of information on a field of much diversity and elaboration, this article should provide a useful work of reference for experimentalists and theoreticians who are directly concerned with the electrical properties of germanium.

B. Donovan

VERTEBRATE ETHOLOGY

Social Behaviour and Organization among Vertebrates
Edited by William Etkin. Pp. xii+307. (Chicago and London: The University of Chicago Press, 1964.) 56*s*.

SOCIAL Behaviour and Organization among Vertebrates is a collective work developed as a result of the activities of the Textbook Committee of the Section of Animal Behaviour of the American Society of Zoologists. It aims at reviewing the activities of the past twenty-five years in the field of the social behaviour of animals as pursued not only by zoologists but also by experimental and clinical psychologists and other students of the behavioural sciences. Besides editing the work, William Etkin contributes four chapters which determine the scope and character of the book. The other five co-authors, Beach, Davis, Lehrman, Scott and Tinbergen, contribute a chapter each.

The book is wide-ranging and, for a co-operative work, well balanced. It gives a good general account, on the whole reasonably objective, of the contribution of modern ethology, and discusses such problems as the neuro-endocrine correlation in invertebrates, the physiological analysis of aggressive behaviour, the control of behaviour cycles in reproduction, and types of social organization in birds and mammals. The book extends far beyond the bounds hitherto considered respectable by the stimulus-response school of psychology so characteristic of North American thinking, and attempts, not always successfully, to get to grips with ethological principles, to interpret them and to relate them to sociological ideas and physiological facts. Topics as diverse as brain structure, "action-specific energy", drive, ritualization, imprinting, and innate releasive mechanisms are given special attention.

The significance of animal play is dealt with, and the widespread importance of the exploratory drive is understood. The views expressed as to the significance of the idea of instinct and the innate are somewhat muddled, and some of the very relevant contributions bearing on these problems, provided by European research workers (notably von Holst and Sokolov), are unaccountably omitted. Topics which seem to demand much more attention than they receive include homing and the orientation problem, and the development and spread of behavioural traditions in animals. A particularly excellent feature of the book is the abundance of clear and relevant illustrations coming from a wide variety of sources, some of them surprisingly and undeservedly unfamiliar. The work should be a valuable antidote for the students now being indoctrinated with the view that the whole of biology can really be summed up in the letters 'DNA'.

W. H. Thorpe

Introduction to Satellite Geodesy

By Prof. Ivan I. Mueller. Pp. xxi+415. (New York: Frederick Ungar Publishing Co., 1964.) 15 dollars.

THIS book was written primarily as a text for students in the Department of Geodetic Science at Ohio State University, where the author is an associate professor. It is, consequently, an extremely specialized work, and in some parts reads more like lecture-notes than a text-book; for example, it begins uncompromisingly with a 9-page list of symbols. The subjects treated fall into two main divisions: the first, of 130 pages, covers the theory of occultations and solar eclipses, and their geodetic uses, in great detail and with many worked examples; the second division, which occupies some 250 pages, is devoted to artificial satellites and their geodetic applications. The subjects covered here include close-satellite orbital theory, methods of observation, the uses of satellites as 'triangulation points' for geometrical geodesy, and dynamical investigations using satellite orbits. The book ends with a specification for geodetic satellites, and the National Aeronautics and Space Administration *Satellite Situation Report* for September 30, 1963.

The author shows a very wide knowledge of the specialized literature and uses it most judiciously. More than 700 papers are listed in his bibliographies and from these he has extracted and presented the methods he likes best. The result is an excellent introductory text-book in satellite geodesy, comprehensive, up to date and well balanced. Readers should not be deterred by the unhappy blurb on the jacket: the publishers were unwise to associate nationalistic sentiment with a subject which, above all others, is world-wide. Though the book will not appeal to the general reader, those working on the subject will welcome it gratefully as a reference book; and any university which contemplates adding some space-age investigations to its syllabus will find here a ready-made course.

D. G. King-Hele

Inorganic Ultramicroanalysis

(International Series of Monographs on Analytical Chemistry, Vol. 15.) By I. P. Alimarin and M. N. Petrikova. Translated by M. G. Hell. (Oxford, London, New York and Paris: Pergamon Press, 1964.) 40*s*.

ULTRAMICROANALYSIS concerns itself with quantities of materials ranging from micrograms to picograms, normal concentrations of reactant solutions being maintained by the use of small volumes of liquid media.

The present monograph collates and systematizes the information on those techniques of inorganic ultramicroanalysis which have been described in the literature since the time of the earlier publications of Benedetti-Pilcher and his colleagues. It does not embrace the radiochemical work of McMillan, Abelson and Seaborg which led to the isolation of minute quantities of the first transuranic elements, neptunium and plutonium. The only mention of radioactive techniques in the book refers to the use of silver-110 and RaE to demonstrate the decrease in the adsorption on the walls of micro-vessels which have been coated with methyltrichlorsilane.

General ultramicro apparatus of capillary type is described in great detail; the use of various microscopes and micromanipulators is illustrated; condenser rods and fibre reactions are demonstrated. Moist chambers, centrifuges, micro-heaters and coloriscopic capillary cuvettes receive comprehensive treatment.

Wilson's qualitative scheme for systematic qualitative analysis is presented in didactic fashion. Micro methods for electrolysis, extraction and ion-exchange separation are lucidly described. Quantitative ultramicroanalysis occupies half the total text of the book. Ultramicro balances of many kinds are described with much theoretical detail, and other techniques for weighing are mentioned briefly. Microtitrations, with either colorimetric or potentiometric endpoints, are described and the amperometric method developed. The volume ends with a concise description of the uses of the Holter–Malmstrom microcolorimeter and of the Beckmann ultramicroanalytical spectrophotometer.

Prof. Alimarin and Dr. Petrikova are to be congratulated on having written an excellent and comprehensive introduction to a subject which is slowly asserting its importance in the fields of research and industrial chemistry. This publication has considerable didactic value and will also be welcomed by lecturers in universities and technical colleges.

D. T. Lewis

Chromatography in Geology

By Arthur S. Ritchie. (Methods in Geochemistry and Geophysics Series.) Pp. viii+185. (Amsterdam, London and New York: Elsevier Publishing Company, 1964.) 50*s*.

THIS slight text of around 50,000 words sails under false colours. It concludes with the statement that "in theoretical geology, chromatographic processes have become recognized as being of the greatest importance"—but all that is said on this topic amounts to no more than three short pages of obscure observations on gels and colloids. A more appropriate title for the book would have been "Chromatography in Inorganic Mineral Analysis", for after introductory chapters on "The Chromatographic Process" and "Chromatographic Techniques" it is with the analysis of soils, minerals and waters that the main section "Applications of Chromatography to Geology" is wholly concerned. It is, however, remarkable that a work concerned with the use of chromatographic techniques in the mineral industry should fail to mention not only the pioneer researches of the late F. H. Burstall and his colleagues at the Chemical Research Laboratory, during the early days of the British atomic energy effort, but also the milliard-dollar uranium industry utilizing the anion exchange resins then introduced. From the point of view of the academic geochemist, the omission of any reference to the importance of chromatographic techniques in recent American studies on palæobiochemistry is equally striking. Perhaps it is understandable that no literature from the U.S.S.R. should be quoted, but to write on the role of gels in mineral genesis without even mentioning Chukrov's Russian-language monograph *Colloids in the Earth's Crust* seems strangely inadequate. The 30-page index which helps to swell the book is for the most part unnecessary.

C. F. Davidson

Annual Rings in Big Sagebrush, *Artemisia tridentata*

By Charles Wesley Ferguson. (Papers of the Laboratory of Tree-Ring Research, No. 1.) Pp. viii + 95. (Tucson: University of Arizona Press, 1964.) n.p.

IT is well known that after felling a woody plant with annual growth rings, it is possible to determine its length of life by counting the growth increments on a transversely cut surface. Experts in dendrochronology can go a stage further, however, for by examining the growth ring pattern in a specimen that has long been dead, they can assess the probable date when it was formerly alive. Furthermore, with proper precautions, the width of successive annual rings can be used to draw conclusions concerning change in climate and ecological conditions as well as about the dates of archæological sites. With these aims in view, the author of this booklet has made an intensive investigation of the annual rings in big sagebrush (*Artemisia tridentata* Nutt.) of the family Compositae, collected from selected areas in the western United States. *A. tridentata* is of special interest because of its capacity to 'invade' areas that are capable of growing, or which have grown, a grass cover. The structure of the plant is of interest to anatomists because of its ability to produce successive rings of interxylary cork in the wood.

C. R. Metcalfe

A COMPARISON BETWEEN THE OLDUVAI HOMININES AND THOSE OF JAVA AND SOME IMPLICATIONS FOR HOMINID PHYLOGENY*

By Prof. P. V. TOBIAS
Department of Anatomy, University of the Witwatersrand, Johannesburg
AND
Prof. G. H. R. VON KOENIGSWALD
Department of Palæontology, Rijksuniversiteit, Utrecht

DURING June 11–13, 1964, we enjoyed the unique opportunity of comparing directly a large selection of Javanese fossil jaws and teeth, as well as a small number of Chinese fossils, with the hominid material discovered by the Leakeys at Olduvai and Natron, Tanganyika. One of us (P. V. T.) had been working at Cambridge for some time on the original African fossils, through the courtesy of Dr. L. S. B. Leakey; the other (G. H. R. v. K.) visited Cambridge from Holland in company with a good number of the Asian fossil originals. Full and free discussion, comparison and measurement took place for three days.

Materials Compared

African: The mandible, parietals, upper molar and sundry additional cranial fragments of the type specimen of *Homo habilis*.

The mandibular fragment, left lower molar, premolar and another dental fragment, from site *BK*I, the lowest living floor in the Olduvai sequence to yield remains of *H. habilis*.

Three unworn, perfectly preserved, isolated teeth of *H. habilis*, and other cranial fragments, found on the same living floor as yielded *Australopithecus* (*Zinjanthropus*) *boisei* (*FLK NI*).

The remains from the lower part of Bed II, namely, the well-preserved mandible with complete lower dentition, as well as parts of left and right maxillæ with 8 upper premolars and molars, and calvaria, of the advanced hominine from *MNK*II.

The teeth of Olduvai 15 and 16, the former of which was reported in paragraph *D* of Leakey and Leakey[1] but not allocated to any taxonomic category, while the latter was provisionally allocated to *Homo habilis*[2]; both of which dentitions one of us (P. V. T.) has since concluded are, in fact, within the range of australopithecine teeth and should therefore not be included with the *Homo habilis* remains.

The mandible of a large-toothed australopithecine from Lake Natron in northern Tanganyika[1].

Casts of a number of South African australopithecine fossils, as well as of the mandible of the Swartkrans hominine ('*Telanthropus*' I).

Asian: The mandibular fragment of *Meganthropus palaeojavanicus* I; a second, unpublished mandibular fragment with two molars, referred to briefly by von Koenigswald[3] and attributed to *M. palaeojavanicus* (*Meganthropus* II).

The Sangiran *B* mandible, classified by Weidenreich[4] as *Pithecanthropus robustus* and by von Koenigswald[3] as *P. modjokertensis*, but included in more recent classifications under *Homo erectus erectus*[5].

The maxilla of '*Pithecanthropus*' IV (claimed by von Koenigswald[3] as a Djetis Beds pithecanthropine).

The 1939 mandible designated by von Koenigswald *P. dubius*[6].

Two groups of teeth assigned by von Koenigswald to *Sinanthropus officinalis*[7] and to *Hemanthropus peii*[8], respectively.

Numbers of other isolated teeth from China and Java, including some thought to have belonged to Peking man.

Observations

(1) *The Lower Bed II Hominine of Olduvai.* The most striking fact to emerge from the comparisons is that the jaws and teeth of the lower Bed II hominine, which had been included as a late paratype of *Homo habilis*[2], are extremely close in morphology to those of *Pithecanthropus* IV and Sangiran *B* (Figs. 1 and 2).



Fig. 1. Right maxilla of Olduvai hominid 13, from the lower part of Bed II, compared with the '*Pithecanthropus*' IV maxilla from Java



Fig. 2. Mandible of Olduvai hominid 13, from the lower part of Bed II, compared with the Sangiran *B* mandible from Java

* Since the two authors do not follow the same classification, we have wherever possible used vernacular names (for example, habiline, pithecanthropine), or names based on type localities (for example, Olduvai Bed II), etc., to designate specimens, or we have stated the synonyms (for example, *Pithecanthropus modjokertensis* (von Koenigswald) 1936, *Pithecanthropus robustus* Weidenreich 1945).

In mesiodistal lengths, the upper and lower teeth of the two forms are almost identical, as Table 1 indicates. However, the Javanese teeth are somewhat broader, in buccolingual diameter.

Table 1

		Olduvai Bed II hominine (Olduvai 13)	Swartkrans hominine ('*Telanthropus*' I)	Javanese hominine (*Pithecanthropus* IV maxillæ and Sangiran *B* mandible)
Maxillary teeth				
P^3	*M–D*	8·3		8·4
	B–L	11·4		12·3
P^4	*M–D*	8·7		8·2
	B–L	11·5		12·0
M^1	*M–D*	12·5		12·1
	B–L	12·6		13·6
M^2	*M–D*	12·8		13·5
	B–L	13·8		15·5
M^3	*M–D*	12·0		10·7
	B–L	13·1		13·8
Mandibular teeth				
P_4	*M–D*	8·9		9·0
	B–L	9·8		10·9
M_1	*M–D*	12·7	11·8*–11·9*	12·8
	B–L	11·6	11·5–11·9	13·0
M_2	*M–D*	13·8	13·3–13·6	13·5
	B–L	12·2	12·3–13·1	13·4
M_3	*M–D*	14·6	14·1, 14·1	14·6
	B–L	12·5	12·4, 12·4	12·5

* Shortened by approximal attrition.

Both sets of tooth measurements were made by the same observer (P. V. T.), using the same technique with the same special instrument, on the same occasion. The total mandibular molar length of Olduvai 13 is 41·1 mm, that of Sangiran *B* 40·9; the total mandibular premolar–molar length ($P_4 - M_3$) is 50·0 for Olduvai 13, and 49·9 for Sangiran *B*. Similarly, for the maxillary teeth, the total molar length is 37·3 (Olduvai 13) and 36·3 (*Pithecanthropus* IV), while the total premolar–molar length (P^3–M^3) is 54·3 (Olduvai 13) and 52·9 (*Pithecanthropus* IV).

The dental dimensions for '*Telanthropus*' I are extremely close to those for M_2 and M_3, but M_1 is shortened by approximal attrition. The values for the left and right '*Telanthropus*' I teeth are given in that order in the third column of Table 1.

The Sangiran, Olduvai 13 and '*Telanthropus*' I specimens agree in having M_3 longer than M_2 and M_1, as in the Montmaurin mandible, but in contrast with the dentitions of Choukoutien, Heidelberg and Ternifine. The large talonid, which is so marked a feature of the mandibular premolars of *Homo habilis*, is encountered as well in the Sangiran *B* mandible; on the right P_4 the absolute talonid length is 2·6 mm in Sangiran *B* and 2·8 in Olduvai 13 and the relative talonid length (talonid index) is 2·27 and 2·04 respectively, indicating a relatively bigger talonid in the Olduvai specimen.

Among the differences between the two forms are the greater depth of the Sangiran mandible (34·3 mm at M_1 as against 26·3 in Olduvai 13), the presence in the Asian form of 4 mental foramina but in the African form of only 1, as well as the aforementioned greater breadth of the Asian teeth.

So far as the cranial capacity is concerned, an estimate of the capacity in the Bed II hominine has not yet been obtained; at the same time, we both feel that the capacity of the various pithecanthropine crania should be re-determined, since reconstruction of missing parts of the calvariæ can now be based on better samples of related crania. For the moment, the resemblance between the two specimens rests on dental and gnathic traits.

It has been pointed out elsewhere[9,10] that the Bed II hominine exhibits the same morphological departures from the australopithecine pattern of teeth and jaws, as do the Bed I habilines, but to a more marked degree. In fact, the reduction of tooth size, both of crowns and roots, would seem to have carried the Bed II specimen, Olduvai 13, to a dental size slightly smaller than the corresponding teeth in the pithecanthropine specimens. This applies as well to the body of the mandible and the size and general morphology of the maxillæ. In these respects, the Bed I habilines are less advanced in a hominizing direction than that of Bed II and are definitely more primitive than any pithecanthropine from Java. In the light of the foregoing, we think it wisest to keep the Bed II hominines separate from the Bed I habilines.

Our general conclusion about the Bed II hominine, Olduvai 13, is that, dentally and gnathically, it would seem to represent an African counterpart of the *modjokertensis* or *robustus* grade of hominid organization which occurs in Java. On the basis of the '*Telanthropus*' I mandible, the only '*Telanthropus*' specimen of which a cast has been available to us, it would seem that '*Telanthropus*' fits into the same group: to this extent we would support Simonetta's[11] attribution of '*Telanthropus*' to *Pithecanthropus* and Robinson's[12] attribution of it to *Homo erectus*.

(2) *The Bed I Hominine of Olduvai.* As regards the Bed I *H. habilis*, we are agreed that this represents a distinct taxon, showing an advance over the australopithecine grade of organization, but not yet on the pithecanthropine (*H. erectus*) grade. Morphologically, the Bed. I *H. habilis* appears to be on the hominid line from *Australopithecus africanus* and possibly leading to a pithecanthropine grade of organization.

The Bed I *H. habilis* is certainly more primitive than any of the pithecanthropine fossils from Java. Dentally, there is a resemblance between it and *Meganthropus palaeojavanicus* (Fig. 3). The *Meganthropus* type specimen is somewhat bigger-toothed than the Bed I *H. habilis*. We had before us a further unpublished mandible from Java, attributed to *Meganthropus*, but unfortunately no casts of Marks's[13] specimen nor the recently published pithecanthropine mandible of Sartono[14], both of which were discovered in the Djetis Beds at Sangiran. The tooth measurements given in Table 2 were made by one of us (P. V. T.) on the same occasion, on the type specimen of *Meganthropus palaeojavanicus* (*Meganthropus* I), on the unpublished mandibular fragment (*Meganthropus* II) with 2nd and 3rd molars, and on the type specimen of *Homo habilis*.

Table 2

		Bed I *H. habilis* (range)	Bed I *H. habilis* (type)	*Meganthropus* I (type)	*Meganthropus* II (unpublished)
P_3	*M–D*	9·5–9·9 ($n=3$)	9·6, 9·9	10·1	—
	B–L	9·5–10·3 ($n=3$)	10·3, 10·1	11·7	—
P_4	*M–D*	10·3–11·1 ($n=3$)	10·3, 11·1	9·8	—
	B–L	10·7–11·3 ($n=3$)	10·7, 10·7	11·9	—
M_1	*M–D*	14·3 ($n=2$)	14·3, 14·3	14·8	
	B–L	12·2–12·4 ($n=2$)	12·2, 12·4	13·6	—
M_2	*M–D*	15·6 ($n=1$)	15·6, —	—	14·4
	B–L	13·5 ($n=1$)	13·5, —	—	13·8

For the type specimens of *H. habilis* and *M. palaeojavanicus*, the premolar-molar length (P_3–M_1) is: *H. habilis* 34·2 (left), 35·3 (right); *Meganthropus* I: 34·7 (right), that is, the *Meganthropus* I value is midway between the values for the left and right sides of the *H. habilis* type jaw.

The P_3 in both African and Asian forms has a high crest connecting the buccal and lingual cusps; but the lingual cusp of *H. habilis* is higher than in *Meganthropus*. Both have a well-developed anterior fovea and in both the talonid is well developed. The talonid length in *H. habilis* is 2·6 and in *Meganthropus* 3·2 mm long, giving talonid indices of 2·15 and 1·91 respectively; in other words, the talonid is both absolutely and relatively somewhat longer in *Meganthropus* than in *H. habilis*. The shape



Fig. 3. Mandible of the type specimen of *Homo habilis* from site *FLKNNI*, Bed I, Olduvai Gorge, compared with the type specimen of *Meganthropus palaeojavanicus* from Java

indices (L/B per cent) are 93·2 (left) and 98·0 (right) in *H. habilis* and 86·3 in *Meganthropus*.

In both forms, P_4 has very similar cusps. Both have a long talonid, measuring in mesiodistal extent 3·8 in *H. habilis* and 3·5 in *Meganthropus*, giving very similar indices of 1·63 and 1·71 respectively. The talonid of *H. habilis* is more distally situated, that of *Meganthropus* is slewed around to a more distolingual position. The shape indices are 96·3 (left) and 103·7 (right) in *H. habilis* and 82·4 in *Meganthropus*.

The first molars are very similar in cusp pattern and both have an anterior fovea, but the M_1 of *H. habilis* is appreciably narrower in buccolingual diameter than its homologue in *Meganthropus* (L/B index in *H. habilis* is 117·2 on the left and 115·3 on the right; in *Meganthropus* 108·8).

The shape indices show that the teeth of *H. habilis* are more markedly elongated and narrowed than those of *Meganthropus*; in fact, the *H. habilis* indices for all three teeth fall completely outside the range of indices for 21 australopithecine P_3's, 20 australopithecine P_4's and 31 australopithecine M_1's, save for one Sterkfontein tooth (*Sts* 24), which has a higher index than the right M_1 of *H. habilis* but not as high as the left M_1.

Robinson[15,16] has suggested that *Meganthropus* is simply an australopithecine. While we agree with him in respect of the African (Garusi, Tanganyika) specimen assigned to *Meganthropus africanus*, we feel that *Meganthropus* of Java shows certain advances over the australopithecine grade of hominid organization. Most striking is the posterior surface of the symphyseal region which is well preserved in the type specimen of *Meganthropus*: the structure is plane with small genial tubercles. There is no deep pit nor massive inferior transverse torus as in the Natron jaw and the large *A. robustus crassidens* (*Paranthropus*) jaws. In this respect, the surface of the post-symphyseal region in *M. palaeojavanicus* strongly resembles that in the pithecanthropines (*Homo erectus*) of Asia and Africa. The somewhat reduced tooth roots are reflected in the relatively high position of the mental foramen, which is just nearer the top edge than the lower edge of the corpus mandibulæ; whereas in *A. robustus* and *A. boisei* it is nearer the lower edge. For example, if we express the projection height of the foramen from the upper edge of the mandibular body as a percentage of the total projection height, we obtain a value of 54·3 per cent in the Natron (australopithecine) jaw; whereas in *Meganthropus* I the value is 49·5 and in an obviously more hominized specimen, namely, that of the Bed II Olduvai 13, the value is 45·9. A further expression of the smaller tooth-roots in *Meganthropus* I is the lesser robusticity of the alveolar portion[7] of the mandible: the most robust part is the lower margin (torus marginalis), whereas in the Natron jaw as well as the large *A. robustus* jaws from Swartkrans, the alveolar portion is far more robust, whereas the lower margin is much less massive, giving a characteristic tapering section from the alveolar girth above to the marginal girth below.

Our feeling about *Meganthropus palaeojavanicus* is that, while it has some strong australopithecine resemblances, it shows several features in which it is somewhat advanced beyond the australopithecine grade of organization. In this sense, it stands in relation to *Australopithecus* as does *Homo habilis*, except that *Homo habilis* has departed further in some respects, such as dental reduction and elongation.

(3) A further parallel between the Asian and African fossils is provided by the more advanced pithecanthropines of the Trinil Beds and of Choukoutien, on one hand, and those of north-west Africa (*Atlanthropus*) and of the upper part of Bed II, Olduvai ('Chellean Man') on the other.

Conclusions

It would seem to us that four grades of hominization can be recognized in the African and Asian sequence of the Lower and early Middle Pleistocene:

1st Grade: *Australopithecus* in South and East Africa; ? in Asia.

2nd Grade: *Homo habilis* in Africa; ? *Meganthropus* in Asia.

3rd Grade: Olduvai 13 and the Swartkrans hominine ('*Telanthropus*') in Africa; the Sangiran *B* mandible and *Pithecanthropus* IV in Asia.

4th Grade: 'Chellean Man' and *Atlanthropus* in Africa; the Trinil Beds hominid (*P. erectus* or *Homo erectus erectus*) and the Choukoutien hominid (*P. pekinensis* or *Homo erectus pekinensis*) in Asia.

There is as yet no general agreement as to the taxonomic status which should be accorded these grades. The 2nd grade is best represented by the Bed I *H. habilis* material, since no cranium or maxilla of *Meganthropus* is known for sure. One of us (G. H. R. v. K.) thinks that this grade should be accorded separate generic or subgeneric status; Leakey, Tobias and Napier[2] have proposed separate specific status within the genus *Homo*; while yet others have proposed either in discussion or in writing (D. R. Hughes, *The Times* of London, June 10, 1964) that sub-specific status within the species *Homo erectus* should be accorded the *H. habilis* remains, that is, that they become *Homo erectus habilis*.

As for the 3rd grade, some have given the hominid represented by *Pithecanthropus* IV and Sangiran *B* separate specific ranking within the genus *Pithecanthropus* (*P. robustus* or *P. modjokertensis*). On the other hand, in one of the most recent classifications[5], it has been lumped into the same sub-species as the Trinil Beds hominid, that is, *Homo erectus erectus*.

We do not propose here to enter into a discussion of the taxonomy of the 4th grade of hominization.

Thus, from the 2nd grade onwards, there are remarkable parallels between the Asian and African sequences. However, we remain unconvinced that there is as yet any unequivocal evidence pointing to the presence in Asia of a frankly australopithecine grade of organization. Simons[17] has suggested that the teeth designated *Hemanthropus peii*[8] are australopithecine. It is not impossible, too, that they may represent the 2nd (habiline or meganthropine) grade of post-australopithecine differentiation; we should feel much happier to have such teeth in a mandible or cranium before accepting either proposition.

This work was supported by the Wenner-Gren Foundation for Anthropological Research, the South African

Council for Scientific and Industrial Research and by the Boise Fund.

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METEOROLOGICAL FACTORS IN RADIOCOMMUNICATIONS AT VERY SHORT WAVE-LENGTHS*

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THE history of the development of radiocommunications shows that there has been a constant interchange between the mathematician or theoretical physicist and the engineer keen on achieving rapid practical results. It is also of interest to note the manner in which the various portions of the electromagnetic wave spectrum have been exploited for communication and other practical aims. The classical laboratory work of Heinrich Hertz on the physical properties of electric waves was carried out at frequencies of a few hundred megacycles per second. In 1896 Marconi demonstrated the transmission of waves of 1,000 Mc/s (corresponding to a wave-length of 30 cm) over distances of a few kilometres. However, the practical use of such short waves did not at the time appear very promising; and in the endeavour to increase the range of transmission, more power had to be supplied to an antenna system of greater height and length, resulting in the generation of waves of lower frequency. The successful achievement of communication over thousands of kilometres greatly surprised those theoretical workers who had assumed that diffraction was the only mechanism by which such waves could overcome the curved surface of the Earth. For the next quarter of a century, the world's radiocommunications were developed at wave-lengths corresponding to the medium- and high-frequency portion of the spectrum (300 kc/s–30 Mc/s). As has long been well known, the propagation of such waves over all but the shortest distances—up to, say, 100 or 200 km—is determined by reflexion from the ionized regions of the Earth's surface.

About 1916, the practical possibilities of transmitting and receiving waves at frequencies of about 50–100 Mc/s were being explored. Again, it was considered that since waves of such frequencies were unlikely to be efficiently reflected from the regions of the ionosphere, the range of transmission would be limited approximately to the optical horizon distance. But once more it was found by practical experiment that the range of transmission could exceed such a distance. and, furthermore, that the amount of this excess varied with atmospheric conditions which themselves depended on season, time and portion of the world in which the experiments were conducted. These effects were encountered to a more marked extent as investigations carried the frequencies used from hundreds to thousands of megacycles per second. Since it was now clear that the ionization of the atmosphere could have little effect in determining the path of waves of such very high frequencies, it was necessary to examine more closely the normal physical properties of the troposphere—temperature, pressure and especially the humidity —in order to explain the phenomena which accompany the propagation of such waves over various distances beyond the normal horizon.

In this way began that portion of the science of radiometeorology, which is concerned with the influence of meteorological conditions on the propagation of radio waves, and particularly on practical radiocommunications. By about 1930 there was experimental evidence sufficient to indicate that radio waves of frequencies in excess of 50 Mc/s could be received at distances well beyond the optical horizon of the sending antenna. When the transmission was over a land path, it was affected by diffraction over hills as well as by refraction in the atmosphere. For cases in which the transmission was over a sea path, the changing atmospheric conditions could result in variable refraction effects with accompanying variations in received signal strength. In particular, it was found that for transmission over sea in warm climatic conditions, such as prevail in the Mediterranean area or the Indian Ocean, the bending of the waves could approach, and even exceed, the curvature of the Earth; and result in what became known as 'abnormal propagation' conditions. Such phenomena of super-refraction have been the subject of extensive research in many parts of the world, but they form part only of the general influence of meteorological conditions on the propagation of waves of very- and ultra-high frequencies and above, and their application to radiocommunication problems.

Propagation of Radio Waves by Tropospheric Refraction

In 1893, Lord Rayleigh considered the problem of the refraction in the Earth's atmosphere of incoming rays of light from stars and other celestial bodies. It has thus been known throughout the history of radio development that the atmosphere can act as a refracting medium for electromagnetic waves passing through it; but this phenomenon was not of any great significance in the use of frequencies below 30 Mc/s. As already mentioned, experimental evidence of such refraction became available in about the 1930's when several radiocommunication links were established in various parts of the world on frequencies of about 40 Mc/s and above, and over distances that were well beyond the limits of the geometrical horizon. In the following decade, the exploitation of frequencies up to several thousand megacycles per second, for both radar and communications, provided ample evidence of similar long-distance transmission around the curved surface of the Earth.

(*a*) *Standard tropospheric wave propagation.* For the past decade or more the propagation of radio waves of

* Substance of a lecture given at the NATO Summer School on Radiometeorology at Lagonissi, Greece, on September 10.

frequencies between 30 and 30,000 Mc/s has been investigated by scientists and engineers in various parts of the world; and the resulting experience has been co-ordinated by the appropriate international radio organizations—URSI (the International Scientific Radio Union) and CCIR (the International Radio Consultative Committee)—in collaboration, as appropriate, with WMO (the World Meteorological Organization). As a result, there has been a large measure of international agreement on the description of the Earth's atmosphere, under what is conveniently termed standard and non-standard conditions (see ref. 8).

The refracting properties of the Earth's atmosphere may be expressed by the parameter 'N' in the following expression:

$$N = (n - 1)\,10^6 = \frac{77{\cdot}6}{T}\left(p + 4810\frac{e}{T}\right) \qquad (1)$$

where n = refractive index of the air, T = absolute temperature (° K), e = water vapour pressure (mbar), p = atmosphere pressure (mbar).

Under all conditions normally encountered in the lower atmosphere, the value of n is a few hundred parts in a million greater than unity. While this absolute value of the refractive index is not of great importance in radio wave propagation, it is now well established that small variations in its value, even of one or two parts in a million, may be a determining factor in the phenomena associated with the transmission of waves through the troposphere. Hence the use of the symbol N as a unit, in terms of which the variations of atmospheric conditions are conveniently expressed.

When considering the path of radio waves transmitted between any two points, it is necessary to realize that the refraction occurs relative to a curved Earth. Hence it had become the practice among scientists and engineers working in this field to make use of a 'modified refractive index', defined as the sum of the refractive index for a given height above sea-level and the ratio of this height to the radius of the Earth. It is convenient to make use of this modified index in terms of a refractive modulus, M, the unit of which is one million times the amount by which the modified refractive index exceeds unity. Thus:

$$M = \left\{(n - 1) + \frac{h}{a}\right\} \times 10^6 \qquad (2)$$

For international research in this field of tropospheric wave propagation, it is furthermore convenient to define for reference purposes a standard radio atmosphere as one in which the refractive modulus, M, varies uniformly with height above the Earth's surface. The gradient of this modulus, which is considered as normal for a well-mixed atmosphere, has a value of 0·12 M units/m (equal to 3·6 M units/100 ft.). In the past, it has been found convenient to associate normal refraction conditions with a standard atmosphere having this gradient of modulus of refractive index; and it has been customary to simplify the calculations by using an effective radius of curvature of the Earth of 4/3 times the actual radius.

More recent research, however, has led to the definition of a 'basic reference atmosphere' in the following form:

$$n(h) = 1 + \{289 \times 10^{-6} \exp(-0{\cdot}136h)\} \qquad (3)$$

where h is the height above sea-level (km). It is to be noted that the refractive index in the first kilometre of such a basic reference atmosphere is very nearly equal to that in an atmosphere corresponding to an Earth of effective radius of 4/3 the actual or geometrical radius. The use of the exponential relationship (3) is more appropriate for the calculation of field strengths under practical conditions, and it has the added advantage of being more accurate when the height of either sending or receiving stations is greater than 1 km.

In later research, it has been observed that the variations of the mean values of the refractive index of the atmosphere may often be approximately represented by the general exponential formula:

$$n(h) = 1 + N_s \exp(-bh) \times 10^{-6} \qquad (4)$$

in which, as before, $N = (n - 1) \times 10^6$ and the subscript s refers to the values at the surface of the Earth, h is the height above the Earth in kilometres, and b is determined by the relation:

$$\exp(-b) = 1 + \Delta N/N_s \qquad (5)$$

where ΔN (a negative quantity) is the difference between the values of N at the surface of the Earth and 1 km above it.

Some investigations have shown that ΔN is in general correlated with the surface value N_s, and typical values according to climate have been deduced as follows:

$\Delta N = -7{\cdot}3 \exp(0{\cdot}0056\,N_s)$ in the United States.
$-9{\cdot}3 \exp(0{\cdot}0046\,N_s)$ in the Federal Republic of Germany.
$-3{\cdot}9 \exp(0{\cdot}0072\,N_s)$ in the United Kingdom.

These formulæ are useful for estimating values of ΔN in cases where only surface meteorological data are available.

(*b*) *Non-standard wave propagation.* It has been assumed here that the Earth's atmosphere is a well-mixed medium, which results in a steady and uniform refraction of radio waves transmitted through it. Many years of practical experience have shown that radio waves are often transmitted to distances well beyond the range to be expected from the assumption of a standard atmosphere. Sometimes in such cases reception is fairly steady; but in other cases the strength of the received signals varies between wide limits. Experience with radio sounding balloons has shown that there are times when sudden changes in atmospheric conditions can result in the formation of a reflecting layer, so that in extreme cases the radio waves are in effect trapped in a wave-guide formed by the layer and the Earth's surface. The existence of such a stable and super-refracting layer at an appropriate height in the atmosphere can thus give rise to a marked increase in signal strength and range of reception. Conversely, on other occasions the atmosphere may be below standard in its refracting properties, and the range of reception is then reduced to that determined by rectilinear propagation. In many cases, the waves arrive at the receiver by more than one path with the accompaniment of interference effects and variation of the resulting signals.

Extensive investigations have been conducted in various parts of the world on the phenomena referred to here as 'super-refraction'. For the condition of partial or total reflexion, it is necessary to have a well-defined and widespread layer with marked changes in refractive index at its boundaries. The coefficient of reflexion increases, first, with the difference in the vertical gradient of refractive index inside and outside the layer; and secondly, with the thickness of the layer in relation to the wavelength of the radiation passing through it. For true reflexion conditions to prevail, there must be marked changes in atmospheric conditions within a height range that is not too large compared with the wave-length. Such considerations have been used to explain the transmission of radio waves in the metre and decimetre wavelength range (30–3,000 Mc/s), and the accompanying reception of unusually strong signals at exceptional distances. This extended range of reception is usually confined to the existence of stable anticyclonic meteorological conditions over the whole of the radio path, and is not acceptable for reliable radiocommunication. While these effects have on occasions in the past been useful in extending the range of detection of some radar systems, in more recent years they have been the cause of interference between television broadcasting systems. Stations which share the same frequency channel, and are separated

by a distance well outside their joint service areas, are occasionally subject to mutual interference due to abnormal propagation conditions over the path between them.

Propagation by Diffusion in a Stratified Atmosphere

For some years past the amount of experimental work on the propagation of radio waves in the metre and centimetre wave-bands has been rapidly accumulating; and in spite of attempts made by both the experimental and theoretical workers to explain their results, it became clear that neither of the simplified conceptions described here was entirely satisfactory. In particular, the rapid and variable fading of the received fields was not easily reconcilable with transmission through either a turbulent and well-mixed atmosphere, or one in which there was an extensive and stable 'wave-guide' formation. Furthermore, it is only in some parts of the world and at certain seasons of the year that the conditions in the lower atmosphere associated with anticyclones can be found to prevail sufficiently to explain the observed results of the radio transmission experiments.

A more detailed examination of the structure of the troposphere, aided to a considerable extent by the knowledge contributed by radio-soundings, shows that it is in general characterized by stratification accompanied by changes in refractive index of several N units. In typical cases, the horizontal dimensions of such layers may be about 1–3 km, with a thickness of 10–20 m, in which the changes in refractive index may have a mean value of 10 units. The thickness of such a layer may change by several metres along its length, with a corresponding change in N of 1 or 2 units in the layer. By examining the thermodynamics of the atmosphere, it seems reasonable to conclude that in place of the simple conditions of turbulence or no-turbulence, the atmosphere comprises both stable stratification and unstable layers. In certain cases, a systematic turbulence can result from thermal convection—perhaps resulting in the existence of several layers. In other cases, the motions of the air result in the formation of eddies or whirlpools within the stratified air. These conditions may, in general, exist simultaneously, so that one may obtain efficient transmission by refraction at the various layers accompanied by fading due to interference effects between waves arriving by different paths, or as the result of transmission through a turbulent atmosphere. Among the difficulties of the experimenter in this field is that of separating the variations in field strength with time from the spatial variations of the diffused signal, which is generally the integral of a number of component fields arriving by different paths and thus with varying differences in phase. Some scientists have attempted an experimental analysis by using a concentrated beam of radiation, which can scan the refractive medium through which the waves are transmitted. For a full exploitation of this technique, scanning in both vertical and horizontal directions would appear to be desirable, and the results displayed on suitable time bases at the receiver. When one considers that such investigations should be accompanied by the simultaneous use of the radio-sonde technique, the complexity of the whole problem is realized.

Propagation Curves for Frequencies between 40 and 1,000 Mc/s

In the preceding sections, reference has been made to the attempt to define the characteristics of the Earth's atmosphere in such a manner as to elucidate the mechanism determining the propagation of radio waves through this atmosphere. Concurrently with this research, extensive investigations have been made in various parts of the world of the experimental facts concerning the field strength of radio waves arriving from sending stations both within and beyond the horizon distance. There is a considerable amount of scientific and technical literature describing the results of such investigations (see refs. 1–10). In view of the need, however, to give practical guidance to the radio engineer concerned with the planning of communication systems including the sound and television broadcasting services, the appropriate international organization—the CCIR, to which reference has been made earlier—has co-ordinated the available material and issued its conclusions and recommendations[8].

These take the form of a series of curves showing on a statistical basis the field strength to be expected under a variety of conditions over distances up to 1,000 km from a sender radiating 1-kW effective power from a half-wave dipole. In the main recommendation (No. 370/1963), the curves were prepared from data obtained mainly in the United States and in Western Europe; and since many more measurements were made for distances up to about 500 km than above, the curves have their greatest reliability within this range. The recommendation already referred to should be read in conjunction with the contemporary *CCIR Report* (No. 239/1963), which describes the details of the construction of the curves, and the manner in which their interpretation depends on distances within or beyond the horizon, the influence of irregularities in the terrain, and the effect of transmission over a mixed land and sea path. The following *Report* (No. 240/1963) contains separate sets of curves, which have been drawn up on the basis of the somewhat limited data relating to the very varied conditions which prevail in the African continent, an area of rapid future development of radio and broadcasting services. For convenience, Africa has been divided into six regions, each corresponding to a fairly well-defined type of climate, ranging from temperate, sub-tropical (continental and maritime) to desert.

In all cases (Recommendation 370, and *Reports* 239 and 240) the sets of curves recommended for provisional use relate to two bands of frequencies, namely: (*a*) 40–250 Mc/s, and (*b*) 450–1,000 Mc/s. The individual curves apply to reception on a dipole antenna at a standard height of 10 m above ground, from transmitting antennæ at heights ranging from 37·5 to 1,200 m above the average level of the ground at distances between 3 and 15 km from the transmitter. Since the effect of irregularities of the terrain over which the waves are propagated is of greater importance at the higher frequencies, some guidance is given in the Recommendation (No. 370) on the estimation of the effect of such irregularities.

The curves are presented on a statistical basis, and show the field strengths to be expected at 50 per cent of the receiving locations, and for 50, 10 and 1 per cent of the time, respectively. In the case of transmission over land paths, the curves apply to temperate climatic conditions as encountered in parts of Europe and North America. As already mentioned, separate sets of curves are presented for the extreme conditions encountered in the African continent (*Report* 240). For propagation over sea, the relevant curves are applicable mainly to the North Sea area. In parts of the world, such as the Mediterranean and Gulf of Mexico, the received field strengths may exceed the value given in the curves, by amounts up to 20 dB at the lower frequencies, and by lesser amounts for shorter periods at higher frequencies.

While these curves represent the considered conclusions of experienced representatives from many countries interested in radiocommunications, it must be emphasized that the recommendations concerning their use should be considered as tentative pending a fuller understanding of the effect of meteorological conditions on the propagation of radio waves through the very varied atmospheric conditions encountered over both land and sea areas throughout the world. At successive assemblies of the experts at the international meetings of both URSI and CCIR, every attempt is made to co-ordinate the results

of further research, and to carry out the corresponding amendments to the recommendations on the application of the most recent knowledge to communication problems.

Influence of Atmosphere on Wave Propagation Curves

The preceding section summarizes the work done by the radio engineer in co-ordinating the results of field strength measurements on an international scale. Similar action was clearly necessary on the part of the meteorologists in presenting their conclusions on the relevant parameters of the atmosphere which may be used to adjust the estimates of field strength to any prevailing conditions throughout the world. In another *Report* (No. 233/1963), the CCIR has presented the results of an investigation of the correlation between propagation beyond the horizon and radio-meteorological parameters. For many years past, numerous measurements in temperate countries have taken into account the variation of refractive index with height above the Earth's surface. These have led to a steadily improving understanding of the bending of radio waves transmitted through the troposphere and of the resulting effect on the field strength obtained at a receiver from a distant transmitter beyond the normal optical range. While it is always desirable to have as complete knowledge as possible of the vertical gradient of refractive index of the atmosphere, experience suggests that for radiation at low angles of elevation above the horizon (less than 3°) the surface value of the refractive index may be a more useful parameter to apply than the vertical gradient of this index. Such a conclusion is, however, to be accepted tentatively pending the results of further research on a world-wide scale on the whole problem of the dependence of radio transmission on meteorological conditions in the troposphere. As an interim measure, therefore, the *CCIR Report* 233 already referred to contains a series of world-wide charts both for the index at the surface and for its vertical gradient, drawn up on the best information available for different times of the day, at each season of the year.

(*a*) *Refractive index at sea-level* N_0. The parameter N has been defined in equation (1) as a convenient unit representing the refractive index of the atmosphere in terms of its temperature, pressure and water vapour content. It is well known that the value of N decreases systematically with altitude; and therefore to avoid confusion between variations of N due to the altitude of an observatory and those due to climatology, it is desirable to reduce the value of N measured at the surface of the ground to the corresponding value N_0 at sea-level. This can be done on the assumption that the exponential relationship described by equation (4) is sufficiently representative of conditions over the range of heights required. Using a limited amount of meteorological information from more than three hundred stations in various parts of the world, values of N_0 were calculated from the formula:

$$N_0 = N_s \exp(0{\cdot}106h) \qquad (6)$$

From this relationship two world-wide contour charts have been prepared showing the mean values of N_0 for the representative months of February and August, and a third chart shows the contours of the yearly minimum monthly mean value of N_0. Owing to the limited number of locations for which measurements were available, the overall accuracy of these charts is of the order of several N units. In some areas, such as the United States, where most accurate and detailed observations have been made, the probable accuracy is correspondingly greater.

(*b*) *Charts of* ΔN. During the general investigation of the influence of radiometeorological phenomena on wave propagation through the troposphere, numerous measurements in temperate countries have taken into account the variation of the refractive index with height. To simplify the problem, the atmosphere has been represented by the difference $\Delta N \times 10^{-6}$ between the refractive index at a height of 1 km and the index at the ground. In a number of instances, theory and experiments agree in showing the existence of a correlation between the variations of the radio field strength observed and the quantity ΔN. While such a correlation has been demonstrated in temperate countries only, and on the basis of weekly or monthly median values, it has been considered worth while to prepare a series of 32 charts for certain areas in both the northern and southern hemispheres and for representative months and times of day (ref. 8). The main purpose of such charts is to indicate the wide variations of radioclimatology likely to be encountered throughout the world; at the same time, they do provide values of ΔN with an accuracy of the order of 5 N units. The general considerations embodied in these charts of values of N and ΔN may be applied to the field strength curves drawn up for the various parts of the African continent referred to in an earlier section.

Future Research in Radiometeorological Relationships

The previous sections have explained the manner in which the meteorologist's description of the lower atmosphere has proved useful to the radio engineer concerned with communications involving the propagation of waves through the troposphere. In so far as statistical results are useful to the engineer, and it must be said that they are most suitable for the planning of international communication services, then a detailed knowledge of the surface values of refractive index, in the form of the parameter N_s, meets the engineer's first requirements. If this information can be supplemented by a knowledge of the gradient of the index, ΔN, between arbitrarily chosen heights of the order of 1 km, over the path in which he is interested, then the communication engineer is grateful. He would be glad to encourage those who are adding to our knowledge of the value of ΔN under a variety of conditions by radiosonde measurements and radar soundings.

But it is to be noted that the radiometeorologists are not entirely agreed concerning the nature of the gradient of the refractive index of the air in the first kilometre or so of the atmosphere. An alternative equivalent gradient has been proposed and defined as that linear decrease of the index with height which would produce the same amount of ray bending as the actual inhomogeneous atmosphere existing over a given radio transmission path. Altogether it is well for the engineer to understand that the meteorologists may have some misgivings if a too-wide application of apparently good correlations between surface index and radio field strengths are accepted for general use. But the radio engineer has a wealth of practical experience of his own past achievements to guide him; and the main assistance he is seeking from the meteorologist is in the extension of this experience into the future and particularly into other areas of the world, where no radio experience is available, and where only limited surface meteorological measurements have so far been possible. To some extent the matter is complicated by the meteorologist's description of the atmosphere as a combination of stable layers and turbulent conditions; the proportions and extent of these clearly vary to a major degree with time and portion of the Earth under consideration. It is therefore to be hoped that the meteorologist will persist in his endeavour to understand more clearly the vagaries of our atmosphere, and will periodically produce improved guidance for the radio engineer in his endeavour to improve the world's communications services to the utmost extent required by our constantly expanding civilization. This is clearly a matter not only for collaboration between research workers in different faculties, but also for the closest

possible international collaboration among the scientists acquiring steadily advancing knowledge and the engineers who have the responsibility of applying this knowledge to useful ends.

REFERENCES

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SEALS

IN view of the considerable amount and variety of research carried out on seal biology during recent years a symposium was held in the Zoology Department, University of Cambridge, on September 9, under the auspices of the Grey Seals Consultative Committee, a body set up jointly by the Nature Conservancy and the Development Commission. The symposium was divided into two halves, the morning being devoted to those species of seal which are the object of a 'fishery' and the afternoon to the population structure, reproduction and behaviour of the grey seal, *Halichoerus grypus*.

The morning session was opened by Dr. E. B. Worthington, scientific director of the Nature Conservancy and chairman of the Grey Seals Consultative Committee. Following this, Dr. L. Harrison Matthews and Dr. R. M. Laws gave brief but compendious accounts of seals in relation to mankind in many parts of the world. Dr. Harrison Matthews discussed the various species which are subject to commercial exploitation; they are now those which gather in herds during the breeding season so that large numbers can be killed economically for oil, fur and leather; only in primitive communities is the flesh utilized. In many species, notably the Antarctic fur seals, exploitation has been controlled merely by economics; it was unregulated until the seals became so scarce that there was no longer any profit in hunting them. Then the fishery ceased and the numbers fortunately recovered. Yet, as he pointed out, most species are admirably adapted for economic exploitation combined with conservation of stocks. They are polygamous, so that there is an excess of bulls which can be cropped without reducing the breeding stock and a management policy on this basis has been strikingly successful in the Aleutians. Dr. Laws followed with a paper on the future prospects of seals as a natural resource, their interaction with fisheries and the possibilities of conservation. He thought all the various species would continue to be cropped, partly because they are valuable to man in various ways and partly also because in some areas and in some circumstances they are competitors with man for fish. Although he thought it likely that the value of fur and of oil would diminish he foresaw that the human population explosion would stimulate the cropping of seals for meat and would also be unfavourable to animals competing with man for fish. Accordingly, he saw no hope for a simple policy of protection and made a strong plea for a management policy of rational exploitation based on reliable information about seal populations. He contrasted the success of this policy in the case of the northern fur seal with the dangers of unregulated cropping as already demonstrated in several species of seal and, of course, scandalously apparent in whaling.

Six papers then followed on different species of seal. Three of them, one from New Zealand and two from the United States, were tape recordings and, although this was no real substitute for the presence of the authors in person, they did extend the interest and scope of the meeting. Mr. M. R. Smith of the University of Canterbury, New Zealand, gave an account of work on the population of Weddell seals in the McMurdo Sound in Antarctica; it was a progress report, but this work is clearly basic and fundamental, for this is a species which has not been and may never be exploited. Mr. F. O'Gorman gave an account of the numbers and distribution of the southern fur seal in South America, and was followed by a recorded talk by Drs. F. Wilke and V. B. Scheffer of the U.S. Fish and Wildlife Service on research under the Fur Seal Treaty. They told a remarkable and hopeful story, for although the Japanese denounced the 1911 Convention (which saved the Pacific fur seal from extinction and enabled stocks to recover) in 1941, because they feared the effect of the seals on their fisheries, and the nations concerned could not agree on a new treaty, they did in 1957 sign an interim convention which provides, among other things, for co-operative research. The speakers showed how fruitful this research is in providing a basis for management and were able to record that the seal population is growing steadily although an annual crop is taken. Mr. W. N. Bonner discussed the utilization of elephant seals in South Georgia. A crop of 6,000 adult bulls is taken annually and it seems that the stock can stand this rate, but only the skin and blubber are utilized. Mr. Bonner gave an account of the various attempts made to utilize the whole carcass and the difficulties that have been encountered. This paper was followed by another recorded talk, by Dr. D. E. Sergeant on Canadian research on harp seals in the north-west Atlantic. His investigation of the population dynamics of this species has shown that it is being over-exploited and has indicated the level at which the annual crop should be set.

The final paper of the morning was by Dr. A. W. Mansfield on the influence of pinnipeds on the fisheries of eastern Canada. This was a long paper discussing the status of four species of seal in the Gulf of St. Lawrence, the harp, hood, harbour or common, and grey. All species incur the enmity of fishermen as competitors for fish, but the first two are the basis of the sealing industry and have been over-exploited. A bounty is paid for the destruction of the harbour seal, but the population of grey seals is not great and the bounty is restricted to those caught in the Miramichi—the great salmon river of New Brunswick. He gave details of the diet of various species, but it is clear that many more are needed before the true effect on fisheries of seal populations can be assessed. He dealt at length with the problem of 'cod worm', a nematode found in the flesh of cod and other gadoids, the adults of which are found in the stomachs of various species of seal. This is also a growing problem in the north-east Atlantic.

The afternoon session was limited to studies of the grey seal, *Halichoerus grypus*, the species which has been the subject of so much controversy in recent years.

Prof. H. R. Hewer and Dr. K. M. Backhouse discussed the exceptional breeding-cycle. In Great Britain the young are born in the autumn and mating occurs shortly afterwards, but the implantation of the embryo is delayed and does not occur until the spring. In Canada and the Baltic the young are born in the spring and the relation between breeding season and gonad development is interesting but obscure. Prof. Hewer went on to discuss the life table which he has recently published[1], and showed that more material now available made little difference to it. This is clearly of great importance for a conservation policy. Dr. J. C. Coulson outlined the social breeding of the grey seal as exemplified on the Farne Islands and other breeding grounds around the coast of the British Isles. He showed that some degree of overcrowding (to our way of thinking) appears to be 'voluntary', and of the various hypotheses advanced to explain this phenomenon he strongly favoured Prof. V. C. Wynne-Edwards's view that this is a mechanism for population control. This is exciting ecology, and many of us were glad to be able to study it further in his joint paper with Mrs. G. Hickling[2]. A paper by Dr. J. Morton Boyd on the mortality of pups on North Rona gave some support to this theory, for it looks as if crowding is a potent factor in causing infantile mortality, and the audience clearly shared Dr. Boyd's hopes for a more intensive study of seal behaviour on this remote islet. Mr. E. A. Smith's observations on the effect of human interference on grey seal colonies added a good deal to our knowledge of the seal problem, particularly by showing that the mother–young relation is not so exclusive as had been postulated. Cows will suckle pups other than their own and pups will suck from females other than their mothers. Human interference, whether it be by hunting or merely by observing, of course also has its effect on colonies, but the impression left on one hearer at least was that seals soon get used to man as part of their environment, as indeed do most other animals. Mr. Oliver Hook gave an account of his explorations in the Baltic Sea in search of grey seals, and it was interesting to hear that there they breed on the ice in spring as they do in Canada and not on shore in the autumn as they do around Britain. It was also interesting to hear that in both Finland and Sweden in the interests of fisheries a bounty is paid for killing seals, and while the value of this to fisheries is uncertain it has enabled a certain amount of biological data to be obtained. Finally, Mr. H. G. Hurrell gave a fascinating account of the mental abilities of his tame grey seal 'Atlanta'. She is clearly an animal of great intelligence, and his account showed how much can be learned by establishing friendly relations with an animal and studying its behaviour as a companion and a pet. As in all good symposia, there was too little time for formal discussion, though Dr. Fraser Darling and Dr. Laws made some valuable points, especially that true conservation seldom implies complete protection. Fortunately the arrangements permitted informal talk, in some cases until the early hours of the morning, and there is no doubt that every one present benefited greatly. The seal problem was put into perspective. Over most of the world, commercial exploitation of stocks is taken as a matter of course, and if left to be governed by economic forces it will in the end be disastrous. But conservation is quite consistent with exploitation, and further research into the biology and ecology of these large mammals will clearly enable us to reconcile these two aspects with increasing efficiency.

F. T. K. Pentelow

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NEWS and VIEWS

The Grassland Research Institute: Dr. W. Davies, C.B.E.

Dr. W. Davies, director of the Grassland Research Institute at Hurley, near Maidenhead, since its inception in 1949, retired in September 1964. Dr. Davies, who was born in London in 1899 of North Cardiganshire farming stock, was educated at Sloane School and at the University College of Wales, Aberystwyth, where he gained B.Sc., M.Sc., and D.Sc. degrees. In 1923 he was appointed grassland agronomist at the Welsh Plant Breeding Station, Aberystwyth, under Sir George Stapledon, a collaboration that by 1939 had established the Station as a grassland centre of international repute. This owed much to the extensive travels which Dr. Davies made to many countries, visits which gave him his unrivalled knowledge of world grasslands, and at the same time made workers in these countries aware of the unexploited potential in their own grasslands. In 1940 Dr. Davies moved with Stapledon to Stratford on Avon, where the Grassland Improvement Station was set up to investigate how best the mainly derelict grasslands of Britain could contribute to wartime food production. This work was consolidated in 1949, when the Ministry of Agriculture and Fisheries (as it was then called), in association with the Agricultural Research Council, established the Grassland Research Institute at Hurley with Dr. Davies as its first director. Here he was able to develop his thesis that grass is a crop being grown to feed animals and must, therefore, be studied as a soil/plant/animal complex; the impact of this thesis on world grassland thought became clear from the papers presented at the eighth International Grassland Congress held at Reading in 1960. His eminence in the sphere of international co-operation in grassland science and practice was recognized by the award of an honorary doctorate of science by the University of New Zealand, and by his election as an honorary life-member of the Royal Agricultural Society of New Zealand, as life president of the European Grassland Federation, as an Honorary Fellow of the Royal Agricultural Society of England, and as honorary councillor of the Spanish High Council for Scientific Research. Dr. Davies was appointed a Commander of the British Empire in 1964.

Dr. E. K. Woodford, O.B.E.

Dr. E. K. Woodford, who has been appointed director of the Grassland Research Institute, Hurley, Berkshire, in succession to Dr. W. Davies, has, for many years, played a leading part in the new science of chemical weed control. After war service in the Indian Army, he resumed his interrupted career as a research agronomist at the Indian Tea Association Research Institute at Tocklai. He returned to the United Kingdom in 1948 to join the Agricultural Research Council research team at Oxford under Prof. G. E. Blackman, which during the war years had undertaken the research work required for the successful introduction into British agriculture of the then new synthetic herbicides based on dinitrophenols and the substituted phenoxyacetic acids. In 1950 the team was reconstituted as the Agricultural Research Council Unit of Experimental Agronomy with Dr. Woodford as assistant director. In the early 1950's practical use of the new herbicides quickly outstripped knowledge of their action and there were many problems requiring research. Dr. Woodford realized the need for collaboration between all concerned and concentrated on building up liaison between the principal interests. It was largely as a result of his initiative that the Weed Control Joint Committee

was formed in 1952, later to become the British Weed Control Council. Dr. Woodford has been intimately concerned with the affairs of the Council and has played a special part in the organization of the biennial conferences also, as chairman of the Recommendations Committee and editor of the *Weed Control Handbook*. Dr. Woodford did much to encourage the formation of the European Weed Research Council, set up in 1960, and was its first president. Research, dissemination of information and liaison formed the principal activities of the Unit at Oxford, and soon after its formation it became clear that a permanent centre was needed for the location of staff and to provide the necessary facilities. In 1960 the Agricultural Research Council established the present Weed Research Organization at Begbroke, near Oxford, with Dr. Woodford as director. The existence and success of the Organization must be credited to a very large extent to Dr. Woodford. With typical foresight he recognized the need for, and was determined to bring into existence, a single organization to co-ordinate research and information in this rapidly moving and highly technical branch of agricultural science. Dr. Woodford's appointment to Hurley is consistent with the pioneering outlook which took him as a boy to Canada in 1930 to learn to farm and where he later obtained an M.Sc. in the University of Alberta in plant physiology. In Canada he had the opportunity of observing the development of scientific organizations unhampered by tradition, an experience which has had a marked effect on his thought and actions during his fifteen years' pioneer work in the field of weed control. Dr. Woodford's outlook and experience, together with his interest in farming and his research work in plant physiology in Canada and later at the Royal College of Science in London, will stand him in good stead in his new appointment.

Physiology in the Queen's University of Belfast: Prof. I. C. Roddie

Dr. Ian C. Roddie, reader in physiology in the Queen's University of Belfast, and consultant in physiology to the Northern Ireland Hospitals Authority, has been appointed at the age of thirty-five to the Dunville chair of physiology in the University in which he graduated in both science and medicine after a distinguished undergraduate career. The investigation into the nervous control of the blood vessels of the human limbs which he began in 1954 did much to clarify and define the independence of the nervous controls of the muscular and cutaneous circulations and disclosed the important role of low-pressure baroreceptors in the reflex control of the muscular circulation. In 1957 he was awarded a Beit Memorial fellowship, but resigned this on appointment to a lectureship. Dr. Roddie spent 1960–61 as a Harkness Fellow of the Commonwealth Fund in the University of Washington and started his present work in which the activity of vascular smooth muscle is assessed by the recording of intracellular electrical potentials. This work promises to provide a more direct answer than has hitherto been available to questions of vascular control. Dr. Roddie was Arris and Gale lecturer to the Royal College of Surgeons in 1962, and Ontario Heart Foundation lecturer this year. He has been awarded the degrees of M.D. with gold medal and D.Sc. for his research work. The Dunville chair of physiology was previously occupied by Prof. A. D. M. Greenfield, who was appointed to the chair of physiology at St. Mary's Hospital Medical School, London, as announced earlier this year (*Nature*, 201, 978; 1964).

Education in France

Under the title *The State and Education in France* the Press and Information Section of the French Embassy in London has issued a pamphlet summarizing the ruling ideas of the new Education Act of 1959, which came into force in October 1960 (Pp. 15. London: Ambassade de France, Service de Presse et d'Information, 1964). It has been somewhat modified since August 1963 and some applications are still at the experimental stage. The first effect of the Act is to raise the school-leaving age for all children from 14 to 16, and this will take effect as from 1967. The new structure provides for comparatively unified instruction for children of 11–15 during the four years of the observation cycle, only the 5–6 per cent maladjusted children requiring special education being excluded. The others are distributed between three types of education: a long general course, a short general course, and an intermediate course of two years, followed by two further years of a terminal course. Twenty comprehensive institutions known as colleges of secondary education are this year being started in an experiment in combining these three types of courses in one building and under the same headship. Technical education begins at the close of the first cycle of secondary education, and the long technical course at present given in the technical *lycées* is being removed, but they will continue to give instruction in technology providing the theoretical basis for more advanced technical training. Private education is for the most part associated with religious institutions, and its 1·7 million pupils in 13,000 institutions and taught by 78,000 teachers (40,000 secular) compares with just more than 9 million in State institutions. Besides agricultural education, depending partly on the Ministry of Agriculture, special institutions are being developed for the mentally retarded, for the maladjusted and spastics, for those with defects of sight, speech and hearing, and for young delinquents. There are also 'social formation' courses organized by the State, and the work of the schools is supported by school medical services and by school and recreational guidance services.

The Science Council of Japan

The annual report of the Science Council of Japan for 1961–62 gives a brief account of the history and functions of the Council, which was formally established in 1949, and reports on its activities during the year (Pp. 95. Tokyo: Science Council of Japan). It is expected that legislation of critical importance to scientific research will be enacted very soon, and the Council believes that it should guide research policy in Japan. The main interest of the report lies in the account of this law, intended to formulate the policies which the Government should follow for the sound development of science. The purpose and social function of scientific research are first emphasized; a second article formulates the basic prerequisites, stressing first the freedom and independence of scientific research workers, and secondly, the importance of making the results freely available. A third article insists that Government administration of research must provide the essential conditions in accordance with the particular field of science and the stage it has reached. Next, the organization for scientific research is outlined in seven articles, covering universities, colleges, research institutions and academic societies, and stressing the importance of free interchange and co-operation and the functions of the Science Council of Japan. Next comes an article stressing the flexibility of budgeting and the importance of varied sources of support. One dealing with the working conditions and treatment of scientific research workers follows, and then four further articles deal with international scientific co-operation and the exchange of scientists and scientific information. The remaining two sections of the law deal with the training of scientific research workers and with the functions of the Science Council of Japan, which will make a statement concerning the responsibilities of science when the new law is enacted. The proposed statement stresses the awareness of each scientist of the aim and social responsibilities of scientific research, and insists that scientists should have the responsibility to protest against any threat to freedom of scientific research, to point out any harmful results pro-

duced by neglect or abuse of scientific research, and to protect the welfare of the people and mankind in general. Generally, the proposed law embodies the common opinion in the Western world regarding the conditions of scientific work and the responsibilities now recognized by scientists themselves.

Solway Barrage

THE proposals for the regional project for the development of the Solway Firth Area have now been issued in a report which stresses the strategic location and character of the Solway region in the British Isles. Dr. R. L. Drew argues that any international crossroads linking Scotland, Ireland and England is bound to pivot on a Solway barrage linking Annan in Dumfriesshire and Bowness in Cumberland. The region is rich in water and the potentials this confers for the location of population and industry based on science, at the increasing levels of energy conservation and utilization demanded by a society using large amounts of energy. The communications aspect, including sea-borne trade and air travel as well as motor ways and roads, is also stressed. Dr. Drew reviews the regional activities which could be based on the project, including space and district heating, market gardening, forestry, agriculture, fishing, food processing and production, and finally its bearing on water supply generally, the conservation of energy, as well as land reclamation and water and power supply. It is estimated that perhaps £250,000 over several years would be required to delineate the full plans. The report is available from Dr. R. L. Drew, Annan, Dumfriesshire.

Planning Economic Policy

UNDER the title *Towards a Common Economic Policy for EEC*, Political and Economic Planning has issued Broadsheet No. 481 (London: July 27, 1964. 5*s*.), containing copies, usually translations, of the basic documents relating to the formation of a common economic policy for the European Community. It is preceded by an introduction by M. Forsyth, and seeks to show the extent to which the Community has progressed in this direction, and to assist the discussion of economic policy in Britain. There are several problems of economic policy which the Community and the United Kingdom share, not least the problem of the extent to which economic planning is now necessary and the form it should take.

Care, Handling and Disposal of Dangerous Chemicals

CHEMISTS spend the major part of their working lives in close proximity to potentially dangerous or toxic substances. Familiarity with the properties of the chemicals they use and an appreciation of the consequences of careless experimentation have ensured that a 'safety code', born of experience and operating almost at subliminal level, protects the mature scientific worker from disastrous accident. The booklet recently published by the Institute of Science Technology, Aberdeen, will provide the inexperienced chemist with a most valuable survey of the more dangerous chemicals and of their interactions with other reagents (*The Care, Handling and Disposal of Dangerous Chemicals*. By P. J. Gaston. Pp. 103. Aberdeen: Institute of Science Technology. Obtainable from Northern Publishers (Aberdeen), Ltd., 11 Albyn Terrace, 1964. 7*s*. 6*d*.). It will also provide a useful 'refresher course' for the more expert laboratory chemist and will guide those officers whose task it is to advise on safety matters. The handling and disposal of hazardous materials or toxic substances, the British Standards Institute marking of gas cylinders, the coding of gas-mask canisters, fire fighting, first-aid, etc., are some of the topics ably and succinctly dealt with in this booklet. There are a few errors. 'Phosphorus' is mis-spelled on p. 49, and on p. 30 diazonium salts are stated to be made by the action of 'sodium nitrate' on a primary amine. On p. 9, it is pointed out that white phosphorus and potassium chlorate form the 'basis' of a powerful explosive. It is very dubious whether these two substances could be mixed without exploding spontaneously! There are some omissions. Some mention could have been made of the disastrous consequences of adding the alkali metals to chlorinated hydrocarbons, or of the interaction of finely powdered zinc, aluminium, etc., with these halogenated organic substances. Particle size has a powerful influence in determining phase interactions. The disposal of azides (p. 28) is always a hazardous business, but they can be destroyed in cooled ammonium acetate, sodium nitrite solution by cautious addition of dilute acetic acid. Similarly the fulminate of mercury is destroyed by sodium thiosulphate to form the tetrathionate. The present publication is, however, a most valuable and useful work and it is hoped that it will be extensively read and studied by the chemical staff of all industrial and university laboratories. Its small cost covers a mammoth insurance for safety.

Polychaetes from Scotland

SEVENTEEN species of polychaete worm hitherto unknown from the Firth of Clyde and the west coast of Scotland, together with a number of others, have been described by Dr. R. B. Clark and P. G. Dawson of the Department of Zoology, University of Bristol (*Annals and Magazine of Natural History*, 6, No. 71; 13th Series, 1963). Three of these have not previously been recorded in British waters: *Harmothoe joubini*, Fauvel, *Clymenella cincta* Saint-Joseph, and an undetermined capitellid probably belonging to the genus *Mediomastus*. Both *Harmothoe joubini* and *Clymenella cincta* have been rediscovered for the first time since their original description. Only two species of *Mediomastus* are known, both from the Pacific coast of the United States. The Clyde specimen does not appear to belong to either, but its damaged condition makes precise determination impossible, and the possibility that it is an abnormal *Heteromastus*, though unlikely, is not excluded. *Pholoe synophthalmica* Claparède is another species new to British waters, but the Clyde specimens are in some respects intermediate between this species and *P. minuta* (Fabricius). In view of the known variability of the latter, it is probably preferable to regard *P. synophthalmica* as a variant of *P. minuta*. Similarly, specimens intermediate between *Aonides oxyceohala* (Sars) and *A. paucibranchiata* Southern have been found, which cast doubt on the validity of the latter species. *Praxillura longissima*, previously known only from a generic record of a single fragment collected in the Clyde, has now been positively identified. It has not been reported elsewhere in British waters.

Distinguishing the Sexes in some Scarabaeid Larvæ

AN excess of females among adults of *Costelytra zealandica* emerging from pastures that had been treated with DDT suggested that susceptibility to the insecticide might differ in the sexes, and made it desirable to have a means of recognizing them in the larval stage. The development of the sexual organs in the pre-adult stages was examined by R. Elliott (*N.Z. Science*, 7, No. 2, June 1964). An apparatus, part glandular and part chitinous, is visible through the integument of sternite 9 in living male larvæ of all three instars. The chitinous part was found to vary in shape and size, with some indication of local differences in the occurrence of different types. A similar apparatus was found in *Pyronota* sp., *Heteronychus sanctaehelenae*, *Pericoptus* sp., and *Aphodius tasmaniae*, differing in detail, the glandular element being poorly developed in the last three. Although female larvæ of *C. zealandica* are usually heavier than the corresponding males, there is no evidence from either laboratory or field observations to suggest that susceptibility to insecticides is directly governed by sex at this stage.

Training Programme in Oral Science at the Massachusetts Institute of Technology

Applications are invited for admission as candidates for a Ph.D. course in oral science at the Massachusetts Institute of Technology commencing September 1965. The course is aimed at preparing the candidates for careers in dental education and dental research, and the curriculum will stress basic sciences and advanced biosciences. The course will take three to four years; all tuition fees will be paid, and candidates will receive a generous stipend. Application is open only to citizens of the United States, or to others who have taken out first papers. Further information can be obtained from Prof. Robert S. Harris, director of Training Program in Oral Science, Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge 39, Massachusetts.

British Scientific Exchanges with East European Countries and the U.S.S.R.

A revised summary of the provisions now in force for visits under the Governmental exchange programmes with Bulgaria, Czechoslovakia, Hungary, Rumania and the U.S.S.R. in the fields of science, engineering, technology and agriculture, has been recently prepared by the British Council. Anyone interested in receiving further information on the facilities available is invited to apply to the Director, Science Department, the British Council, 59 New Oxford Street, London, W.C.1.

University Entrance Requirements in Britain

A *Compendium of University Entrance Requirements for First Degree Courses in the United Kingdom* has been issued by the Committee of Vice-Chancellors and Principals of the Universities of the United Kingdom (Pp. 161. London: Association of Commonwealth Universities, 1964. 8*s*. 6*d*.) in accordance with recommendations of a sub-committee reporting in 1962. It is more comprehensive than the previous edition, in that it contains details of the entrance requirements of the Universities of Scotland, the Queen's University of Belfast, and the new universities of Kent, Lancaster and Warwick. It does not include the entrance requirements of the colleges of advanced technology, but these are expected to appear in a future edition, after the colleges have received their charters as universities. The course requirements are expressed in terms of the General Certificate of Education examination in 66 tables which afford easy comparison of the different university requirements, and those courses not included in the tables are listed separately. Appendixes cover the distinctive requirements of Oxford and Cambridge, and the Scottish universities, and of the courses or requirements in those of the new universities which do not fall into the usual pattern. The compendium is designed to assemble and present the details of university entrance requirements for the benefit of schools, where sixth form curricula have to be planned and sixth form boys and girls advised on their choice of subject. It should enable the schools readily to discover the extent to which the requirements of universities are common for any particular course of study.

University News:

Keele

The following appointments to lectureships have been announced: V. H. Dundas-Grant (education); L. W. F. Page (geography); Dr. D. O. Hughes (chemistry).

Leeds

In the Proctor Department of Food and Leather Science, Dr. H. G. Muller has been appointed lecturer with special concern for cereal chemistry, and Mr. T. W. Houston has been appointed to a senior research post to initiate meat research. Mr. Houston's post has been made possible through a grant from the Pig Industry Development Authority.

London

Dr. D. C. Bradley, senior professor of chemistry in the University of Western Ontario, has been appointed to the chair of inorganic chemistry tenable at Queen Mary College. Dr. G. V. Groves, lecturer at University College, has been appointed to a readership in physics tenable at that College. The title of reader in insect ecology has been conferred on Dr. T. R. E. Southwood in respect of his post at the Imperial College of Science and Technology.

Southampton

Mr. G. R. Fisher, at present lecturer in the Department of Econometrics and Social Statistics, University of Birmingham, has been appointed to the newly-created Leverhulme chair of econometrics at the University of Southampton, with effect from January 1, 1965.

Announcements

Dr. Gerhard Herzberg, director of the Pure Physics Division, National Research Council of Canada, has been awarded the Frederic Ives Medal for 1964, "in recognition of his extraordinary contributions to research, teaching, and scientific administration". The Frederic Ives Medal is awarded every two years by the Optical Society of America for distinguished work in optics.

A meeting of the Nutrition Society will be held at the Royal Veterinary College, London, on December 5. Further information can be obtained from Dr. I. Macdonald, Physiology Department, Guy's Hospital Medical School, London, S.E.1.

A symposium on "Membrane Problems" will be held in the State University of New York at Buffalo during December 8–11. Further information can be obtained from Mrs. Muniak, c/o Theoretical Biology Unit, 210 Winspear Avenue, Buffalo, New York.

A symposium on "The Scientific Basis of Maximum Permissible Levels of Radiation Exposure", arranged by the British Institute of Radiology, will be held in London on December 8. Further information can be obtained from the Secretary, British Institute of Radiology, 32 Welbeck Street, London, W.1.

A special session of the Scientific Meeting of the Palæobotanical Society will be held at the Birbal Sahni Institute of Palæobotany, Lucknow, during December 7–12. During the session a symposium will be held on "Floristics and Stratigraphy of Gondwanaland". Further information can be obtained from R. S. Sharma, Birbal Sahni Institute of Palæobotany, 53 University Road, Lucknow 7.

Addendum. The following list of references has been supplied by Prof. C. Lindegren for his joint communication with Miss Yong Nyu Bang entitled "Origin from Mitochondria of the Double-walled Viral Membrane in *Saccharomyces*" (*Nature*, 203, 431; 1964):

[1] Lindegren, C. C., and Bang, Y. N., *Antonie van Leeuwenhoek*, 27, 1 (1961).
[2] Lindegren, C. C., Bang, Y. N., and Hirano, T., *Proc. N.Y. Acad. Sci.*, 24, 540 (1962).
[3] Hirano, T., Lindegren, C. C., and Bang, Y. N., *J. Bacteriol.*, 83, 1363 (1962).

Erratum. In the article "*Para-O*-Methylation of Dopamine in Schizophrenic and Normal Individuals", by Frederick A. Kuehl, jun., Martin Hichens, Robert E. Ormond, Dr. M. A. P. Meisinger, Paul H. Gale, Vincent J. Cirillo and Dr. Norman G. Brink, which appeared on p. 154 in the July 11 issue of *Nature*, lines 8 and 9 of the right-hand column of p. 155 should read "ratio of 4-hydroxy-3-methoxy to 3-hydroxy-4-methoxyphenethylamine was found to be 4 : 1".

NEW ZEALAND DEPARTMENT OF SCIENTIFIC AND INDUSTRIAL RESEARCH

THE following staff changes have been announced by the New Zealand Department of Scientific and Industrial Research.

Mr. W. A. Joiner

MR. W. A. JOINER retired recently as deputy director-general of the New Zealand Department of Scientific and Industrial Research. Mr. Joiner, who was born in Scotland in 1902 and moved to New Zealand when he was nine, was educated at Wellington College and Victoria University of Wellington, where he was awarded a senior scholarship in chemistry, a Joe Jacob Joseph scholarship and a National Research scholarship, and gained his M.Sc. with honours in chemistry. Appointed as assistant analyst in the Dominion Laboratory in 1923, he specialized in fuel research, and his work on the carbonization of Waikato coals led to the establishment of a briquetting plant at Huntly in 1930. In 1934 he took a postgraduate course in chemical engineering at University College, London, and also investigated problems of coal hydrogenation at the Fuel Research Station and the Imperial Chemical Industries Works at Billingham. In 1935 he was awarded a diploma of chemical engineering by University College, London. On his return to New Zealand, he initiated a survey of the country's coal resources. In 1937 he was appointed chemical engineer in the Dominion Laboratory, and in 1941 assistant director. One of his major projects was the development of a special grass-seed dryer to overcome the problem of the poor germination of such seed when exported in a moist condition. He also played a leading part during the Second World War in the developing of mobile gas producers and in the dehydration of fruit and vegetables. In 1946 he succeeded Mr. Andrew as director of the Dominion Laboratory, and in the following year was appointed assistant secretary of the Department of Scientific and Industrial Research, in charge of the branches dealing with chemistry and geology. He became deputy secretary in 1950.

Dr. E. I. Robertson

DR. E. I. ROBERTSON, director of the Geophysics Division, has been appointed assistant director-general of the Department of Scientific and Industrial Research. Dr. Robertson was educated at Hutt Valley High School and Victoria University College, from which he graduated M.Sc. He was awarded a Shortcliffe fellowship, but because of the Second World War, during which he served in the Navy for four years, he delayed going to the University of London until 1945. There he gained his Ph.D. in geophysics. Returning to New Zealand in 1948, he took up a position as a geophysicist in the Department of Scientific and Industrial Research. In 1951 he was appointed director of the Geophysics Division of the Department, in which capacity he was responsible for scientific programmes in Samoa, Rarotonga and at Wairakei. During the International Geophysical Year, 1957–58, he played a leading part in New Zealand's contribution to the work, being chairman of the Inter-departmental Committee. Dr. Robertson has been the scientific adviser to the New Zealand Navy for several years, is chairman of the Ross Dependency Research Committee, and is a Government representative on the Council of the Royal Society of New Zealand.

Dr. F. F. Evison

DR. F. F. EVISON, superintendent of the Seismological Observatory, has been appointed director of the Geophysics Division. Dr. Evison was born in Christchurch and educated at the Victoria University of Wellington, where he gained his M.A. and B.Sc. degrees. In 1949 he went to the Imperial College of Science and Technology, London, and was awarded a Ph.D. for his work in geophysics and a diploma of the Imperial College in 1950. On returning to New Zealand he joined the staff of the Geophysical Survey Section of the Geophysics Division, and in 1960 was appointed superintendent of the Division's Seismological Observatory. In 1956 Dr. Evison was awarded a Nuffield fellowship at the University of Cambridge. He has made a special study of the Earth's crust in Antarctica and New Zealand, using earthquake waves, and has investigated the cause of earthquakes. In 1963 Dr. Evison was visiting professor in geophysics at the University of Minnesota.

Dr. R. D. Adams

DR. R. D. ADAMS has been appointed superintendent of the Seismological Observatory. Dr. Adams was born in Wellington and educated at the Victoria University of Wellington, where he gained both his M.A. and M.Sc. degrees with honours. Appointed to the New Zealand Defence Science Corps, he went to the University of Cambridge, where he was awarded a Ph.D. for his thesis on seismology. Following his return to New Zealand, Dr. Adams worked for two years at the Naval Research Laboratory, Auckland, before joining the staff of the Seismological Observatory in 1960, as a scientific officer. Dr. Adams has carried out a magnetic survey of the region between New Zealand and McMurdo Sound, Antarctica. He has undertaken research on problems of earthquake mechanism, and investigated the use of dispersive earthquake waves to examine the Earth's crustal structure in the New Zealand region.

Dr. L. Corkill

DR. L. CORKILL, director of the Crop Research Division of the New Zealand Department of Scientific and Industrial Research, has been appointed director of the Department's Grasslands Division, in succession to the late Dr. P. D. Sears. Dr. Corkill was educated at Auckland and Massey Universities, where he gained both B.Sc. and M.Agr.Sc. degrees. Appointed as a plant breeder in the Grasslands Division, he worked under the general direction of Sir Bruce Levy and was responsible for the development of many new and improved strains of pasture plants. He was associated particularly with short-rotation ryegrass and with long-rotation ryegrass, which has just been released to farmers. In 1957 Dr. Corkill was awarded a D.Sc. by the University of New Zealand for his researches on plant breeding. Later that year he was appointed director of the Crop Research Division. In 1958, together with Dr. Sears, Dr. Corkill visited Japan under the auspices of the Food and Agriculture Organization to advise the Japanese Government on pasture establishment on new land previously under scrub and on a breeding programme for plants best suited to this environment.

Dr. H. C. Smith

Dr. H. C. Smith, senior principal scientific officer in the Plant Diseases Division, has been appointed director of the Department's Crop Research Division. Dr. Smith was educated at Massey Agricultural College, where he gained an M.Agr.Sc. degree, specializing in plant pathology. In 1951 he was awarded a National research scholarship at the University of Cambridge. There he undertook research work for his Ph.D. on *Phytophthora*. On completing his Ph.D. degree in 1953, he returned to New Zealand and was appointed mycologist at the Plant Diseases Division sub-station at Lincoln. In 1960 Dr. Smith was awarded a Canadian National research fellowship, which he held at the Canadian National Research Council's Plant Research Institute at Ottawa, where he studied virus diseases of grasses and cereals, and in particular barley yellow dwarf virus which he had already been investigating in New Zealand. His work on this disease has led to the development of effective control measures of great economic significance to barley and wheat growers in New Zealand.

Dr. J. M. Hoy

Dr. J. M. Hoy has been appointed director of the Entomology Division, Nelson, in succession to the late Dr. W. Cottier. Dr. Hoy was educated at Otago Boys' and King's High Schools, Dunedin, and at Lincoln College, where he gained B.Agr.Sc. in 1942. In 1943 he became entomologist in charge of malarial control for the Royal New Zealand Air Force in the south-west Pacific area. In this capacity he spent a year in the forward Pacific war area. On his return from overseas in 1945 he joined the Entomology Division of the Department of Scientific and Industrial Research as assistant entomologist, Nelson, where he was concerned with the systematic investigation of the larvæ of grass grubs and the possible utilization of nematodes to control pasture insects. In 1953 Dr. Hoy was appointed officer-in-charge of Entomology Division's sub-station at Palmerston North. This unit made an intensive investigation of manuka blight, and Dr. Hoy was responsible for the discovery that the causal insect, *Eriococcus*, was Australian in origin. For his work on scale insects he was awarded a D.Sc. by Lincoln College early in 1964. In March 1963 Dr. Hoy joined the headquarters staff of Entomology Division at Nelson to lead the research team concerned with present work on the control of grass grub and subterranean grass caterpillar. Dr. Hoy is immediate past-president of the New Zealand Entomological Society and is vice-president of the Nelson Branch of the Royal Society of New Zealand.

ORGANIZATION AND ADMINISTRATION OF INDIAN SCIENCE

In successive annual reports on the Commonwealth Scholarship and Fellowship Plan the Commonwealth Education Liaison Committee has expressed concern at the reluctance of some scholars to return home on completion of their tenure. This was discussed at some length in the third report, and the difficulty appears to have become more acute during the past year. It is appreciated that the temptation to remain in Britain, for example, may be considerable when the scholar can see no immediate prospect of obtaining employment in his home country at the level he regards as suitable to his qualifications, or when opportunities for further research are severely limited at home.

Some light is thrown on this reluctance to return home, so far as India is concerned, by a memorandum on Indian Scientific Policy submitted by the Institute of Political and Social Studies to the Review Committee of the Council of Scientific and Industrial Research on National Research Laboratories, and published in *Minerva* (11, 519; Summer, 1964). It is also illuminated by the comments which Dr. K. A. C. Mendelssohn made in a broadcast talk "Science in India" discussing his visit to India in 1963, which was published in *The Listener* for September 24 (72, 457; 1964).

The main submission of the Institute of Political and Social Studies is that the Review Committee, to which its memorandum is addressed, should concentrate on creating a suitable climate for serious scientific research in the national research laboratories. It is considered in the memorandum that the previous Minister for Scientific Research and Cultural Affairs was at least partly aware of the problem, and it quotes his references, when addressing a recent conference of scientists and educationalists, to the lack of an atmosphere of activity sustained by critical appreciation and the existence of a bureaucracy in the scientific field, tending to a false sense of prestige due to status and office. These criticisms fully confirm Dr. Mendelssohn's observations. In general, the authors of the memorandum believe that the organization of research has proliferated too widely and rapidly, with excessive attention to buildings and equipment, etc., compared with training and expanding the initial nucleus of research staff. There are now 27 national laboratories and other allied research institutions, housed and substantially equipped and with further facilities in prospect; in spite of this, the memorandum asks the fundamental questions whether a suitable research leadership has emerged in each of these institutions, and whether a climate has developed which spurs the research worker to do his job well. The whole implication of the memorandum is that neither question can be answered in the affirmative without hesitation or reservations.

The Institute of Political and Social Studies, however, goes further and directs attention to specific points. It questions whether the laboratories are recruiting scientific workers, particularly in the senior grades, who combine aptitude for research with basic competence in the particular field. Enquiry here could go far to explain the reluctance of Indian scholars and Fellows to return from abroad. The mere fact that the memorandum suggests that it would be worth while to consider setting up a high-level selection body for recruiting scientists on the pattern of the Union Public Service Commission indicates that all is not well with present methods of recruitment. It also directs attention to the vital importance of ensuring that the research worker can rely on obtaining guidance and on critical appreciation of his efforts. It asks key questions about direction of the laboratory, both as regards the formulation and study of problems and the publication of results.

The questions about relations with industry, about the establishment of regional research laboratories, about duplication at the national level, are both searching and constructive. The memorandum gives firm support to the younger workers; like Dr. Mendelssohn, the Institute has evidently formed the opinion that the younger men do not always receive a fair chance and that conditions are not conducive to serious research by many competent scientists. The core of its criticism is, in fact, that the direction of many of the laboratories is often defective, if not incompetent, and that this position is at least partly due to the form of organization under the Council of Scientific and Industrial Research.

Perhaps the most encouraging feature of the memorandum is the evidence it provides that the Institute of Political and Social Studies has clear ideas as to what is needed, in respect of the conditions on which effective research depends, of the organization of research, and of the qualities required in its direction. Dr. Mendelssohn's broadcast talk supplies evidence that there are other Indian sources also competent to give the Review Committee all the guidance it should need as to the organization of the National Research Laboratories. At the outset, however, he makes it only too apparent that the causes run deep and that there is widespread frustration and discontent in universities as well as in Government research laboratories, largely but not wholly due to the effect of the 20 per cent cut in their budgets imposed as an emergency measure in 1963. Since the director could not dismiss his staff and their salaries had to be paid, this involved a reduction of 60–70 per cent in running expenses.

Dr. Mendelssohn picks out the quality of scientific publication, and the reluctance of Indian scientists to return to India after training in the West, as evidence that all is not well with science and technology in India. His observations satisfied him that there was justification in some of the reasons given for this reluctance—in the lack of appreciation, insufficient means for research, difficulties in obtaining equipment, pettiness and jealousy among colleagues. It seems only too clear that Dr. Dedijer's plea for a clearer understanding of science and its implications and for the formulation of scientific policy, addressed more particularly to the emerging nations, applies no less to India. Too many of her politicians, as Dr. Mendelssohn recognizes, are not science-minded, and the trouble starts in the universities, modelled on Western institutions of the pre-scientific era.

While it is true that the whole purpose of the Commonwealth Scholarship and Fellowship Plan will be defeated if many of the Scholars and Fellows do not return to their own country when tenure of their scholarship and fellowship expires, it must not be forgotten that this is largely a matter for the country which sends the scholars and Fellows. Fortunately, as Dr. Mendelssohn shows, there are hopeful signs. India, he recognizes, has scientists who are second to none. He is impressed by her reserve of capable brains and by the younger generation of scientists whose love for their subject is matched by their capacity for hard work, and often by their competence. Moreover, there are, here and there, as at the Tata Institute in Bombay and the Atomic Energy Research Laboratories at Trombay, those who have the strength of character to oppose the Government, and he does not despair of the real impetus coming, as it must, from India itself.

What is needed is to create the conditions in which the young generation of Indian scientists can develop their talents and work for their country, and what Indian professors and directors of research need is the art of talking to their own Government. These are the two key conditions which will determine the readiness of Indian scholars abroad to return to their country. This should be remembered both in discussing the future of the Commonwealth Scholarship and Fellowship Plan, and in assessing the extent and character of the technical assistance given to India. With sympathy and insight much could be done indirectly, rather than by direct action, to help Indian politicians to understand the situation and to appreciate the nature of the action required, if not merely India's science but her general economic and social development are to proceed on the scale and at the rate which has been planned.

MARINE RESEARCH AT ZANZIBAR

AN important aspect of the International Indian Ocean Expedition has been the part played by the various marine research stations in the area. Not only have they offered facilities for research vessels and made their own individual contributions to the Expedition, but, perhaps more important, their previous research activities have provided much valuable information on which the assault on the Indian Ocean could be planned. The research station at Zanzibar, although hampered during much of 1963 by the absence of its research vessel, R.V. *Manihine* (for refit and survey), nevertheless continued some promising lines of research and initiated some new ones*. Three new biologists were recruited during 1963, bringing the scientific complement up to four research officers in addition to the director.

In the absence of the R.V. *Manihine*, the deep long-line investigation, work on demersal fishes, and hydrological work had to be abandoned. On the other hand, a new vessel, the 58-ft. *Jodari*, built by and for the use of the Government-sponsored Zanzibar Fisheries Development Co., Ltd., and the purchase of a 450 × 45 fm. purse-seine net, enabled investigations to be made of day-time fishing for scombroid fishes. Of the latter, five types of shoal have been distinguished on their species composition, the majority of shoals containing bonito and frigate mackerel in the proportions of 50 : 50 to 97 : 3. The food of the tuna examined consisted chiefly of small fishes (*Stolephorus* and *Atherina*) with occasional small crustaceans and cephalopods.

Two further boats were operated by the Zanzibar Fisheries Development Co. (39 ft. and 55 ft.), both used in night purse-seining for sardine-like fishes. As elsewhere, night fishing with lights was limited to about twenty days between full moons. Initial difficulties were encountered in finding areas free from coral. Of the clupeid species, *Sardinella jussieu* (Lacépède), *S. perforata* (Cantor) and *S. sirm* (Walbaum) (the latter an offshore species) were the most abundant, but several small carangids were commonly caught (for example, *Decapterus dayi* Wakiya). The purse-seines also took significant numbers of tuna-like fishes. Highest landings were recorded during the periods May–July and November–December.

Prawns collected during three cruises off the Tanganyika coast, supplemented by specimens from elsewhere in the Indo-Pacific Ocean, were reported on at the meeting of carcinologists at Zanzibar, organized by the Scientific Council of the Commission for Technical Co-operation in Africa South of the Sahara in April. Three useful papers on plankton were published during 1963, resulting from collections made in the Zanzibar area during 1960–61; one strongly denies the popular conception of the 'impoverishment of tropical seas'.

Interest in the work of the Zanzibar station is evidenced by the amount of financial assistance received from outside sources: Unesco for library material; the Rockefeller Foundation for hydrological equipment; the Food and Agriculture Organization for deep long-line gear; the Freedom from Hunger Campaign for purse-seine net; the Munitalp Foundation for hydrographic winch, etc. It is to be hoped that the valuable contributions made by Zanzibar to knowledge of the Indian Ocean and its fisheries will continue to be recognized.

P. J. P. WHITEHEAD

* East African Common Services Organization. Annual Report of the East African Marine Fisheries Research Organization for 1963. Pp. iv+19. (Zanzibar: East African Marine Fisheries Research Organization, 1964.) 4s.

CO-ORDINATION CHEMISTRY

THE eighth international conference on "Co-ordination Chemistry" brought together some 750 research workers in Vienna during September 7–16 and gave rise to two cognate symposia, one in Czechoslovakia and the other in Hungary.

The symposium in Hungary, held at Tihany, Lake Balaton, during September 14–17, under the auspices of the Hungarian Chemical Society, with Prof. G. Schay as president and Dr. M. T. Beck as secretary, was attended by about 60 Hungarian chemists and some 100 visitors from countries as distant as the United States and Japan. The papers were preprinted in full in two octavo volumes totalling 886 pages, and authors were allowed 15 min to introduce their papers followed by 10 min discussion. The papers were drawn equally from countries east and west of the 'Iron Curtain', and the conference afforded an excellent opportunity for Western chemists to meet their counterparts from the East. The two volumes make very interesting reading, containing many authoritative reviews with new work from well-known authors. They will be published together with the discussion in the near future, and are warmly recommended to those who did not have the good fortune to attend the conference.

Tihany is a beautifully situated holiday resort on Lake Balaton and, appropriately to this wine-producing area, the lectures and discussions took place in the 'Bar' of the Tihany Motel. Participants from abroad were accommodated at the Tihany Hotel, about 100 yards away. Forty papers were presented and raised much interesting discussion, so that in many cases the discussion time was much too short. Briefly, the papers touched on the following points, and only the authors who presented papers are mentioned.

Outer sphere association of complex cations and simple anions has been examined by charge transfer spectra (C. K. Jørgensen, Switzerland) and optical rotatory dispersion (S. Kirschner, United States). Perhaps the most remarkable fact is the high degree of association which can occur. R. Larsson (Sweden) reported successive stability constants for up to four thiosulphate ions with d-$[Coen_3]^{3+}$ forming a super anion of charge 5– and requiring, for the later stages of its formation, the association of negative ions in aqueous solution of ionic strength 2.

The four papers on homogeneous hydrogenation (J. Halpern, United States; V. A. Tuloupov, U.S.S.R.; J. C. Bailar, jun., United States; and L. Simándi, Hungary) contained much new material leading to a lively discussion and firmly pointing the way to selective and specific catalysts. This was followed by papers on ligand interaction with 5f orbitals (B. Jezowska-Trzebiatowska, Poland) and some acetylacetone complexes of the uranyl ion (J. Szőke, Hungary).

A major part of the programme concerned the chemistry of aqueous solutions of complex ions. L. G. Sillén (Sweden) made an earnest plea for care in interpreting stability constant measurements and emphasized the value of a computer for checking data and calculating constants. This was followed by papers concerned with the quantitative aspects of the formation of polynuclear complexes in aqueous solution. P. Szarvas (Hungary) discussed the equilibria between isopolytungstic acids and tungstate-carboxylic acid complexes, A. E. Martell (United States) the formation and possible structures of polynuclear species derived from polyfunctional organic ligands and tri- and tetra-positive ions, and A. Gergely (Hungary) the equilibria between neodymium(III) ion and d-glycosaminic acid.

J. Bjerrum (Denmark) reviewed and correlated the great variety of different complex formation curves obtained from ligands with Cu(I), Ag(I), Au(I) and Hg(II), including new data on organic phosphines and arsines as ligands. This was followed by a paper on the formation of azido complexes in non-aqueous solvents by V. Gutmann (Austria), and a comprehensive account of the formation of mixed dimethylglyoxime (DMG) complexes of general type $M(DMG)_2X_n$ (M = Mn(II), Fe(II), Co(II), Co(III), Cu(II) and Pd(II); X = halide ion, OH, and other ligands; n = 1 or 2) (K. Burger, Hungary). Only nickel failed to form such complexes, and this was advanced as the main reason for relative insolubility of $Ni(DMG)_2$ in water.

Solvent extraction of metal ions through complex-formation with organic ligands and the enormous enhancement of the extraction by some ligand mixtures were described in papers by H. Irving (England), D. Dyrssen (Sweden), and L. Bakos (Hungary). W. K. Wilmarth (United States) discussed the base catalysed reduction of μ-peroxo bridged complexes of cobalt, and D. Konrad (Czechoslovakia) the extraction of halogen from halogenated hydrocarbons by complexes of transition metals in low valent states. The retardation of hydroformylation and hydrogenation of olefines on a cobalt catalyst was attributed by L. Markó (Hungary) to the formation of cobalt sulphide by means of cobalt carbonyl derivations such as $Co_3(CO)_9(S)$ and $Co_3(CO)_9(S)(SR)$, the sulphide being stable under carbonylation conditions.

Polymeric and polynuclear complexes formed the subject of a number of papers. The formation of oxygen bridged complex fluorides by the thermal condensation of hydroxofluoro complexes of arsenic and antimony was discussed by L. Kolditz (East Germany), and thermal degradation of the infinitely bridged complexes of methylpiperazines with cobalt halides to the binuclear $[diamine_2Co_2X_4]$ by E. Körös (Hungary). The structures and thermal decomposition of polymeric alkoxide bridged complexes were the subject of papers by D. C. Bradley (Canada), F. Majdik (Hungary) and Gy. Pfeifer (Hungary). Gy. Bor (Hungary) gave a detailed account of the application of infra-red spectroscopy to determining the structure of polynuclear metal carbonyls, and showed that the "method of local symmetry" is invalid. Infra-red and Raman spectral investigations were also carried out to determine the type of bonding in mixed halide–cyanide complexes of gold(III), for example $[AuCl_2(CN)_2]^-$ (L. H. Jones, United States).

Polynuclear complexes containing metal–metal bonds were reviewed and classified by R. S. Nyholm (England), who also described a number of new compounds in which a covalent metal–metal bond was an essential link, for example $[CH_3C(CH_2AsMe_2)_3Cu.Mn(CO)_5]$.

E. Thilo (East Germany) attributed the catalytic effect of metal ions on the hydrolysis of polyphosphates and pyrosulphate to the chelation of the cations by the polyanion. A very thorough tracer investigation of the alkaline hydrolysis of pentamminecobalt(III) carboxylates by H. Taube (United States) showed that it proceeded by two paths, at low temperatures and high $[OH^-]$ by C–O bond breaking and at high temperatures (90°) and low $[OH^-]$ (0·01 M) by breaking of the Co–O bond. The rate-determining step in strong acid hydrolysis of *cis*-$[Cr(C_2O_4)_2(H_2O)_2]^-$ involved the protonation of the complex with splitting away of $HC_2O_4^-$ (H. L. Schläfer, West Germany).

Equilibrium studies of the stabilities of mixed complexes in aqueous solution were given by I. Leden (Sweden) and by E. J. Marcus (Israel). It is often found that the mixed complexes are more stable than would be expected on the basis of the stabilities of the corresponding binary complexes, for example, HgClI exists in greater concentration than would be expected statistically from the

stability constants of $HgCl_2$ and HgI_2. Marcus generalized that the more unlike the ligands the more stabilized the mixed complex provided the two ligands form complexes of the same bonding type, and calculated the stabilization in terms of a polarized ion model.

The remaining six papers were concerned mainly with the effect of ligands on electron transfer reactions and the redox properties of complexes (C. H. Brubaker, jun., United States; G. M. Nord-Waind, Denmark; W. Langenbeck, East Germany; and L. Kiss, Hungary), concluding with a systematic review of the mechanism of catalysed redox reactions including catalysis by so-called 'inert' complexes (M. T. Beck, Hungary).

J. CHATT

A SIMPLE MASS SPECTROMETER FOR THE ANALYSIS OF ARGON AT ULTRA-HIGH VACUUM

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THE most modern methods of determining the ages of rock formations are based on radioactivity[1,2], perhaps the most widely used technique so far being that utilizing the decay of potassium-40 to argon-40. Potassium-40 is a long-lived rare isotope of potassium (0·0119 per cent atomic) and by virtue of a branching decay produces both argon-40 and calcium-40. A potassium–argon age measurement on a mineral involves two steps: (*a*) determination of the potassium concentration by acid digestion of the mineral followed by flame-photometry; (*b*) evaluation of the argon concentration by fusion of the mineral in vacuum followed by mass spectrometric, isotope dilution measurements on the evolved argon. While many laboratories have the facilities for potassium analysis, equally many are deterred from setting up methods of argon analysis because of the considerable expense usually thought to be involved in the acquisition of a suitable mass spectrometer. However, we have recently been experimenting with a simple, inexpensive mass spectrometer, the *MS*10, bought from Associated Electrical Industries, Ltd., and wish to report here its successful application to potassium–argon dating.

The instrument uses a 2-in. radius of curvature with 180° deflexion. A magnetic field of 1,830 gauss is produced by the small permanent magnet, and electrostatic scanning of the various ion beams is brought about by a motor-driven potentiometer in the source controls which causes the appropriate variations in accelerating voltage. When argon-40 is focused on to the collector slit the accelerating voltage is about 100 V. Ions are generated by the usual electron impact method of ionization[3], the ionizing electrons being emitted from a heated rhenium filament prior to being accelerated by falling through a potential difference of 70 V. Stable ion beams are produced with the trap current regulated at 50 μamp and these are detected with a d.c. amplifier having a 10^{11} Ω input resistor. The output is fed to a Honeywell–Brown potentiometric pen recorder, where the noise-level is less than an equivalent ion current of 2×10^{-15} amp.

Use of the static method[4] of analysis in mass spectrometry produces a great increase in sensitivity over the more usual dynamic mode. In the former technique a small fraction of gas from the inlet system is admitted to the tube, where it is then completely isolated and analysed. This requires that before such an analysis may be carried out the vacuum inside the mass spectrometer must be sufficient to ensure that isolation of the tube from the pumping system during a static analysis does not cause a significant rise in pressure. To satisfy such a requirement we have set up the *MS*10 so that it may be baked out inside an oven while being pumped out. The instrument is designed for regular baking at temperatures up to 400° C, but we have not found it necessary to exceed 200° C. With overnight baking little difficulty has been experienced in producing pressures of about 5×10^{-10} mm mercury, and these have been maintained, without further baking, over periods of several months. The pumping system uses a silicone oil, Dow Corning '705', in a metal diffusion pump—the latter being separated from the mass spectrometer tube by a liquid air trap, and being backed by a simple rotary pump. No difficulty has been experienced in reducing the background at the critical mass 36 to zero. At pressures of the order 5×10^{-10} mm mercury, the only significant peak occurs at mass 28 and is presumed to be due to nitrogen and carbon monoxide.

A very simple inlet system has been used and may be seen in Fig. 1. It is a simple matter to evacuate the inlet side so that any background contributions in the inlet at masses 36 and 38 are not detectable by the mass spectrometer and any at mass 40 is only just seen on the most sensitive range. Samples left overnight in the inlet system show no variation in 36/38 and 40/38 ratios with time.

It has frequently been reported by mass spectrometry workers that in static analysis the gases being analysed during a particular measurement are contaminated by a previous sample. Reynolds[4] has suggested that during an analysis ions are driven into the walls of the mass spectrometer tube. The composition of the embedded gases

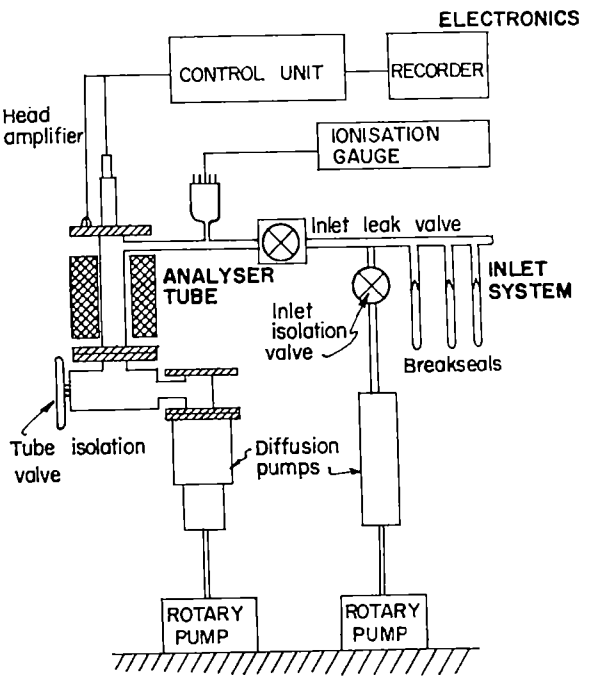


Fig. 1. Schematic diagram of experimental arrangement for static analysis of small volumes of argon on *MS*10 mass spectrometer

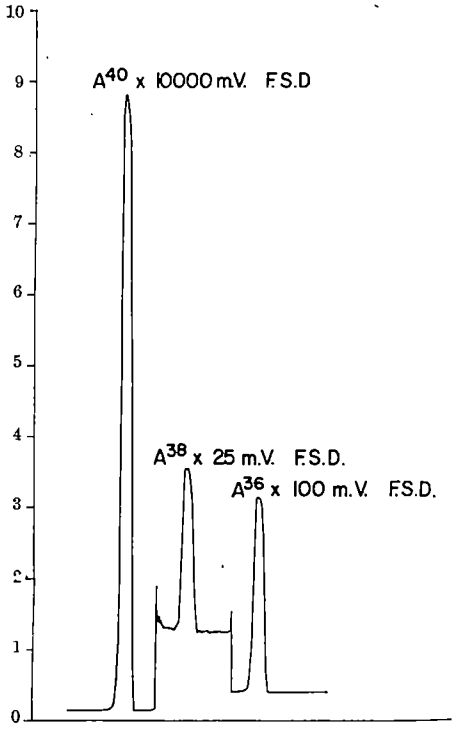


Fig. 2. Recording of atmospheric argon showing the resolution in the mass 36–40 range

would then be representative of that sample. During a subsequent analysis it appears that ion bombardment of the walls causes the embedded gases to be re-emitted into the sealed-off chamber, thus contaminating the new analysis and causing changes of the measured ratios with time. This 'memory' effect has long been troublesome and apparently is not removed completely by baking. In the *MS*10, for argon analysis, we find no trace at all of a memory effect. Measurements of the isotope ratios of an argon sample trapped in the tube for several hours show no variation with time. Peak heights barely change. This is an extreme advantage possessed by the instrument. We attribute this effect to the low accelerating voltages (100 V) used when examining the argon isotopes. That is, in this instrument, the positive ions bombard the walls of the mass spectrometer with a much lower energy than is usual. In most mass spectrometers several thousand volts are used for ion acceleration.

The isotope ratios obtained with the *MS*10 are critically dependent on the ion repeller voltage in the source. Ratios of $(^{40}Ar/^{36}Ar)_{at}$ varying from about 300 to 220 may be reproducibly produced by systematic settings of the repeller plate voltage. However, the measured ratio remains fixed for a given gas with a given repeller voltage setting. Thus, as there is no call to adjust the repeller voltage the discrimination is constant and easily found from analyses of atmospheric argon samples. The results of static analyses give good agreement with dynamic runs when the former are submitted to a root-mass correction to allow for fractionation through the inlet leak valve.

For argon analyses of this nature it is merely necessary to be able to resolve completely the peaks at masses 40, 38 and 36. This is not a severe requirement, and the *MS*10 is able to meet it. The resolving power is about 50. A typical recording of the mass spectrum of atmospheric argon is shown in Fig. 2. The argon-40 and argon-38 beams contribute no significant amount to each other. A more important feature to consider is the possible existence of 'tailing' of the argon-38 beam at the mass 36 position. As can be seen from Fig. 2, this is a negligible effect—the contribution two mass numbers away due to a beam at mass 38 is approximately 10^{-4} times of the size of the argon-38 beam.

Table 1. Table including Comparison of Analyses carried out in Toronto with others made in Various Laboratories on Same Mineral Sample

Sample	K (%)	Atm. (%)	^{40}Ar (p.p.m.)	Lab.	Age (m.y.)
Colmonell Biotite No. 3	5·44	36·0	0·213	Toronto	483
	5·34	1·5	0·203	Cambridge	467
Colmonell Biotite No. 4	4·98	10·3	0·192	Toronto	476
	4·85	2·7	0·186	Cambridge	477
Colmonell Biotite No. 5	6·13	3·0	0·238	Toronto	479
Muscovite XXII		3·9	0·704	Toronto	
			0·710	Geochron	
Muscovite X		2·8	2·76	Toronto	
		6·5	2·76	Geochron	
		1·7	2·73	Geochron	
Laxfordian Biotite 20165		0·9	1·353	Toronto	
		0·6	1·376	Oxford	
Biotite XXIII	7·09	3·5	0·6096	Toronto	933
Hornblende XXIII	0·221	53·0	0·0192	Toronto	940
Goulding Biotite fine	5·96	13·5	0·466	Toronto	865
Goulding Biotite coarse	7·73	4·8	0·590	Toronto	849

$\lambda_\beta = 4{\cdot}72\ 10^{-10}$ yr.$^{-1}$. $\lambda_e = 0{\cdot}584\ 10^{-10}$ yr.$^{-1}$.

The older a mineral, the greater its argon content, the easier the mass spectrometric analysis. We have carried out fifty accurate argon analyses on mineral samples in the course of four months' work. The youngest sample we have analysed so far is a 50 m.yr.-old biotite. One g of this sample produced approximately $1{\cdot}4 \times 10^{-5}$ c.c. at N.T.P. for analysis by isotope dilution with argon-38 as the diluent. This represented far more gas than was actually needed for analysis. The manufacturers claim the sensitivity to be 5×10^{-5} amp/mm mercury, and our work indicates a figure similar to this. In Table 1 some of our results are shown, and comparisons with those obtained on the same samples by other laboratories using standard mass spectrometers are made. The close agreement in age between biotite XXIII and hornblende XXIII is noteworthy. These co-genetic minerals differ in potassium content and hence in argon content by a factor of thirty, suffered totally different atmospheric argon contaminations and still produced identical ages.

Our experience leads us to conclude that it is straightforward to use the *MS*10 mass analyser for isotopic dilution determination of the minute amounts of argon-40 generated in rocks by the radioactive decay of potassium-40. Isotope ratios can be measured with an accuracy of $\pm 0{\cdot}5$ per cent after calibration of the amplifier and recorder system. It is clear from our experiments that 1 g of a biotite 1 m.yr. in age would yield definitely sufficient argon to be measured by the *MS*10. This demonstration could have useful consequences. Laboratories at present unable to afford the more elaborate mass spectrometers could doubtless now consider

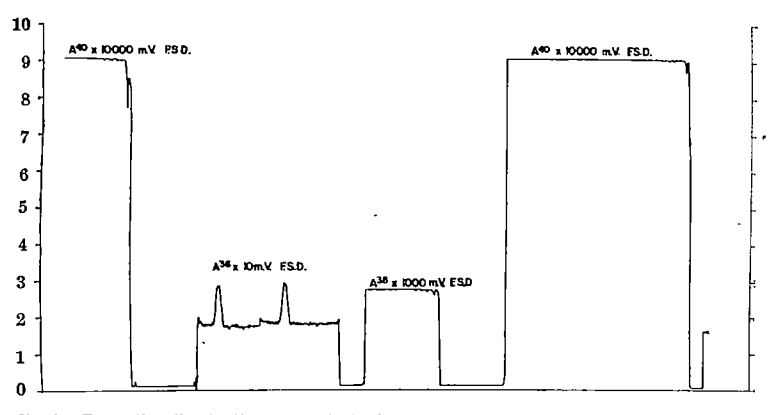


Fig. 3. Recording illustrating argon isotopic ratios in a typical analysis of a Grenville biotite

the variety of simple background analysers available of which one at least has been shown to be entirely suitable.

We thank Profs. R. M. Farquhar, J. T. Wilson, G. D. Scott and H. L. Welsh and Dr. J. A. Miller for their advice and help. This work was supported by grants from the National Research Council of Canada and the Geological Survey of Canada.

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ACTIVE TRANSPORT OF ALKALINE EARTH IONS AS PHYSIOLOGICAL BASE OF THE ACCUMULATION OF SOME RADIONUCLIDES IN FRESHWATER MOLLUSCS

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TRANSPORT of calcium ions against an electrochemical potential gradient has been described in a variety of biological objects such as the proximal tubuli[1], the giant axon[2], the small intestine[3], the endoplasmic reticulum and other sub-cellular fractions[4,5]. In freshwater molluscs it was also suggested, but is not yet proved, that such an active transport may exist[6–8]. This aspect of calcium metabolism is of interest because molluscs are one of the most important groups of calcium-binding organisms, and because freshwater molluscs can serve as indicators for the radiocontamination of their habitat, for example, for strontium-90 (ref. 9) and radium-226 (ref. 10).

Active transport[11] of a substance implies essentially the intervention of metabolic energy. The necessity of this intervention will be established when an ionic substance such as Ca^{++} moves from a compartment having low chemical activity to one with high activity, and when this movement is not induced by electrical field or solvent drag[16].

In order to verify the existence of active uptake, we have to investigate: (1) whether a difference in concentration of calcium exists, and whether this calcium is diffusible[12] between internal and external *milieu*; (2) whether a potential difference between these two media could account for the calcium-fluxes observed[8].

Lymnaea stagnalis (L.) from a laboratory culture was used in most experiments.

The concentrations of calcium and strontium are measured by flame-spectrophotometry (Unicam *SP* 900), the radioactivity of strontium-85 and barium-133 with a single channel pulse-height analyser in a sodium iodide well crystal and the radioactivity of calcium-45 by liquid scintillation with a Packard 'Tri-Carb'. The potential differences were measured with a mV-meter (10^{11} ohm internal resistance, radiometer *pHM* 22). The movable electrodes were made from glass capillaries extended at their larger end with polyethylene tubing and filled with 3 M potassium chloride agar. These electrodes were in turn connected to the mV-meter over calomel–potassium chloride bridges.

The dialysing membrane used was a cellulose membrane supplied by Fisher Scientific Co. (Cat. No. 8–667–1), with an average pore diameter of 25 Å. Before use, it was saturated with an aqueous 5 per cent calcium chloride solution and rinsed with demineralized water until the washing water contained less than 0·01 mg/l. calcium. This membrane did not show an adsorption of calcium-45 from the blood of animals loaded with calcium-45. The statistical errors of the mean values given are the fiducial limits[15] at 95 per cent ($= \bar{x} \pm t.s_{\bar{x}}$).

(*a*) *Concentration of calcium in the blood.* The total concentration of calcium in the blood averages 150–200 mg/l., but a net uptake of calcium is possible if only 5 mg/l. calcium are present in the water. Such a concentration gradient could only exist on the basis of diffusion if the epithelium of *L. stagnalis* were permeable to calcium ions and if calcium in the blood were present in a bound form so that the diffusible blood calcium remained below 5 mg/l. The concentration of diffusible calcium in the blood can be determined by compensation-diffusion[13] of blood against solutions of calcium, without the risk that some calcium-binding components could dissociate[14]. Blood is collected in ice-cooled tubes, and 5 ml. (Ca = 166 ± 5 mg/l.) was dialysed in a basket against 5 ml. calcium chloride solution of 166 or 114 mg/ml. (at 2° C) in hermotically closed polyethylene bottles of 15 ml. capacity. Equilibrium is fully established after 24 h. In four experiments the solutions of 166 mg/l. calcium were still at the end of dialysis at 166 ± 8 mg/l., thus not changing their concentration significantly.

The solution of 114 mg/l. calcium changed to 137 ± 3 mg/l. during dialysis. If all the blood calcium is diffusible one can calculate that the final concentration should be 140 mg/l.; 94–100 per cent calcium in the blood must therefore be in a diffusible form, and diffusion alone could never result in a net movement of calcium from the water to the blood of the animals.

(*b*) *Concentration of strontium in the blood.* Four groups of 10 *L. stagnalis* were placed in 200 ml. water containing originally 40 mg/l. calcium and 5 mg/l. (Sr + ^{85}Sr). The blood contained no measurable strontium before the experiment.

The radioactivity of the blood in these animals, corresponding to 14 mg/l. (Sr + ^{85}Sr), was four times higher than that of the surrounding water after 48 h. This concentration gradient of strontium indicates an active uptake since in five experiments on compensation diffusion of 3-ml. blood dialysed against 5-ml. of a solution of 170 mg/l. calcium + 14 mg/l. strontium, a homogeneous distribution of strontium-85 is obtained. The results of these experiments were for the radioactivity outside the dialysing basket 831 ± 5 c.p.m./ml., and for the radioactivity inside this basket 848 ± 7 c.p.m./ml. Not more than 3 per cent of the strontium in the blood could thus be non-diffusible. Hence it appears that the concentration gradient for strontium as well as for calcium cannot be obtained nor even maintained by diffusion alone. If we consider the animal as having a constant volume, solvent drag alone[16] could not cause the observed accumulation. Indeed, it would be necessary to remove the solvent from the animal to maintain this constant volume. An active uptake of ions would have to occur in conjunction with the efflux of solvent in order to preserve the high internal concentration of Ca^{++} and Sr^{++} and to permit a net uptake of these ions from the external medium.

(*c*) *The potential difference between the animal and the water.* Histological preparations showed multiple blood-spaces directly under the epithelium, for example, in the

mantle of the snails. A small wound on the top of the shell produces extrusion of a little drop of blood. Since this blood is highly conducting (1,200 mg/l. Na^+) an electrode can be placed in this drop and a reference-electrode in the water. The shell is maintained dry (for example, by silicone grease) and kept outside the water, whereas the soft parts of the snail are left in the water. Thus the potential difference (*PD*) between the two sides of the epithelium that separates blood from water can then be determined. The *PD* disappears at once when the reference electrode is brought through the epithelium. This indicates that no other electrical barrier between the inner side of the epithelium and the drop of blood exists. Between two wounds on the shell, the *PD* was also zero. The *PD*'s measured varied from -5 to -30 mV (inside negative) with an average of about -15 mV.

If one applies the equation of Ussing[16]:

$$\frac{\text{outflux}}{\text{influx}} = \frac{\text{internal concentration}}{\text{external concentration}} \times e^{\text{exponent } \frac{Z.F.\ (V \text{ int.} - V \text{ ext.})}{R.T.}}$$

where e, napierian base; F, faraday (96,500 coulombs/g. equiv); R, 8,3144 joules; T, 293° K; V, PD (V); Z, charge of the ion (+ 2 for Ca); concentration considered as = activity; and assumes stationary conditions where outflux equals influx, at an external concentration of 10 mg/l. calcium and a PD of $-$ 15 mV, one finds that such a PD could maintain an internal concentration of about 31 mg/l. Since the internal concentration is as high as 150 mg/l., this PD cannot be responsible for the accumulation of calcium in the animal.

Other freshwater molluscs such as *Planorbis corneus* (L.) and *Lymnaea auricularia* (L.) can also concentrate calcium-45 and strontium-85 in their blood to levels similar to those attained by *Lymnaea stagnalis*.

(*d*) *Experiments with barium*-133. Investigations with barium-133 were carried out at low concentration in the water (0·01 mM) to avoid lethal toxic effects. Ba seems also to be taken up under these conditions, but the blood-level in *L. stagnalis* did not exceed that of the surrounding water after 76 h, although the whole animal was about 10 times more radioactive than the same volume of water.

In conclusion, the concentrating ability of these freshwater snails for some radionuclides seems to be due to an active absorption of some alkaline earth ions from dilute solutions.

We thank R. Kirchmann and Dr. G. Gerber for their advice and help.

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SIMULTANEOUS MEASUREMENTS AND SPECTRAL ANALYSIS OF MICROPULSATION ACTIVITY

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A PREVIOUS article[1] described a series of simultaneous measurements of telluric micropulsation activity involving recordings made in Austin, Texas, Puerto Rico and Trinidad. Initial results were reported concerning power spectra and coherency of the signals in the frequency-range of 0·01–0·25 c/s. Further processing and analysis of the data have led to additional interesting observations and the correction of some of the initial results.

The investigation consisted of an experimental programme during October 1962. Micropulsations of the Earth's electric field were recorded simultaneously at three stations: Austin, Texas (30° 18′ N., 97° 47′ W.), Camp Tortuguero, Puerto Rico (18° 29′ N., 66° 25′ W.), and the U.S. Naval Air Station, Trinidad, West Indies (10° 42′ N., 61° 38′ W.). The stations at Austin and Puerto Rico were operated by the Electrical Engineering Research Laboratory of the University of Texas. The Trinidad station was operated by the Communications Sciences Laboratory, U.S. Air Force Cambridge Research Laboratories. Approximate distances between stations were: Trinidad–Puerto Rico, 600 statute miles; Puerto Rico–Austin, 2,100 miles; and Trinidad–Austin, 2,700 miles.

At all three locations electrodes were placed in the ground approximately 0·5 km apart along magnetic north–south and east–west lines and connected to a low pass filter used for rejecting 60 c/s interference. After broadband pre-amplification the signal passed through an active band-pass filter. The half-power points of the filter were 0·01 and 1·5 c/s with attenuation rates of 6 dB per octave below the passband and 12 dB per octave above. After final amplification the signals were recorded on paper charts at a recording speed of 60 mm/min. Accurate timing was supplied by a stable-frequency standard, synchronized with *WWV*.

Recordings were made continuously through the month of October 1962. Twelve data samples approximately 1 h each in length were selected for analysis, hand digitized and put on to punched cards for computation. The data were sampled at 2-sec intervals. Great care was taken in the digitization process to ensure that all channels were sampled simultaneously.

The analysis of the signals included the computation of the resultant electric field vector, power spectra, coherencies and coherency phase angles. The analysis was

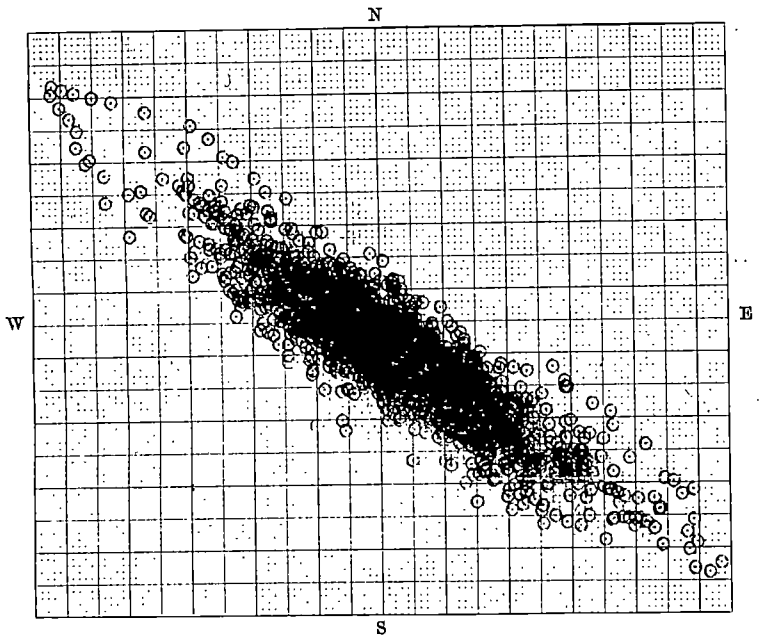


Fig. 1. *X–Y* plot of electric field data—Trinidad, October 28, 1962, 1200–1300 G.M.T.

done on an IBM 7090 using programmes developed by Simpson and utilizing the Daniell method of spectral resolution.

In order to determine the direction of the resultant electric field vector the digitized data were displayed on an *X–Y* plotter. For each sampling point the north–south voltage was plotted as the ordinate and the east–west voltage as the abscissa. All the data points for each hour-long sample were plotted together. Fig. 1 is an illustration of the result for the signal recorded on October 28, 1962, at Trinidad. Note the strongly elliptical polarization, an effect common to almost all the samples examined. For each sample the average value of the *E* vector was estimated by passing the best-fitting straight line through the data points. Table 1 is a compilation of the results for the 12 data samples which were digitized. Blanks represent plots for which it was difficult to determine the major axis of the ellipse, due primarily to low signal-to-noise ratio. Remarkably little variation in the vector direction is noted even though the data samples cover a period of 17 days and the hours 0700–2230 G.M.T. Fig. 2 is a map of the recording station locations showing the 12 sample average electric field direction. The limits of the vector over the samples are also shown.

The little variation noted in the direction of the electric field and the orientation of the resultant fields as shown in Fig. 2 indicate the strong influence of local topography and geology on telluric currents. For the case of Trinidad, an island situated about 50 km from Venezuela, the vector clearly parallels the dominant physical feature, the coast of South America, the boundary between conductive sea-water and more resistive continental mass. The *E* field at Austin, situated about 200 km inland from the Gulf of Mexico, is oriented roughly normal to the coast-line. On the island of Puerto Rico the orientation is roughly parallel with the West Indies chain. Although these islands are themselves quite a small topographical feature they are the visible portion of a large ridge of volcanic origin which is mostly submerged.

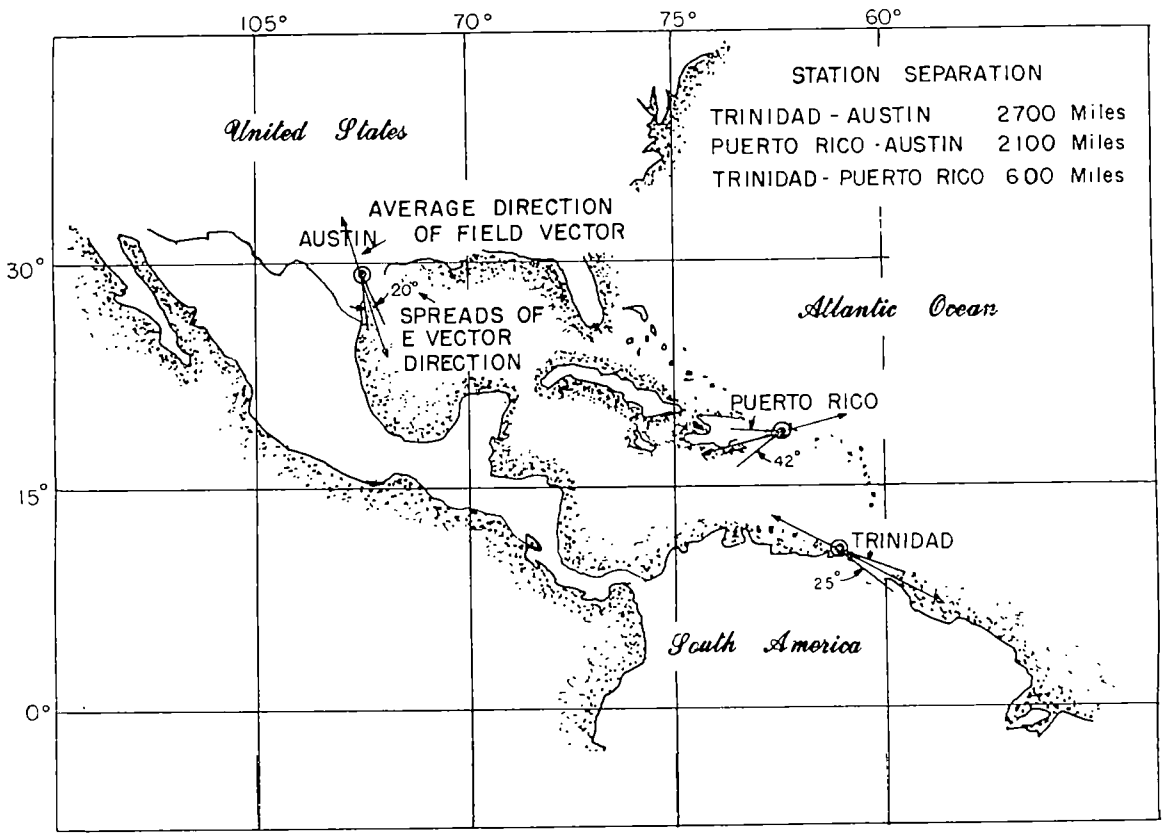


Fig. 2. Orientation of electric field vectors at Austin, Puerto Rico and Trinidad

Table 1

Sample	Date	G.M.T.	Average E vector direction, degrees west of magnetic north: Trinidad	Puerto Rico	Austin
A	13/10/62	1030–1115	67	131	32
B	15/10/62	0930–1040	55	—	30
C	16/10/62	1030–1145	53	97	—
D	17/10/62	0800–0915	40	95	24
E	20/10/62	1210–1355	54	85	15
F	24/10/62	1025–1155	52	98	35
G	24/10/62	1500–1600	62	100	26
H	28/10/62	1200–1300	54	93	27
I	30/10/62	0700–0815	55	92	29
J	30/10/62	1245–1400	55	—	—
K	30/10/62	1615–1715	53	93	19
L	30/10/62	2130–2230	55	94	—
	Averages		55° +12 −15	98° +30 −13	26° +10

The foregoing results may be partially explained by considering the idealized case shown in Fig. 3*a*. Two regions, land with conductivity σ_L and sea-water with a conductivity σ_S, are separated by a sharp boundary. In the vicinity of the boundary the tangential component of the electric fields and the normal components of the induced currents will be continuous, that is:

$$E_{T_L} = E_{T_S} = E_T \qquad (1a)$$

$$J_{N_L} = J_{T_S} = J_T \qquad (1b)$$

E and J are related through the familiar expression:

$$E = \frac{J}{\sigma} \qquad (2)$$

where σ = conductivity of the medium in mhos/m. In practice the conductivity of the sea (approximately 5 mho/m) is considerably larger than that of the land mass (approximately 1–10 m mhos/m).

Consider now Fig. 3*b*. As measured on the land, the electric field $\bar{E}$ will have components normal and tangential to the boundary. From the continuity relationships the

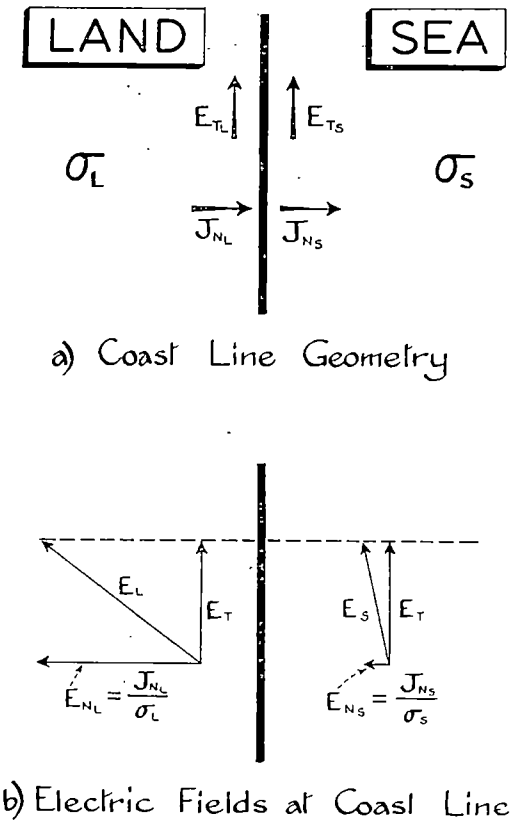


Fig. 3. Idealization of the 'coast-line' effect

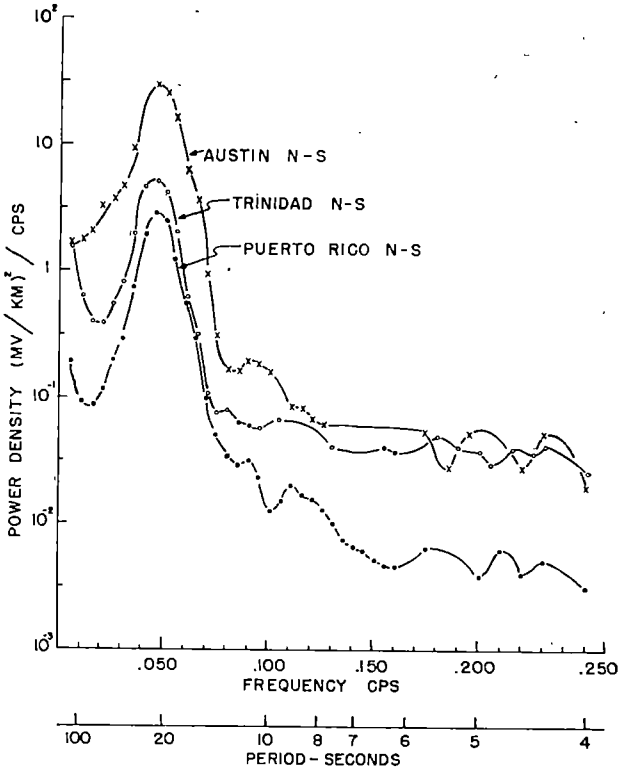


Fig. 4. Telluric current power spectra for simultaneous data, October 13, 1962, 1030–115 G.M.T.

tangential electric field measured in the sea will be the same as that for the land. Since the normal induced current must also be continuous and applying expression (2), the normal electric field as compared with that on land will vary inversely with the conductivity ratio. Since the sea-water conductivity is much larger than that on land the normal component will be much smaller. If σ_L is 0·1 per cent of σ_S, as would not be uncommon, then E_{N_S} will be 0·1 per cent of E_{N_L}. The result is that in any case except where E_L is directly normal to the boundary the resultant field as measured in the sea will be predominantly tangential to the boundary. This 'coast-line effect' is well illustrated at Trinidad. At Austin, although the site is about 200 miles from the coast the data probably reflect the influence of the sea and the propensity for the currents to flow towards the good conductor. The Austin situation is further complicated by the presence of 1/2–1 ohm-m sediments in the Gulf Coast region, resulting in an unclear land–sea boundary.

Additional power spectra and coherency computations have shown that those previously reported (ref. 1, Figs. 3–5) were in error. Corrected power spectra and coherency curves are shown in Figs. 4–6. Fig. 4, power spectra for data recorded simultaneously on October 14, 1962, displays distinct peaks at the pc frequency at all three recording locations. Note the identical peak frequency and the near-identical shape of the curves representing data recorded as much as 2,700 miles apart. Fig. 5 shows power spectra of data recorded at Austin on three separate days. The energy peak at the pc frequency is seen to vary by a factor of 3. Figs. 4 and 5 indicate that theories attempting to describe pc-type oscillations must satisfy the requirement of little latitude dependence while explaining the vast day-to-day variation in peak frequency.

Fig. 6, plots of the coherency between micropulsation data recorded on October 24, 1962, further demonstrates the simultaneity of events observed at different locations. High coherency is noted over a broad range of frequencies and even in the Trinidad–Austin case displays peaks in excess of 0·8. The fine structure observed appears on many

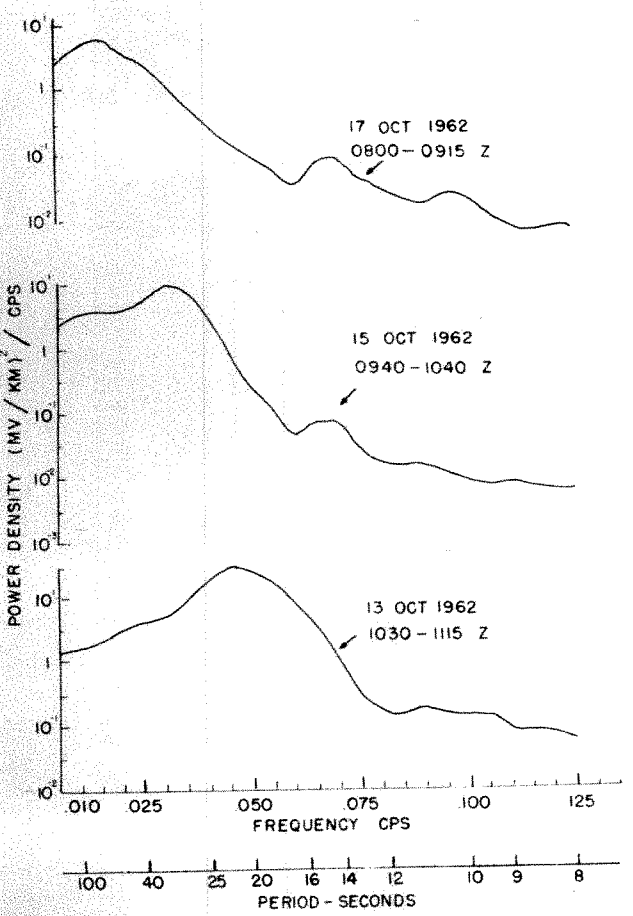


Fig. 5. Telluric current power spectra: Austin north–south recordings

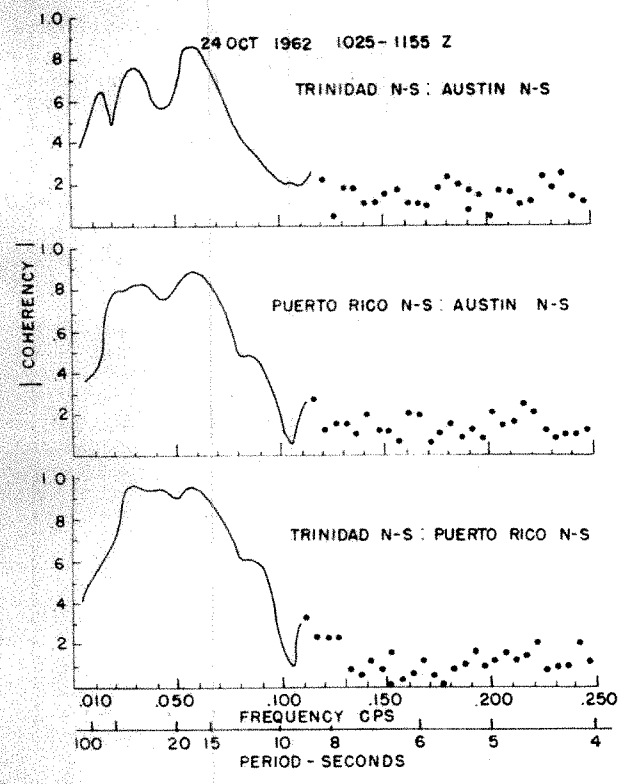


Fig. 6. Coherency analysis of Trinidad–Puerto Rico–Austin data

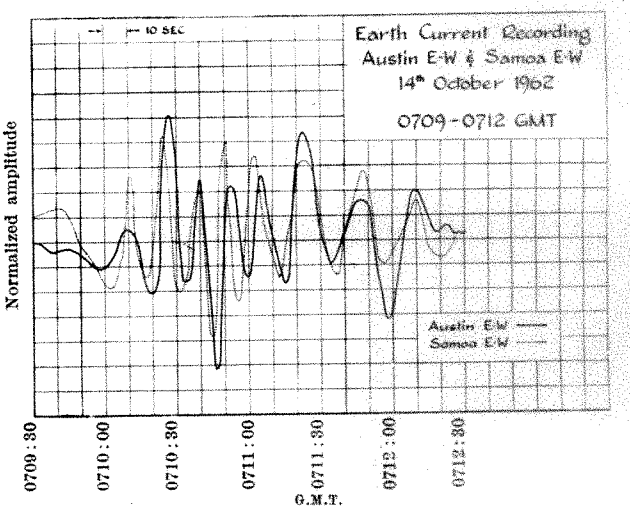


Fig. 7. Simultaneous micropulsation data, Austin east–Samoa east

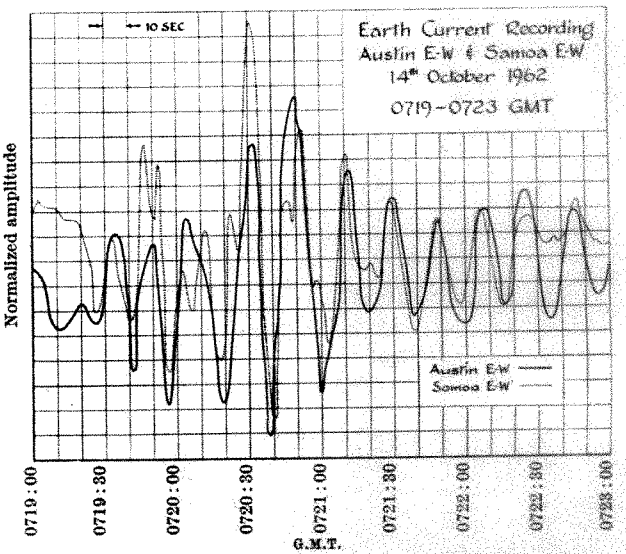


Fig. 8. Simultaneous micropulsation data, Austin east–Samoa east

of the computed coherency curves, but it is felt that insufficient data have been processed to allow meaningful comment concerning this phenomenon.

Telluric current micropulsations were also recorded during October 1962 on the island of Samoa in the South Central Pacific Ocean by an NRA, Inc. (New York, N.Y.), group. Comparison of data obtained at Samoa with those obtained at the University of Texas-*AFCRL* stations has yielded information concerning the widespread occurrence of some effects. Figs. 7 and 8 are plots of pt-type events that were simultaneously recorded at Samoa and Austin, Texas. For each case the Samoa east–west and Austin east–west data are plotted together normalized so that the peak amplitudes are roughly similar. The cycle-for-cycle similarity is striking considering that the records were obtained at stations separated by approximately 73° in longitude and 45° in latitude. At times in these figures the two signals appear perfectly synchronized. At others, such as 0709 : 50 (Fig. 7) and 0722 : 10 (Fig. 8), the Samoa signal appears to lead Austin. As yet there is no satisfactory explanation of this 'wandering' phase shift; more observations are required to evaluate the effect.

We thank Dr. S. Goldblatt of NRA, Inc., for permission to use the results obtained at Samoa.

[1] Komack, R. L., *et al.*, *Nature*, **201**, 460 (1964).

EXPERIMENTAL POST-TRAUMATIC DYSTROPHY IN THE RABBIT

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As a result of affections of the brain, of the spinal cord, the heart or the lungs, and especially of traumata, dystrophic phenomena for man frequently occur in the arm. If a trauma is the cause, then it is called post-traumatic dystrophy.

Hackethal[1] has shown that bruising or constriction of the n. ischiadicus in the rabbit leads to almost identical phenomena in the hind leg. His theory is that with the trauma bruising of the nerve takes place which gives rise to an ascending neuritis, which again leads to propulsive pressure on the nerve in the foramen intervertebrale.

Post-traumatic dystrophy phenomena, however, lead one to suspect a neurovegetative disregulation in the affected area. Cerebro-spinal nerves also contain vegetative fibres. To test this experimentally it is necessary in the first place to produce a lesion in the vegetative nervous system, and, if possible, with complete avoidance of any injury to the cerebro-spinal nerve fibres. Following Coujard's method[2], phenol was used as the damaging agent. The lesion of the ganglion stellatum and the plexus pelvicus caused post-traumatic dystrophy to appear in the hind legs of the rabbit along with irritation of the ganglion stellatum.

For the sake of simplicity the rabbits were treated only with phenol mixed in paraffin with a low melting-point—the injection being near the mouth of the bladder. Following this, diffusion to the plexus pelvicus occurred.

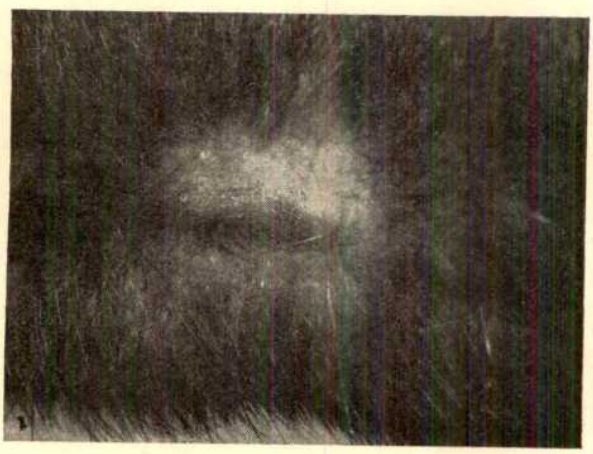


Fig. 1. Post-traumatic dystrophy in the rabbit starts with falling fur on the dorso-lateral side of the hind-leg

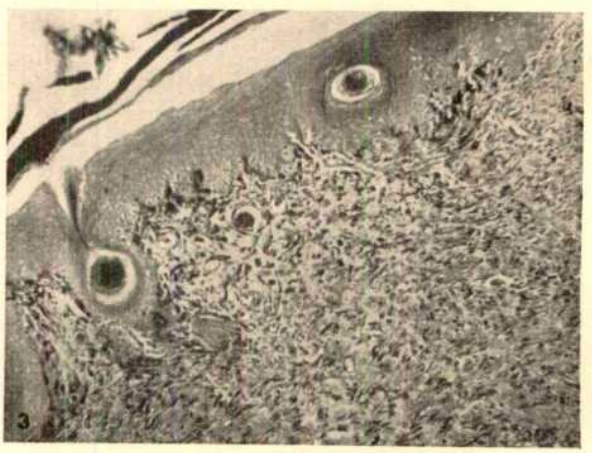


Fig. 3. The skin of the dorso-lateral side of the hind-leg in a dystrophic animal. Wide capillaries in the subcutis. Champy–Coujard technique

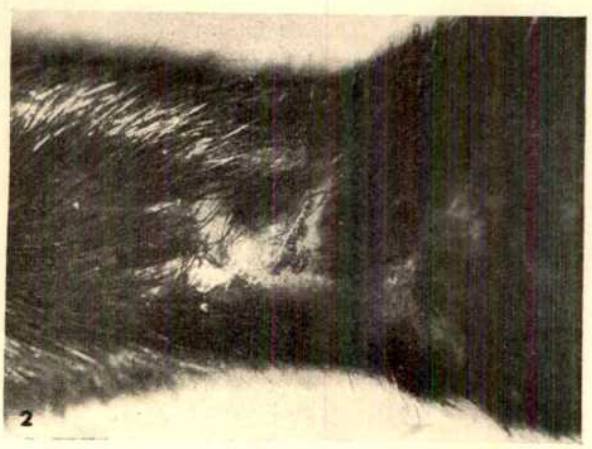


Fig. 2. Later skin lesions occur

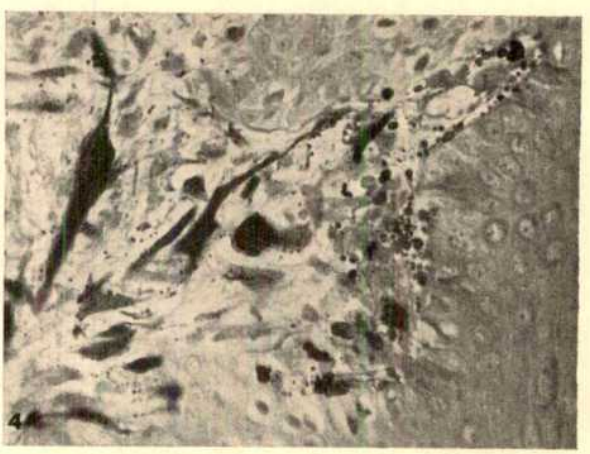


Fig. 4. Positive, black-stained interstitial cells of Cajal near capillaries in a dermal papilla. Champy–Coujard technique

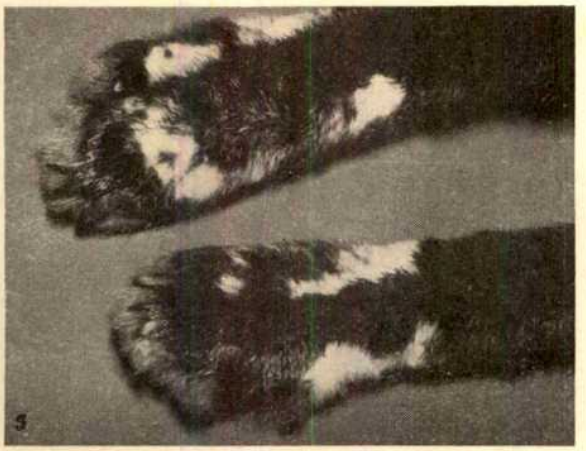


Fig. 5. The predilection places are marked after recovery, as the fur regenerates unpigmented

motor end-plates may also be detected by means of cholinesterase reaction.

In order to obtain an impression of the order in which the phenomena occur they have been set out graphically against time. Rabbit 9980 (Fig. 6) is an example of this. It is clearly visible that the phenomena in the skin arise first (within one week). The macroscopic and microscopic changes in the skin are so closely correlated that in the histogram the findings are combined. Then the reflexes decrease (three weeks after the dose of phenol). A muscle biopsy four weeks after the dose of phenol shows that, with strongly decreased reflexes, the motor end-plates, rendered visible by means of the cholinesterase reaction, are still about normal. The state of the peripheral vegetative nervous system in the muscles, however, is already considerably disturbed. After seven weeks clinical dystrophy is maximal, the reflexes have disappeared, yet the end-plates are still normal. The vegetative disturbance is now quite manifest, though only after eight and especially after 10–12 weeks, do the end-plates show a seriously disturbed picture together with strong reactions in the

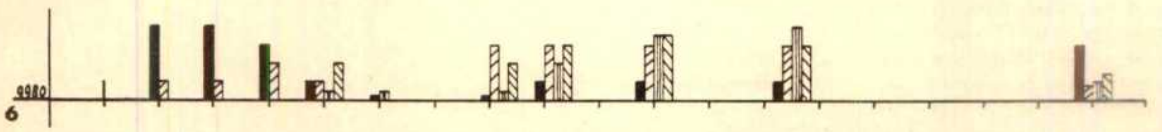


Fig. 6. Diagrammatic representation (rabbit 9980). Black, reflexes (high, normal); hatched, right down to left, clinical dystrophy and microscopic skin alterations; hatched (vertical), abnormal motor end-plate; hatched, left down to right, reactivity autonomic periphery in the muscle

Numerous post-mortem investigations have shown that the phenol did not penetrate to the cerebro-spinal plexus lumbo-sacralis.

Symptoms of post-traumatic dystrophy in the rabbit are: fur falling out, beginning on the dorso-lateral surface of the lower foot (Fig. 1), discoloration of the skin, œdema, and, later, wound formation (Fig. 2). Sometimes the area affected expands on the sole of the lower paw while, on occasions, the forepaws are also affected.

After some time a decrease of muscle reflex occurs in the hind legs. This reflex is occasioned by exercising a sudden pressure on the muscles of the lower legs while the hind legs are stretched. This decrease of the spread reflex indicates impotence in the muscles of the lower part of the foot, a phenomenon which also occurs with human patients (powerlessness in the hands). During later stages of the disease, club foot regularly occurs. With man the loss of power in the hand leads to a kind of claw-like appearance. In the rabbit, after some time a general recovery may take place. The regenerating fur is unpigmented (Fig. 5).

The first symptoms appear in the skin, commencing with loss of hair and œdema. In histological sections the strongly expanded capillaries in the skin are especially striking (Fig. 3). In sections of tissue, stained according to the Champy–Coujard method (a mixture of OsO_4 and ZnI_2), the peripheral vegetative nervous system appears to be in a very reactive stage (Fig. 4). This is especially evident from a far stronger coloration of the network of nervous fibres and interstitial Cajal cells, situated immediately below the cutis and within the internal cutis papillæ, mainly immediately along and around the capillaries. The basal layer of cells in the cuticle sometimes shows a black granulation, which has also been observed with some human patients, while the so-called Langerhans cells show a stronger reactivity. This is presumably connected with a strong increase of sensitivity to touch of the areas of skin affected.

In the further stages of the disease dystrophic changes also occur in the muscles. The findings of the muscle biopsies are described by Dr. Wiertz-Hoessels (following article). To sum up, it may be said that in the muscles, too, the reactivity of the peripheral vegetative nervous system increases while in the later stages changes in the vegetativum. After 18 weeks the symptoms have greatly decreased, the reflexes are normal again, and the condition shown by the muscles also subsides.

It appears that as a result of these experiments it can be stated that post-traumatic dystrophy can be provoked in an entirely vegetative manner. In contradistinction to Hackethal's ascending neuritis theory we would assume that the disease may be introduced as follows: the trauma, both a brief and a severe one (for example, a fracture) as well as a prolonged and moderate one (for example, excess strain), causes a local state of irritation to which the vegetative nervous system is sensitive. Should the level of irritation exceed a certain value, which is defined by individual sensitivity (vegetatively unstable persons), then a more general state of irritation occurs in which presumably the central part of the vegetative nervous system in the diencephalon is concerned. In the spots of least resistance the trophic supply becomes irregular and symptoms of post-traumatic dystrophy occur, with man in the hand and the shoulder, with the rabbit on the dorsal surface of the paws and the whole hind legs.

From this we would deduce, as a provisional conclusion, that the first therapy of a trauma should be directed to the stimulation of the blood supply to the area affected. With human patients, Thornban[3] observed as early as after three days over-filling of the subcutaneous capillaries when post-traumatic dystrophy was developing, otherwise not. Some rabbits, too, after four days already showed post-traumatic dystrophy. This is entirely in accordance with the view that the task of the peripheral part of the vegetative nervous system is above all a trophic one.

This work is being carried out in collaboration with Prof. J. F. Folkerts (Valeriuskliniek, Amsterdam) and Dr. E. L. M. J. Wiertz-Hoessels (Institute of Veterinary Anatomy, Utrecht). It was supported by the European Community for Coal and Steel in Luxembourg (Folkerts, Krediet, Sneep, Wiertz-Hoessels), and since 1963 by the Netherlands Organization for Pure Research (Z.W.O.), Den Haag (Folkerts, Krediet, Wiertz-Hoessels).

[1] Hackethal, K. H., *Medizin*, 1 (1958).
[2] Coujard, R., *Acta Neuroveg.* (Wien), **21**/1–3 (177–184).
[3] Thornban, W., *Acta Neuroveg.* (Wien), **25**/1–62.

INFLUENCE OF TROPHIC DISTURBANCES ON SKELETAL MUSCLE INNERVATION

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TROPHIC disturbances may occur in man in connexion with traumata but also as a result of affections in the central nervous system or chest organs. In the patients examined, the cause was, respectively: neuritis of the median nerve, amputation of the right index as the result of an industrial accident, and a left hemiplegia in consequence of thrombosis cerebri. All are patients of Prof. J. F. Folkerts, Neurological Department, Free University of Amsterdam (Valerius Clinic). In laboratory animals—rabbits—similar symptoms were observed after section of the sciatic nerve, while it was possible to stimulate the symptoms experimentally by irritation of the stellate ganglion and the plexus pelvicus by means of phenol. The symptoms also appeared in the rabbit, once after surgical research into pregnancy and once after injection of methylene blue in the foreleg.

The trophic disturbances known as post-traumatic dystrophy (see preceding article), which in man are indicated as 'hand–shoulder syndrome', consist, among other things, of discoloration of the skin and falling out of the hair, pain, rigidity of the joints, œdema and paralysis of the muscles. With the experimentally stimulated syndrome in animals, falling out of the fur is first observed. A fixed sequence is proved of the most liable areas. In contrast to man, in the rabbit it is in the first instance the lateral and dorsal sides of the lower foot of the hind-leg. The symptoms of these animals are comparable with those in man.

With those affections, extensive disturbances are evident in the cerebro-spinal and in the neuro-vegetative innervation of the skeletal muscles in the region concerned.

In view of the character of the clinical symptoms, changes may be expected in the first place in the vegetative innervation of the muscles. This occurs mainly by way of the blood vessel- and capillary-innervation. As Meyling[1] has pointed out, the post-ganglionic ortho- and parasympathetic nerves do not end directly on the tissues which they innervate, but on a linked network of the interstitial cells of Cajal. This is formed by small ganglion-like cells with long varicose prolongations. The network is not adrenergic or cholinergic, but preponderantly either one or the other[2]. Slides of material treated with the Champy–Coujard technique gave results which may be better interpreted than those of material treated with the cholinesterase reaction. The latter was applied by us according to Koelle in the modification of Gerebtzoff, whereas, besides this, we applied silver impregnation (Bielschowsky-Gros and Bodian), intravital methylene blue staining (Coers and Woolf), and gold chloride staining (Gairns) to obtain an idea of the total innervation. In normal circumstances interstitial cells occur around the capillaries, the prolongations of which envelop the vascular tissue more or less densely (Fig. 1). When a post-traumatic dystrophy has developed, the aspect proves to be quite different. The innervation pattern is irregular with the Champy–Coujard reaction (Figs. 2 and 3). The capillaries which mostly have a very irregular course are, on the whole, strongly innervated. Moreover, it is striking that between the somewhat irregular muscle fibres numerous black cell elements occur, sometimes larger than the normal interstitial cells, though all belonging to this group. In contrast with the normal case, they very seldom have contact with capillaries. These cells with their prolongations sometimes form networks all over the muscle tissue. Sometimes they have granular contents and have a remarkable resemblance to the interstitial cells that Meyling[1] found in the normal intestinal wall after vital methylene blue staining. In the patients we repeatedly saw similar cells occur in the connective tissue round the muscle fibres and sometimes in contact with prolongations of pericapillary interstitial cells.

As will be pointed out in a more extensive article in preparation with Prof. J. F. Folkerts and Dr. P. Krediet, it was evident that in the rabbit changes in the adrenergic system were correlated with macroscopic changes of the

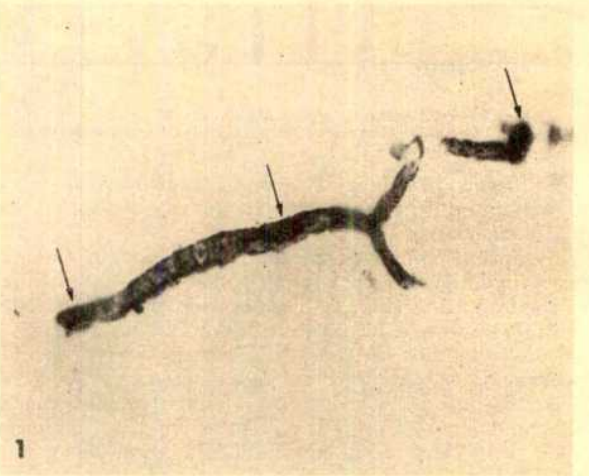


Fig. 1. Adrenergic innervation of capillaries in the rabbit. Prolongations of interstitial cells of Cajal envelop the vessel; at ↗ a cell body. (Champy–Coujard method)

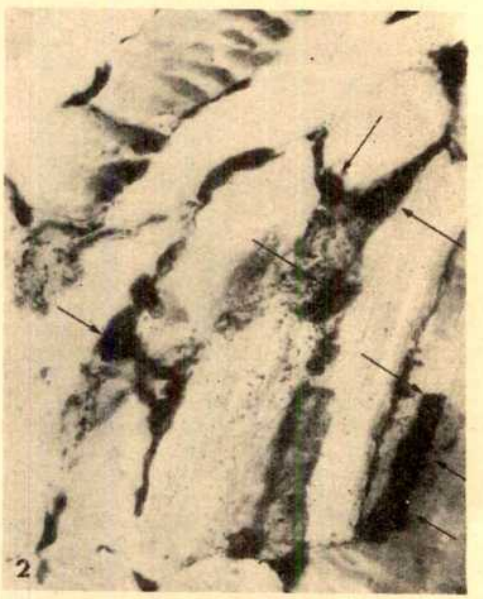


Fig. 2. Abnormal adrenergic muscle innervation in the rabbit in a case of post-traumatic dystrophy. At ↗ an interstitial cell. (Champy–Coujard method)

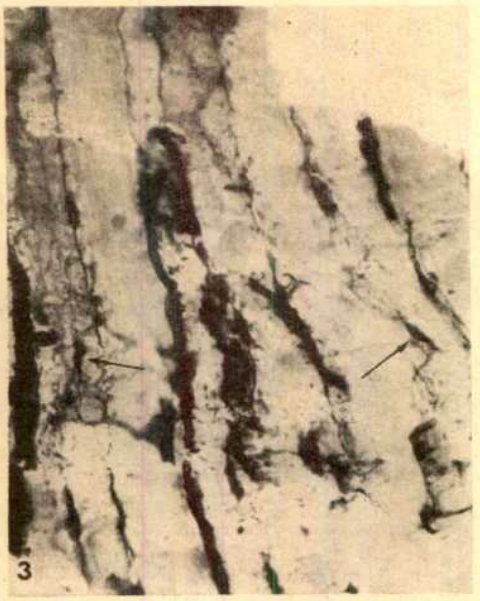


Fig. 3. Abnormal adrenergic muscle innervation in man in a case of post-traumatic dystrophy. Interstitial cells occur without contact with the capillaries (↗). (Champy–Coujard method)

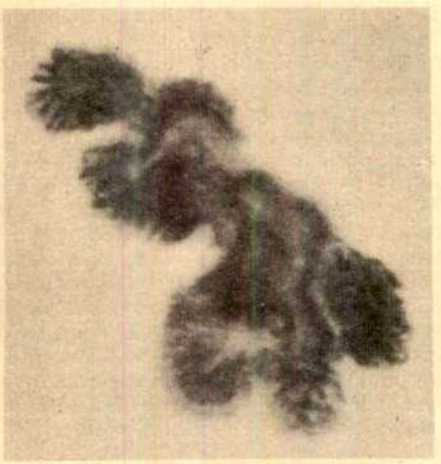


Fig. 4. Normal human motor end-plate, with several synaptic grooves and an irregular sub-neural apparatus of Couteaux. (Cholinesterase reaction)

skin and a pathological spread reflex. In the meantime it appeared that also in the cerebro-spinal innervation changes had taken place: here, attention was only given to the motor end-plates. With human patients where dystrophy had existed for a long time, a number of end-plates proved to be normal (cholinesterase reaction). Most, however, were more or less degenerated (Figs. 4 and 5). In the laboratory animals where dystrophy, after repeated phenol injections, had existed for a few months, preponderantly changed end-plates have been found as well (Figs. 6, 7 and 8). Not only with the cholinesterase reaction but also with methylene blue staining the motor end-plates look modified. The muscle tissue itself also, locally, has a pathological aspect. In some cases real paralysis of the hind-leg proved to exist while the motor end-plates were intact to a large extent. So the dysfunction of the end-plates must have a cause other than their morphology. Moreover, it may be mentioned that the motor end-plates may degenerate in very various ways. No single fixed sequence is to be observed in the progress. In later experiments, muscle biopsies have been taken after a short time. So far as we can now judge it would appear that a larger percentage of normal motor end-plates are to be found, though the adrenergic innervation shows a considerably varied pattern.

The fact that paralysis may occur with preponderantly intact motor end-plates, on which neither on the sciatic nerve nor on the spinal cord deviations appear, shows that we are dealing with functional disturbances. These probably precede morphological disturbances. This would explain why shortly after the beginning of the dystrophy still many, but afterwards considerably less, motor end-plates are intact. The functional disturbances of the

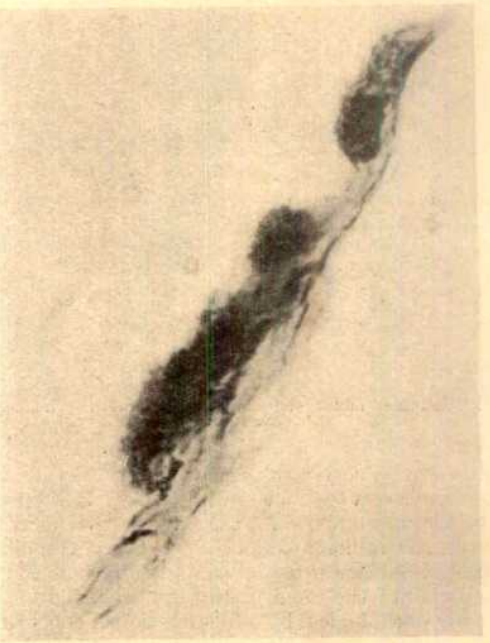


Fig. 5. Abnormal motor end-plate in man. (Cholinesterase reaction)

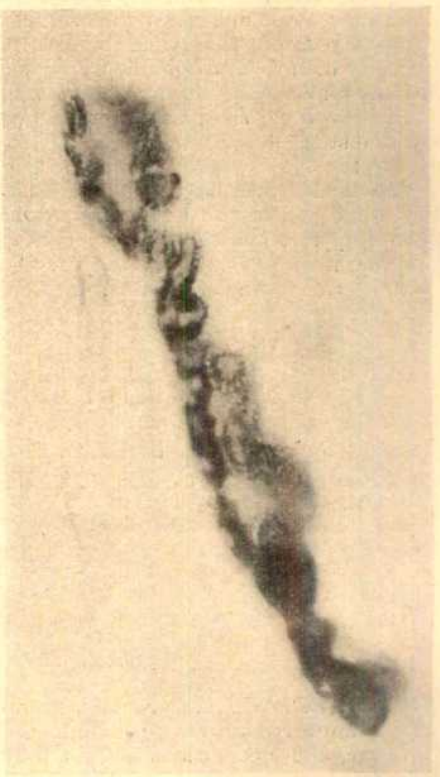


Fig. 6. Normal motor end-plate of the rabbit. The synaptic grooves are in communication with each other. There is a tiny sub-neural apparatus of Couteaux. (Cholinesterase reaction)

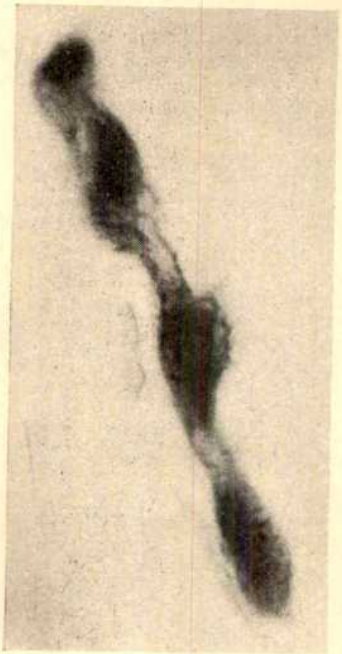


Fig. 7. Abnormal motor end-plate in the rabbit. (Cholinesterase reaction)

muscle, however, correlate very distinctly with deviations in the adrenergic vegetative innervation. If we now consider the strong influence that the vegetative nervous system has on the 'milieu intérieur' or, as Coujard[3] points out, the trophic influence it exercises on the tissues, it may be supposed that the end-plates are able to become secondarily pathological by a dysfunction of the vegetative nervous system. Morphologically, the myoneural synapse is still intact, though it is no longer able to function normally because its environment has been disturbed. In accord with this is Prof. Folkerts's observation, with one of the dystrophic patients, that while the electromyogram had been greatly disturbed, in the biopsy no such serious pathological anatomical changes in the muscle fibres were to be found. However, the adrenergic innervation had been seriously disturbed.

Exactly on the spot where the motor end-plate is located, a close contact can be demonstrated between the cerebro-spinal and the vegetative nervous system, as can be shown in Fig. 8, which illustrates a silver preparation of a normal muscle on which a capillary lies around the end-plate. In a number of Champy–Coujard preparations, too, the capillary loop as well as its vegetative innervation is to be seen round the sole nuclei of the end-plate. Meyling[4] also found in electron-microscopic preparations that the capillaries push with the axons into the sarcolemma.

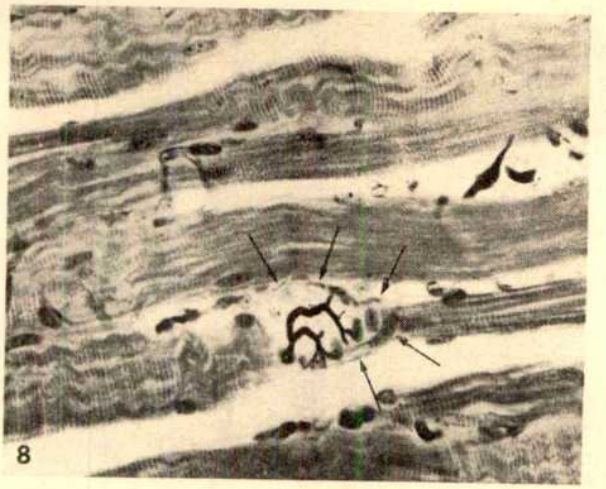


Fig. 8. A motor end-plate, surrounded by a capillary loop in a normal muscle. Bodian silver stain. The capillary is indicated by arrows

Judging from the results at present at our disposal, we gain the impression that trophic disturbances, which may be evoked by different causes, first influence the vegetative innervation in the muscle, not the cerebro-spinal as might have been expected. These symptoms may already be observed after a few days. In accordance with this is Thornban's[5] observation that changes in the capillary system of patients occur a few days after the injury. It is not until later that, secondarily, caused by the abnormal vegetative innervation, changes in the cerebro-spinal system also arise. First the function of the motor end-plate is disturbed; afterwards it also changes histologically.

This work is being carried out in collaboration with Prof. J. F. Folkerts (Neurological Department of the Free University, Amsterdam) and Dr. P. Krediet (Institute of Veterinary Anatomy, Utrecht). It was supported by the European Community for Coal and Steel in Luxembourg (Folkerts, Krediet, Sneep, Wiertz-Hoessels), and since 1963 by the Netherlands Organization for the Advancement of Pure Research (Z.W.O.), The Hague.

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TOXICITY OF *Phalaris tuberosa* FOR SHEEP

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PHALARIS TUBEROSA is a valuable component of some of the improved pastures in south-eastern Australia. However, under conditions not yet fully defined, sheep grazing this grass have sometimes developed a peracute disease which causes sudden collapse and death[1–3], or a more chronic syndrome, phalaris staggers[1,4,5], which is characterized by neurological disorders. Recent observations on the peracute syndrome in sheep grazing *P. tuberosa* in the Australian Capital Territory have led to the development of a new hypothesis as to the ætiology and pathogenesis of poisoning due to *P. tuberosa*.

An outbreak of the peracute disease in autumn 1963 afforded the opportunity to examine clinical cases and to perform immediate necropsies. Five sheep were seen to collapse while exhibiting severe clinical signs. Two died quickly after the initial collapse, but three recovered. Two of the recovered animals died after subsequent attacks. All five sheep showed clinical signs of acute heart failure. Of those examined as soon as detected, three had ventricular fibrillation, and the other two had arrhythmic tachycardia which proceeded rapidly to fibrillation; the heart then stopped beating. The mucous membranes and skin became cyanotic during the period of fibrillation and cardiac arrest, but the animals remained conscious throughout, their reflexes responded to stimuli, and they continued to breathe. Recovery from this acute attack in three of the five sheep was initiated by isolated, infrequent heart beats, which accelerated to tachycardia over about 2 min, and then the animal could be forced to rise and walk away. Another clinically identical case was observed to die rapidly during an acute attack in autumn 1964.

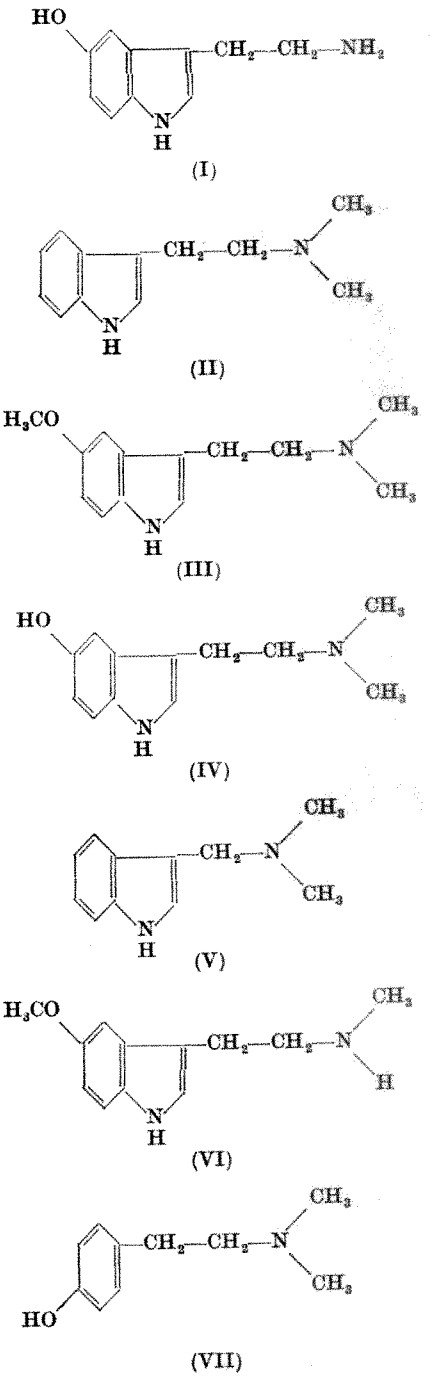
Necropsy performed immediately on the three sheep which did not recover from their observed clinical episode revealed severe congestion of the abdominal viscera, particularly of the liver and kidneys, and petechial hæmorrhages in the epicardium and duodenum of one sheep. No other lesion was observed by either macroscopic or microscopic examination. In addition to these, four sheep which were grazing *P. tuberosa*, and which had been observed by stock attendants to collapse and die rapidly, showed abdominal visceral congestion, and two of them had epicardial hæmorrhages when examined soon after death. In all affected sheep examined, the heart had stopped in systole. The necropsy findings were indicative of acute heart failure of sudden onset.

These observations indicated that a substance with specific cardiopharmacological properties might be present in *P. tuberosa*. Culvenor *et al.*[6] reported the presence of a group of particularly interesting alkaloids from *P. tuberosa*, which are *N,N*-dimethylated indolealkylamines closely related to serotonin, 5-hydroxytryptamine (I). The major alkaloids found in *P. tuberosa* were *N,N*-dimethyltryptamine (II) and 5-methoxy-*N,N*-dimethyltryptamine (III), with variable amounts of 5-hydroxy-*N,N*-dimethyltryptamine (bufotenine) (IV) and traces of other indole derivatives. In addition, (II) and gramine (V) were found in the related grass, *P. arundinacea*[6], which had earlier been shown[7] to contain 5-methoxy-*N*-methyltryptamine (VI) and hordenine (VII). These and related methylated indolealkylamine and β-phenylethylamine derivatives have also been isolated from several other plant genera and from lower vertebrates, but have not been found in mammals[8].

Serotonin is highly active pharmacologically. It causes contraction of smooth muscle, influences cardiac activity and is concerned in the normal functioning of brain. It is probably the chemical transmitter in the parasympathetic component of the autonomic nervous system, performing an analogous function to that of acetylcholine[8,9]. Consequently, the hypothesis was formulated that the methylated tryptamine alkaloids present in *P. tuberosa* produced disease by interfering with the metabolism and pharmacological functions of serotonin.

Parenteral administration of solutions of pure 5-methoxydimethyltryptamine, dimethyltryptamine or gramine has been found to kill sheep, guinea-pigs, rats and mice due to acute heart failure, characterized clinically by arrhythmia, ventricular fibrillation and cardiac arrest. Less than lethal doses may cause cardiac arrest, from which the animal recovers in a fashion similar to the manner in which sheep recover from peracute poisoning with *P. tuberosa*.

The cardiac effects in sheep of the alkaloids present in *P. tuberosa* have been investigated more critically using a nine-lead electrocardiogram as described for the horse by



Steel[10]. Sample tracings are shown in Fig. 1. When it was ascertained that the main abnormality was a striking ventricular arrhythmia, most of the electrocardiographic data were obtained by continuously repeating tracings made from leads I, II and III. In order to minimize muscular spasms caused by injection of dimethyltryptamine and 5-methoxydimethyltryptamine and so prevent distortion of the ECG tracing by muscle potential, sheep were anæsthetized with 'Kemithal' and maintained on oxygen and nitrous oxide during the experiment. ECG recordings were taken after anæsthesia was induced, and prior to the administration of the alkaloid in order to obtain the normal pattern for each sheep.

All three alkaloids tested, 5-methoxydimethyltryptamine, dimethyltryptamine and gramine, caused a marked tachycardia with pulse-rates varying from 170 to 270/min (Fig. 1, *A* and *E*). This was usually followed by the occurrence of isolated and multiple ventricular extra-

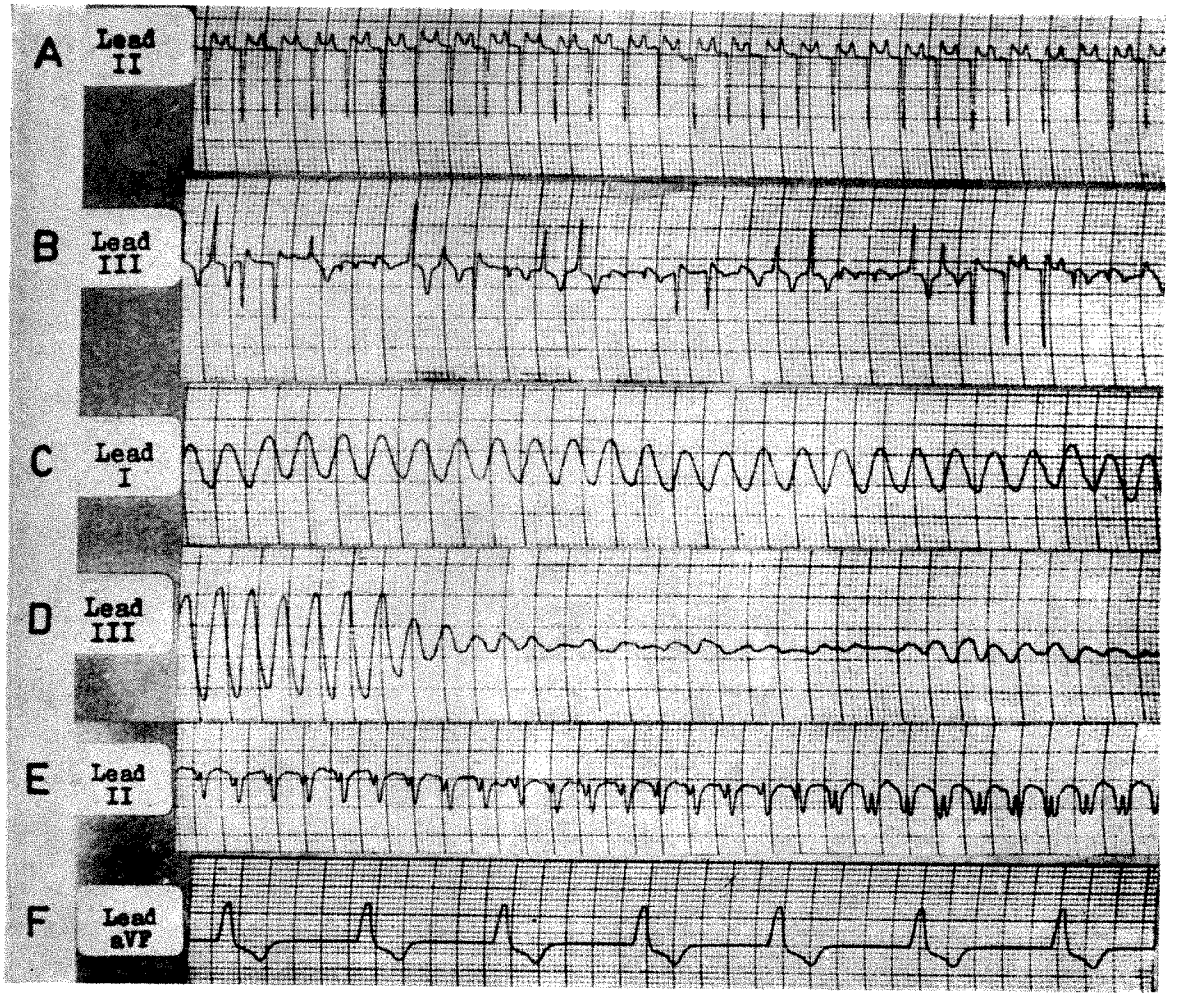


Fig. 1. *A*, Unanæsthetized sheep given 10 mg gramine/kg intravenously. Lead II showing paroxysmal ventricular tachycardia. Heart-rate 270/min. *B*, Lead III for *A* showing ventricular extrasystoles arising from multiple ectopic pacemakers and producing a chaotic heart action. The sheep survived. *C*, Anæsthetized sheep given 5 mg 5-methoxydimethyltryptamine/kg intravenously. Lead I showing the sine-like undulation characteristic of ventricular flutter. *D*, Lead III for *C* showing the transition of ventricular flutter into terminal ventricular fibrillation. The sheep died. *E*, Anæsthetized sheep given 5 mg 5-methoxydimethyltryptamine/kg intravenously. Lead II showing an intraventricular conduction defect during a period of tachycardia in which the heart-rate was 240/min. The *QRS* duration was 0·11 sec on the left side of the tracing and 0·13 sec on the right. The sheep died. *F*, Anæsthetized sheep given 10 mg dimethyltryptamine/kg intravenously. Lead aVF showing intraventricular block and a heart-rate of 60/min. The sheep died

systoles (Fig. 1, *B*), and succeeded by long or short episodes of paroxysmal, ventricular tachycardia. The heart action then became chaotic in some cases followed by ventricular flutter and fibrillation (Fig. 1, *D*). In other instances, after an initial arrhythmic tachycardia, the heart-rate slowed markedly to 60–70/min with the development of intraventricular block before a final, slow, ventricular fibrillation (Fig. 1, *F*).

The neurological syndrome of phalaris staggers may take an acute form from which recovery is possible, or may terminate in a chronic, degenerative disease. The acute form is common in New South Wales and the Australian Capital Territory, whereas the chronic form of the disease is said to be more common in South Australia. The most severe form of acute phalaris staggers results in lateral recumbency of the sheep with tetanic spasms and extensor rigidity of the legs, the digits are over-extended, resulting in splaying and dorsi-flexion, the head is dorsi-flexed, the pupils dilated, the face wrinkled, the jaws may be clamped and there is usually profuse, ropy salivation. Animals affected less severely with the acute syndrome show hyperexcitability, marked incoordination of gait often characterized by high-stepping with the front legs, splaying and dorsi-flexion of the digits, convulsive spasms, nodding of the head, salivation and twitching of the ears and tail. All the signs are exaggerated by enforced movement or fright, and the animal may fall as it runs. Sheep affected with chronic phalaris staggers may show all the signs shown by acute cases, but in addition show evidence of residual nervous lesions as, for example, the loss of co-ordination of the front legs, with the animal moving on its knees.

Parenteral administration of small doses of the alkaloids of *P. tuberosa* to unanæsthetized sheep and to small laboratory animals causes profound disturbances of the central nervous system, producing the same group of neurological signs displayed by sheep with acute phalaris staggers. The most potent of the alkaloids in producing central nervous system disorder is 5-methoxydimethyltryptamine, followed by 5-hydroxydimethyltryptamine, and dimethyltryptamine. Each of these alkaloids passes quickly into the brain from the blood, and produces neurological signs within 13–16 sec of injection into the jugular vein of sheep. The smallest dose used, 0·1 mg/kg, of 5-methoxydimethyltryptamine produced hyperexcitability, salivation, head-nodding, ear-twitching, tail-twitching, urination, dilatation of the pupils, stamping, circling, incoordination and backward movement. A greater variety of signs was shown by adult sheep given 0·5 mg 5-methoxydimethyltryptamine/kg intravenously. These sheep showed the development of the following clinical signs: hyperexcitability, licking of the lips,

salivation, chewing movements, ear-twitching, tail-twitching, urination, head-nodding, face-wrinkling, pupillary dilatation, flaring of the nostrils, stamping, circling, backward movement, collapse either in lateral recumbency with extended, paddling legs or in ventral recumbency with the hind legs stretched out backwards, the digits were over-extended appearing splayed and dorsi-flexed, breathing was stertorous and the rectal temperature rose to 108°–109° F. The animals took about an hour to recover. Larger doses, 1·5–2 mg, 5-methoxydimethyltryptamine/kg may prove fatal, the animal falling rapidly in lateral recumbency, with extensor rigidity, tetanic spasms, opisthotonos and dyspnœa. Death occurs from acute heart failure. Subcutaneous administration of the alkaloids produced the same syndrome with slower onset, less severity and longer duration. However, doses of 1–2 mg/kg of 5-methoxydimethyltryptamine may still prove lethal by this route. Oral administration of 5-methoxydimethyltryptamine produced central nervous system signs in sheep in about 6 min, showing that the alkaloid is rapidly absorbed from the gastro-intestinal tract.

It is considered that, according to the dose and mode of administration, the group of neurological signs exhibited by sheep with acute phalaris staggers can be produced by the alkaloids present in *P. tuberosa*. Chronic phalaris staggers with persistent, degenerative central nervous system lesions has not yet been reproduced by administration of the tryptamine alkaloids.

The hypothesis that the dimethylated tryptamine alkaloids are the toxic principles of *P. tuberosa* responsible for all manifestations of its toxicity is being investigated. The alkaloids have been found to inhibit the catabolism of serotonin by monoamine oxidase, and to exert powerful pharmacological effects on the central nervous system and the heart. The results presented here strongly support the hypothesis that the alkaloids present in *P. tuberosa* are responsible for the peracute syndrome of poisoning and for acute phalaris staggers.

We thank Dr. C. C. J. Culvenor of the C.S.I.R.O. Chemical Research Laboratories for gifts of the alkaloids.

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SEQUENTIAL AND REVERSIBLE INHIBITION OF SYNTHESIS OF RIBONUCLEIC ACID IN THE NUCLEOLUS AND CHROMOSOMES: EFFECT OF BENZAMIDE AND SUBSTITUTED BENZIMIDAZOLES ON DIPTERAN SALIVARY GLANDS

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THE object of the work recorded here was to examine the relationship between nucleolar and chromosomal synthesis by means of chemical treatment. The chemicals used were 5,6-dichloro- and 4,5,6-trichloro-1-(β-D-ribofuranosyl)benzimidazole (DRB and TRB, respectively), and benzamide (the substituted benzimidazoles were a gift of Merck Sharp and Dohme, Rahway, New Jersey). Of these, TRB acts as an antagonist of purine ribosides[1].

Salivary glands were dissected from larvæ of the chironomid *Smittia parthenogenetica* at a stage[2] where the cells are fully grown but secretion increasingly accumulates in the lumen. The glands were incubated for different periods at 25° in a chemically defined medium[2], into which the chemicals were added at various concentrations (given as wt./ml.). The glands were then offered uridine (nominally 5 : 6 tritium, Radiochemical Centre, Amersham) usually for 30 min in medium containing chemicals as in the previous incubation, or without chemicals in some of the recovery experiments. Control glands were incubated similarly without chemicals.

Autoradiographs were prepared as described elsewhere[2]. Most of the radioactivity incorporated in the nucleus is removed from the preparations by ribonuclease[2]. The incorporation appears to be mainly into internal positions of the RNA, since the incorporation of tritiated uridine or tritiated guanosine is appreciably the same whether or not an excess of non-radioactive cytidine or adenosine, respectively, is present to reduce interconversion among nucleotides. Accordingly, the distribution of ^{14}C-uridine (in the absence of non-radioactive cytidine) to the other major nucleotides is negligible[3].

Selective inhibition of chromosomal synthesis. In chromosomes the synthesis was markedly inhibited after incubation of glands for 2 h with 10 μg DRB. The inhibition was greater with 20 μg, and total in practically all cells with 40 μg ($1·25 \times 10^{-4}$ molar) or more, as shown in Fig. 2. In the nucleolus the synthesis was slightly inhibited by 40–80 μg DRB (Fig. 2), and was reduced to only about three-quarters of the incorporation in controls by 200 μg (at this concentration DRB did not dissolve completely).

In time-sequence incubations with 20 μg DRB, the inhibition of chromosomal synthesis increased progressively to near completion at 2 h as found in the previous experiments, while the nucleolar synthesis was still not inhibited at 4 h. With 200 μg, synthesis in the chromosomes was already totally inhibited at 30 min, while in the nucleolus the synthesis was progressively inhibited from little at 30 min to about half the incorporation in controls at 4 h. The kinetics of inhibition of the chromosomal and nucleolar syntheses are different.

The specific inhibition of chromosomal synthesis is also produced by benzamide at higher doses. In time-sequence incubations with 1·3 mg benzamide ($10·7 \times 10^{-3}$ molar), chromosomal synthesis was suppressed at 30 min, while the nucleolar synthesis was gradually reduced and was only slightly reduced by 4 h. The two kinetics of inhibition with benzamide show a similar difference as with DRB.

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Table 1. Stimulation of Incorporation of Uridine into Nuclear RNA

Chemicals present during incubation with tritiated uridine	Incorporation (arbitrary values)
DRB (30 or 200 μg)	(+ + + +) × 100 see Fig. 5
No chemical	(+ + + +) × 10 see Fig. 4
No chemical, control	+ + + + see Fig. 1
Benzamide (0·5 mg) + DRB (50 μg) + TRB (50 μg)	+ + +
Benzamide (1 mg) + DRB (100 μg) + TRB (100 μg)	+
Benzamide (1·5 mg) + DRB (150 μg) + TRB (150 μg)	0

All glands, except controls, were previously incubated for 40 min with benzamide (1·5 mg), DRB and TRB (150 μg each).

Benzimidazole, the parent compound of DRB, does not cause a selective inhibition of chromosomal synthesis, but rather affects all nuclear synthesis uniformly. Glands incubated for 2 h with $1{\cdot}25 \times 10^{-4}$ molar benzimidazole showed slight overall nuclear inhibition of synthesis. With $10{\cdot}7 \times 10^{-3}$ molar benzimidazole the glands showed severe inhibition (and signs of damage to cells).

1,1,3-Tricyano-2-amino-1-propene (tricyamp) stimulates the incorporation into nuclear RNA by more than one order of magnitude[4]. 50 μg tricyamp (a gift of Upjohn Co., Kalamazoo, Michigan) and 150 μg DRB (not completely dissolved), offered simultaneously during incubation for 2 h, affected nuclear synthesis in opposite directions.

Sequential inhibition of chromosomal and nucleolar synthesis. The nucleolar synthesis, which resisted inhibition by DRB and benzamide when given separately, was inhibited by a mixture of benzamide (1·5 mg), DRB and TRB (150 μg each, not completely dissolved). After incubation for 15 min total inhibition was observed only in chromosomes, and in chromosomes as well as in the nucleolus after 30 min. Preliminary experiments, in which one inhibitor at a time was left out of the mixture, suggested that TRB is the most important of all three chemicals necessary to prevent nucleolar synthesis.

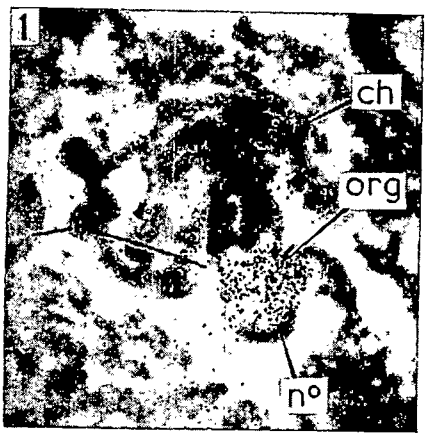


Fig. 1. Autoradiograph of squash preparation of control gland incubated first in standard medium for 2 h, and then in standard medium with tritiated uridine (40 μc./ml.; 3·0 c./m.mole) for 30 min. Exposure: 25 days (× 400). *ch*, chromosomes; *n°*, nucleolus; *org*, nucleolus organizer

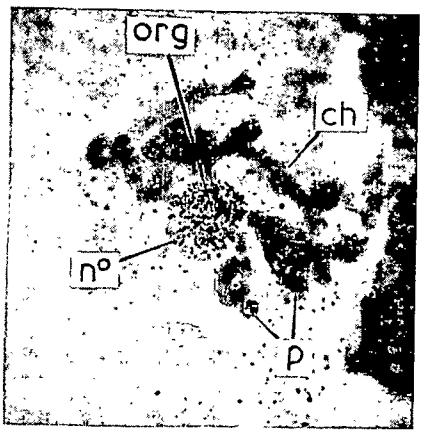


Fig. 2. Same as Fig. 1, except that 80 μg DRB was present throughout the incubations. *p*, pigment

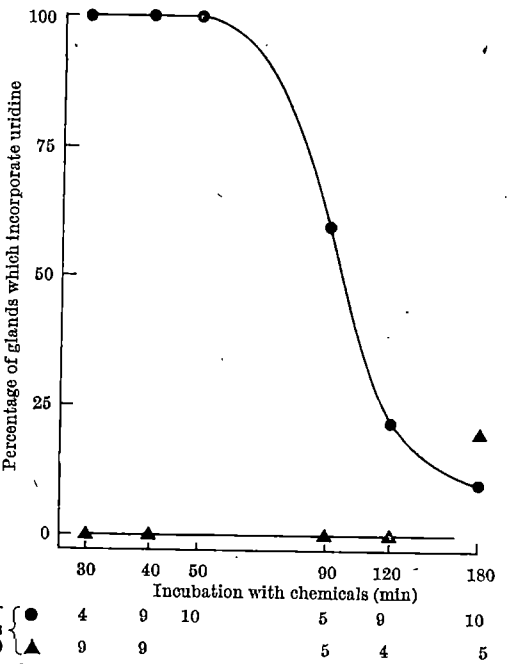


Fig. 3. Recovery of incorporation of uridine into nucleolar RNA after previous incubation with benzamide (1·5 mg), DRB and TRB (150 μg each) for different periods. Tritiated uridine was offered in the absence (●) or presence of the chemicals (▲)

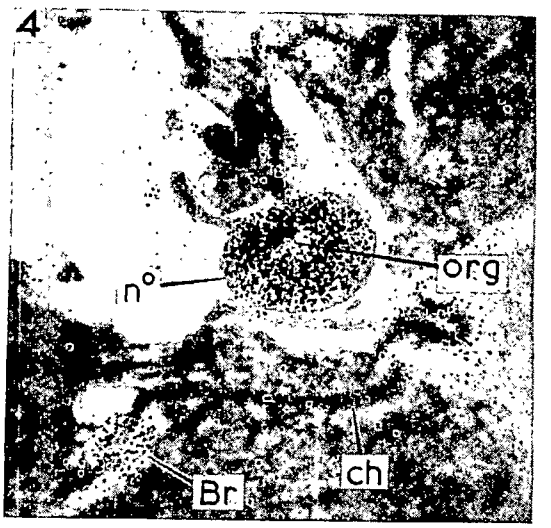


Fig. 4. Autoradiograph of gland incubated with standard medium containing benzamide (1·5 mg), DRB and TRB (150 μg each) for 40 min, and then with standard medium containing tritiated uridine (60 μc./ml.; 2·1 c./m.mole) alone for 30 min. Controls for this gland show a similar picture as in Fig. 1. Exposure: 20 days (× 400). *Br*: Balbiani ring

Restoration of nuclear synthesis. Nuclear synthesis was restored soon after withdrawal of the mixture of chemicals. As shown in Fig. 3, recovery fails after incubation for 90 min; thereafter the proportion of cells that recover falls, the amount of incorporation per cell becomes more variable, and signs of damage to cells appear.

As summarized in Table 1, incorporation in the recovered cells is frequently stimulated by about one order of magnitude, which is particularly clear in glands incubated for less than 90 min. The stimulation affects mainly the nucleolus (Fig. 4).

Stimulation by up to two orders of magnitude was generally found in the presence of 30 or 200 μg DRB

(Fig. 5). The stimulation is greater in the nucleolus than in chromosomes. Radioactive RNA now appears in the proximity of the nucleolus and chromosomes, and in the cytoplasm near to the nucleus. Because of probable displacement during squashing of the preparations, the origin in the cell of this RNA cannot be precisely determined. Incorporation in cytoplasm is clear.

The over-stimulation caused by DRB was unexpected, since the intention was to permit only restored nucleolar synthesis, while continuing to maintain inhibition of chromosomal synthesis. To examine further this possibility, DRB was replaced by different mixtures of benzamide, DRB and TRB (Table 1, lines 4 and 5), but the general effect was to reduce incorporation.

To summarize, on withdrawal of all three chemicals which inhibit synthesis, synthesis is recovered and is accompanied by stimulation, possibly as a 'rebound' phenomenon. The stimulation is accentuated if DRB alone remains present. Over the two observed levels of stimulation the nucleolus shows a greater stimulation than the chromosomes, which at the lower level of stimulation incorporate less than in the controls. The three chemicals which at high concentration inhibit synthesis permit restoration of synthesis at low concentration. However, as the concentration increases the overall synthesis ceases to be stimulated before it gradually disappears; concurrently, the greater stimulation in the nucleolus disappears. It would seem, therefore, possible to isolate a stimulated nucleolar synthesis only at the lower level of stimulation obtained by total withdrawal of chemicals, using shorter radioactive incubation than in Fig. 4 (compare with Fig. 2 where non-stimulated nucleolar synthesis is isolated).

The nuclear incorporation is restored first in the nucleolus (Fig. 6), as judged from the different stages of cell incorporation which are evident in glands after prolonged incubation with chemicals. It can also be seen that the incorporation starts in the nucleolus around, but not inside, the organizer (easily recognized in the chromosome that traverses the nucleolus), and that the radioactive RNA then moves towards the nucleolar periphery[5,6].

One conclusion which emerges is that the nucleolar synthesis displays an independence lasting for at least several hours of inhibition of chromosomal synthesis. The existence of a nucleolar synthesis was concluded originally from the kinetics of normal incorporation[7]. In the normal cell, as well as in the cell which recovers from inhibition, a significant incorporation into nucleolar RNA precedes that into chromosomal RNA.

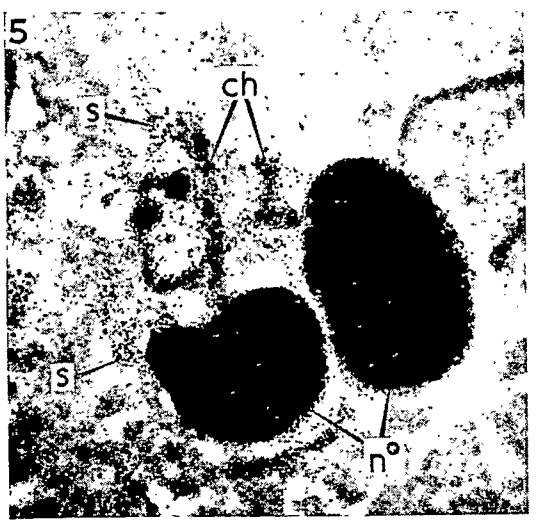


Fig. 5. Same as Fig. 4, except that the medium with tritiated uridine also contained 30 μg DRB. *s*, radioactive RNA

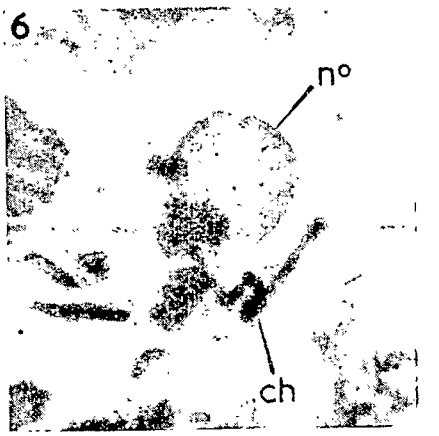


Fig. 6. Autoradiograph of gland incubated for 1·5 h with the same chemicals as for Fig. 4, and then in standard medium with tritiated uridine (60 μc./ml.; 3·0 c./m.mole) alone for 20 min. Exposure: 30 days (×400)

Kinetically, the nucleolar and chromosomal syntheses are different, both with respect to the kinetics of inhibition and of the stimulation that goes with reversal of inhibition. During inhibition the nucleolar synthesis is more resistant, and during restoration it is more stimulated, than the chromosomal synthesis. One explanation would be a different behaviour of the precursor pools for the two syntheses, as suggested by previous studies[8].

The restoration of synthesis begins in the nucleolus proper and not inside the organizer, as in normal synthesis[5,6] even during brief periods[7]. In the first step of stimulation shown in Fig. 4, the stimulation affects the nucleolus but not the organizer which incorporates no differently from the chromosome. (The reverse is true in the first step of inhibition shown in Fig. 2, where the incorporation is as much inhibited in the organizer as in the chromosome, but much less so in the nucleolus.) Conversely, substances, such as proflavine and actinomycin, that affect the nucleolar synthesis very specifically do not particularly affect synthesis in the organizer more than in the chromosome. All the evidence tends to indicate that in the nucleolus the synthesis is dissociable from the organizer, and therefore appears to be not immediately dependent.

A practical application comes from the virtual isolation of the nucleolar synthesis which is achieved within the cell (Fig. 2). Using this approach, the newly synthesized nucleolar RNA was characterized[9] avoiding cell fractionation. It would appear that a stimulated nucleolar synthesis can also be isolated. It is suggested that inhibition of chromosomal synthesis, particularly with low doses of DRB or TRB, can provide a tool for the reversible depletion of messenger (chromosomal) RNA without much affecting the nucleolus. It now seems probable that the inhibition of chromosomal synthesis, and the concomitant underproduction of moulting hormone, observed in an insect endocrine gland after feeding benzamide[10] in fact involved an effect of this kind.

This work was supported by the Damon Runyon Memorial Fund for Cancer Research (*DRG*–579) and the British Empire Cancer Campaign for Research.

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ANTIGEN BARRIER OF THE MOUSE PLACENTA

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THE placenta of mammals is a homograft since it differs genetically from the host. However, although it is intimately united with the maternal tissue, it is not rejected until parturition, a period usually far in excess of the time taken to elicit a homograft reaction. Several hypotheses have been put forward to account for the remarkable success of the conceptus as a homograft. They are: (*a*) the fœtus is antigenically immature; (*b*) the immunological reactivity of the mother is non-functional during pregnancy; (*c*) the uterus is an immunologically privileged site; (*d*) there is an anatomical barrier between mother and fœtus. These hypotheses have recently been fully reviewed by Billingham[1]. The first three of these hypotheses have been conclusively shown by various workers to be unsatisfactory, and Billingham concludes by stating that: "The only reasonable hypothesis . . . is that mother and fœtus are completely separated by an anatomical barrier". Such a barrier must logically reside in the placenta, where fœtal and maternal tissues come into intimate contact with one another.

The purpose of this article is to describe a structure which is considered to function as a barrier in preventing fœtal transplantation antigens from reaching the maternal tissues. A detailed account of our findings will be reported elsewhere. Our reasons for suggesting we have identified this barrier are as follows:

(1) *Electron-microscopical examination of the placenta.* The 16-day placenta from a *BALB/c* ♀ × *C57BL* ♂ mating was fixed in 1 per cent osmium tetroxide, buffered in veronal acetate to *p*H 7·3 for 1 h at 4° C. Dehydration was by a graded series of alcohols before embedding in 'Araldite'. Sections cut at approximately 80 mμ were stained for 10 min with a solution of lead citrate. It was found that the great majority of trophoblast cells were surrounded by a layer of amorphous, electron-dense material varying considerably in thickness from 0·1μ to 2·0μ. It was thick and conspicuous around the trophoblast giant cells which were in contact with the maternal decidual cells. Schiebler and Knoop[2] have described a similar distribution of this intercellular material in the rat placenta. The morphological evidence that this material overlies the free surface of the labyrinthine trophoblast bordering the maternal lacunæ is not so convincing. High-resolution electron-micrographs, however, show a very thin, ill-defined, filamentous layer in this position.

(2) *Histochemical identification of the intercellular material.* An exhaustive histochemical analysis of this material showed it to be of a mucoprotein nature, weakly sulphated and probably rich in hyaluronic and sialic acids. In its staining reactions it conforms in all respects to the material described in the placenta under the name of 'fibrinoid', which is reviewed in detail by Pearse[3]. In particular, the material showed a very strong positive reaction with the periodic acid–Schiff technique following diastase digestion, and with the Hale colloidal iron reaction (carried out as described by Barker and Anderson[4]). The latter reaction, as modified for the electron microscope by Gasic and Berwick[5], was performed in order to confirm that the amorphous layer seen in the electron-micrographs corresponded to the material examined histochemically. A positive reaction to this test can be visualized in the electron microscope by the deposition of cubic crystals of ferric-ferrocyanide at the sites of uptake of colloidal iron. In the mouse placenta the crystalline deposits appeared between the trophoblast cells, furthermore they also lined the maternal lacunæ in a position corresponding to the filamentous material already mentioned.

(3) *Comparison of the amounts of fibrinoid in hybrid and inbred placentæ.* From the histochemical analysis it was found that the periodic acid–Schiff reaction (following diastase digestion) and the Hale's colloidal iron reaction demonstrated the presence of fibrinoid most clearly. Consequently they were used as 'marker' stains in the following quantitative estimations of fibrinoid in hybrid and inbred placentæ. The following strain combinations of inbred mice were used: A_2G × *C57BL*, *BALB/c* × *C57BL*, *BALB/c* × *CBA*. The first of these strain combinations had been used previously by one of us (W. D. B.) to demonstrate clearly the influence of immunological dissimilarity of mother and fœtus on the size of the placenta[6]. The mice were mated so that each strain of each combination served as the mother. The placentæ were removed on the 16th day of pregnancy, fixed and sectioned at 8μ. Near mid-line sections of each hybrid placenta were compared for fibrinoid content with the similar sections of the corresponding inbred placenta—thus A_2G ♀ × *C57BL* ♂ compared with A_2G ♀ × A_2G ♂, etc.

In every case there was considerably more fibrinoid present, assessed subjectively by independent observers, in the hybrid than in the inbred placentæ. To eliminate the effects of heterozygosity, blastocysts obtained from A_2G × A_2G matings were transferred to the uterus of a pseudopregnant *C57BL* female. The placenta obtained by this method contained a greater amount of fibrinoid than did the *C57BL* × *C57BL* placenta obtained from a normal pregnancy. This indicated that the increase in fibrinoid is entirely due to the genetic dissimilarity between mother and fœtus. The origin of the placental fibrinoid is uncertain. Both the decidua[7] and the trophoblast[8] have been suggested as contributing the major amount, but since fibrinoid is present around extra-uterine trophoblast (see following), it seems to us more likely to be of trophoblastic origin.

(4) *Extra-uterine trophoblast.* It is now well established that mouse blastocysts will develop in an extra-uterine site to give rise to highly invasive trophoblast and normal embryos[9–11]. The success of the graft was in no way affected by genetic variation between donor and host

mouse. Indeed, mouse blastocysts will develop with a considerable degree of success beneath the kidney capsule of an adult male rat[12]. Simmons and Russell[13] have shown that presensitization of a host mouse by a skin graft from a genetically dissimilar donor strain does not affect the well-being of a subsequent trophoblast graft, whereas embryonic implants are promptly destroyed. These observations demonstrate that trophoblast transplanted to abnormal sites still retains its ability to prevent the sensitization of the host. Electron-microscopical examination of trophoblast which had been actively invading host kidney for between two and ten days revealed that the fibrinoid layer was conspicuously present around the cells of the graft (Kirby and Malhotra, unpublished results). Recent histochemical investigations show that the material has the same composition as that in the placenta.

(5) *Histochemical comparison of placental fibrinoid with the intercellular matrix of the hamster cheek pouch.* The hamster cheek pouch is an immunologically privileged site. Homo-specific and, not infrequently, hetero-specific tissue grafted into the cheek pouch quickly becomes vascularized and is accepted for prolonged periods of time. From their investigations of this phenomenon, Billingham and Silvers[14] suggest that transplantation antigens from the graft are prevented from reaching the host tissues by some sort of absorptive activity of the connective tissue matrix in the pouch skin. In view of the suspected functional similarity with respect to the antigen barrier between this absorptive matrix of the cheek pouch and placental fibrinoid it seemed of interest to determine if the two materials were chemically similar. Histochemical tests indicated that the intercellular matrix of the hamster cheek pouch dermis and placental fibrinoid were chemically very similar.

These observations lead us to conclude that the fibrinoid material in the mouse placenta prevents the escape of fœtal transplantation antigens into the maternal system. In view of the very thin layer of fibrinoid lining the maternal lacunæ, it might be argued that there is not sufficient material in this site to act as a barrier to antigen escape. Even if this is so, it does not invalidate our hypothesis. The route of transplantation antigens into the host is incompletely understood—tissue/tissue contact and lymphatic drainage are known to be important in this respect. That the vascular system is an ineffective route of antigen transfer is amply demonstrated by homografts into the hamster cheek pouch, in which, although the foreign tissue becomes richly vascularized, it nevertheless fails to sensitize the host.

The analogy between the hamster cheek pouch matrix and placental fibrinoid is justified only with respect to the blockade of transplantation antigens. Thus, whereas pouch skin homografts are susceptible to a state of specific immunity, elicited by proxy, for example, by a homograft of normal skin, the placenta seems insusceptible to a similar immunological state[13].

Fibrinoid lying between the maternal and fœtal tissues in the placenta has been described for a number of species including the rat[2,7], guinea-pig[15], the carnivora[16] and man[17]. In this last-named case the authors suggested that "the fœtal and maternal tissues usually are set off from one another by a local deposition of fibrinoid substance, the well-known layer of Nitabuch, which may behave somewhat as an immunological no-man's-land, walling the fœtus off from chemical interaction with its host". It is tempting to suggest that the phenomenon of antigen barrier by a mucoprotein material, commonly called fibrinoid, may be common in mammalian placenta.

It will be of interest to remove the investing fibrinoid from the trophoblast by enzymatic digestion and to determine if such 'divested' trophoblast will sensitize allogeneic hosts.

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CHEMICAL ASPECTS OF INHIBITORS OF $Rh_0(D)$ ANTIBODY

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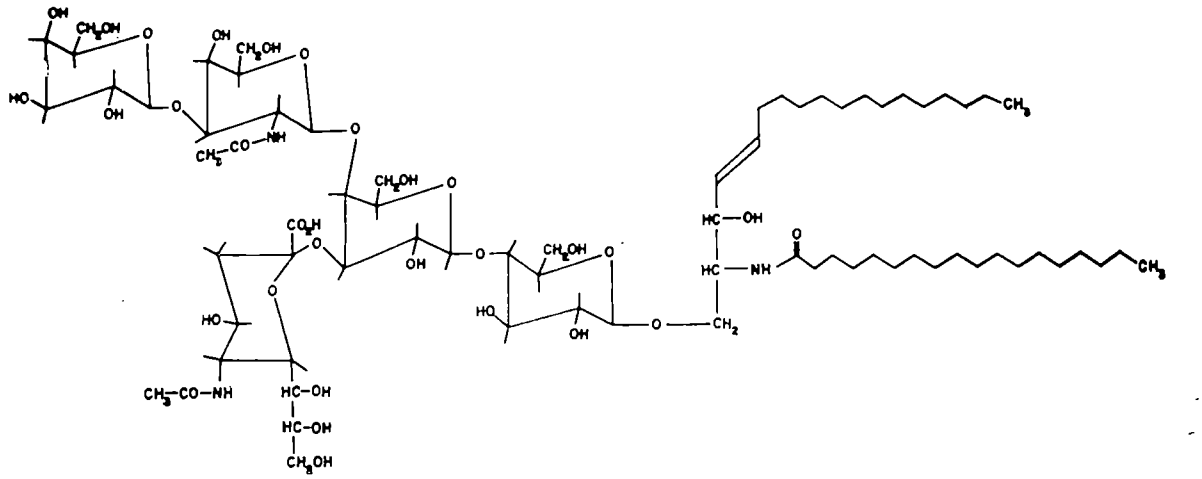
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SINCE Landsteiner and Wiener[1] first described the $Rh_0(D)$ antigen, its chemical nature has been reported as lipid, protein or carbohydrate. The difficulties involved in the isolation of the antigen from erythrocytes have been the main reason why most attempts to identify it have utilized inhibition of agglutination of $Rh_0(D)$ cells in specific antiserum by substances isolated from other sources. Final evidence of antigenic specificity, of course, lies in the capacity of an inhibitory agent to stimulate specific antibody on injection.

The present article is concerned with the chemical nature of sialogangliosides from brain tissue, and a glycopeptide from a species of *Pseudomonas*[2], which inhibited the agglutination of $Rh_0(D)$ erythrocytes by specific antiserum, and stimulated the production of $Rh_0(D)$ antibody, in rabbits. The chemical structure of these substances is as yet incompletely known, but the immunological and chemical information available is sufficient to suggest that some substance similar to them exists in $Rh_0(D)$ cells. This, in turn, allows the suggestion of a possible structure for this antigen which can be reconciled, to some extent, with substances reported by others.

Howe[3] developed the concept in which the influenza virus receptor, Rh and ABO antigens are parts of a complex. Moskowitz and Treffers[4] and Morgan and Watkins[5] described the destruction of $Rh_0(D)$ antigen by periodate. Bigley, Chandler and Dodd[6] demonstrated inhibition of $Rh_0(D)$ antibody by eluates of erythrocytes treated with mumps virus or periodate. The failure of Mäkelä *et al.*[7] to repeat these results with mumps virus was shown in this laboratory to be due, not to differences in the hæmagglutinating capacity of their virus, but to its lack of enzymatic activity on urinary mucoprotein inhibitor[23]. This is related to the neuraminidase activity of the virus, which is the key to the production of inhibitory eluates. The possibility that *N*-acetylneuraminic acid (NANA) might be related to the $Rh_0(D)$ antigen suggested the examination

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Galactose(1→3)N-Acetylgalactosamine(1→4)Galactose(1→4)Glucose Ceramide

(3 ↑ 2)

NANA

Fig. 1. Ganglioside G_1 of Kuhn and Wiegandt. This ganglioside corresponds to ganglioside 1-*G* used in the present work

of sialogangliosides and NANA for the capacity to inhibit the specific antibody. Previously, Werner and Odin[8] had reported that NANA occupied a terminal position in gangliosides and mucoproteins, and Faillard[9] described a brain ganglioside containing 13·2 per cent NANA resistant to neuraminidase, which Gottschalk[10] considered to indicate a different linkage.

Ganglioside preparations. Gangliosides were prepared from acetone powders of human brain[11], by the methods of Kuhn and Wiegandt[13] who isolated a series of oligosaccharides from bovine gangliosides, obtaining in disaccharide form, each linkage existing in monosialogangliosides. One of the acetolysis products NANA (2 → 3) galactose (4 → 1) *N*-acetylgalactosamine is neuraminidase-resistant. The resistance of this NANA residue resides in the steric hindrance provided by the *N*-acetylgalactosamine residue linked at the C-4 of galactose which is *cis* to the C-3 linked NANA. NANA

Gal *N*ac(1→4)Gal(1→4)Glu—1→*R**

(3 ↑ 2)

NANA

G_0 (*FM*)

Gal(1→3)Gal *N*ac(1→4)Gal(1→4)Glu—1→*R*

(3 ↑ 2)

NANA

G_1 (1-*G*)

Gal(1→3)Gal *N*ac(1→4)Gal(1→4)Glu—1→*R*

(3 ↑ 2)

NANA(2→8)NANA

G_3 (3-*G*)

NANA(2→3)Gal(1→3)Gal *N*ac(1→4)Gal(1→4)Glu—1→*R*

(3 ↑ 2)

NANA(2→8) NANA

G_4 (4-*G*)

**R*, ceramide.

Fig. 2. Ganglioside structures as proposed by Kuhn and Wiegandt. Notations in brackets refer to corresponding preparation used in the present study

(2 → 3) galactose and NANA (2 → 3) lactose, which are not substituted in the galactose (C → 4) position, are susceptible to neuraminidases from *Vibrio cholerae*.

The structures proposed by Kuhn and Wiegandt[13] are represented in Figs. 1 and 2. Their gangliosides G_0, G_1, G_3 and G_4 correspond in composition and chromatographic properties to the preparations *FM*, 1-*G*, 3-*G* and 4-*G*, respectively as used here. Ganglioside 2-*G* is similar to Kuhn's G_2, a positional isomer of G_3.

Homogeneous preparations of *FM*, 1-*G*, 3-*G* and 4-*G* were prepared from the crude mixture, gangliosides-I (ref. 14). Gangliosides 4-*G* and 3-*G* were converted to 1-*G* by neuraminidase. The NANA residues of *FM* and 1-*G* are resistant to the enzyme. Mild acid hydrolysis of 1-*G* produced galactose and the ganglioside *FM*. Calf and rabbit gangliosides are analogous to gangliosides-I. Neuraminidase-treated gangliosides-I was prepared from gangliosides-I by treatment with neuraminidase, dialysed, passed over 'Dowex-50-[H+]' and precipitated with acetone. It contained mostly 1-*G* and some *FM*.

Inhibition of Rh_0(D) antibody by gangliosides and NANA. Previous reports have shown that crude gangliosides containing NANA, and NANA alone, from several sources inhibited specific agglutination by Rh_0(D) antibody[15]. The data in Table 1 are representative of the results obtained with these substances and the homogeneous ganglioside preparations described here. It has been emphasized that the procedure for inhibition previously described[15] is essential for these results. For the data in Table 1, a 1 : 16 saline dilution of anti-D serum (Ortho) was used, representing the highest dilution of the serum producing complete agglutination of all the trypsinized, group O Rh_0(D) erythrocytes added. The use of this minimal 4+ dilution of serum has been stressed as an absolute condition for the sensitivity of the inhibition test, since lower dilutions with an excess of antibody require a disproportionate amount of inhibitor. These data were repeated with five different commercially prepared antisera and with sera from three different iso-immunized females. Red cells were obtained from Ortho Pharmaceutical Corporation, Rariton, New Jersey, and from the blood bank at University Hospital, Columbus, Ohio.

The data in Table 1 show that the homogeneous human gangliosides, 1-*G*, 3-*G* and 4-*G*, were superior inhibitors to the mixed gangliosides-I, ganglioside *FM* and gangliosides from calf and rabbit brains, while gangliosides lacking NANA did not inhibit at all. Crude NANA completely inhibited at a concentration much greater

Table 1. INHIBITION OF $Rh_0(D)$ ANTIBODY BY GANGLIOSIDES AND NANA

Inhibitor	Inhibition (μg/0·1 ml.) Complete	Partial
Gangliosides-I	1,000	1·9
Ganglioside *FM*	500	250
Gangliosides 1-*G*		
(1)	0·50	0·03
(2)	0·95	0·015
Ganglioside 3-*G*	0·13	0·0008
Ganglioside 4-*G*	62·5	15·6–7·8
Neuraminidase-treated gangliosides-I	0·95–0·47	0·06
Tay-Sachs ganglioside	62·5	0·47
Calf gangliosides	1,000	62·5
Rabbit gangliosides	500–250	31·3
Asialoganglioside*	>1,000	>1,000
Neuraminlactose†	62·5	31·3
(*d*+) Lactose	>1,000	>1,000
N-Acetyl glucosamine	>1,000	125
Glucosaminic acid	>1,000	>1,000
NANA‡		
Bovine submaxillary gland 85 per cent pure	1,740	1,740
Crude bovine crystallized-once	500	125
Crude bovine mild acid hydrolysis	1,000	1·9
Human plasma three times crystallized	>1,000	>1,000

* Kindly supplied by Dr. Roscoe Brady.

† Purchased from General Biochemicals, Chagrin Falls, Ohio.

‡ See ref. 11 for a description of these samples.

than for the gangliosides, but after mild hydrolysis, inhibition was only partial, while pure NANA from human plasma did not inhibit. It is suggested that NANA in the cruder preparations is more polymerized and forms a less dissociable complex with antibody. It is evident from the data that gangliosides 1-*G* and 3-*G* are the best inhibitors, requiring concentrations as low as 0·5–0·13 μg/ml. Gangliosides 4-*G* and *FM* were less effective.

The inhibition noted by NANA was confirmed by Boyd and Reeves[16], who also noted that colominic acid, a polymer of NANA produced by certain strains of *E. coli*, was also inhibitory. Inhibition by NANA was not observed by Springer *et al.*[17] or by Johnson and McCluer[18]. However, Dodd *et al.*[19] demonstrated that inhibition by NANA is dependent on the amount of antibody, time of incubation and the reversibility of the reaction. Incubation of NANA and antiserum for 30 min or longer prior to the addition of cells usually results in a lack of inhibition, which may account for the results of Springer *et al.*, McCluer and Johnson, and of Yokoyama *et al.*[20]. The latter incubated NANA and antiserum for 2 h at 23° C before the addition of red cells. The capacity of gangliosides to inhibit agglutination of $Rh_0(D)$ cells by specific antibody was confirmed by Yokoyama *et al.*[20], who also noted inhibition of anti-*A* and anti-*N* sera. No inhibition occurred with asialogangliosides. In all the work in this laboratory, inhibition was specific for anti-$Rh_0(D)$ except that 1-*G*, 3-*G* and mixed gangliosides-I, after treatment with neuraminidase, weakly inhibited anti-*c* in concentrations of 1,000 μg/0·1 ml. or more, which was not considered highly specific.

$Rh_0(D)$ specificity of anti-ganglioside serum. Bogoch[21] described the production of ganglioside antibodies by intravenous injection of ganglioside solutions. Yokoyama *et al.*[20] could not repeat this, but produced antibodies with gangliosides in a complete Freund adjuvant, injected along with foreign proteins; and with sheep or rabbit red cells coated with gangliosides. They did not find antibody for $Rh_0(D)$ cells although antibody to gangliosides was present.

Although the results are limited because of the small amount of ganglioside available, rabbit antiserum to ganglioside 1-*G* injected intramuscularly and subcutaneously in complete Freund's adjuvant, contained antibodies to both ganglioside and $Rh_0(D)$ cells. Two doses were given, 50 mg followed by 20 mg 21 days later. Apparently, such doses are not suppressive as with polysaccharide antigens, since Yokoyama *et al.*[20] also used 20 mg in adjuvant and 6 mg intravenously three weeks later. Antibodies for ganglioside were demonstrated as hæmagglutinins for ganglioside-coated human red cells, essentially by the procedure described by Yokoyama *et al.* For $Rh_0(D)$ antibody, normal and trypsinized $Rh_0(D)$ cells were used as previously described[6,15]. As indicated in Table 2, no antibody for any of the test cells was present before immunization. Rh antibody and antibody to the ganglioside appeared eleven days after the first injection, the maximum titre of the anti-ganglioside being 1 : 32 and 1 : 256 for trypsinized $Rh_0(D)$ cells. The latter was specific for the D antigen, the serum being negative with cells lacking it, such as dce/dce, dCe/dce and dcE/dce. Six rabbits injected with adjuvant produced no antibody specific for D cells.

Further evidence of the specificity of these antibodies is shown in Table 2 by absorption of the serum with *D*-negative cells coated with ganglioside, and with normal *D*-positive and negative cells for 10 min at room temperature using one volume of serum and cells. Absorption with *D*-negative cells failed to reduce the anti-*D* titre and the one-tube drop in the anti-ganglioside titre was not considered significant. However, *D*-negative cells coated with ganglioside 1-*G* removed all antibodies for this antigen and reduced the titre for trypsinized *D*-positive red cells from 1 : 256 to 1 : 8. Absorption with normal, group O, $Rh_0(D)$ cells removed all antibodies.

Apart from the weak inhibition of anti-*c* mentioned earlier, gangliosides 1-*G*, 2-*G* and 4-*G* were specific for anti-D. These three, as well as gangliosides-I and calf ganglioside, failed to inhibit agglutination by anti-A serum in concentrations as high as 1,000 μg/0·1 ml., nor did they inhibit the heterophile antibody in sera of individuals with infectious mononucleosis. The so-called *D*-like antigen present in both Rh positive and negative cells[28] was not involved since this antigen is destroyed by trypsin, and trypsinized cells were used both for agglutination tests and absorption.

Structural aspects of the $Rh_0(D)$ specificity of gangliosides. These results suggest that neuraminidase-resistant NANA residues, the 2 → 8 linked NANA, and galactose (Figs. 1 and 2), all contribute to the determinant structure of these substances for $Rh_0(D)$ antibody. The monosialo-(1-*G*) and the disialo-gangliosides (3-*G*) (Fig. 2) are more inhibitory than the trisialoganglioside (4-*G*) (Fig. 2), and since both 3-*G* and 4-*G* can be converted to 1-*G* by the action of neuraminidase, the basis of the determinant

Table 2. ANTIBODIES FOR $Rh_0(D)$ ANTIGEN AND HOMOLOGOUS ANTIGEN IN ANTISERUM TO GANGLIOSIDE 1-*G*

Time (days)	Test cells	Serum absorbed with	Titre (reciprocal of serum dilution)
0	NH		—
	H Tryp		—
	N–H Gang		—
11	NH		4
	H Tryp		16
	N–H Gang		32
14	NH		2
	H Tryp		32
	N–H Gang		16
	H Tryp	1 × with N–H	32
	N–H Gang		8
19	NH		—
	H Tryp		64
	N–H Gang		4
24	NH		8
3 days after booster	H Tryp		256
	N–H Gang		4
	NH	1 × with N–H	8
	H Tryp		256
	N–H Gang		2
27	NH		8
6 days after booster	H Tryp		256
	N–H Gang		8
	NH	1 × with N–H Gang	—
	H Tryp		8
	N–H Gang		—
	NH	1 × with NH Gang	—
	H Tryp		—
	NH Gang		—

NH = O, $Rh_0(D)$ positive red cells. H Tryp = human O, $Rh_0(D)$ positive red cells treated with trypsin. N–H Gang = O, Rh negative (dce/dce) cells treated with ganglioside. N–H = O, Rh negative red cells (dce/dce). NH Gang = O, $Rh_0(D)$ cells treated with ganglioside.

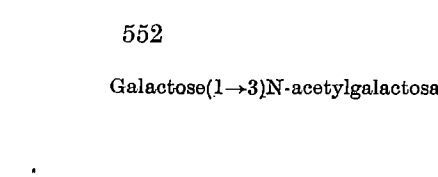


Fig. 3. Proposed immunological determinant of Rh_0(D) antigen

would seem to be the latter structure. The 2 → 8 linkage also seems to contribute since the second residue of 3-*G* increased the inhibitory capacity. It is of interest here that colominic acid contains 2 → 8 linkages[22]. The role of the terminal galactose of 1-*G* is indicated by the fact that the ganglioside *FM* (Fig. 2), which also contains the enzyme-resistant NANA, is less inhibitory than 1-*G*. The structure shown in Fig. 3 is suggested to be either identical with, or very similar to, the Rh_0(D) determinant of *D*-positive erythrocytes.

The third NANA residue in 4-*G* is terminally linked, and is neuraminidase-susceptible, being removed prior to the second NANA of 3-*G*. It decreased inhibition of anti-Rh_0(D), possibly by combining first with antibody, and the steric fit of this end of the molecule, structurally different from the NANA linkages of 1-*G*, interferes with the more effective binding of the latter. It was suggested that this third NANA linkage might offer an excellent substrate for viral neuraminidase. Since the enzyme converts 3-*G* and 4-*G* to 1-*G*, neuraminidase should cause a decrease in the viral–hæmagglutination-inhibiting capacity of the mixed gangliosides-I, and increase its anti-*D* inhibition, both of which were confirmed by the experimental results. Similarly, human Rh_0(D) cells treated with neuraminidase have lost the capacity to combine with indicator virus and are more agglutinable by antibody. Johnson[23] noted that urinary mucoprotein virus receptor inhibited Rh_0(D) antibody after treatment with trypsin, suggesting that neuraminidase and trypsin labile NANA perhaps acts as a viral receptor and that its removal exposes the more complementary resistant residues.

Rh_0(D) antibody inhibition by lipids, nucleotides and L-sugars. Some of the variety of substances reported to have similar Rh_0(D) specificity can be related to the structure proposed here. Elinin, a lipoprotein fraction of erythrocyte stroma, was associated with specificity for Rh as well as A and B antibodies[24,25], but further fractionation of the protein removed this capacity. Carter[26] described a water-insoluble lipid inhibitory for anti-Rh, extracted only from Rh-positive cells, which was not confirmed by Moskowitz *et al.*[25], while Hackel *et al.*[27] indicated that the Rh antigens may be partially nucleotide structures. Recently, Wolf and Springer[29] demonstrated inhibition of anti-Rh_0(D) by a fraction obtained from cells containing this antigen by the method of Calvin *et al.*[24]. It is interesting that this substance, like the gangliosides 1-*G* and 3-*G*, was also somewhat inhibitory for antibody to the *c* antigen.

Lack of information about these agents prevents any attempt to relate them to the structures reported here. However, Boyd *et al.*[30] noted weak but specific inhibition of Rh_0(D) antibody by the simple monosaccharides, L-glucose, L-mannose or D-gulose, and found that streptomycin (a natural glycoside of *N*-methyl-L-glucosamine) and rutinose ((6-) (β-L-rhamnosyl)-D-glucose) were more inhibitory. It was suggested that the terminal unit of the D antigen might be a sugar belonging to Group 4 of Mäkelä's classification[31]. NANA (5-acetoamido-3,5-dideoxy-D-glycero-D-galactomonulosaminic acid), although classified as a D-sugar because of the configuration of carbon-8, actually has the ring structure of an L-sugar, so that inhibition by NANA and substances containing it might be considered to be consistent with Boyd's suggestion.

Inhibition of Rh_0(D) antibody by a glycopeptide isolated from Pseudomonas. It was found[32] that a glycopeptide isolated from an as yet unidentified species of *Pseudomonas* inhibited Rh_0(D) antibody, and that rabbits immunized with it produced both saline and incomplete antibodies for Rh_0(D) cells. Flocculation, and eventual precipitation, were obtained when this substance was layered over some, but not all, anti-*D* sera. Absorption of anti-*D* sera reduced the titre for *D* cells significantly; but did not affect the titres of anti-*A* sera for *A* erythrocytes. Curiously, NANA inhibited agglutination of *D* cells by anti-serum to the glycopeptide, although it does not contain NANA. Furthermore, Dr. N. Bell and Dr. C. Randles of this department have shown that treatment of both glycopeptide and ganglioside 1-*G* (neuraminidase-resistant) with a crude bacterial enzyme preparation removed the inhibitory capacity from each of these substances. The effective enzymatic action is unknown.

The structure of this glycopeptide is unknown except that Lazen[2] has shown that it contains 56 per cent carbohydrate and 0·5 per cent of the amino sugar, *N*-acetylglucosamine. Obviously, only very general comparisons of the structure of this substance and gangliosides with reference to common inhibitory portions can be made. However, information provided by Dr. Randles shows that both substances contain glucose and galactose in 1,4 linkages, and free acidic groups. It is also possible that amino-acids in the glycopeptide could provide functional groups analogous to the acetylamino group of NANA and glycopeptide linkages with sugars, while evidence also indicates cross-linkages analogous to the cross-linkage from the sugar chain to NANA.

We thank Mrs. Virginia B. Geyer for serological work; Dr. Howard M. Johnson (now of the Taft Sanitary Engineering Center, Cincinnati, Ohio) for performing the viral inhibition investigations involving urinary mucoproteins and the ganglioside preparations; Dr. Chester I. Randles, Dr. A. G. Lazen (now at the Weismann Institute of Science, Rehovoth, Israel) and Miss Nancie L. Bell of this department for their help and their preparations of the *Pseudomonas* glycopeptide.

This work was supported by grant *AI*–03887–04 from the National Institute of Allergy and Infectious Diseases.

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THE LIPID-PROTEIN UNIT IN MYELIN

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AN investigation of the electrophoretic characteristics of the material obtained from the total solubilization of whole rat brain homogenate by lysolecithin[1] suggested that the principal component of the mixture was derived from myelin. Further work on rat brain homogenate, from which soluble proteins had been removed by buffer extraction at *p*H 8·6, followed by lysolecithin solubilization of the residue, indicated that it is probable that a ternary complex of lipid-protein-lysolecithin is formed[2], but indications of inhomogeneity were observed. This could be interpreted either as resulting from the separation of different lipid-protein-lysolecithin complexes from an originally uniform material or as being due to the presence of several classes of such complexes derived from the various kinds of membranes present in the brain. The development of subcellular fractionation techniques which result in the preparation of a highly uniform myelin fraction has allowed a more precise approach to the problem of myelin-lysolecithin interaction. The results suggest the possibility that myelin is composed of lipid-protein units, which are removed as such in the solubilization of myelin by detergents. Further evidence concerning the unitary nature of the lipid-protein complex is given, together with information as to its size and composition.

The Preparation of Rat Brain Myelin. The myelin-rich fraction was prepared from the crude mitochondrial fraction of rat brain as described previously[3]. This fraction was then re-suspended in distilled water, left to stand in the ice-bath for 20 min to release axonal contents, and re-sedimented at 20,000*g* for 25 min. Such treatment increased the cholesterol : protein ratio from 0·63 to 0·77, and electron microscopy of the material indicated almost complete disappearance of the axonal contents.

Solubilization of Myelin by Lysolecithin. Various volumes of a solution of lysolecithin (10 mg/ml.) in 0·05 M $NaHCO_3$ were added to aliquots of a suspension of myelin (2 mg/ml.) in the same salt solution, and the mixtures made up to a fixed volume. They were incubated at 37° C for 4 h and the turbidity was measured in the range 400–750 mμ on a Perkin–Elmer model 137 ultra-violet spectrophotometer. After storage of the solutions for a further 12 h at 4° C the turbidity curves were re-measured. No significant change was found; a typical set of such data is shown in Fig. 1, the turbidities being read at 550 mμ. The lower end of the turbidity (t) curve was found to show a linear relationship between $\log_{10}t$ and weight of lysolecithin (w) added. The weight of lysolecithin required to make $\log_{10}t$ just equal to the constant minimum value of this quantity observed at all larger additions of lysolecithin is defined as the minimum amount of lysolecithin required for maximum solubilization = w_m. Three estimations gave w_m = 36 ± 2 mg per 100 mg dry wt. of myelin.

Similar myelin-lysolecithin preparations were centrifuged at 14,000*g* for 30 min and the protein in the supernatant estimated by ultra-violet absorption[4] (the turbidity of these solutions at 550 mμ was equal to, or less than, that of the fully solubilized preparation (0·03), indicating that all large particles had been removed). The fraction of protein solubilized when plotted against the weights of lysolecithin used (Fig. 2) indicated that w_m = 36 mg of lysolecithin per 100 mg dry wt. of myelin.

Electrophoretic Study of Lysolecithin-solubilized Myelin. Samples of myelin suspended in 0·05 M $NaHCO_3$ were solubilized with various amounts of lysolecithin both less and more than w_m. After incubation the solutions were made up to a 1 per cent w/v myelin concentration and dialysed against the buffer for 48 h at 1° C. Where less than w_m was used the unsolubilized myelin was centrifuged off at 10,000*g* for 30 min. Solutions containing greater than w_m tended to precipitate lysolecithin slowly in the cold and were warmed before introduction to the electrophoresis cell. Electrophoretic investigations were made with a Perkin–Elmer Tiselius electrophoresis apparatus. At *p*H 8·6 (25° C), I = 0·1, it was found that irrespective of the amount of lysolecithin used a single peak only was observed. Where amounts of lysolecithin in excess of w_m were used the peak area increased proportionally (Table 1).

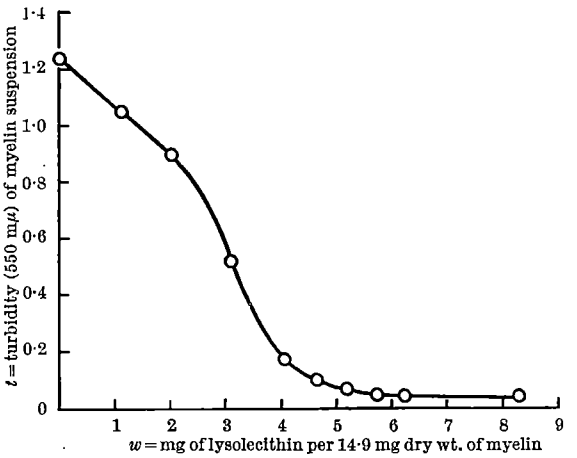


Fig. 1. The turbidity (t) at 550 mμ of myelin suspension treated with various amounts (w) of lysolecithin. Myelin, 14·9 mg dry wt. per 10 ml.

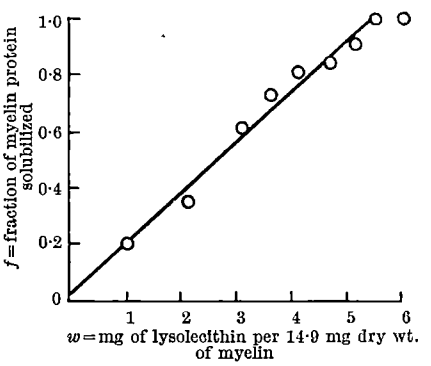


Fig. 2. The fraction (f) of myelin protein solubilized by various amounts (w) of lysolecithin. Myelin, 14·9 mg dry wt. per 10 ml.

Table 1. Electrophoretic Pattern Areas in cm²/10 mg Dry Wt. of Myelin per ml.

Wt. of lysolecithin per ml.	cm²	Mobility cm² V⁻¹ sec⁻¹
3·67	1·02	−5·7
5·48	1·21	−5·4
7·30	1·51	−5·2

Composition of Myelin. Aliquots of the suspensions of rat central myelin in distilled water were taken into 0·005 M NaOH and the ultra-violet absorbance measured at 215 and 225 mμ[4] on a Perkin–Elmer model 137 ultra-violet spectrophotometer. The absorbance difference was used to find the protein concentration from a calibration curve prepared from similar measurements on dilutions of Armour's Protein Standard Solution. Other aliquots of the myelin preparation were used to estimate the dry-weight concentration of myelin. Ten sets of dilutions on five batches of material gave the mean protein content as 33·1 per cent dry weight of myelin, *S.E.M.* ±1·82.

The detailed lipid analysis of the myelin is not relevant here and is in the process of being reported[5], but the following data are referred to in the discussion below: 100 mg dry wt. of myelin represents: cholesterol 48·6 μmoles; phospholipids 45·4 μmoles; amino-acids 253 μmoles; galactolipids 14·4 μmoles. This estimate of amino-acids is based on average amino-acid residue molecular weight from amino-acid analysis of myelin protein = 129.

Ultracentrifugal Study of Lysolecithin-solubilized Myelin. Solutions were prepared as for electrophoresis and examined in a Spinco model *E* analytical ultracentrifuge at 20° C. Sedimentation velocity experiments were performed with a rotor speed of 59,780 r.p.m. and sedimentation equilibrium experiments using short columns (3 mm) of solution according to the method of van Holde and Baldwin[6]. Results were calculated by their methods using partial specific volumes ($\bar{v}$) determined by direct pyknometry of the solutions.

A single symmetrical Schlieren peak was observed in sedimentation velocity experiments with various amounts of lysolecithin in excess of w_m, the minimum weight of lysolecithin producing maximum solubilization. However, the ratios of weight-average molecular weight (M_w) to the z-average molecular weight (M_z) determined at sedimentation equilibrium show (Table 2) that some degree of polydispersity exists in the solutions.

Table 2. Ultracentrifugal Data on Solubilized Myelin

Wt. of lysolecithin in mg/mg dry wt. myelin	M_w	M_w/M_z	$\bar{v}$
0·547 (*c.* $1·5 \times w_m$)	$3·44 \times 10^5$	2·75	0·894
0·745 (*c.* $2·0 \times w_m$)	$5·18 \times 10^5$	3·08	0·940
0·920 (*c.* $2·5 \times w_m$)	$7·53 \times 10^5$	3·09	0·944

Discussion. The fact that myelin is completely solubilized by lysolecithin and by other detergents (compare Hulcher[7]) implies that myelin contains relatively small protein units. Since only a single component is observed in both electrophoresis and ultracentrifugation it would seem probable that lysolecithin forms an addition complex with a lipid-protein unit present in the mature myelin. If lysolecithin sequestered one or more lipid components it is very unlikely that this would result in two or more particles identical in both mass and electric charge.

The direct proportionality of the fraction of total protein released to the amount of lysolecithin used indicates that only one kind of process is involved in the solubilization. Consequently, the complex shape of the curve relating the turbidity of the partly solubilized suspensions of myelin to the amount of lysolecithin must be due to changes of size and shape of the unsolubilized fragments. In a myelin suspension prior to the addition of lysolecithin a considerable range of fragment size is to be expected. The smaller pieces, having a relatively larger surface area, will tend to take up lysolecithin to form the soluble complex more readily. The turbidity will be due mostly to the larger fragments and will, therefore, diminish more slowly at first (Fig. 1) and then more rapidly as attrition of the larger particles results in their conversion to smaller and less light-scattering fragments. When these particles are small enough the Debye relationship should hold: $t = Knv^2$, where n = number of particles per unit volume and v = volume per particle. Part of t is due to the scattering action of the fully solubilized material and hence can be corrected, proportionally to the turbidity at w_m, to give a corrected turbidity T. If the action of lysolecithin is to progressively remove lipid-protein units from the surface (area = a) of the myelin fragments, then the smaller the surface area the smaller will be the amount of lysolecithin involved per fragment: $-da/dw$ = constant, hence $(w_m - w)$ will be proportional to a. Because the surface area is proportional to $v^{2/3}$ then it follows that $T = Kn(w_m - w)^3$. If the action of lysolecithin does not result in the division of larger fragments into more or less similar-sized pieces, then n will be constant, in which case T should be proportional to $(w_m - w)^3$. Fig. 3 shows that the relationship does hold for a typical set of data.

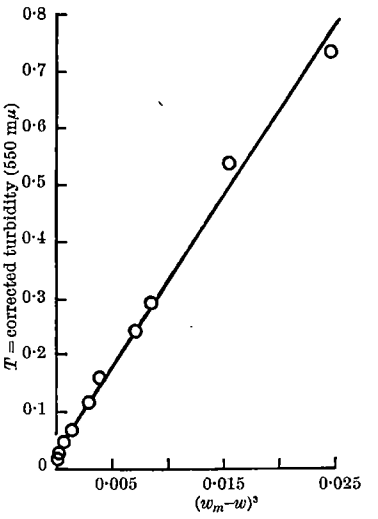


Fig. 3. The relationship between the corrected turbidity (T) of myelin suspensions treated with various amounts (w) of lysolecithin and $(w_m - w)^3$, w_m being the minimum weight of lysolecithin required for maximum solubilization. Myelin, 14·9 mg dry wt. per 10 ml.

The decrease of electrophoretic mobility with increase of amount of lysolecithin implies an increase of particle size, since the uncharged lysolecithin would not alter the absolute charge of the complex. This is in accord with the increase of pattern area noted in Table 1.

It is concluded that these experiments on the solubilization of myelin are consistent with the hypothesis that myelin is composed of relatively stable lipid-protein units which are removed by the action of lysolecithin to form a single species of a complex micelle composed of protein-lipid-lysolecithin.

From the data of Table 2 it appears that the increase of molecular weight is greater than that to be expected from lysolecithin addition. Two possibilities suggest themselves: first, that the addition of lysolecithin modifies the structure of the molecular unit as a whole so as to permit the inclusion of progressively more water or, secondly, that, although the addition of lysolecithin to the lipid-protein units increases their hydrophilic character and hence allows disaggregation, the dilution of negative charge density by inclusion of the electrically neutral lysolecithin will allow an increasing cohesion between the lipid-protein-lysolecithin particle. In either case the increment of molecular weight δM will be equal to $KM\delta m + \delta m$, δm being the mole increment of lysolecithin. Consequently

$\ln(M/M_0) = Km$ where M_0 = molecular weight of the lipid-protein unit in native myelin and K is a constant. The data of Table 2 fit such a relationship very well to give $M_0 = 109{,}000$. At the moment there is no definitive evidence to favour either of the two hypotheses concerning the exponential increase of molecular weight, but it may be noted that if M_z/M_w be taken as a measure of polydispersity this alters very little, implying that the molecules simply become larger rather than more aggregated. Furthermore, the partial specific volume, although initially less than that of lysolecithin alone ($\bar{v} = 0{\cdot}921$), finally becomes greater, an effect which could arise from progressive inclusion of water rather than aggregation of similar units.

On the assumption of an overall hydration of the native lipid-protein unit of 0·3 g H_2O/g dry wt. of unit and with the analytical data shown above, the molecular weights of the various components are as follows: protein = 28,000 (215 amino-acid residues), cholesterol = 16,000 (41 molecules), phospholipid + galactolipid = 40,000 (50 molecules). The protein molecular weight is of the same magnitude as that found for mitochondrial structural protein[8], with which myelin structural protein might well be related. An α-helical configuration of the protein, with the usually accepted dimensions for this conformation, would form a protein base of area 3800 Å^2, while the lipids in monolayer, with the dimensions given by Vandenheuvel[9], would require about 4000 Å^2. The lipid-protein unit, therefore, can be visualized as a flat plate of protein in the α-helical configuration about 60 × 60 Å^2, covered on one side by a micro-monolayer of lipid to give a thickness of about 40 Å. Such units could form parts of both myelin and other membranous structures; it may be noted that the unit is similar in size to the micelles observed through the electron microscope by Sjöstrand[10] in mitochondria.

Two conclusions are drawn from the results of this investigation: (1) the procedures outlined in this article may be generally applicable for the investigation of cell-membrane structure, and (2) it appears that there may be a sufficiently strong adhesion between lipid molecules and a protein molecule for the complex to be regarded as a unit, and hence for membranes to be regarded as aggregations of such units. In this case the Danielli hypothesis[11] of membrane structure may need modification, since it places emphasis primarily on the properties of a continuous bimolecular layer of lipid as determining the properties of a membrane.

More extended accounts of the information summarized here are in preparation. These will include supporting data obtained from the study of a number of vertebrate myelins and erythrocyte membrane. We thank the Multiple Sclerosis Society for a grant to one of us (N. A. G.) and the Medical Research Council for the provision of the Spinco analytical ultra-centrifuge and a grant for special research (D. B. G.).

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FLEECE EVOLUTION IN DOMESTIC SHEEP

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FURTHER specimens of ancient skin and wool have added to earlier findings[1-3] in such a way that a hypothesis covering what appear to be the main stages in fleece evolution can be formulated.

The new material described is as follows:

(*a*) A piece of cloth from the Danish Bronze Age dated *c.* 1200 B.C. supplied by Margrethe Hald.

(*b*) Six pieces of leather and four specimens of wool supplied by Prof. Y. Yadin, of the Hebrew University, Jerusalem, from the 'Cave of the Letters' near the Dead Sea[4]. These belonged to Jewish rebels against Roman rule who took refuge in the caves in A.D. 135. Observations on this material were completed while I was at New England University, Australia.

(*c*) Some cloth supplied by A. T. Lucas, of the National Museum of Ireland, from a body found in a bog in Co. Kildare, and dated as from "the first few centuries of this era".

(*d*) A sample of Anglo-Saxon cloth from a grave at Fonaby, Lincs, dated sixth or seventh century A.D. and supplied by Miss E. Crowfoot through the courtesy of Mrs. Sonia Hawkes.

(*e*) Five Roman, ten Norse and three medieval textile specimens (all but one from Scotland) from the National Museum of Antiquities of Scotland[5]. These were sampled by Miss A. S. Henshall through the courtesy of Mr. R. B. K. Stevenson.

One of the specimens of leather (No. 51/2) from Israel had been sewn, suggesting that it had come from some clothing; the remainder came from water bottles. The four specimens of wool comprised one apparently unspun (7/17/2 dyed maroon), one of yarn (7/10 maroon) and two of cloth. One of these (2/33) was dyed green, and the other (2/87) was pale yellow. Such a colour often occurs as a discoloration in archæological specimens of wool, but this was brighter than the yellow discoloration of the Altai wool[3].

To the naked eye the individual fibres in the wool specimens appeared to be fine, and that most fibres were, in fact, fine was confirmed by microscopic examination, but the microscope revealed the unsuspected presence of 15–20 per cent of fibres of medium diameter. Only one naturally pigmented fibre was seen, and the distribution of pigment granules in it was sparse. There was variation between individual fibres in the extent of dye uptake, particularly in the green wool, but no evidence of bilateral asymmetric dyeing was seen. There was no evidence of blue or yellow colouring: the individual fibres appeared uniformly blue-green under the microscope, although the green colour almost certainly resulted from a mixture of blue and yellow dyes.

The scale pattern of the surface cuticle of the fibres was clear, particularly in the medium fibres, in which an irregular-waved mosaic pattern could be discerned, the wave being indicated by the frequent diagonal orientation of the scale margins. Some of the medium fibres in the leather, however, had an irregular mosaic pattern lacking a wave. This accords with recent work by Burns[6], which

suggests that a waved pattern derives from mosaic pattern as a result of wear in the fleece. Thus, what had been thought to be a fundamental difference apparently has no evolutionary significance.

The wool from the two Jewish cloth specimens was non-medullated, but a few of the medium fibres from the unspun wool, and the yarn specimen, had a fragmental medulla. Some fibres from the yarn specimen had an interrupted medulla, while a few had an unbroken medulla for an appreciable length, and could therefore be regarded as fine hairs. For example, a fibre 44μ in diameter had a medulla 12μ wide. It is not common for medium fibres in modern breeds to have an unbroken medulla; but the foregoing measurements compare with fibres in the Soay sheep ranging from one 29μ in diameter with a medulla 6μ wide to one 46μ in diameter with a medulla 16μ wide. They were not as fine as a fibre in a parchment from the Dead Sea Scrolls apparently from a hairy sheep[7], which was only 16μ in diameter, yet had a medulla 12μ wide. Nor were they as coarse as the relatively fine hairs of the Altai sheep[3], which had a diameter about 80μ and a medulla about 40μ wide.

Diameter measurements were made of 100 fibres from each wool specimen, and the mean diameters were as follows: unspun wool, 24μ; yarn, 30μ; green cloth, 24μ; and yellow cloth, 25μ. These compared with approximate values from the Soay (frequency in brackets) of 20μ (3), 24μ (2) and 30μ (3), also 38μ in a sample of Cheviot wool, a breed probably partly derived from the Soay. The range of fibre diameter was similar in each specimen, being from about 10μ to 48μ, but that of the yarn ranged up to 54μ. The diameter frequency had a skew distribution in each instance, the greatest number of fibres being in the fine diameter range. This explains why the specimens appeared fine to the naked eye. The frequency distribution of fibre diameters in the specimen of unspun wool is shown in Fig. 1. A similar skew distribution was found in the Soay, Shetland and in the Cheviot, but not in the Southdown and Suffolk Down, nor in the Romney and Leicester Longwools, which had a more symmetrical diameter distribution.

This skew distribution very probably arises from an appreciable difference in diameter between the primary and secondary fibres. In fact, in the distribution shown in Fig. 1 there is a suggestion of a second peak among the medium fibres which may be due to the primaries. Here the mean diameter of the fine fibres was 21μ, and that of the medium fibres was 38μ. It is noteworthy that the mean diameter of fine fibres approaches 20μ and that of medium fibres approaches 40μ in different breeds of sheep and at different periods in history.

It would be useful if one could calculate an S/P ratio from the number of fibres in each peak of a bimodal distribution of fibre diameters like that in Fig. 1. But, in fact, the ranges of the diameter of the primary and secondary fibres are likely to overlap, and so in the intermediate diameter range any division made between the two would be arbitrary. Calculations made with two possible and apparently suitable points of division on the diameter distribution of the yellow cloth gave values of 1·6 and 8, that is, outside the S/P range of most modern breeds. The clearest division between fine and medium fibres was that shown in Fig. 1, and an S/P ratio of 4·5 was obtained from this distribution. The yarn specimen and the green cloth gave S/P ratio values of 4·5 and 5·6, respectively. These values are what one would expect from a medium wool and so support its identification as such. An S/P ratio of 5 was estimated from the medium-woolled type of follicle grouping found in parchment of similar date from the nearby site of Murabba'at (shown in Fig. 4 of ref. 1). The (identified) fibres in this parchment had a similar range of diameter to those in the foregoing wool specimens: secondaries 16μ–24μ and primaries 32μ–42μ. An S/P ratio of 5 compares also with a mean value (from the skin) of 4·5 for the Cheviot breed[8], and

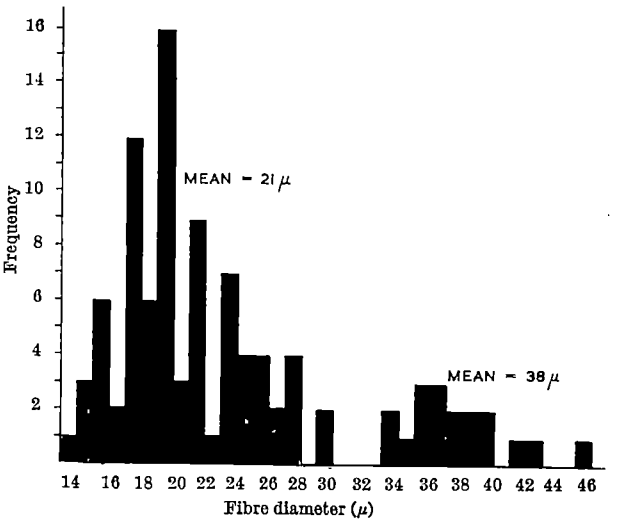


Fig. 1. Distribution of fibre diameters in un-spun wool from cave near Dead Sea, A.D. 130

individual values of 3·8 and 4·7 for the Soay[9]. But as a reminder that the above S/P ratios obtained from the wool could have been fortuitous, one might mention that S/P ratios of 4·8 and 2·1, respectively, were obtained from the wool of these two Soay sheep.

Four of the leather specimens (51/2, 61/2/1, 61/2/21 and 7/68) were relatively thick and hard with a dark-brown skin surface (grain side), which suggests vegetable tanning. Two (61/2/18 and 19) were pale brown in colour, and were thin and soft like chamois leather which is oil tanned, but these specimens could have been degraded. All the specimens gave a modified staining reaction showing increased basophilia that has been associated with vegetable-tanned leather[7]. But this staining reaction has since been found to occur also in untanned material entirely as a result of burial.

Whereas leather has hitherto yielded fewer follicle remains, when sectioned, than parchment, only one of these Jewish specimens (6/2/19) had no remains. Although one might expect that the cloth specimens would comprise the best wool available, the leather could have been from any type of animal. In fact, four of the specimens were from sheep of the same medium-woolled type as those from which the wool specimens must have come, and one was apparently a fine type. One medium-woolled specimen had medium follicle remains and fibres 42μ and 46μ in diameter. The three other leather specimens had fine and medium follicle remains, one of which had fibres 10, 16, 18 and 20μ in diameter, and another of which had fibres 12, 15, 36 and 46μ in diameter. The fine type, on the other hand, had relatively large complete groups of fine follicles, and the fibres that could be measured had the following diameters: 16μ (2), 18μ (5), 19μ (2), 20μ (2) and 26μ; there was not one fibre or follicle of medium diameter. This fine-woolled type therefore seems to be the same as that found in the parchment from the Dead Sea Scrolls; the specimen illustrated by Ryder[1] had large groups of mostly empty follicles; the few fibres that could be measured had diameters as follows: 10μ (2), 13μ, 26μ and 28μ.

The findings from the Jewish specimens of skin and wool indicate a predominance of a medium-woolled sheep like that which predominated in parchment from the Dead Sea Scrolls and from Murabba'at. This type o sheep is attested by the range of fibre diameters in bot the wool and the skin. The existence of a primitive medium-woolled sheep in that time and place is therefore amply confirmed, and this invalidates the suggestion[3], based on the investigation of the Altai skin, that the medium type in the Dead Sea Scrolls could have been

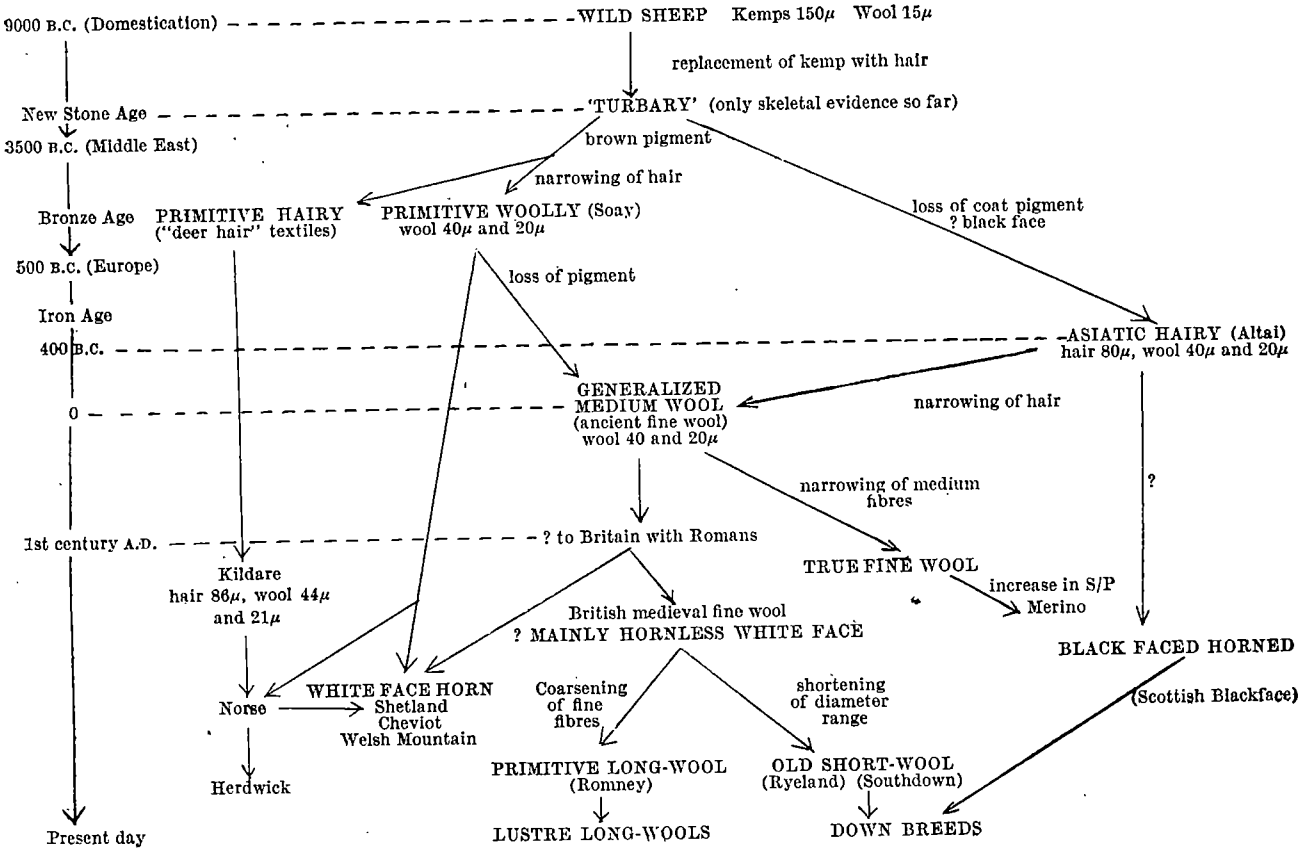


Fig. 2

merely a hiray type in which the coarser fibres had narrowed preparatory to shedding.

As with the Altai specimen, the wool in this investigation added much to the findings from the skin; in particular, it emphasized the much greater fibre diameter variation in the past compared with modern medium wools (Down and long-wool breeds). The observations recorded here indicate that the four specimens among the Dead Sea Scrolls described as being fine to medium[1] could have been medium wools. Two more, identified as fine wools from a few fibres of extreme fineness (12μ) alone, could have been medium wools. But the existence of the fine wool is confirmed by one specimen from the Dead Sea Scrolls, and one among the present specimens, with large complete groups of fine fibres only. So far, therefore, from the Dead Sea area the following totals have been identified: hairy sheep or goat, 4; medium wool, 21; fine wool, 2.

The hypothesis that can be put forward from these findings is that this medium wool seems to be a generalized fleece type that appears to lie in an intermediate position along the evolutionary scale between the more primitive hairy type (represented by the Altai sheep of 400 B.C.[3]) and the apparently more highly evolved fleece types of to-day. The medium-woolled type could have arisen from the hairy type by a narrowing of the primary fibres so that medium fibres grew instead of hairs. The Altai sheep, for example, did not have a very coarse fleece, and had an S/P ratio of 5 similar to that of the medium wool, so it could readily have given rise to the medium wool by a replacement of the hairs with medium fibres, which were already present in small numbers in the Altai wool. Further support for such a link can be gained from the finding of 'fine hairs' in the Jewish medium wool. Affinities with the Soay have been indicated in Fig. 2 to show that the crossing and admixture of different types are likely to have been important. The main change along this possible link is a loss of pigment from the coat.

The generalized fleece of the medium wool could in turn have given rise to various modern fleece types as follows. It could have given rise to the fine-woolled type by a further thinning of the primary fibres so that medium fibres were replaced by fine fibres. The two fine wools discussed in the present account suggest that this change was already taking place in the Near East about A.D. 100, and the S/P of 7 in the specimen from the Dead Sea Scrolls suggests that the S/P ratio had already begun to increase towards the average of about 20 found in the modern fine wool, the Merino.

Before considering how this medium wool could have contributed to the fleeces of modern British sheep I must briefly state how it could have reached Britain. It is likely that the Ancients regarded this medium wool as fine wool: in the investigation recorded here the presence of a proportion of medium fibres was completely unsuspected until a microscopic examination was made, so convincing was the impression of fineness given by the wool to the naked eye. It is therefore possible that this medium wool was, in fact, the renowned fine wool of the Near East that spread around the Mediterranean[10]. This may have been among the sheep that the Romans brought to Britain. Until the Romans came, the best-woolled sheep in Britain are likely to have been of Soay type, that is, medium-woolled, but brown. Crosses between the white Roman medium-woolled sheep and the brown, horned Soay could have given rise to the white-faced, horned breeds such as the Cheviot and Welsh Mountain[11], which, if coloured, have a brown pigment.

The persistence of the generalized medium wool into the Middle Ages as a distinct type is suggested by the English medieval parchments identified as coming from fine-, and fine- to medium-woolled sheep[12]. When these were examined originally the possibility of a wide range of diameter within a medium wool was not realized; parchments having fibres less than 18μ in diameter were regarded as indicating fine-woolled sheep, and fibres

18–35μ in diameter were described as fine-to-medium; wool 35–55μ in diameter (comparable with modern long-wools) was termed medium.

The present investigation provided a clue to the identity of the unidentified parchment illustrated in Fig. 2 of ref. 12. This had primary fibres ranging from 34μ to 48μ and secondary fibres ranging from 14μ to 24μ, which compare with the generalized medium-woolled type, and the brown pigment suggests a sheep more akin to the Soay.

In Britain, the generalized medium type could on its own have given rise to the short-wool fleece type (for example, modern Down) by a shortening of the range of fibre diameter, and it could have given rise to the diameter range found in the long wools by a coarsening of the fine fibres. The existence of this generalized medium fleece makes it unlikely that short-wools were derived from long wools, as was suggested by Fraser and Hamada[13]; instead, both appear to have had a common ancestor.

The specimens now to be described were examined as a first attempt to check the aforementioned hypothesis. It is intended to describe these more fully elsewhere. The only Bronze Age specimen examined so far in the work recorded here was one of the Danish textiles in which the presence of hairs or kemps was first erroneously thought to indicate an admixture of deer hair. In fact the proportion of hairs (50–88μ in diameter) and medium fibres (32–44μ in diameter) in this specimen was low, being together only about 5 per cent compared with more than 15 per cent in the typically hairy Kildare specimen described here. Although the total range of diameter was 8–88μ, the mean in the two yarns was as low as 16μ and 19μ, respectively. This fleece structure is not typical of a hairy, medium or fine type, but it occurred in later times (see following) and will be described at the moment as a hairy medium wool. More prehistoric specimens must be examined before suggestions can be made about this stage of evolution. Although in hand-spinning the wool is usually spun direct from the staple, one could imagine an attempt being made to remove the hairs. But this was not done with the Kildare specimen mentioned here, and could not explain the smaller proportion of medium fibres in the Bronze Age specimen*. Although, like the Soay, the fibres had a dense distribution of pigment granules, these were of the black rather than the red type, as the cloth appeared black in contrast to the chocolate colour of Soay wool.

The Kildare wool and the Roman specimens were of similar date to those from Israel. The Kildare specimen was from a hairy sheep (fibre diameter range 10–130μ) and some of the coarsest fibres are likely to have been kemps. There was moderately dense pigmentation, which varied between fibres. Such a fleece type lies further back than the Soay on the evolution diagram (Fig. 2) and is probably represented to-day by the Herdwick breed, which is the hairiest of the British white-faced horned group.

The Roman specimens from Scotland yielded seven yarns: three of these were white and four had slightly to moderately pigmented fibres, suggesting Soay influence. Two of the wools (one pigmented, one not) showed the same skewed-to-fine distribution as the Jewish medium wool, and three (two pigmented, one not) had the same distribution with a few relatively fine hairs (for example, 60μ in diameter with a medulla 30μ wide). Whereas the means of these five specimens ranged from 18μ to 23μ, the remaining two specimens had means of 17μ and 28μ, and the diameter distribution of these specimens tended to be symmetrical on each side of the mean. Thus, the Roman specimens show a variation of type from a slightly hairy medium wool, through the generalized medium wool, to a true yet pigmented medium wool (mean 28μ) similar in diameter distribution to the modern long-wool, and to a white short-wool (mean 17μ and non-pigmented), although the diameter range of this particular specimen was like that of the Merino rather than that of a modern British short wool.

The York specimen, although described as Roman[5], may be somewhat later in date[14]. This yielded two similar types of heavily pigmented wool with means of about 24μ and 33μ. The diameter range was 8μ to 64μ and both wools had a few hairs like the Bronze Age and Roman specimens mentioned here, that is, they were not as hairy as the Kildare wool. The Saxon wool from Fonaby had a finer diameter range (mean 18μ) than the generalized medium wool, but had the same skewed-to-fine distribution. Its moderate-to-dense pigmentation suggests Soay type.

The first group of Norse wools came from the Orkney Hood[5]. These comprised nine yarns, all with slight or moderate pigmentation. Five of these had fibres within the range 10–80μ, and had a skewed-to-fine distribution with only a few hairs like the slightly hairy Roman and York specimens, but four were true hairy types with hairs (or kemps) ranging up to 114μ, that is, more like the Kildare wool. The second group of Norse specimens from Kildonan comprised four yarns, all slightly to moderately pigmented. Two of the yarns had fibres in the diameter range 14–60μ with means of 25μ and 28μ and a few fine hairs as previously described. The distribution of the remaining Kildonan specimens was more difficult to interpret. One of the other yarns had a continuous distribution of fibre diameters of 16–58μ with a mean of 36μ. Some fibres were medullated, and so this may be another hairy medium wool. The fourth yarn had a diameter range of 12–46μ with a mean of 28μ, and although superficially symmetrical around this mean, as in a true medium wool, the distribution was slightly skewed towards the fine part of the range, identifying it with the generalized medium wool.

A medieval cloth from Closeburn yielded two moderately to heavily pigmented yarns with a fibre diameter range of 10–54μ and means of 23μ and 26μ. It had a skewed-to-fine diameter distribution which, with the pigment, suggests a Soay-type sheep. The two yarns in another medieval specimen, from Loch Trieg, had fibres with only slight pigmentation ranging from 8μ to 34μ in diameter and with means of 18μ and 20μ, respectively. The diameter distribution was symmetrical and typical of a short wool, which shows that some of the medieval parchments[12] could have been from true fine wools. A third medieval fabric, from Kelso, had fine and medium fibres with slight pigmentation, but these were too degraded to be measured.

These textiles, therefore, provided evidence supporting the hypothesis in that the generalized medium wool was found in Britain, and the suggested divergence from it towards the long wool and short wool was apparently occurring, unexpectedly, as early as Roman times. Further evidence was gained of the persistence of the Soay type in Scotland at least until the Middle Ages. An interesting finding is the predominance of hairy wool among the Norse specimens, as this supports the opinion that the Danes made a separate introduction of sheep akin to the Herdwick. This would explain the difference of the Herdwick from the remainder of the British white-faced horned group of breeds and its similarity to some modern Scandinavian sheep, notably in the high gene frequency for hæmoglobin A (ref. 15). Although it does not affect the argument, it should be mentioned that the later part of the Norse occupation of the northern and western parts of Scotland was, in fact, contemporary with the medieval period farther south.

When the third main group, the hairy black-faced horned sheep, arrived in Britain is still a mystery[11]. Hairy sheep lacking pigment in English medieval parchments such as that dated 1403 illustrated in Fig. 1 of Ryder[12] possibly belonged to this group, but its source (possibly originally Asia) is unknown.

An attempt has been made in Fig. 2 to illustrate the changes discussed here. The evidence for some of the affinities shown is discussed by Ryder[11], and other known changes have been omitted for simplicity and relevance.

* *Note added in proof.* In the moult of the Soay the hair sheds later than the wool, so wool plucked during the moult contains little hair.

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AUXIN TRANSPORT IN ROOTS

Vicia faba

IT has been tacitly assumed, since the work of Went[1] and van der Weij[2], that the response of roots to gravity is mediated by the auxin, indolyl-3-acetic acid (IAA), which originates in the root tip and is transported thence to the elongating cells, more being deflected to the lowermost part of the organ where it inhibits growth and induces positive geotropic curvature. However, there is little experimental evidence in roots for such auxin movement or deflexion under gravity, and a series of researches carried out in this laboratory have strongly indicated that IAA may not be a key substance regulating either the extension growth of roots[3–5] or their response to gravity[6,7]. For these reasons work was started some two years ago on IAA transport in segments of the roots of *Vicia faba*, a species much used in the aforementioned investigations.

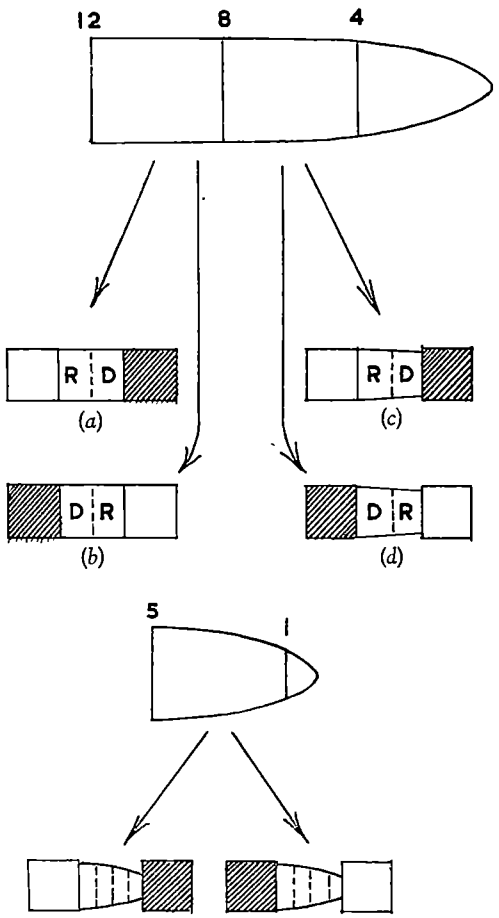


Fig. 1. Diagram to scale showing the methods used in studying the movement of ^{14}C-labelled IAA in 4-mm root segments of *Vicia faba* radicles. The figures represent the distances from the root tip in millimetres. The donor blocks are shaded

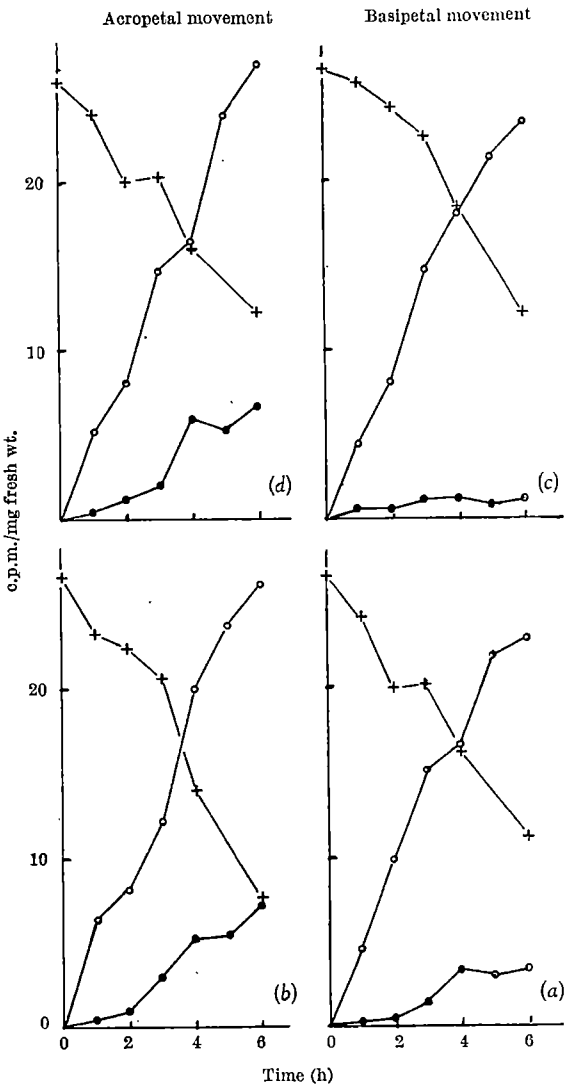


Fig. 2. Result of a typical experiment using segments cut between 4 and 8 mm (above) and between 8 and 12 mm (below) from the root tip. (*a*), (*b*), (*c*) and (*d*) correspond to the four treatments shown in Fig. 1. +, IAA concentration in donor block; ○, IAA concentration in half of segment at donor end; ●, IAA concentration in half of segment at receiver end. No radioactivity could be detected in the receiver block

The movement of radioactive IAA, ^{14}C-labelled in the carboxyl group (1·75 mc./m.mol), has been examined in 4-mm segments cut from various regions of the primary

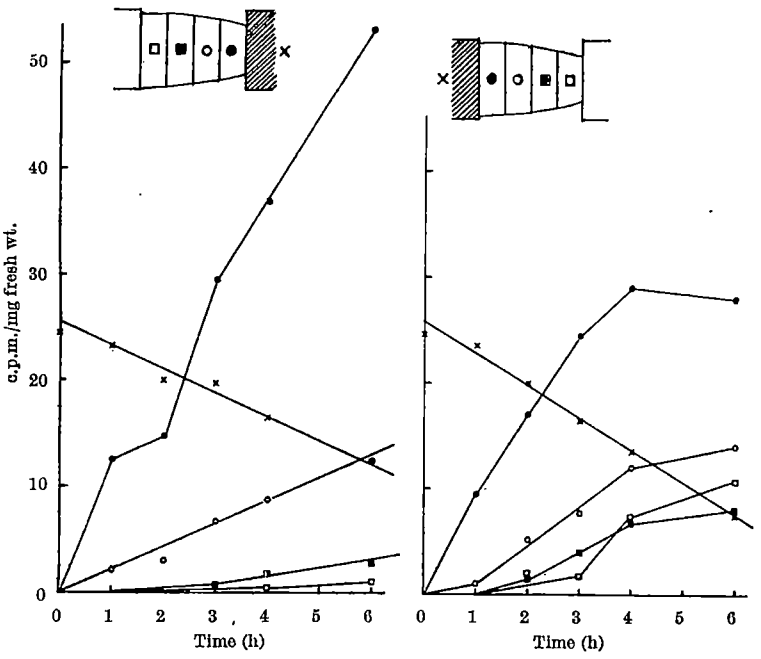


Fig. 3. Result of an experiment using extreme apical (1–5-mm) segments. The IAA content of quarter segments was determined, the keys to the graphs being shown at the top of the figure. ×, Concentration in donor block

radicle, using the technique of McCready[8] exactly as described in his papers. The IAA was obtained by alkaline hydrolysis of the nitrile and was shown by paper chromatography to have a high degree of purity. The donor blocks were of 0·02 ml. volume and contained 0·2 μg of IAA (≡ 5,450 c.p.m.). Sufficient replicates were set up to allow sampling at hourly intervals and counts were made of radioactivity in both donor and receiver block and also in the segments. In most experiments the segments were cut transversely into 2 or 4 equal parts, which were assayed separately so that the IAA gradient down the segment could be accurately ascertained.

In a root, cell differentiation proceeds rapidly as one moves away from the tip and segments cut near the tip are bound not to be homogeneous. Comparisons of acropetal and basipetal movement of auxin applied to the proximal or distal ends respectively of exactly similar segments could be as much a reflexion of uptake or absorption differences at the two ends as of a polarity of movement in the segment. To get more information on this the classical technique was modified and segments were cut in pairs from the seedling root and IAA movement in both directions followed in both segments (Fig. 1). In this way we could examine acropetal and basipetal movement into approximately the same tissue at a point in the root where the two segments had been contiguous. In segments very near the root tip such a technique could not be used because the two segments would not be of comparable dimensions. For these apical root segments (Fig. 1) only basipetal movement could be examined in the apical tissue; no attempts have yet been made to follow acropetal movement into the extreme 1-mm tip, which is constituted almost entirely of root cap.

Fig. 2 shows the result of a typical experiment using contiguous segments cut at 4 and 8 mm from the root tip, that is, in the region of active extension growth. The first thing which is striking about the results is that during the course of 6 h no appreciable passage of IAA occurred completely through these 4-mm segments since no counts significantly above background were ever detected in any receiver blocks. On the other hand, movement into the donor end of the segment was rapid and, as will be seen from a comparison of concentrations in the donor block and its adjacent half of the segment, this was against an overall concentration gradient from about 4 h onwards. This accumulation of IAA showed little change in characteristics with position along the root axis (that is, at 4, 8 or 12 mm from tip).

In the absence of measurable transport through the whole segment, the progress of accumulation in the half of the segment remote from the donor block can be used as a measure of transport rate in the root tissue. Accumulation here is a very small fraction of that in the half at the donor end, but it provides clear evidence that acropetal movement is greater than basipetal in both segments. The curves also show that, while acropetal movement varies little with position of the segment on the axis, basipetal movement (that is, away from the tip) is much smaller in the more apical segments.

These differences, although still small, become more marked in the 1–5-mm segment. Fig. 3 shows the results of another typical experiment in which the progress of IAA movement in both directions has been followed during 6 h in these segments, which are composed of the root meristem and the region of early cell expansion. These segments were divided into four 1-mm pieces for the analysis of concentration gradient changes. The curves show clearly that uptake of IAA into the extreme apical end is at the same rate as, but continues longer than, that into the basal end. On the other hand, the gradient of concentration in the basipetal direction is at all times much steeper than in the acropetal direction, which clearly demonstrates the distinctly preferential movement of IAA towards the apex.

The nature of this very marked accumulation of IAA in root tissue has been examined by experiments of the sort outlined here, but carried out either under nitrogen

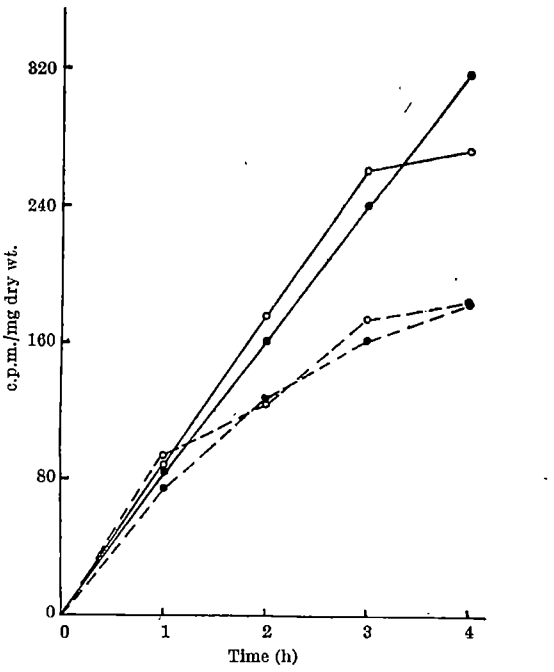


Fig. 4. Effects of pretreatments with 2,4-dinitrophenol on IAA movement into 4–8 mm and 8–12 mm segments. ——, Untreated segments; – – – –, segments pretreated 30 min with 10^{-4} M dinitrophenol; ●, acropetal movement—treatment (*d*) (Fig. 1); ○, basipetal movement—treatment (*a*) (Fig. 1)

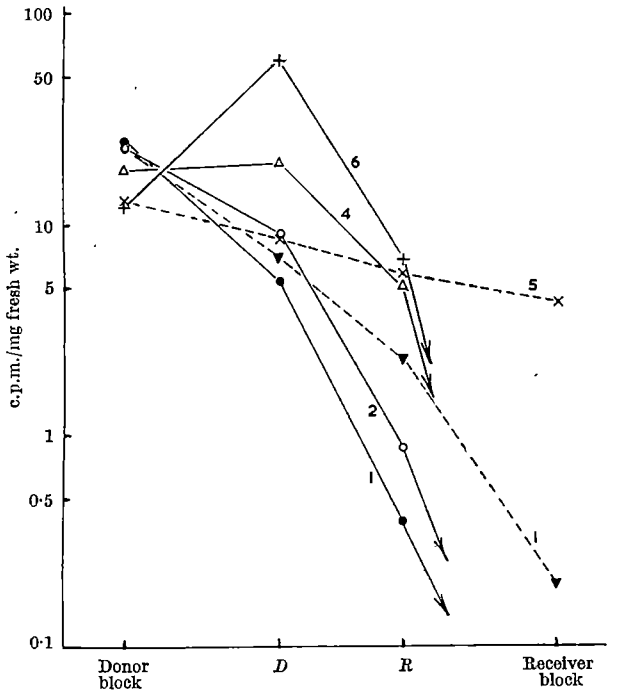


Fig. 5. Time-course of movement of IAA in 8–12-mm root segments and in agar cylinders of the same dimensions. ——, Root segments—treatment (*b*) (Fig. 1); – – – –, agar cylinder. The numbers by the graphs represent the time (h) after the application of the donor blocks

gas or with roots pretreated with solutions of potassium cyanide and 2,4-dinitrophenol (DNP) at concentrations which normally prevent active solute accumulation. All three treatments yield virtually the same result, which is illustrated by Fig. 4 (segments pretreated for 30 min with 10^{-4} M dinitrophenol). It will be seen that for movement in either direction into a 4-mm segment uptake during the first hour was unaffected by DNP, this presumably being diffusion into the free space in the tissues. Thereafter accumulation is depressed and, after 4 h, the overall concentration in the tissues is still not greater than that in the donor-block. The difference between the two sets of curves gives a measure of the active accumulation of IAA.

The rate of movement of IAA in the root tissue has been compared with the rate of diffusion in cylinders of 1·5 per cent agar of the same dimensions. Analyses have similarly been made of IAA concentrations in the donor and receiver blocks, and also in the two halves of the agar cylinder. The two sequences of 'flow' during the period of 1–6 h are compared in Fig. 5. There is no doubt of the restrictive influence of the accumulation process on the flow of IAA through root tissues, which is not otherwise inconsistent with diffusion. There is no evidence whatsoever for an active polar transport. The difference observed in acropetal and basipetal movement in these root segments can be most plausibly explained in terms of a positive gradient of accumulation potential as one moves towards the root tip. Since accumulation provides a 'sink' for diffusive flow this gradient would automatically retard basipetal movement and accelerate acropetal movement.

'These results throw further doubt on the classical theories of auxin control of root growth, in particular the role of IAA. The phenomena described seem to be associated more with the prevention than the promotion of IAA movement in root tissues and add further support to the suggestion that a quite different molecule may be the main growth regulator involved.

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Lens culinaris

The transport of auxin is less well defined in roots than in other parts of the plant[1–3]. On the whole, endogenous auxin seems to be translocated in both directions along the main axis[4] and sometimes transport towards the root tip may be greater than away from it[5]. In *Lens* roots, I[6] found evidence of a completely cyclic transport involving transverse and longitudinal movements, both to and from the tip.

It is clear that our knowledge of the situation is very imperfect, and in the work to be described the powerful techniques of ^{14}C-labelling have been brought to bear on the problem. In principle the basic method used was the classical technique of Went[7] and van der Weij[8]. An agar block containing β-indolyl-3-acetic acid with carbon-14 labelled IAA was applied to one end of a segment cut from a root and the transport estimated by observing the labelled IAA accumulating in another agar block applied to the opposite end of the segment. This is essentially the method described by McCready and McCready and Jacobs[9].

Plants of *Lens culinaris* grown under uniform conditions[10] were selected for uniform length of root (18 ± 0·6 mm). Segments 5 ± 0·2 mm long were used. The agar gel was made with Oxoid 'Ionagar No. 2' at 1·6 per cent w/v. All experiments were carried out at 25° C in dim green light. Thin-layer chromatography[11] showed that the labelled IAA finally used was at least 97·4 per cent radiochemically pure. Each experiment was repeated six times.

In the first series of investigations six different experimental conditions were used (for details see Fig. 1) in order to investigate the effect of orientation of segment in relation to gravity on the labelled IAA transport. The results are shown in Table 1, from which it can be concluded: (*a*) That the rate of transport is extremely low in all treatments. (*b*) In some cases (namely, treatments 1, 4 and 5) there is a small significant accumulation of

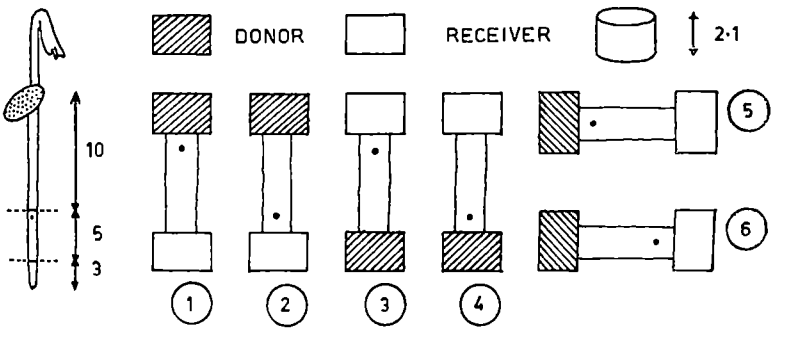


Fig. 1. Diagram showing principle of transport experiments (Table 1). The apex of each segment is distinguished from the base by the convention of marking it with a black dot

labelled IAA in the receiver block. (*c*) This accumulation occurs only in those applications at the proximal end of the segment and is not related to orientation with respect to gravity. (*d*) There is thus a weak polar movement of applied labelled IAA towards the root tip.

Table 1. RADIOACTIVITY IN THE RECEIVER BLOCKS
(in c.p.m./mg dry wt)

Donor block IAA labelled in —COOH. Specific activity : 8 mc./mM 97·4 per cent radiochemically pure. Initial concentration: 12·5 μM. Standard: 1,580 c.p.m. Background : 25 c.p.m. Time of diffusion : 240 min

	Exps. (see Fig. 1)*					
	1	2	3	4	5	6
C.p.m.	97	51	47	89	92	44
±	12	8	7	10	11	8
$\frac{\text{(Receiver)}}{\text{(Initial donor)}} \cdot 10^2$	6·1	3·2	2·9	5·6	5·8	2·7

* Fragments of *Lens* roots (5 ± 0·5 mm) were used

The poor translocation of the labelled IAA in the root segments cannot be explained by a weak penetration of the labelled IAA into these segments. It was previously demonstrated that the uptake of labelled IAA by the root segments can be very high. In Table 2 it can be easily observed that the characteristic decrease of the radioactivity in the donor blocks is not exactly correlated with the weak increase in the receiver blocks (Table 1).

Table 2. RADIOACTIVITY IN THE DONOR BLOCKS
(in c.p.m./mg dry wt.)

	Exps. (see Fig. 1)		
	1	4	5
Initial radioactivity, D_0	1,580 ± 25	1,580 ± 25	1,580 ± 25
Final radioactivity (after 240 min), Df	1,312 ± 20	1,307 ± 18	1,325 ± 19
Radioactivity lost from the donors			
$\Delta D = D_0 - Df$ in c.p.m.	268·0	273·0	255·0
$(\Delta D/D_0) \times 10^2$ in per cent	16·9	17·3	16·2

Table 3. TIME-COURSE OF MOVEMENT OF LABELLED IAA INTO RECEIVERS
(Radioactivity in c.p.m./mg dry wt.)

	Time of diffusion (min)			
Exps.	240	480	720	960
1	97 ± 12	150 ± 37	164 ± 40	167 ± 54
4	89 ± 10	146 ± 20	173 ± 32	170 ± 42
5	92 ± 11	157 ± 29	175 ± 42	181 ± 57

In the second series of experiments the time-course of labelled IAA transport was analysed using essentially the same technique. It can be seen in Table 3 that the initial accumulation in the receiver blocks began to slow down between 4 and 8 h and had practically ceased by 12 h. These results can easily be explained by the unavoidable drying-up of the root segments and also, as will be seen below, by the *in vivo* destruction of the labelled IAA.

In a third series of experiments two kinds of labelled IAA were used, one labelled in the carboxyl group (I) and the other in the α-position of the indole ring (II). In these investigations an agar plate containing labelled IAA (in a Petri dish) was used instead of individual agar blocks. The segments (Exp. 4, Fig. 1) were placed in a special support by a technique already described[13] (Fig. 2). In Fig. 3 is plotted the time-course of accumulation of the two molecules in the donor block. In both cases the rate remains steady with time over an 8-h period but the rate of accumulation of labelled IAA (II) was about twice that of labelled IAA (I).

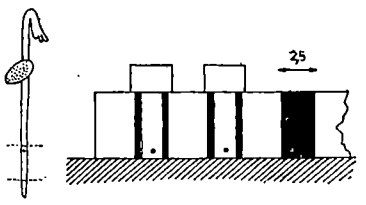


Fig. 2. Diagram showing principle of rapid transport experiments (results Fig. 3). The donor blocks are replaced here by an agar plate which contains labelled IAA. White, receiver; hatched, donor

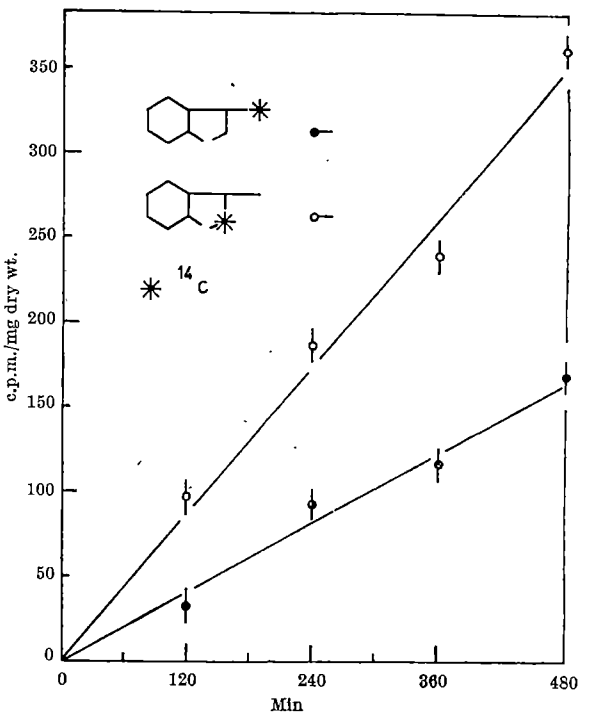


Fig. 3. The time-course of movement of carbon-14 from IAA supplied in the donor plates. ●, IAA labelled in —COOH; ○, IAA labelled in α-ring. Radioactivity in the receiver blocks in c.p.m./mg dry wt.

These results suggest that IAA during its transport is destroyed in *Lens* root segments. It has already been shown that *Lens* roots contain an active enzyme system which catalyses the inactivation of IAA both *in vivo* and *in vitro*. It was also demonstrated that the IAA-oxidases from *Lens* roots can not only remove the side-chain but also open the ring of IAA although the first process is faster than the second[15].

Since the translocation of auxin was analysed only by measurements of the transport of carbon-14, the difference between the rate of accumulation of labelled IAA (II) and labelled IAA (I) can easily be explained in terms of IAA destruction, the decarboxylation occurring more readily than the opening of the ring. The weak translocation of IAA in the root of *Lens* may be closely related to its rapid destruction. It might be that only a little of the radioactivity in the receivers is due to the accumulation of labelled IAA and that most of it is in related compounds formed *in vivo* by the action of the IAA–oxidase system.

In general conclusion it is clear that the applied labelled IAA is very weakly transported in *Lens* root tissue although the techniques used succeed in revealing a polarity of movement towards the tip.

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LETTERS TO THE EDITOR

PHYSICS

Detection of the Onset of Instability in a Cylindrical Magnetohydrodynamic Flow

DURING the past few years the classical small perturbation analysis for the prediction of criteria of stability in hydrodynamic flows has been extended to cover several cases where the fluids are electrical conductors and magnetic fields are present[1].

The experiment described here concerns the circular flow between stationary cylinders of a conducting liquid which is driven by the interaction of an axial magnetic field with radial currents. If the gap between the cylinders is small compared with their radii, the drop in voltage across the gap for simple laminar flow is approximately:

$$\Delta V = \frac{Id}{2\pi L R_1 \sigma}\left\{1 + \frac{M^2}{12}\right\} \qquad (1)$$
$$= \Delta V_\Omega + \Delta V_\eta$$

where I is the total current passed between the cylinders (if the current in the liquid is axially and azimuthally constant), d is the gap, R_1 is the inner cylinder radius, L is the cylinder length, σ is the conductivity, and $M = \sqrt{\frac{\sigma}{\eta}}\,Bd$ is the Hartmann No., η is the viscosity and B the axial magnetic flux density (all in M.K.S. units). ΔV_Ω and ΔV_η are the drops in voltage due to ohmic and viscous dissipation, respectively. The small perturbation analysis gives equations identical to those for pressure-driven circular flow with an axial magnetic field. For these the theoretical stability criteria have been established[2]. There is a critical value of k for any given M: where

$$k = Re \cdot \sqrt{\frac{d}{R_1}}$$

$$\text{and } Re = \frac{\rho u_m d}{\eta},\ u_m = \frac{\Delta V_\eta}{Bd}$$

The magnetic field has a stabilizing influence, and lengthens the cells of the post-critical flow. If B is fixed,

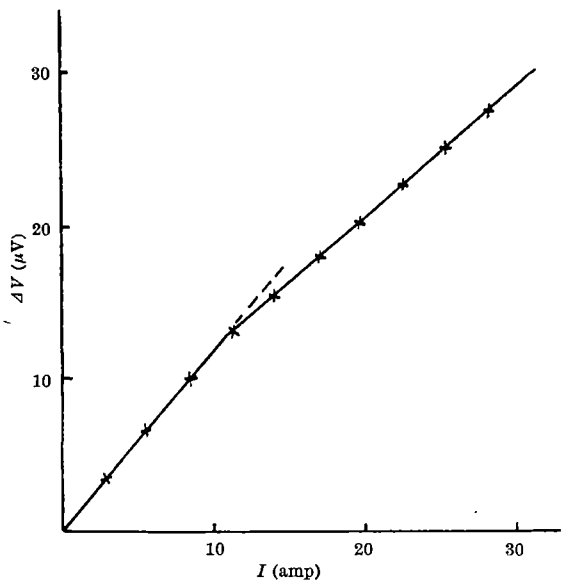


Fig. 1. B, 0·3; M, 9·3

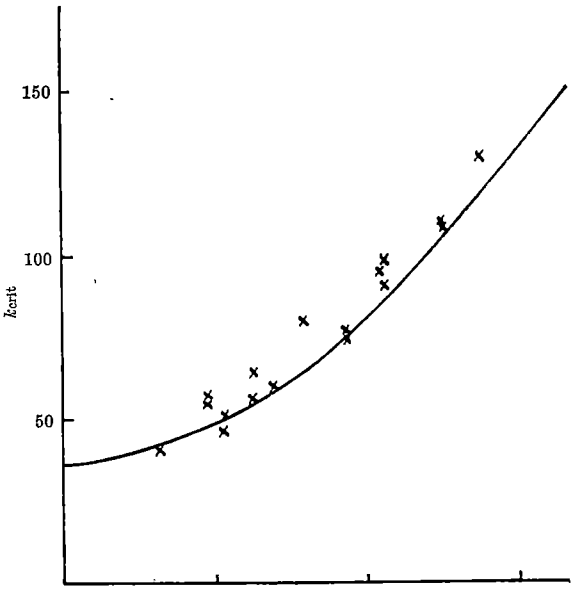


Fig. 2

ΔV should fall progressively below the value given by expression (1) above the critical point.

In the apparatus $R_1 = 0{\cdot}026$ m, $L = 0{\cdot}05$ m, $d \simeq 0{\cdot}0012$ m (three values were used). The maximum field obtainable was 0·5 webers/m². The cylinders were of high conductivity copper and the liquid was mercury. Fig. 1 shows the results of a typical experimental run. Fig. 2 shows the experimental values of k_{crit} and the theoretical curve for perfectly conducting walls. The chief source of experimental error was the variations in d due to the mercury–copper amalgam layer. The experimental points tend to lie above the theoretical curve, and this is probably due to the small gap assumption.

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Electron Transfer in Irradiated Vacuum Cavity Chambers

GREENING[1] has explained the residual current in an ionization chamber, when the pressure is reduced to very low values, in terms of the transfer of slow electrons between the electrodes. He has proposed a theory for the saturation characteristics of evacuated chambers under irradiation. On the basis of a review of experimental data, he suggested an electron energy spectrum emitted from the walls, namely, the low-energy part of the electron spectrum from 0–41 eV could be represented by $KE^{\frac{1}{2}}e^{-E/S}$ per unit energy interval and the high-energy part of the electron spectrum from 41–∞eV by $\frac{64{\cdot}5K}{E^2}$ per unit energy interval, where E is the electron energy and K is a constant. Generally it is necessary to know the directional distribution of the electrons emerging from the wall before the saturation characteristic of a vacuum

chamber can be calculated from Greening's theory. Notwithstanding this, it is possible to calculate the saturation characteristics of a spherical vacuum chamber without any knowledge of the directional distribution of the electron emitted from the walls, because of the threefold symmetry in this case. A spherical vacuum chamber, with the radii of the inner and outer electrodes being 3·13 cm and 5·08 cm respectively, has been constructed and a theoretical saturation characteristic calculated, using the electron energy spectrum suggested by Greening, and is represented by the curve labelled *A* in Fig. 1.

The copper vacuum chamber was irradiated in the γ-ray field from a 2,000-c. cobalt-60 unit. The wall thickness of the outer electrode, 2·64 mm of copper, was sufficient to establish electron equilibrium. The polarizing voltage was supplied by dry batteries and a potential divider, and the current was measured on a vibrating reed electrometer. The pressure during the measurement was 0·9–1·1 × 10^{-5} mm of mercury, which according to Taylor[2] is well below the pressure at which ionizing events will contribute significantly to the current. The temperature was 23·2° ±1° C. The experimental results are represented in Fig. 1 by curve *B*.

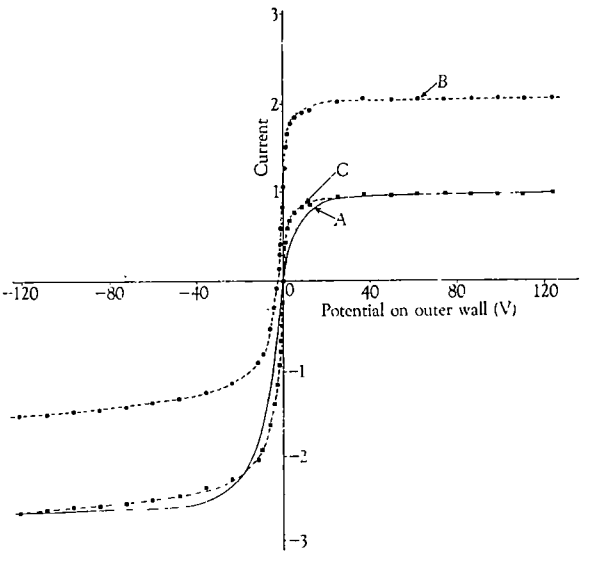


Fig. 1

The experimental curve is displaced from the theoretical curve, and in particular, it is noted that there is a finite current when zero polarizing voltage is applied. This is provisionally attributed to the attenuation of the γ-radiation by absorption and the inverse square law giving rise to unequal emission of electrons per unit area from the two electrodes, whereas the theory assumes equal electron emission per unit area. The current at zero voltage has therefore been subtracted from the measured values of the current and the experimental values replotted in curve *C*. The theoretical curve, *A*, and the adjusted experimental curve, *C*, have been normalized on the value of the current at +120 V to the outer electrode. The experimental curve *B* has been normalized on the same value as curve *C*.

The experimental results are now in general agreement with the theoretical predictions. At the higher values of the polarizing voltage (above 100 V), the agreement between experiment and theory is very close. In this region, the theoretical results are only slightly influenced by the shape of the electron energy spectrum. It therefore appears that Greening's theoretical suggestions are substantially correct. At lower values of the polarizing voltage the theoretical curve is in large measure determined by the shape of the electron energy spectrum. It is noted that the experimental curve *C* has a steeper ascent toward the plateau than the theoretical curve *A*, which suggests the electron energy spectrum occurring in practice tends to be more concentrated in the lower energies than that used in the theoretical calculation. Similar results were obtained when a telecæsium-137 unit was used to irradiate the chamber, but the agreement between theory and experiment at −120 V to the outer electrode was not so close. Thus, while Greening's explanation of the residual current in ionization chambers at low pressures and his basic theory are correct, the energy spectrum he postulated appears to be too heavily weighted at the higher energies, at least in this particular case.

We thank Mr. D. E. A. Jones for allowing us to use the radiation facilities at Mount Vernon Hospital, Northwood, and Prof. J. F. Fowler and Mr. J. Yarwood for their advice.

This work was supported by the Department of Scientific and Industrial Research.

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GEOLOGY

Evidence for a Considerable Depression of the Vegetation Belts during the Upper Pleistocene on the East African Mountains

Preliminary analyses were made of a core from the sediments of Sacred Lake, which lies in the montane rain forest zone at an altitude of 2,440 m at 37° 32′ E. and 0° 03′ N. on the north-east flank of Mount Kenya. A carbon-14 age determination of 15,400 ± 180 B.P. (GrN–3614) was obtained from the −12·30 m level. To base this date on the half-life 5,730 ± 40 years, it was multiplied by 1·03, thus converting it to 15,862 ± 185 B.P. (All other dates mentioned have likewise been converted.) On the assumption of a constant sedimentation rate, the age of total core is calculated to be 18,626 B.P. The European chronostratigraphy was used for calibration with the pollen stratigraphy of the core.

Pollen sum. The pollen sum consists of pollen from the regional vegetation belts of the mountain, namely, the Gramineae, Compositae, species of the ericaceous belt, forest trees and shrubs. Pollen produced by the aquatic plants and plants of the edge of the lake is excluded from the sum, being only of local importance. Five hundred pollen grains from each level were counted. It was possible to recognize the following typical vegetational belts of the mountain[1] in Fig. 1: (*a*) the montane forest belt consisting of the lower semi-tropical rain forest, the montane rain forest and the *Hagenia–Hypericum* zone of the tree-line at about 3,350 m; (*b*) the ericaceous belt above the forest which occurs up to about 3,660 m; (*c*) the alpine belt of grassy moorland and other Compositae which extends from about 3,660 m to 4,500 m.

As in the case of the equatorial Andean Mountains[2] and at the Cherangani Hills of East Africa[3,4], high percentages of Gramineae indicate open vegetation with conditions of the Afro-alpine belt. *Hagenia* pollen gives a good indication of the position of the tree-line and high percentages of the rest of the forest indicate more favourable humidity and temperature conditions. *Podocarpus* pollen is over-represented in the deeper levels because of its high pollen production and long-distance transport.

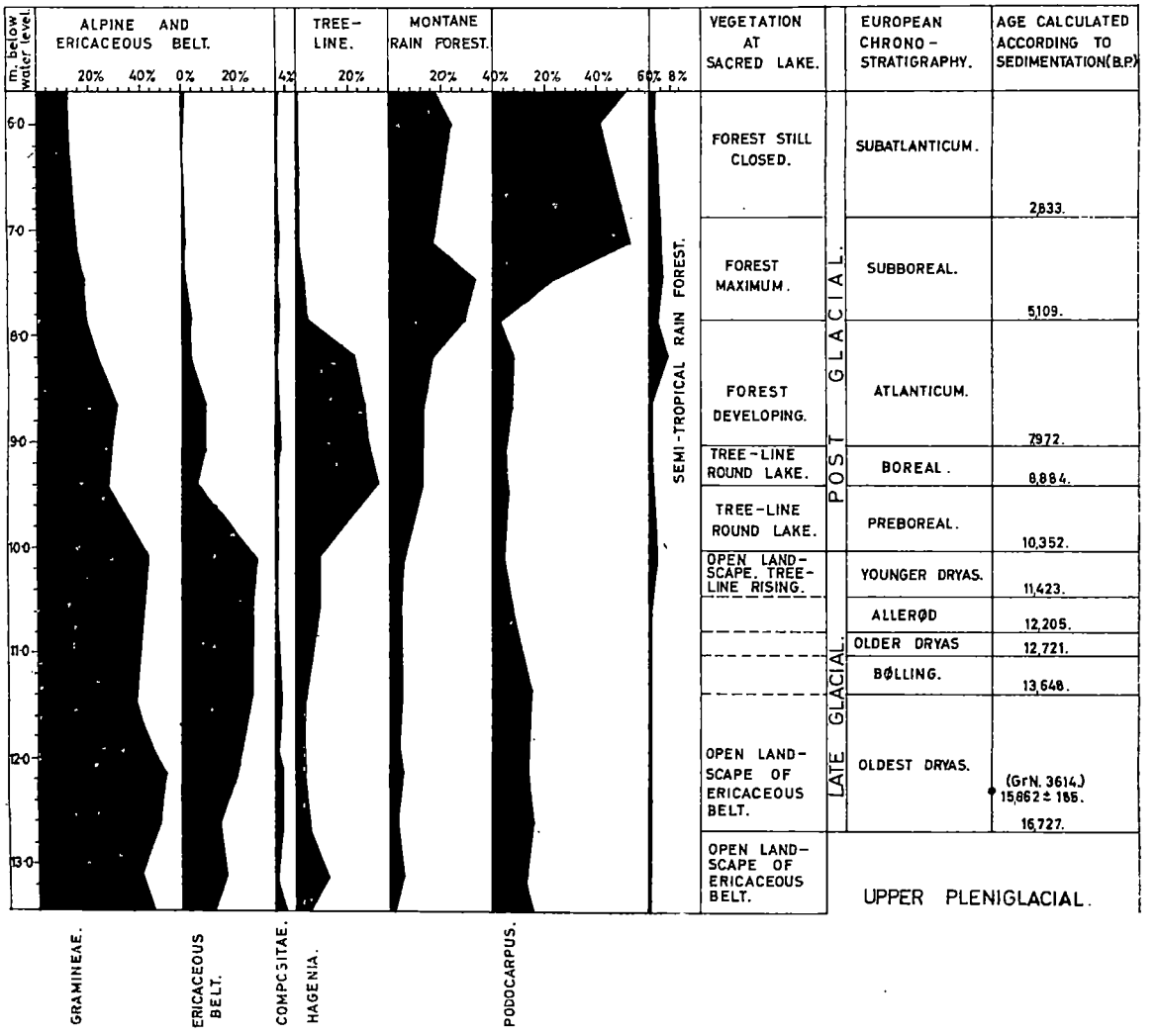


Fig. 1. Species identified: (*a*) Ericaceous belt: *Artemisia, Cliffortia, Stoebe, Myrica, Ericaceae, Anthospermum.* (*b*) Montane Rain Forest: *Rhamnus, Afrocrania, Proteaceae, Ilex, Macaranga, Olea, Rhus, Olinia, Cassipourea, Urera, Neoboutonia, Dombeya, Chrysophyllum, Pygeum, Apodytes, Juniperus, Xymalos Toddalia, Maytenus.* (*c*) Semi-tropical Rain Forest: *Celtis, Cylicomorpha, Trichilia, Trema, Ehretia, Galiniera, Chlorophora, Lovoa, Canthium, Bersama, Clausena*

Pollen diagram. The dated sample falls within the oldest *Dryas* period of the European time-scale. The vegetation round Sacred Lake during this period consisted mainly of Gramineae and species from the ericaceous belt, indicating an open landscape exposed to severe diurnal changes of temperature and humidity. This phase was contemporaneous with the retreat of the Ruwenzori glaciers of East Africa as dated by Livingstone to be 15,141 ± 298 B.P. (Isotopes Inc.). The Würm glaciers in Europe and the Wisconsin glaciers in North America also diminished in size from that time onward[5]. So far the equivalents of the Bølling and Allerød oscillations have not been detected. Analyses at shorter intervals will have to be made in this connexion.

The most remarkable point of the diagram is found at the level −10·10 m, where a significant change occurs in practically all the curves. From here upwards the pollen of the grasses and ericaceous belt shows a clear decline whereas *Hagenia* rises rapidly and the montane rain forest starts to show a definite development round the lake. This horizon has a calculated age of 10,583 B.P. and is coeval with the end of the Late Glacial and the beginning of the Preboreal period of Europe. During Preboreal times *Hagenia* is dominant on the site and is accompanied by the tall Compositae shrubs which are typical at the upper limit of the forest. This succession indicates a rapid rise in temperature. Conditions remain practically the same during Boreal times (from levels −9·42 to −9·07 m). During the period which is coeval with the climatic optimum of the Atlanticum of Europe, conditions are very favourable for the growth of the forest. It only becomes closed, however, at a later stage as is clearly shown by the forest maximum and the definite minima of the ericaceous belt and *Hagenia* at about −7·50 m. This establishment of the montane rain forest occurs after 5,100 B.P., which date is generally accepted to be the beginning of the Subboreal in Europe. It indicates more favourable conditions during the Subboreal than during the Atlanticum.

In the upper part of the diagram, the forest is still closed. The rapid rise of the *Podocarpus* curve indicates climatic changes which occurred during the Subatlanticum. The semi-tropical rain forest is represented in the diagram from the beginning of the Preboreal and shows a definite maximum during the latter half of the Atlanticum.

Conclusions. The results of the pollen analysis of the Sacred Lake sediments now give definite proof that the time-scale of the Late Glacial and Post Glacial periods of Europe can be applied in tropical East Africa. It shows that similar climatic changes occurred simultaneously in these regions.

Contemporaneous with the cold Oldest Dryas of Europe, there was a considerable depression of the vegetation belts of Mount Kenya. At the level of the dated sample, the pollen spectrum clearly shows a vegetation round Sacred

Lake similar to that found at present above the tree-line in the open ericaceous belt. This indicates a 1,000–1,100-m downward shift of this vegetation and a probable drop in temperature of about 8° C in this high mountain area, if the temperature calculation method for the equatorial Andean Mountains is applied[2].

A deeper core has since been made at Sacred Lake. Judging by the age of the deepest level, namely 33,350 ± 1,000 B.P. (GrN–4194), the total core probably represents the period from before the Paudorf interstadial of the Interpleniglacial of Europe to the present time.

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Relationship of Palæomagnetic Reversals and Micropalæontology in Two Late Cænozoic Cores from the Pacific Ocean

Recent investigation has revealed palæomagnetic reversals preserved in the clayey radiolarian ooze of two deep-sea cores from the equatorial Pacific Ocean. The cores are:

MSN 12*G*; position 3° 01′ N., 174° 02′ W.; depth, 5,230 m.

MSN 142*G*; position 5° 20′ N., 146° 13′ W., depth, 5,089 m.

The cores are illustrated, their lithologies briefly described, and Radiolaria listed by Riedel and Funnell[1].

Palæomagnetism. The uppermost parts of both cores are thought to be normally magnetized with respect to the present Earth's field. As the cores come from equatorial positions and were not orientated on collection it is not possible to be certain that this is so. There is, however, no evidence of erosion or non-deposition at or near the tops of either sequence. The first reversal occurs in *MSN* 12*G* between 74 and 95 cm, and in *MSN* 142*G* between 78 and 87 cm. Other reversals occur below these levels in both cores. Assuming that the uppermost magnetic vector is normal, the magnetization of the cores is as follows:

MSN 12*G*; 6–74 cm (N, 5 samples), 95–116 cm (*R*, 7 samples), 119, 123 cm (N), 133 cm (*R*), 135 cm (N), 138–146 cm (*R*, 7 samples).

MSN 142*G*; 15–78 cm (N, 8 samples), 87–107 cm (*R*, 3 samples), 116, 125 cm (N), 134, 142 cm (*R*).

The palæomagnetic results below the first reversed zone (*R*1) do not seem to be consistent with the hypothesis that reversals of the Earth's magnetic field have occurred about every million years, unless extremely slow ratios of deposition are assumed for the lower portions of these cores. However, recent results from continental rocks[2–5] are also inconsistent with this hypothesis, and it seems likely that below the *R*1 zone reversals occurred more rapidly than was formerly suggested.

Micropalæontology. In *MSN* 12*G*, Radiolaria between 0 and 43 cm are similar to Recent assemblages and indicate a Quaternary age. Specimens of *Pterocanium prismatium* occur rarely at 90–92 cm, and commonly from 127 cm to the bottom of the core. Some re-worked Eocene, Miocene and possibly Oligocene forms are present throughout. In *MSN* 142*G*, a Quaternary age is indicated by the Radiolaria between 0 and 76 cm, but from 94 cm to the bottom of the core *Pterocanium prismatium* is rather common. In this core some re-worked Miocene forms are present throughout.

In both cores examined the first reversal appears to correspond more or less exactly with the upper limit of occurrence of *Pterocanium prismatium*. Lithologically there does not seem to be any break in accumulation at this level in *MSN* 12*G*, which consists throughout of a vaguely mottled, brown clayey siliceous ooze, but in *MSN* 142*G* there is a burrow-mottled gradation, between buff or brown ooze above and grey-brown ooze below, at approximately 85 cm.

In recent investigations of the palæomagnetic polarity of basalts from the continents and oceanic islands, normal magnetization has been found to characterize all rocks up to 0·98 m.y. B.P., prior to which a period of reversed magnetism (*R*1) is found extending back to 1·9 m.y.[2–5]. The reversal at 0·98/0·99 m.y. would appear to fall within the Quaternary, as defined in accordance with the recommendations of the International Geological Congress 1948 (refs. 4 and 6). If, as seems most likely, the first reversal recorded in the cores corresponds to that found on land, an intra-Quaternary age of 0·98/0·99 m.y. is implied for the level of the reversal in the cores.

The upper limit of occurrence of *Pterocanium prismatium* has hitherto been regarded as approximating to the Tertiary–Quaternary boundary[1,7]. In the Pacific it corresponds rather closely with the disappearance of *Discoaster* spp. from the calcareous nannoplankton, a phenomenon which has also been attributed to the Tertiary–Quaternary boundary in the Atlantic[8].

The association of a first magnetic reversal with the upper limit of occurrence of *Pterocanium prismatium* in the two Pacific cores suggests that this level has an age of very slightly less than 1 m.y. and that it is intra-Quaternary in terms of current stratigraphical definitions.

The work of one of us (C. G. A. H.) was supported by American Chemical Society petroleum research grant No. 700–*A*.

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New Species of *Schizaea* Spore from the Upper Mesozoic of Kashmir, India

During the study of samples collected from Wakkachu Traverse, Ladhak District, Jammu and Kashmir, I obtained a new type of striated bean-shaped spore belonging to the family Schizaeaceae together with a number of other spores and pollen grains having an upper Mesozoic affinity. The detailed results of this work will be published elsewhere. The samples were crushed and treated with hydrofluoric acid followed by concentrated nitric acid and hydrogen peroxide solution. The polyspores were separated by a heavy liquid (specific gravity, 2·3) and mounted in glycerine jelly.

The spore is large, bilateral and bean-shaped; monolete, laesura long; exine sculptured with unbranched broad striations. Striations 4μ thick are spaced about 3·5–4μ



a *b*

Fig. 1. *Schizaea kashmiriensis* sp. nov. *a*, Camera lucida drawing; *b*, photomicrograph (× 400)

and parallel to each other and run along the long axis of the spore. Length, 85·8μ; breadth, 39μ; broad end, 42·9μ; narrow end, 31·2μ (Fig. 1).

From the available knowledge of the morphology of the recent and fossil Schizaeaceae spores, bilateral bean-shaped, striated, monolete spores are met with only in the genus *Schizaea*. No *Schizaea* spores previously described, recent or fossil, from Tertiary or Mesozoic beds[1–10], resemble the spore described here. The new species is named *Schizaea kashmiriensis* sp. nov.

I thank Mr. A. K. Ghosh for advice, and Shri L. L. Bhandari for the samples collected by him.

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GEOCHEMISTRY

'Impact Contamination' of the Mokoia Carbonaceous Chondrite

In the course of inspection of a specimen of the stone from New Zealand, in the Museum of Paris, a rather unusual yellow chondrule-like grain, 1·7 mm in diameter, was detected on what appeared to be fresh fractured surface, some 23 mm from the nearest visible crust on the specimen. On closer examination the grain proved to consist of resinous material soluble in ether, and with volatiles of 92·7 per cent; 4·1 per cent fixed carbon and 3·2 per cent ash. The terrestrial origin of this blob of resin is supported by the ample fragments of wood, spores, Radiolaria, and other typical micro-fossils.

The foregoing observation indicates that in the course of the impact of a given meteorite, foreign bodies can penetrate to considerable depth, particularly in the case of the porous and soft carbonaceous stones. Beside this physical penetration process, illustrated by this work, distillation of surrounding plant remains could be brought about by the hot stone, and the products may become deeply absorbed as a result of vacuum forming through the cooling-down of the meteorite and condensation of vapours of water, etc., in its pores. I consider that this factor of 'impact contamination' should be investigated in greater detail, in order to assess the value of searches for molecules whict are generally believed to be exclusively or almosh exclusively biogenic.

I have found[1] that the Cold Bokkeveldt meteorite contained no optically rotating molecules, within the limit of sensitivity of the equipment used, which was of the order of 1 p.p.m. The same conclusion was reached regarding the Mokoia meteorite[2], with measurements made approximately with the same range of sensitivity. B. Nagy[3], using more refined experimental methods, detected optic rotation in the Orgueil meteorite, and G. W. Hodgson and B. L. Baker[4], utilizing elaborate experimental techniques, found also porphyrins in the same meteorite. Although the actual amounts detected were not mentioned by the foregoing workers it appears to me that they may have been of the order of perhaps 0·01 p.p.m. of the whole stone. One should ask the question whether such data are within the range of terrestrial impact and storage contamination. The fact that, according to the aforementioned authors, the Brudenheim and Peace River non-carbonaceous chondrites proved to contain no porphyrins has relatively little significance on account of the much less porous nature of these stones. The absence of chlorins in Orgueil and old terrestrial carbonaceous sediments, claimed by the authors as evidence for the extra-terrestrial origin of the porphyrins, may be due to the fact that, in the course of distillation of plant matter following the impact, the chlorins would be decomposed by the hot surface crust of the meteorite, and therefore mainly the more resistant porphyrins would penetrate to the deeper zones of the stone.

The foregoing questions could be investigated with a more positive approach through simulation of actual falls of meteorites; silicates of the composition of chondrites could be impregnated with synthetic organic polymers, carefully tested for traces of optically active molecules or porphyrins; afterwards such 'synthetic meteorites' could be ejected from high-flying aircraft or preferably satellites, and the degree of contamination of falls to different types of terrain could be examined. The extent of 'storage and handling contamination' could be also determined on the samples in question.

I consider that we must be on uncertain ground in our search for molecules. which are at present believed to be exclusively living products, until the effects of 'impact' and 'storage' contaminations are experimentally evaluated, unless, among the 20 or so carbonaceous stones hitherto not investigated, one is found which contains the substances in considerably higher concentration than in the Orgueil meteorite.

I thank Prof. J. D. Bernal for his advice and Prof. J. Orcel for providing me with facilities to examine the meteorite collection of the Museum of Paris, and placing material at my disposal for experimental work.

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Depth of Source of Lead

We have recently introduced the idea of the liquid fraction value. As defined elsewhere[1–4], this term indicates the quantitative fraction which a liquid at a certain stage of solidification occupies relative to the amount of initial liquid which for the liquid fraction value is taken as unity. As self-evident from the

definition, the liquid fraction value never surpasses unity, and diminishes steadily as solidification or crystallization proceeds.

In addition, it has been pointed out[5] that, if several assumptions are made, there is a possibility of reducing the value of the liquid fraction obtained to the dimension of depth within the Earth. These assumptions are as follows: (1) the mantle was formed as an aggregate of crystals separated from the liquid with starting composition like chondritic oxide; (2) the solids (crystals) separated from the liquid in the course of formation of mantle precipitated successively inwards; (3) the transfer of material by convection or diffusion did not occur on a large scale after the formation of the mantle; (4) the magma represents the material produced by partial melting within the mantle. If these conditions are assumed, the original depth (ΔR) of source of a primary magma can be related to its liquid fraction value (f_w) relative to initial chondritic oxide melt by:

$$f_w = \frac{\left\{1 - \left(\frac{R-\Delta R}{R}\right)^3\right\}\rho'}{\left\{1 - \left(\frac{\gamma}{R}\right)^3\right\}\rho} \qquad (1)$$

where R and γ = the radii of the Earth and its core, respectively, ρ = the mean density for the mantle plus crust, and ρ' = the mean density for the spherical zone shallower than ΔR from the Earth's surface. From the results provided by Bullen[6] on the density distribution within the Earth, the diagram as shown in Fig. 1 was obtained in conformity with equation 1.

Based on the foregoing assumptions, the liquid fraction values obtained from the abundances of lanthanides in a South African eclogite, and a Polar Ural peridotite, were reduced to dimension of depth. As a result of such a calculation, we have obtained[5] $\Delta R = 160$ km and 400 km, respectively, for the eclogite and the peridotite in question. It may be said that this result conforms with petrological observations.

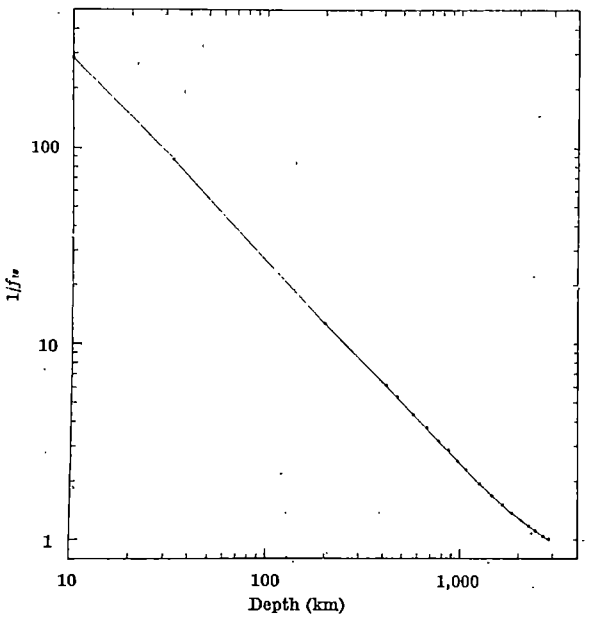


Fig. 1. Diagram for relation of liquid fraction value to depth (after ref. 3)

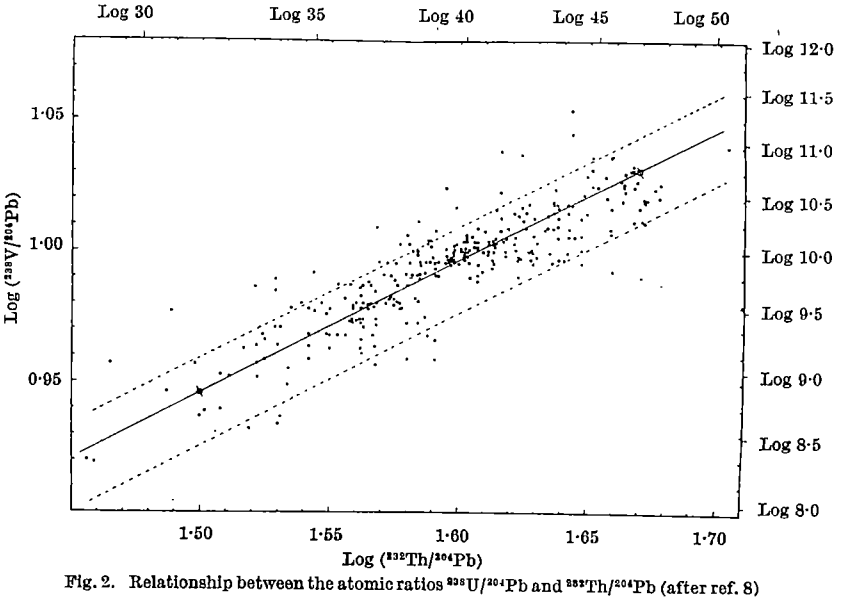


Fig. 2. Relationship between the atomic ratios $^{208}U/^{204}Pb$ and $^{232}Th/^{204}Pb$ (after ref. 8)

In this communication, an attempt has been made to evaluate the liquid fraction values for the source materials of leads and to reduce them to depths by assuming the conditions already mentioned. However, it is thought[7] that the source of lead originated from such material which had been produced by partial melting shortly after the formation of mantle material, and pocketed *in situ*. Meanwhile, the following theoretical equation holds good[2]:

$$\log (C_{Th}/C_{Pb}) = \log (C^0_{Th}/C^0_{Pb}) - (k_{Pb} - k_{Th}) \log f_w \qquad (2)$$

where C refers to the concentration of subscripted element in a melt, C^0 to the concentration in initial chondrite-like oxide melt, and k to the partition coefficient. Since $C^0_{Th} = 0{\cdot}048$ p.p.m. (refs. 2 and 10), C^0_{204} (the initial concentration of lead-204) $= 0{\cdot}00199$ p.p.m. (ref. 2), $k_{Pb} = 0{\cdot}292$ (ref. 2), and $k_{Th} = 0{\cdot}0733$ (ref. 3), equation (2) becomes:

$$\log (C_{Th}/C_{204}) = 1{\cdot}383 - 0{\cdot}219 \log f_w \qquad (3)$$

where (C_{Th}/C_{204}) refers to the weight concentration ratio between thorium and lead-204. At this point, let us direct our attention to Fig. 2, which shows the abundance relationship among uranium, thorium, and lead-204, estimated[8] from the isotopic composition of lead of ores. (The abundance ratio in this diagram refers to atomic ratio.) According to the data shown in Fig. 2 the average value for log [$^{232}Th/^{204}Pb$] is 1·5965, which is equivalent to log $(C_{Th}/C_{204}) = 1{\cdot}6522$ whereas log $(232/204) = 0{\cdot}0557$. Introducing log $(C_{Th}/C_{204}) = 1{\cdot}6522$ into equation (3) gives us log $f_w = -1{\cdot}233$ ($f_w = 1/17{\cdot}1$). According to Fig. 1, $f_w = 1/17{\cdot}1$ corresponds to $\Delta R = 150$ km. This suggests that the most frequent seats of original sources from which lead in ores was derived are at about 150 km. It should be noted that this dimension coincides with the depth of low velocity layer. As seen in Fig. 2, the points are very rare for log [$^{232}Th/^{204}Pb$] $< 1{\cdot}50$ and for log [$^{232}Th/^{204}Pb$] $> 1{\cdot}668$. These values correspond to $f_w = 1/6{\cdot}3$ and 1/36, and, in turn, to depth = 400 and 75 km. This fact indicates that the original source material for lead of ores is seated rarely below 400 km and above 75 km. Emphasis must be placed again on the fact that the range of depth of 75–400 km is involved by the range of position thermodynamically inferred for the origin of magma[9]. These conclusions are thought to suggest that the approach newly presented, and the model necessary for it, deserve further consideration.

In the foregoing calculation, the effect of convection was regarded as virtually negligible. If convection on a large scale exists within the mantle, the resultant values of depth for particular leads do not necessarily indicate the present situation of their original source. Even if such is the case, it will not be necessary to modify the conclusion that the common range of depth of magmatism is 75–400 km.

The foregoing results may be interpreted also as suggesting that material of the continental crust which has an average value of f_w of about 1/80 (refs. 1 and 4) is being developed from a layer which was composed of primary liquid-type materials corresponding to $f_w = 1/6{\cdot}3 \sim 1/36$ (1/17·1 on an average). This interpretation does not necessarily contradict our model.

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MINERALOGY

Far Infra-red Spectra of Montmorillonite, Kaolinite and Illite

ALTHOUGH infra-red spectra of many of the common rock-forming minerals have been presented by several investigators[1–5], few results are available for the diagnostic analysis of clay minerals in the far infra-red spectral region (below 400 cm^{-1}). This communication presents the far infra-red spectra of montmorillonite, kaolinite, and illite for the portion of the spectrum between 90 and 400 cm^{-1}.

Absorption maxima in this region most likely reflect the presence of complex molecular librations and low frequency stretching and bending modes. Inasmuch as relationships between the different clay structures and their far infra-red spectra are essentially unknown, to speculate at length on probable assignments of different absorption bands would be premature. Only qualitative results have so far been obtained.

The samples examined were obtained from the following sources: montmorillonite and kaolinite from American Kaolin Products Co., Aiken, South Carolina; illite (Fithian) and bentonite from American Colloid Co., Skokie, Illinois. Relative purity of the samples was checked by both X-ray diffraction and conventional infra-red methods.

The infra-red patterns for the conventional range are presented in Fig. 1. Referring to these spectra, it is apparent that the samples utilized are of reasonable purity. Fithian illite, even though known to be a mixture of mica, expansible layers, quartz and kaolinite, was chosen because references to it and its characteristics are common in the general literature of clay mineralogy. Bentonite, owing to its usually high montmorillonite content, served as a check on those absorption bands obtained with the 'normal' montmorillonite sample.

An examination of the spectra in Fig. 1 shows that quartz (indicated by the characteristic doublet at 800 and 780 cm^{-1} and absorption at 700 cm^{-1}) is a common impurity in all samples. X-ray diffraction studies indicate quartz to be present in amounts less than 10 per cent in all samples. The presence of some kaolinite (< 10 per cent as confirmed by X-ray diffraction techniques) is indicated in both the montmorillonite and illite samples. On the basis of X-ray diffraction and infra-red studies, it is felt that the major absorption bands present in the far infra-red region (90–400 cm^{-1}) are likely characteristic of the clay minerals studied and not of the impurities present.

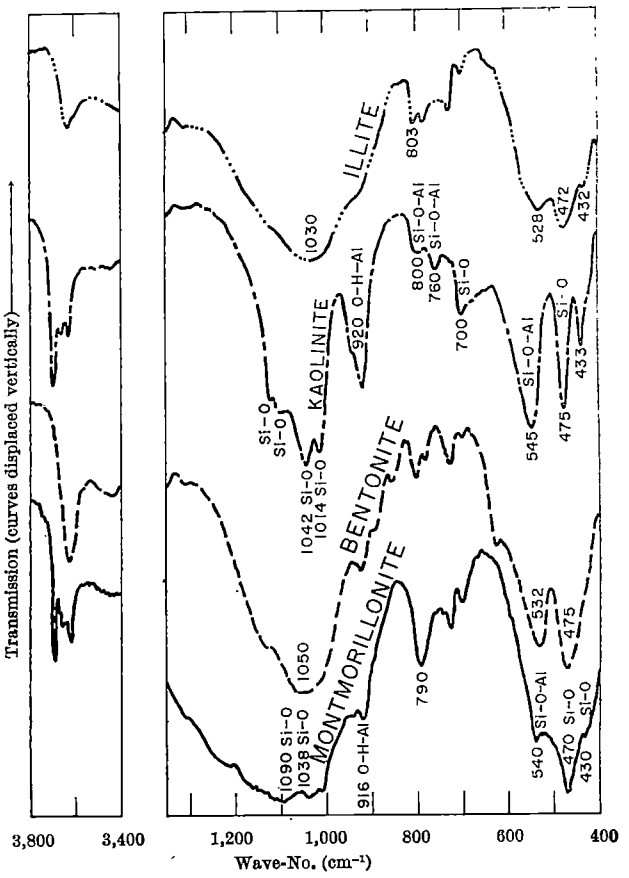


Fig. 1. Spectra of clay minerals investigated (conventional range). Assignment of absorption peaks indicated where known

The samples were prepared by grinding with a mortar and pestle (with ethanol) to a size of 5 microns or less. Mineral oil ('Nujol') was added to form a thick mull (to enhance any weak absorption bands) and a portion of the mull was then pressed between two 0·51 mm thick polyethylene plates. Measurement of the spectra in the range 33–800 cm^{-1} was made on a Beckman *IR*-11 spectrophotometer.

Afterwards, the thick mull was thinned by the addition of more 'Nujol', a portion of this mixture pressed between polyethylene plates and a scan again made from 150 to 800 cm^{-1} (Beckman *IR*-11). This latter step was made to bring the more intense bands 'on scale'. A second portion of the thin mull was pressed between thin (2–3 mm) CsI plates and run in the conventional range (400–4,000 cm^{-1}) on a Beckman *IR*-9 unit.

The spectra of montmorillonite and bentonite and of kaolinite and illite are shown in Figs. 2 and 3 respectively. Assignments (Fig. 1) for the main absorption bands in the 400–600 cm^{-1} region are indicated where known[6]. It is not possible at present to make definite assignments of the absorption peaks beyond 400 cm^{-1}. The primary purpose of this communication is to present the far infra-red spectra of these compounds.

Although possible weak absorption peaks were indicated, the region below 80 cm^{-1} showed more noise than signal to be diagnostic. Absorption in the range 530–555 cm^{-1} (noted in all samples) has been assigned[6] to the Si-O-Al group for montmorillonite and kaolinite; that in bands

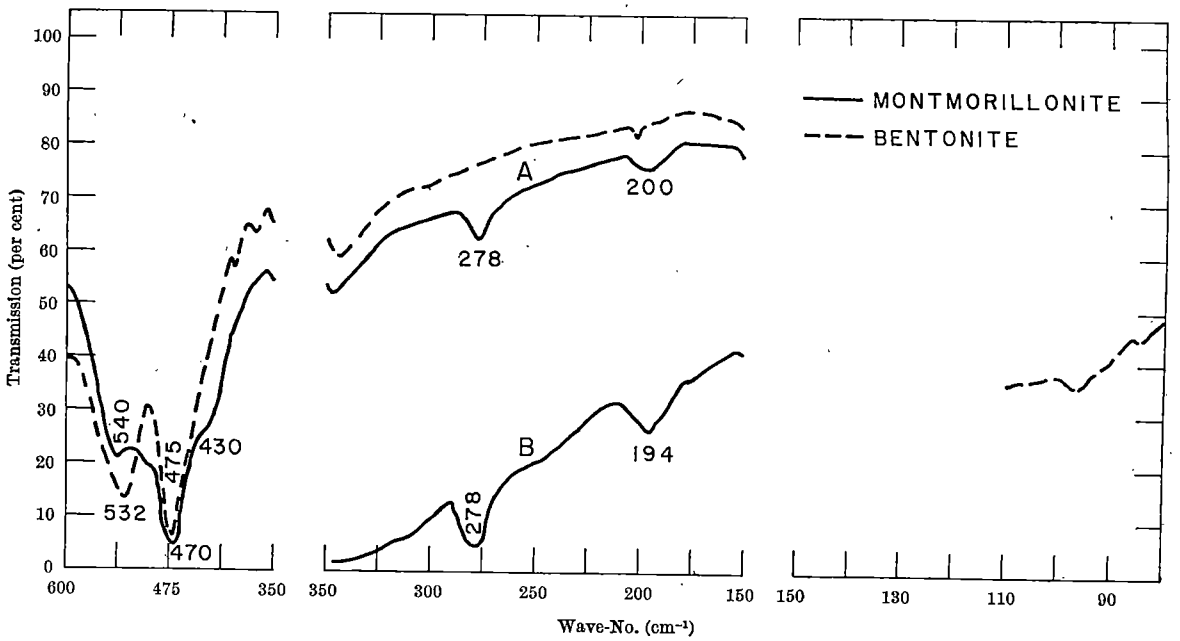


Fig. 2. Far infra-red spectra of montmorillonite and bentonite. Curve *A* and 350–600 cm^{-1} thin mull between polyethylene plates; curve *B*, thick mull between polyethylene plates

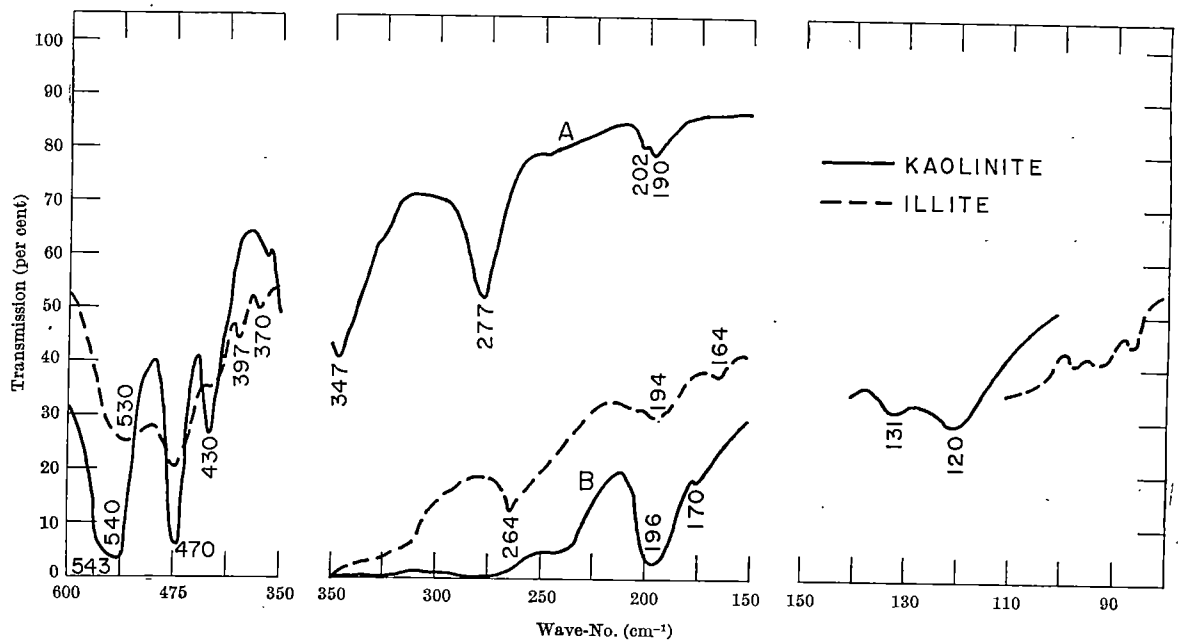


Fig. 3. Far infra-red spectra of kaolinite and illite. Kaolinite, curve *A* and 350–600 cm^{-1} thin mull; curve *B* and 90–150 cm^{-1} thick mull. Illite thin mull 350–600 cm^{-1}, thick mull 80–350 cm^{-1}

470–475 cm^{-1} and 430–433 cm^{-1} has similarly been assigned to Si-O for the same minerals. It is likely that these vibrational modes in the illite, owing to its compositional make-up, are due to the same atomic groupings. Bentonite, being dominantly montmorillonite, would be expected to show an absorption pattern similar to montmorillonite.

Normally in the range 400–4,000 cm^{-1}, it is difficult to distinguish montmorillonite and illite in the same sample. The identification of these two minerals is commonly based on the presence of strong partially overlapping absorption bands centred at 1,040 and 1,030 cm^{-1} respectively. Examination of the far infra-red spectra of these two clay minerals reveals the presence of one major and several minor and distinct non-overlapping absorption bands (illite-major 264 cm^{-1}, minor 164, 397 cm^{-1}, etc.) which might allow positive easy identification of these minerals.

Although several of the major bands of kaolinite and montmorillonite are similar, the relatively strong absorption of kaolinite at 120 and 131 cm^{-1} (these peaks are absent in montmorillonite) should likewise allow positive identification and separation of this pair.

The absorption maxima at 344–348 cm^{-1} in montmorillonite and kaolinite might be a fundamental vibrational mode, but more likely they are difference bands—in kaolinite 543 – 196 = 347, in montmorillonite 540 – 194 = 346. The band at 194–200 cm^{-1} was noted in all spectra and although assignment of this peak cannot be made at present, its ubiquitous nature suggests its probable relation to some common structural feature of the phyllosilicates.

At present the far infra-red spectra of clay minerals are of limited usefulness for the purposes of quantitative

analyses. However, their potential value for qualitative work is clear. Investigations of the far infra-red spectra (below 400 cm^{-1}) of the different clays and rock-forming minerals could open a whole new range of possibilities in the investigation of rock-forming compounds.

I thank Kenneth E. Stine of Beckman Instruments, Inc., Fullerton, California, for making it possible to obtain runs on the *IR*-11. This work was supported by the Office of Naval Research No. *NONR*–2119(04).

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CHEMISTRY

Determination of Carbon in Steels by Measurement of the Prompt γ-Radiation emitted during Proton Bombardment

CONVENTIONAL techniques of activation analysis usually require the measurement, some time after the completion of the irradiation, of the radiation emitted by a radioactive nuclide manufactured during the irradiation, with or without prior separation of the radionuclide to be determined, and sensitive analytical methods have been devised for most elements using thermal neutrons as bombarding particles[1,2]. However, for the light elements, low cross-sections and inconvenient half-lives of the product nuclides often preclude the use of thermal neutron irradiation as the basis of an analytical method, and charged particles, γ-photons[2,3], and neutrons of non-thermal energies[4] have been used instead.

A further possible application of the techniques of nuclear spectroscopy to analytical problems would be to measure the prompt radiation emitted during the de-excitation of the excited states of certain nuclei, rather than the radiation emitted by a radionuclide after the irradiation stage has been completed. Irradiation and detection could then be carried out simultaneously, thus offering the opportunity of more rapid analysis and, further, since radiation corresponding to energy transitions in excited states of stable nuclei could be measured by this means, it would no longer be necessary to choose a nuclear reaction which provided a suitable radionuclide.

Measurement of prompt γ-rays is complicated by the radiation of radioactive nuclides which build up during the course of the irradiation, but in view of the potentialities of prompt γ-ray measurement, particularly for the determination of the light elements, a programme of work has been instituted in this laboratory with a view to assessing the feasibility of the method. Charged particles were used for irradiation in spite of their low penetrating power, as the prompt γ-rays could be measured with relatively simple counting equipment and the coulomb barrier prevented nuclei of high atomic number reacting, thus simplifying the γ-ray spectrometry required. This work will be reported in detail elsewhere, but we give as an example of the technique the determination of carbon in steels using low-energy proton irradiation.

The determination of carbon by means of the reaction $^{13}C(p,\gamma)^{13}N$ measuring the 10 min positron emitter ^{13}N is well known[5], and the reaction has a resonance at the rather low proton energy of $E_p = 0{\cdot}456$ MeV. During reaction a 2·3-MeV γ-ray is emitted[6], and this was measured

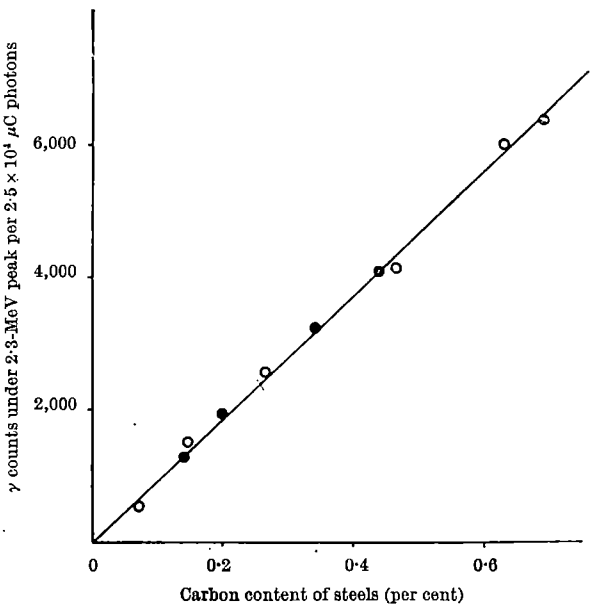


Fig. 1. Counts under the 2·3-MeV γ peak as a function of the carbon content of the irradiated steels. ○, Spectrographic standards; ●, analysed samples supplied by A.E.R.E., Chatham

in these determinations. Although the thick target yield is not high ($7{\cdot}5 \pm 0{\cdot}5 \times 10^{-10}$ disintegrations/proton at $E_p = 1{\cdot}00$ MeV (ref. 7)), the low energy of the bombarding particles results in a relatively low background, and the prompt γ-rays can be determined easily.

Targets were in the form of steel disks, and were of known carbon content. Five were a set of commercial spectrographic standards (Bureau of Analysed Samples, Ltd., Nos. *SS* 31–35 Plain Carbon Steels), and the others were cut from lumps supplied by the A.E.R.E. Outstation, Chatham. The spectrographic standards were 1·75 in. diameter by 0·5 in. thick, and fitted directly on to the O-ring of a specially constructed target holder; the other samples were smaller (0·75 in. diam. × 0·1 in.) and fitted into the well of a brass target assembly.

Targets were wobbled and air cooled, and a liquid nitrogen trap was placed in the vacuum system just ahead of the target to reduce interference from the carbon background of the accelerator, but investigations showed that the two machines used for this work (5-MeV and 3-MeV Van der Graaff electrostatic generators) did not produce appreciable interference at the rather high levels of carbon (> 700 p.p.m.) present in the samples. The detector was a 3 in. × 3 in./NaI (Tl) crystal at 0° angle of observation placed 1½ in. away from the irradiated face of the target, and was connected to a 100 channel pulse height analyser. The beam falling on the target was monitored and the total proton dose recorded by a beam integrator. Throughout these experiments 0·8 MeV protons were used for the irradiation, and targets were irradiated to the same constant dose of 25,000 μC. Irradiations took about 13 min, and the time could have been reduced, if required, by increasing the beam current.

Results obtained from proton irradiations are summarized in Fig. 1, where the counts under the 2·3-MeV prompt γ peak are plotted against the known carbon content of the steels. Data for the two sizes of steel disks are plotted on the same graph, for although targets and holders imposed slightly different counting geometries in the two cases the effect was too small to be significant.

From Fig. 1 it can be seen that good correlation was obtained between the prompt γ-rays counted and the carbon content of the steels, down to the lowest value determined (700 p.p.m.), indicating that measurement of prompt γ-rays can be used as the basis of an analytical method for carbon.

We thank the Nuclear Physics Division, A.E.R.E., for making accelerator time available, and for the generous loan of electronic equipment; and also Dr. G. A. Jones for advice, Mr. T. E. Sparrow for machine operation, Mr. H. I. Shalgosky for providing steels of known carbon content, and Messrs. H. D. Hitchcox and A. H. G. Boram for preparing samples for irradiation.

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Infra-red Absorption of the Hydroxyl Radical

Although the vibration–rotation structure of the hydroxyl radical emission spectrum in the infra-red has been observed both from flames[1] and from the chemiluminescent reaction[2] of hydrogen atoms with oxygen molecules:

$$H\cdot + \cdot O_2\cdot \rightarrow HO_2\cdot$$
$$H\cdot + HO_2\cdot \rightarrow 2\ \cdot OH\dagger$$

(where ·OH† denotes a vibrationally excited hydroxyl radical), there has been no report of the infra-red absorption spectrum of ·OH. The purpose of this communication is to suggest that the absorption of both ·OH and ·OD has been recorded without the identity of the carrier being discovered.

In investigations[3–5] concerned with the infra-red absorption of water molecules trapped in noble gas matrices at 4° K, there have been observed two sharp absorption lines, at 3,574 cm^{-1} in H_2O experiments and 2,635 cm^{-1} in the case of D_2O, both of variable intensity relative to other spectral features. These frequencies are close to the zero lines of the gas phase, calculated from electronic spectra[6] to be 3,569·6 cm^{-1} and 2,632·5 cm^{-1} for ·OH and ·OD, respectively.

Again, in experiments[7] in which were frozen at 4° K the products from a discharged mixture of 50 per cent hydrogen (or deuterium) and 50 per cent argon reacted with a small amount of nitric oxide, an absorption band at 3,596 cm^{-1} (or 2,680 cm^{-1}) was observed besides the other absorption bands conclusively assigned[5,8,9] to nitroxyl, HNO. (In connexion with the assignment in ref. 9 of 3,380 cm^{-1} as the N—H stretching fundamental of HNO, better agreement with the infra-red emission from the hydrogen atom–nitric oxide reaction[10] would result from the alternative assignment of the feature at 3,450 cm^{-1} as this fundamental.)

There are three points to be considered in the proposed assignments for the hydroxyl radical: the mechanisms of formation, the frequency shifts in the solid matrices, and the absence of vibration–rotation structure.

In the case of the hydrogen atom reaction with nitric oxide, the hydroxyl radical could result from reaction of hydrogen atoms with nitrogen oxide impurities stated[7] to be present:

$$H\cdot + \cdot NO_2 \rightarrow \cdot OH + \cdot NO$$

or

$$H\cdot + N_2O \rightarrow \cdot OH + N_2$$

the former bimolecular reaction being particularly rapid[11]. Less easily solved is the mechanism of hydroxyl production in experiments in which 0·2–0·35 per cent water in argon is simply deposited on a window at 4° K (refs. 3–5). Possibly significant are the facts that the 3,574 cm^{-1} absorption feature is absent when H_2O in argon is deposited at 20° K (refs. 12, 13), and that the thermal conductivity of solid argon is a maximum at 8° K (ref. 14), enabling greater deposition rates without loss of optical transmission and isolating power. A possible mechanism is that the friction of the gas mixture flowing about 5 m/sec through glass tubing toward the cold window could produce static electricity in the form of discharges in the gas, noble gases having comparatively small breakdown potentials. The principal effect of these discharges would be to cause dissociation of water in the gas phase either directly or sensitized by metastable argon atoms in the $^3P_1$ state. The energy of the metastable argon atoms could also be transferred to the water in the solid[15] through the intense band systems existing in solid noble gases[16]. The necessity for the discharge to be weak or sporadic is indicated by the results[5] of an experiment in which the products deposited at 4° K from a leak-tester discharged mixture of 0·5 per cent H_2O in argon showed no infra-red absorption attributable to hydrogen–oxygen species.

The observed frequencies of the matrix-trapped hydroxyl radicals are in all cases blue-shifted, being 3–4 cm^{-1} for H_2O and D_2O in argon[3–5] and 26 and 48 cm^{-1} for H_2O and D_2O in the argon–hydrogen mixed matrix[7]. The shift in the former case is small, and comparable to the shifts experienced with water itself[3,5]. In the latter case, the presence of about 50 per cent hydrogen in the solid causes the lattice sites to be smaller, thereby augmenting repulsive interactions of the trapped radical and hence tending to increase its stretching frequency, again in general concord with spectra of both water[3,5] and formaldehyde[17] bond-stretching fundamentals for these substances in noble gas matrices.

By analogy with HCl in argon at 4° K (ref. 18), one might expect to observe additional lines due to some reorientation process of ·OH, particularly in the argon matrix. However, no absorptions near 3,611 cm^{-1} or 2,655 cm^{-1}, the expected positions of $R(0)$ for H_2O and D_2O, have been observed, despite the intense absorptions at the Q-branch positions. That the observed matrix absorptions are blue-shifted is again relevant in that rotation of a solute molecule is unlikely if matrix conditions cause the molecule to be compressed.

Finally, the presence of the paramagnetic hydroxyl radicals in solid argon matrices also containing water provides a simpler mechanism for ortho-para nuclear spin conversion than the postulated oxygen impurity[3,4] certainly present (unless deliberately added) in a much smaller concentration than hydroxyl.

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Hydrogen-bonding Patterns and Ice Nucleation

INVESTIGATIONS of hydrogen-bonding patterns of organic crystal surfaces in these laboratories have shown that the ability to nucleate ice can be predicted reliably if the complete crystallographic structure is known. Both 'parametric' fit and the density of hydrogen-bonding groups are essential factors conferring nucleative ability, and it is on these principles (which will form the subject of a more detailed presentation in the near future) that a new organic highly effective nucleating material has been forecast for the first time.

A consideration of the crystal structure[1] of the α-isomer of 1 : 3 : 5-*cyclo*hexanetriol dihydrate (α-phloroglucitol dihydrate) led us to expect high nucleative power, which was confirmed by cloud chamber tests. Counts of up to 10^{11} nuclei/g were obtained at a temperature of −12° C, although owing to the high solubility of the compound tests could not be run above −8° C. In comparison, the most active organic nucleators known at the present time (phloroglucinol dihydrate and α-phenazine) produce 10^{11}–10^{12} nuclei/g under identical conditions, whereas silver iodide is slightly more active still.

Each oxygen atom in the 111 plane of the rhombohedral α-phloroglucitol dihydrate corresponds to an ice oxygen, the disregistry being 4·8 per cent. The density of oxygen groups is very close to that of ice and about 50 per cent greater than on the 100 plane of phloroglucinol dihydrate. α-Phloroglucitol, being a polyalcohol, shows a much higher solubility in water and it is also more difficult to sublime without decomposition. It does not, however, possess a sweet taste like phloroglucinol as stated in Beilstein.

The compound was prepared by the method of Wislicenus[2] in which phloroglucinol is hydrogenated with sodium amalgam. After removing all traces of phloroglucinol as well as the β-isomer of phloroglucitol, the correct m.p. of 184° C was obtained.

Because of its alicyclic nature and polyalcoholic character, α-phloroglucitol is probably non-toxic.

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High-pressure Polymorph of Zirconium Pyrophosphate

ZIRCONIUM pyrophosphate is one of a group of isostructural compounds of the Type $X^{+4}\ P_2O_7$ where X may be zirconium, hafnium, titanium, silicon, tin or uranium[1,2]. It is cubic (Type $K6_1$) with zirconium octahedrally, and phosphorus tetrahedrally, co-ordinated with oxygen. The structure may be compared with that of NaCl (Type B1) by considering the zirconium ions and P_2O_7 groups to be at sodium and chlorine sites, respectively.

Reagent grade ZrP_2O_7 containing ~12 per cent of water, which corresponds to $ZrP_2O_7 \cdot 2H_2O$, was obtained from Bios Laboratories, Inc., New York. This material was heated in air at 700° C for 4 h, and its identity as anhydrous cubic ZrP_2O_7 was confirmed by X-ray powder diffraction ($a_0 = 8{\cdot}26$ Å in agreement with the observation by Harrison *et al.*[3]). The anhydrous ZrP_2O_7 was subjected to pressures between 25 and 100 kbars and temperatures of 750° and 1,000° C in a 'belt'[4,5] and a 'girdle' apparatus[6]. The samples were quenched under pressure and then examined optically and by X-ray powder methods. The conditions and results of these experiments are given in Table 1.

One hundred per cent conversion to a new quenchable high-pressure polymorph of ZrP_2O_7 was effected at 55 and 100 kbars. The new phase is colourless under the microscope and white in powder form. It is uniaxial positive with $\varepsilon = 1{\cdot}773$, $\omega = 1{\cdot}755$, both $\pm 0{\cdot}003$, and $\varepsilon - \omega = 0{\cdot}018$. The crystallites have an average size of about 20μ, but a few crystals as large as 40μ were observed. The crystallites are anhedral so there is no direct indication whether this phase is tetragonal or hexagonal. X-ray powder diffraction data are given in Table 2. The density of the new phase is $3{\cdot}62 \pm 0{\cdot}02$ as determined by float-sink methods using Clerici's solution. The high-pressure phase, therefore, is 15·7 per cent denser than the cubic form, which has a density of 3·13, calculated from its lattice parameter.

The high-pressure phase inverts to the cubic form within 3 h when heated at ambient pressure in air at 1,000° C.

Table 1. HIGH-PRESSURE HIGH-TEMPERATURE EXPERIMENTS ON ZrP_2O_7

Pressure (kbars)	Temperature (°C)	Time (h)	Product
25	1,000	18	Cubic ZrP_2O_7
35	750	19	Cubic ZrP_2O_7
55	750	20	High-pressure polymorph
100	1,000	3	High-pressure polymorph

The 100-kbar run was made in a 'girdle' apparatus; the others were made in a 'belt' apparatus

Table 2. X-RAY POWDER DIFFRACTION DATA FOR HIGH-PRESSURE POLYMORPH OF ZIRCONIUM PYROPHOSPHATE
(Mn-filtered FeKα_1 radiation, $\lambda = 1{\cdot}93597$ Å, 57·3 mm camera)

d(Å)	I	d(Å)	I
4·76	10	1·302	2
3·72	50	1·281	5
3·465	2	1·272	1
3·316	15	1·253	15
3·207	2	1·227	25
3·155	60	1·214	10
2·948	40	1·167	4
2·597	3	1·156	20
2·445	3	1·135	2
2·411	4	1·115	20
2·319	6	1·099	2
2·160	3	1·087	20
2·041	100	1·074$_B$	2
1·873	10	1·061	30
1·806	1	1·055	1
1·748	30	1·054	1
1·632	1	1·052	10
1·615	50	1·034	5
1·586	5	1·029	20
1·521	1	1·022	70
1·485	5	1·005	1
1·462	50	0·9954	2
1·449	1	0·9928	7
1·420	30	0·9862	30
1·392	1	0·9817	3
1·382	15	0·9796	2
1·353	1	0·9730	10
1·328	3		

Table 3. MOLAR REFRACTION AND SPECIFIC REFRACTIVITY OF THE CUBIC AND HIGH-PRESSURE POLYMORPHS OF ZrP_2O_7

Polymorph	Mean refractive index (n)	Density (d) g/c.c.	Molar refraction $\left(\frac{n^2-1}{n^2+2}\cdot\frac{M}{d}\right)$	Specific refractivity $\left(\frac{n-1}{d}\right)$
Cubic	1·661	3·13	31·31	0·211
High-pressure	1·761*	3·62	30·17	0·210

* $\sqrt[3]{\omega^2\varepsilon}$

M = molecular weight

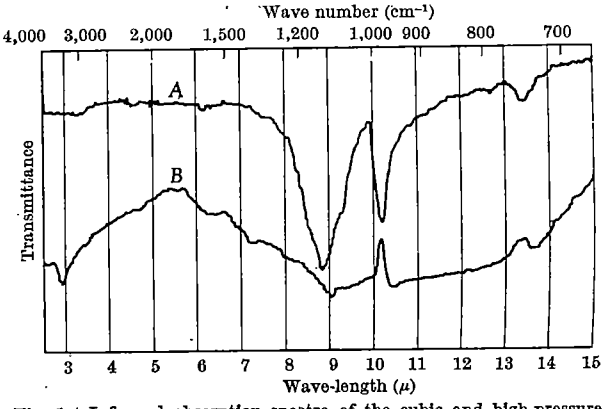


Fig. 1. Infra-red absorption spectra of the cubic and high-pressure polymorphs of ZrP_2O_7. Spectra obtained with Perkin–Elmer Infracord recording spectrophotometer using the KBr disk technique. *A*, cubic form preheated at 700° C; *B*, high-pressure form prepared from (*A*) at 55 kbars and 750° C

It persists metastably at ambient pressure, however, when heated in air at 600° C for 65 h.

Recent studies of polymorphic pairs of oxides related by re-constructive transformations of first co-ordination have shown that the effect of an increase in cation co-ordination from 4 to 6 is to decrease the molar refraction by ~12 per cent and to shift the main infra-red absorption band ~23 per cent in the direction of increasing wave-length[7–9]. Applying these principles to the polymorphs of ZrP_2O_7, it is deduced from the small difference in molar refraction and the slight displacement toward longer wave-lengths of the main infra-red absorption bands at 8·8, 10·2 and 13·4μ (cf. Table 3 and Fig. 1) that there is no difference in the primary cation co-ordination of these polymorphs and that the increase in density is due to closer packing of the co-ordination polyhedra. The two structures probably differ in secondary co-ordination and may thus be related by a reconstructive transformation of higher co-ordination[10].

There is some indication that the refractive index of the cubic form increases with pressure; reagent preheated to 400° C has $n = 1{\cdot}661 \pm 0{\cdot}002$ (Na light) whereas the cubic form pressurized at 35k bars and 750° C has $n = 1{\cdot}666 \pm 0{\cdot}002$ (Na light). The infra-red spectrum of the cubic form is in agreement with that given by Steger and Leukroth[11].

It will be interesting to subject the other known members of this isostructural group to high pressures and temperatures to determine whether a group of pressure-dependent polymorphs isostructural with the high-pressure polymorph of ZrP_2O_7 can be synthesized.

This work was supported by U.S. Air Force Systems Command through contract *AF*33(616)–7471.

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BIOCHEMISTRY

Nucleoprotein Synthesis in the New-born Mouse

The proceedings of the first World Conference on Histone Biology and Chemistry were summarized in a recent article by Bonner and Ts'o[1]. Part of the reported discussion dealt with the probable relationship between DNA replication and histone synthesis. It was pointed out that 5-fluorodeoxyuridine blocks DNA synthesis but allows the synthesis of histone to proceed in an essentially normal manner. This evidence was cited to support the view that DNA replication and histone synthesis are not necessarily coupled although they usually accompany one another. Similar conclusions have been reached by Plaut[2], who investigated the pattern of nucleoprotein synthesis in *Drosophila* polytene chromosomes by means of the pulse-labelling technique. He found that the labelling patterns resulting from brief exposures to tritiated precursors of DNA (H^3 thymidine) and protein (H^3 lysine, H^3 histidine) were distinctly different. In summary, DNA labelling was often restricted to certain sites along the chromosome, while protein labelling was found to be more or less continuous. The present report, based on the results of cytophotometric investigations, furnishes further evidence in support of the view that DNA replication and histone synthesis may not be coupled.

Table 1

	DNA–Feulgen	Histone–fast green
New-born liver	3·54 ± 0·19 (49)	1·96 ± 0·07 (52) 4·64 ± 0·17 (13)
New-born kidney	3·30 ± 0·04 (49)	1·93 ± 0·07 (34) 3·50 (6)

In the mouse, the onset of parturition appears to be accompanied by a virtual cessation of mitosis in fœtal tissues; significant restoration of mitotic activity does not occur until after suckling has begun[3]. Cytophotometric determinations of nuclear contents of DNA and histone were carried out as part of an attempt to elucidate the probable nature of this 'mitotic arrest'. Male animals were killed a few hours after birth and pieces of kidney and liver were fixed in 10 per cent neutral formalin. Following fixation and washing, the tissue samples were dehydrated, embedded, sectioned, and mounted together on the same slides (sections of liver from an adult male were used for comparative purposes). Slides were stained either by the Feulgen reaction for DNA[4], or by the alkaline fast green procedure for histone[5]. The cytophotometric procedures have been described previously[6,7]. The results are summarized in Table 1. The mean DNA–Feulgen and histone–fast green contents are given in arbitrary units (sample size is indicated in parentheses). The Feulgen data suggest the existence of only one nuclear class. The mean value is characteristic of normal diploid cells in the G_1 period of interphase. The fast green measurements, on the other hand, are indicative of a mixed population with some nuclei apparently containing the post-synthetic level of histone expected of cells in the G_2 period. From these results it may be concluded that synthesis of histone can precede DNA replication.

The results derived from three rather different analytical approaches are therefore in agreement and strongly suggest that DNA replication and histone synthesis are not invariably coupled.

This work was supported in part by U.S. Public Health Services research grant *CA*–05591 from the National Cancer Institute.

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Possible Cause of Electrophoretic and Chromatographic Heterogeneity of Pituitary Hormones

There is evidence that pituitary hormones can exist in more than one biologically active form. Electrophoresis[1] or chromatography[2] of thyrotrophic hormone has been found to yield a number of active fractions. Several components with corticotrophic potency were obtained from adrenocorticotrophic hormone (ACTH) from various sources[3–5], while fractionation of ovine prolactin also revealed various active forms of the hormone[6,7]. Similar results have been obtained with preparations of sheep[8,9] and human[10] interstitial cell-stimulating hormone (ICSH), as well as with alkali-treated

growth hormone from beef pituitary glands[11]. A comparative study of the electrophoretic behaviour of pituitary hormones has been published by Ferguson and Wallace[12], who reported that preparations of pituitary hormones were invariably electrophoretically impure and that more than seventy components could be detected in some crude extracts of pituitary tissue by electrophoresis on starch gel.

The appearance of more than one active form of the hormone may in some cases be due to slight differences in amide content[4,13] or in amino-acid composition and sequence between the protein molecules. There are also indications[14,15] that, due to proteolytic activity in pituitary extracts, a partial degradation of polypeptide hormones may sometimes occur, leading to the appearance of multiple components. However, the additional fractions obtained in that case are usually less active than the undegraded material[15].

It is difficult to attribute the high degree of heterogeneity of pituitary hormones, as shown by electrophoresis or chromatography, solely to differences in primary structure. Recently, indications have been found that the presence of several biologically active components in a hormone preparation was due to aggregation effects[16,17]. It was shown that the monomer of prolactin had a different electrophoretic mobility from that of aggregated forms of the hormone and that these forms were also chromatographically distinct[16].

In view of these findings we propose that association phenomena may, in general, contribute to a large extent to the charge multiplicity of pituitary hormones. We also suggest that the term 'isohormones' be used to designate the different active forms of a certain hormone.

As aggregation of prolactin occurs when solutions of the hormone are treated with alkali[17], it seemed of interest to see whether disulphide-interchange reactions were involved in the polymerization. We have therefore investigated the effect of reduction on the molecular weight of bovine prolactin[18]. Investigations in the ultracentrifuge, by gel filtration and gel electrophoresis, showed that reduction and alkylation[19] of the hormone led to polymerization, and not to depolymerization, of the protein. The finding that reduced prolactin can also exist as a polymer indicates that disulphide-interchange is not necessarily involved in the association of the protein molecules. These results will be reported more fully elsewhere.

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Use of Mutants to study the Electron Transport Pathway of Nitrate Reductase

NITRATE-INDUCIBLE TPNH (reduced triphosphopyridine nucleotide) cytochrome *c* reductase is believed to participate in nitrate reduction for the following reasons: (1) Sorger[1] and Kinsky and McElroy[2] showed that it is induced and repressed simultaneously with nitrate reductase by the same specific inducers and repressors. (2) Kinsky and McElroy[2] observed that cytochrome *c* competes with nitrate in purified preparations of nitrate reductase. (3) These investigators also found that inhibitors of TPNH cytochrome *c* reductase inhibit nitrate reductase. (4) They further observed that the ratio of TPNH cytochrome *c* reductase to nitrate reductase remains constant throughout a fifty to seventy-fold purification by two procedures. (5) Sorger[1,3] and Pateman *et al.*[4] found that mutants at a single locus, lacking nitrate reductase, also lack nitrate-inducible TPNH cytochrome *c* reductase. (6) Sorger[5] noted that the ratio of TPNH cytochrome *c* reductase to nitrate reductase remains constant during heat inactivation (at 31·1° C and 42·3° C) of crude extracts made from induced mycelium.

One class of mutants, represented by *nit*-1 in *Neurospora*, and by *ni*-3 in *Aspergillus*, lacks nitrate reductase but retains nitrate-inducible TPNH cytochrome *c* reductase[1,3]. These mutants can be used in elucidating the mechanism of action and the organization of nitrate reductase because they have an incomplete enzyme, capable of carrying out only the first steps in the electron transport sequence from TPNH to nitrate. The experiments to be described are part of an attempt to elucidate the nature of the physical organization of the nitrate reductase electron transport pathway.

If TPNH cytochrome *c* reductase is an enzyme physically independent of the rest of the electron transport chain from TPNH to nitrate, one would expect *nit*-1 of *Neurospora* to have an inducible TPNH cytochrome *c* reductase identical to that of wild type. The reason for this is that *nit*-1 is blocked beyond cytochrome *c* reduction in the electron transport sequence from TPNH to nitrate; therefore, a mutation affecting an enzyme which catalyses a reaction beyond this step in the sequence should not affect TPNH cytochrome *c* reductase structurally. If, on the other hand, the entire electron transport sequence is carried out by a single protein or by an aggregate of enzymes, TPNH cytochrome *c* reductase from *nit*-1 might be expected to be qualitatively different from the corresponding enzyme of wild type. If only one enzyme is involved, a mutation would be expected to alter its properties. If an aggregate carries out nitrate reduction, a mutation affecting an enzyme in the aggregate might be expected to alter the properties of the whole aggregate.

Extracts from induced wild type and induced *nit*-1 mycelium were prepared by freezing the mycelial pads in liquid nitrogen and grinding the frozen material with chilled powdered glass in an ice-cold mortar and pestle. Enzyme in the paste was dissolved in ten volumes of 0·1 M phosphate buffer, *p*H 7·0 at 0°–4° C, and afterwards centrifuged at 20,000*g* in the cold to remove cell debris. Aliquots (0·5 ml.) of each extract were pipetted into test tubes in an ice bath and subsequently immersed in a constant-temperature bath set at 41·0° or at 30·5° C. At desired time intervals, samples were removed and stored on ice until the experiment was concluded. At this point, the samples were assayed for TPNH cytochrome *c* reductase activity by the method of Kinsky and McElroy[2].

The results in Table 1 show that *nit*-1 and wild-type TPNH cytochrome *c* reductase differ in their heat sensitivity at 41·0° and at 30·5° C.

If nitrate reductase is an aggregate, rather than a single protein, sucrose density gradient centrifugation might cause the separation of the components of the aggregate.

Table 1. Heat Inactivation of Wild Type and of *nit*-1 Nitrate-inducible TPNH Cytochrome *c* Reductase

Experiment No.	Minutes in water bath	TPNH cytochrome *c* reductase activity remaining: Wild type	*nit*-1	Wild type / *Nit*-1	$\frac{\chi^2}{(o-e)^2/e^*}$
1 (41·0 ± 0·5° C)	0	29·0	13·0	2·2	0·00/2·2
	10	7·5	2·2	3·4	1·21/2·2
	15	5·0	1·2	4·1	3·61/2·2
	20	5·0	1·0	5·0	7·84/2·2
	30	3·5	0·6	5·8	12·96/2·2
				Sum χ^2 (4 d.f.)	11·7
				0·02 > *P*† > 0·01	
2 (30·5 ± 0·5° C)	0	47	27	1·7	0·00/1·7
	60	34	23	1·6	0·01/1·7
	120	29	11	2·6	1·21/1·7
	180	33	7	4·7	9·00/1·7
	240	26	4	6·0	18·49/1·7
	300	27	3·5	7·7	36·00/1·7
	360	17	3	5·8	16·81/1·7
	720	9	1·5	7·3	31·36/1·7
				Sum χ^2 (7 d.f.)	66·4
				P < 0·01	

*e is the initial ratio of wild type to *nit*-1 TPNH cytochrome *c* reductase activity. o is the observed ratio of these two activities.

†P is the probability that the differences between e and o are due to random variation.

One unit of TPNH cytochrome *c* reductase activity is defined as a change of 0·01 *O.D.* units per min at 550 mμ at 24° ± 2° C.

Table 2. *s* Values of TPNH Cytochrome *c* Reductase and Nitrate Reductase of Fresh and of Frozen and Thawed Preparations from Wild Type and from *nit*-1 Mycelium

Extract	Treatment	TPNH cytochrome *c* reductase Peak 1 Activity	*s*	Peak 2 Activity	*s*	Nitrate reductase Activity	*s*
Wild type	fresh	9	10·6	23	6·8	20	6·8
,, ,,	frozen and thawed	11	10·6	10	4·5	0	—
nit-1	fresh	10	10·6	34	4·5	0	—
,, ,,	frozen and thawed	11	10·6	77	4·5	0	—

For the definition of units of activity of TPNH cytochrome *c* reductase see Table 1.

One unit of nitrate reductase activity is defined as a TPNH and nitrate-dependent production of 10 mμM of nitrite in 15 min at room temperature.

s values were calculated by the method of Martin and Ames[5] using catalase as the standard enzyme:

$$\frac{s \text{ of } x}{s \text{ of reference}} = \frac{\text{Distance of } x \text{ from meniscus}}{\text{Distance of reference from meniscus}}$$

Nit-1-induced and wild-type-induced mycelia were extracted as described here and either used within 6 h of extraction or frozen in liquid nitrogen and stored for two to three weeks at −20° C before use. Sucrose gradients (5 to 20 per cent w/v) were made by the procedure of Martin and Ames[5] and centrifuged at 38,000 r.p.m. for 11 h in an *SW*39 swinging bucket head in a 'Spinco' model *L* refrigerated centrifuge. Fractions (0·16 ml.) were collected by downward displacement through a hypodermic needle; they were assayed for TPNH cytochrome *c* reductase by the method of Kinsky and McElroy[2] and for nitrate reductase by a modified version of the assay of Evans and Nason[7]. The modification was to terminate the reaction by precipitating pyridine nucleotides with barium acetate and subsequently to precipitate proteins by the addition of 95 per cent ethanol. The suspension was then centrifuged, and the clear supernatant assayed colorimetrically as usual, with sulphanilamide and N-1 naphthyl ethylene diamine. The results shown in Table 2 indicate that fresh wild-type-induced extract has a heavier TPNH cytochrome *c* reductase than frozen and thawed preparations of the same material. Also, unlike the frozen and thawed preparation, the fresh wild-type material had a nitrate reductase peak. Both frozen and thawed and fresh extracts of wild-type-induced mycelium had nitrate reductase activity before they were subjected to density gradient centrifugation.

The simplest interpretation of these data is that nitrate reductase is an aggregate of TPNH cytochrome *c* reductase and another polypeptide, and that freezing and thawing labilize this aggregation. The labilized aggregate therefore dissociates in the gradient. *Nit*-1 extracts, which have no nitrate reductase, have a TPNH cytochrome *c* reductase pattern in the gradient like that of frozen and thawed wild type. The simplest interpretation of this observation is that TPNH cytochrome *c* reductase in *nit*-1 is like that of wild type, but the aggregate with the mutationally altered protein (that would normally transport electrons from the branch point to cytochrome *c* reduction to nitrate) is unstable in the gradient. It therefore dissociates, even without freezing and thawing, into TPNH cytochrome *c* reductase and an enzymatically inactive protein. The relatively greater heat lability of the *nit*-1 TPNH cytochrome *c* reductase agrees with the above hypothesis.

This work was carried out in the Department of Biology, Yale University; details of the experiments will be published in full.

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Incorporation of ^{14}C-Glucose and ^{14}C-Glucose 6-phosphate into Glycogen and CO_2 by Rat Liver Slices

It has been postulated that the hexokinase reaction is not a necessary step in glycogen synthesis. This is supported by experiments in liver slices[1,2] and in the diaphragm[3], as well as in cell-free systems[4,5]. The hexokinase reaction was assumed to occur only in the degradation pathway of glucose, and a different pathway was postulated for glycogen synthesis, circumventing the hexokinase reaction[4,5].

It seemed worthwhile, therefore, to compare radioactive glucose and radioactive glucose 6-phosphate with respect to both their production of ^{14}C-CO_2 and their incorporation into glycogen in the same liver. Liver slices obtained from male rats were used. The slices were incubated in Hastings buffer solution (medium I, ref. 6), enriched with glucose-1-^{14}C or glucose-1-^{14}C 6 phosphate. At the end of the incubation period the slices were removed to determine the radioactivity of glycogen. Glycogen was isolated with 30 per cent KOH and precipitated with ethanol, and its concentration in the slices was measured at the beginning and at the end of the incubation period by the method of Montgomery[7]. ^{14}C-labelled CO_2 was trapped with 60 per cent KOH and transformed into $Ca^{14}CO_3$ to measure its radioactivity.

It can be seen in Table 1 that glucose is a better precursor of glycogen than glucose 6-phosphate, measured by the incorporation method. The glycogen concentration of the tissue at the end of the incubation period is also greater with glucose than glucose 6-phosphate, as was shown by Niemeyer and Figueroa[1]. There is evidence that glucose 6-phosphate penetrates inside the cell since inorganic phosphate is liberated from the ester (about 10 μmoles) by the slices, and glucose 6-phosphatase does not diffuse out into the medium[8]. Glucose 6-phosphate increases oxygen uptake, while additional glucose does not when incubated with rat liver slices[1]. Additional evidence is presented in this work by the observation of the production of ^{14}C-CO_2 from radioactive glucose 6-phosphate. In fact, the results showed that consistently more ^{14}C-CO_2 is produced from ^{14}C-glucose 6-phosphate as compared with ^{14}C-glucose (Table 2).

Table 1. INCORPORATION OF ^{14}C-GLUCOSE AND ^{14}C-GLUCOSE 6-PHOSPHATE INTO GLYCOGEN BY RAT LIVER SLICES

About 100 mg of liver, in slices, was incubated in 2 ml. Hastings medium. Glucose or glucose 6-phosphate concentration was 0·03 M (100,000 c.p.m. each). 95 per cent O_2–5 per cent CO_2 was used as the gas phase. The flasks were shaken in a bath for 60 min at 37·0° C.

Exp.	Initial glycogen*	Final glycogen* Without substrate	Glucose	G-6-P	Incorporation† Glucose	G-6-P
1	71	50	182	48	1,400	143
2	—	—	—	—	519	76
3	117	66	293	261	1,480	113
4	347	287	333	243	1,805	149
5	355	—	438	287	1,770	159
6	149	151	367	238	1,570	748
7	134	84	448	248	2,030	796

*Glycogen is expressed as μg/100 mg of wet tissue.
†Incorporation corresponds to counts/min incorporated in 100 mg wet tissue.

Table 2. ^{14}C-CO_2 PRODUCTION FROM ^{14}C-GLUCOSE AND ^{14}C-GLUCOSE 6-PHOSPHATE BY RAT LIVER SLICES

Experiments 2–7 refer, respectively, to the animals so numbered in Table 1. Experimental conditions as in Table 1

Exp.	Glucose*	Glucose 6-phosphate*
2	376	504
3	47	378
4	122	196
6	164	202
7	261	361
8	489	635

* Figures correspond to c.p.m. incorporated into carbon dioxide by 100 mg of wet tissue.

This is in agreement with the evidence of Di Pietro *et al.*[9], who found in a rat liver homogenate a greater production of ^{14}C-CO_2 with ^{14}C-glucose 6-phosphate than with ^{14}C-glucose using uniformly labelled hexose as well as labelled in carbon 1 or 6.

Therefore, the hexokinase reaction constitutes, without doubt, a limiting step in glucose degradation. On the other hand, since glucose forms glycogen at a higher rate than glucose 6-phosphate, the hexokinase reaction does not constitute a limiting stage in glycogen synthesis. These results, as well as those of Beloff-Chain *et al.*[3], Petrova[4], Niemeyer and Figueroa[1], and Figueroa *et al.*[2,5], may be satisfactorily explained if the hexokinase reaction is not a necessary step in glycogen synthesis.

Recently, Viñuela *et al.*[10] reported the identification in rat liver of two enzymes that phosphorylate glucose. One of them, called hexokinase, has a *Km* about 10^{-5} M for glucose, and the other one, called glucokinase, has a *Km* about 10^{-2} M. Although, in the conditions of our experiments (concentration of glucose 0·03 M), hexokinase is working at maximum velocity and glucokinase is only partially saturated, the argument presented here may be applied to both hexokinase and glucokinase.

This work was supported by grant 59–*I*–35 from the "Comisión de Ayuda a la Investigación Científica" financed by the University of Chile and the Rockefeller Foundation (grant 60038) in a joint programme. We thank Dr. H. Niemeyer for advice.

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Heterogeneity of *H* Chains of Myeloma Proteins: Susceptibility to Papain and Trypsin

PROTEOLYTIC digestion of proteins has been one of the methods used in determining protein structure[1–3]. Closely allied proteins such as γ-globulins revealed after enzyme cleavage significant differences in products. It was shown that the main fragments (*S* and *F*) of normal human 7*S* γ-globulins obtained by papain digestion are heterogeneous[4–6]. These investigations were now extended to abnormal γ-globulins (7*S* and β_2A myeloma γ-globulins) in order to compare the structural make-up of the subcomponents of their *S* and *F* fragments. During the course of the investigation an *F* fragment, extremely labile to proteolytic digestions, was found in one of the 7*S* myeloma γ-globulins studied.

All the proteins were purified by starch-block electrophoresis[7] and screened for their purity and type by immuno-electrophoresis and ultracentrifugation. Group-specific antigens of both the *L* and *H* chains were typed by Dr. H. G. Kunkel. The two β_2A myeloma globulins studied were of the polymer variety. All samples were digested by papain at *p*H 7·5 for 18 h[4] and subjected to starch-gel electrophoresis in discontinuous system of buffers[8] at *p*H 8·3 room temperature, and 3–4 V/cm/h for a total of 18 h.

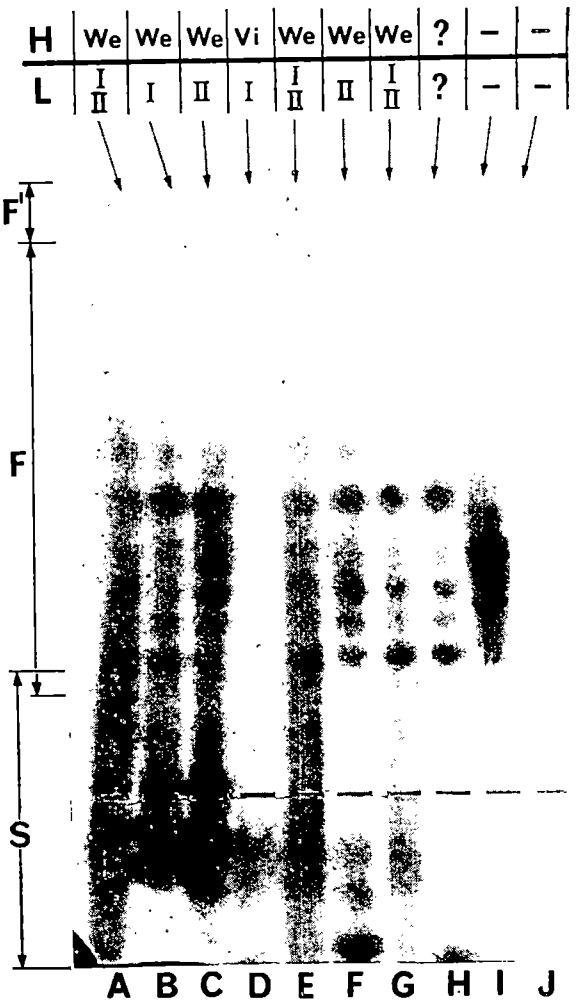


Fig. 1. Vertical starch-gel electrophoretic patterns of normal 7*S* γ-globulins, 7*S* myeloma γ-globulins and β_2A myeloma γ-globulins after papain digestion. *A*, *E*, normal 7*S* γ-globulins (Cohn Fraction II); *B*, *C*, *D*, *F*, *G* and *H*, 7*S* myeloma γ-globulins; *I*, *J*, β_2A myeloma γ-globulins, *L*, light-chain type; *H*, heavy-chain type; *F* and *F'*, fast fragments; *S*, slow fragment; (—), not typed; (?), not typable

Typical starch-gel electrophoretic patterns of normal and myeloma γ-globulins after papain digestion are shown in Fig. 1. Fig. 1*A* and *E* show typical patterns of the 7*S* γ-globulin (Cohn Fraction II). The *F* fragment is separated into 10–13 distinct sub-components. The *S* fragment of the same γ-globulin displays only a moderate heterogeneity (cf. ref. 4), in contrast to 7*S* myeloma proteins (Fig. 1*B*, *C*, *F*, *G* and *H*). Two of the myeloma proteins (Fig. 1*D* and *H*) were strikingly similar in their *S* fragment.

The patterns obtained with the β_2A myeloma globulins (Fig. 1*I* and *J*) were radically different from those of the 7*S* myeloma proteins described above. Sub-components of the *F* fragment have different mobilities and distribution with respect to those of 7*S* myelomas. In each of the β_2A myelomas tested so far, the three fast sub-components characteristic of 7*S* γ-globulin (*F'*) are missing. Although it was difficult to demonstrate the sub-components of the *S* fragment on starch-gel electrophoresis, these were shown to be present on immuno-electrophoresis.

In contrast to the multibanded nature of the *F* fragment seen in Cohn Fraction II and most myeloma proteins, a striking exception was noted with one of the 7*S* myeloma γ-globulins (Fig. 1*D*), where the *F* fragment is completely destroyed by papain. Time sequence digestion was carried out on this protein with papain[4] and trypsin[9]. Complete dissolution of the *F* fragment by papain was achieved between 2 and 4 h as shown in Fig. 2 in contrast to trypsin (Fig. 3), where digestion for 24 h was required to obtain the same result. Ultracentrifugal analysis confirmed this finding. Thus it appeared that this fragment is similar to that described recently by Takatsuki and Osserman[10]. Typing of the intact protein for the *L* and *H* chain types[11] (courtesy of Dr. Kunkel) has shown that this protein belongs to *L* chain type *I* and *H* chain type *Vi*. Grey and

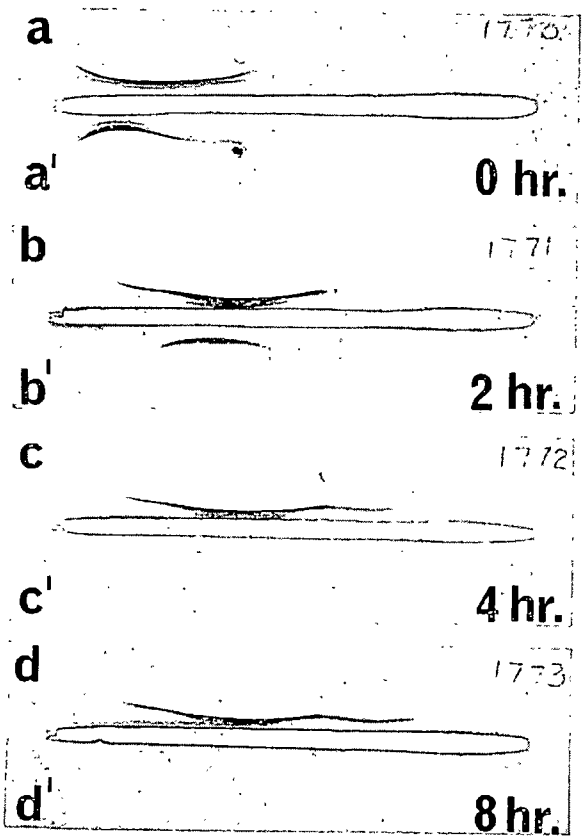


Fig. 2. Immunoelectrophoretic patterns of papain digests of 7*S* γ-globulin (Cohn Fraction II) and 7*S* myeloma γ-globulin (cf. Fig. 1*D*, Patient M. N.) carried out in time sequence. Samples removed after 0, 2, 4 and 8 h of digestion. *a*, *b*, *c*, *d*, normal 7*S* γ-globulin; a^1, b^1, c^1, d^1, myeloma protein (Patient M. N.). Specific rabbit anti-*F* antiserum was used to develop precipitin arcs

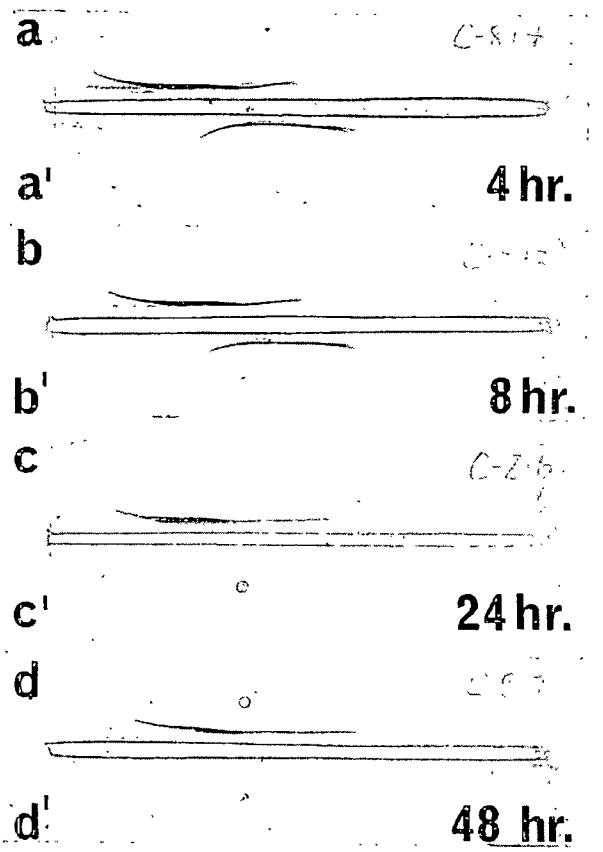


Fig. 3. Immunoelectrophoretic pattern of trypsin digests of 7*S* γ-globulin (Cohn Fraction II) and 7*S* myeloma γ-globulin (cf. Fig. 1*D*, Patient M. N.), carried out in time sequence. Samples removed after 4, 8, 24 and 48 h of digestion. *a*, *b*, *c*, *d*, normal 7*S* γ-globulin; a^1, b^1, c^1 and d^1, myeloma protein (Patient M. N.). Rabbit antiserum containing anti-*F* and anti-*S* antibodies was used to develop the precipitin arcs

Kunkel[11] have shown that the myelomas of the *Vi* type they used have papain-stable *F* fragments. Whether the availability and/or accessibility of the critical peptide bond(s) to enzyme digestion depend on the primary amino-acid sequence or on the conformation of the chains with respect to each other is under investigation. It is well known that a single amino-acid substitution may induce profound configurational changes in the protein structure. One may also anticipate an increase in the number of basic amino-acids in this protein since the intact protein migrates to the cathode in the starch gel. In contrast, the myeloma γ-globulin shown in Fig. 1*H* also migrates backwards in similar fashion, yet its *F* fragment (*H* chain) is stable to papain digestion, even though its *S* fragment appears electrophoretically to be very much the same as the *S* fragment of the papain-labile protein. It is of interest to mention here that it was impossible to classify this protein (Fig. 1*H*) according to any known immunological *H* or *L* chain type (personal communications by H. G. Kunkel).

In the light of the current reports that *H* chains of the γ-globulins appear to be markedly heterogeneous, specific antigenic determinants and properties residing on these chains now include: (*a*) *Gm* groups; (*b*) group specific *H* chain types[11–13] of which there are now four classified, *We* (γ_2b), *Vi* (γ_2c), *Ge* and γ_2a; as well as (*c*) those which are not typable; (*d*) individually specific *H* chain antigens[11]; (*e*) enzyme labile *H* chains—to papain and trypsin as shown here and to papain as demonstrated by Takatsuki and Osserman[10]. The *H* chains of both these susceptible proteins may be of the same group-specific antigenic type.

Undoubtedly new properties of the *H* chains will soon be forthcoming and permit further sub-classification of myeloma γ-globulins.

This work was supported by U.S. Public Health Service research grants *AI*-05785 and *HE*-07495 from the National Institute of Allergy and Infectious Diseases and the National Heart Institute, and in part by the Michigan Leukemia Foundation of Michigan.

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Reduction and Re-oxidation of the Disulphide Bonds of Soy Bean Trypsin Inhibitor

Several examples have recently been reported of the partial or complete recovery of the native conformation and the biological activity of a protein following the reduction of all disulphide bridges under strongly denaturing conditions, such as 8 M urea or 5 M guanidine hydrochloride. The examples include insulin[1], ribonuclease[2], lysozyme[3], and pepsinogen[4]. In each case the recovery was induced by removing the reducing agent and denaturing solvent and allowing the disulphide bridges to re-form spontaneously through re-oxidation of sulphydryl groups by atmospheric oxygen.

Since the loss of the native molecular organization is generally very extensive for reduced, denatured proteins, these results are of importance as a direct test of the hypothesis that the overall configuration of proteins is primarily determined by their amino-acid sequence[5].

Investigations in this laboratory have recently demonstrated that soy bean trypsin inhibitor is likewise capable of a major degree of recovery of its native structure after treatment of this kind. The organized structure of this protein has been shown to be rather refractory to the action of 9 M urea at neutral *p*H and 25° C[6]. Exposure to alkaline *p*H or elevated temperatures in this solvent results in a time-dependent transition to a molecular state which is much more loosely organized, as judged by changes in intrinsic viscosity, optical rotation, and rotational relaxation time[6].

Soy bean trypsin inhibitor (STI) consists of a single polypeptide chain of molecular weight 21,000 which is cross-linked by 2 disulphide bridges[7]. Treatment of 1 per cent STI (Worthington) solutions in 9 M urea, 5 per cent β-mercaptoethanol for 20–30 min at 57° resulted in quantitative reduction of cystine groups to cysteine, as determined by titration with *p*-chloromercuribenzoate[8]. The product of the combined reduction and denaturation under these conditions exists in an advanced state of molecular disorganization, as indicated by the high value of intrinsic viscosity (21·2 ml. g^{-1}), and low value of rotational relaxation time ($\rho_h^{25} = 1{\cdot}6 \times 10^{-9}$), as determined by fluorescence polarization[9]. However the non-vanishing value of the latter parameter suggests that some structural restraints may survive under this condition.

Removal of urea and mercaptoethanol by passage through a 'Sephadex *G*–25' column, eluting with 0·1 M NaOAc, *p*H 5·6, yields the reduced protein in water. This material has little or no inhibiting capacity (< 2 per cent of native) for trypsin, as determined by assay with the substrate tosyl arginine methyl ester at *p*H 7·5, 25° (ref. 10).

Air oxidation of a 0·1–0·3 g/l. solution in 0·1 M phosphate, *p*H 8·0 for 48 h at 25° results in quantitative reformation of disulphide bridges to yield a re-oxidized product with 30–40 per cent of the trypsin-inhibiting capacity of the original (Table 1). Ultracentrifugal examination of the product, after re-concentration by pervaporation, shows two components of sedimentation coefficient 4·7 and 2·2 (1 per cent STI in 0·1 M potassium chloride, 0·01 M phosphate, *p*H 7). The value for the latter component is close to that for native inhibitor.

Table 1. Properties of Reoxidized STI Preparations

Preparation	Per cent reduction	Per cent activity re-oxidized material	Per cent activity monomeric fraction*
STI–*R*–1	90	42	—
STI–*R*–3	100	43	81
STI–*R*–4	100	38	85
STI–*R*–5	100		100
STI–*R*–6	93		61

Reduction was carried out in 9 M urea, 0·1 M phosphate, *p*H 7·7, for 15–20 min at 58°. Re-oxidation was carried out in 0·1 M phosphate, *p*H 8·0 for 48 h at 25°. The concentration of STI was 0·1 to 0·3 g/l.
*Obtained by 'Sephadex *G*–100' fractionation.

The two components could be separated by fractionation on a 'Sephadex *G*–100' column. The elution pattern of re-oxidized STI showed two major components and sometimes a minor (< 10 per cent) trailing component. The leading component (*F* I) and the second elution peak (*F* II) had sedimentation coefficients of 4·7 and 2·2 respectively (1 per cent STI in 0·1 M potassium chloride, 0·01 M phosphate, *p*H 7).

Almost all the inhibitory activity of the re-oxidized material was present in fraction *F* II, the specific activity of which was 80–100 per cent of the native protein. There was some indication of a slight (< 10 per cent) activity in *F* I, but the possibility of contamination with *F* II has not been rigorously excluded.

The monomeric fraction (*F* II) probably closely resembles the native material in structure, in view of its sedimentation properties and biological activity. It would, however, be premature to equate it with the original in all structural aspects. It is clear that the process, competitive with re-formation of the native molecule, involves aggregation, possibly through formation of intermolecular cystine bridges.

It may be concluded that soy bean trypsin inhibitor may be added to the list of proteins which are capable of spontaneous re-formation of a conformation resembling the native after an extensive loss of structure.

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Esterase Activity of Pepsin

It is known that many proteolytic enzymes—trypsin, chymotrypsin, thrombin, papain and others—hydrolyse peptide, amide and ester bonds. Pepsin, however, is generally believed to hydrolyse the peptide bonds only, without exhibiting the esterase activity[1]. This conclusion is based on observations which suggest that pepsin does not hydrolyse the ester bonds in several esters of N-substituted amino-acids and peptides, but no special investigation of the esterase activity of pepsin has been published.

In view of the possible significance of esterase activity in the action of pepsin, we have examined its effect on some esters. The N-substituted aromatic amino-acid esters differing in the structure of the alcoholic component were chosen as substrates and the following were used: N-acetyl-L-tyrosine ethyl ester, L-phenylalanine benzyl ester (the corresponding N-substituted phenylalanine ester could not be used as it was insoluble in the experimental conditions), and N-acetyl-L-phenylalanyl-L-phenyllactic acid. Effect of pepsin on the N-acetyl-L-phenylalanyl-L-phenylalanine methyl ester, which could be expected to be hydrolysed, was investigated as well.

N-Acetyl-L-phenylalanyl-L-phenylalanine methyl ester (APPMe) and N-phenylalanyl-L-phenyllactic acid (APPL) were prepared by the method of mixed anhydrides from N-acetyl-L-phenylalanine and L-phenylalanine methyl ester and L-phenyllactic acid, respectively.

Twice-crystallized pepsin (Nutritional Biochemical Corporation) was used. Some experiments were performed with pepsin and pepsinogen purified on a DEAE-cellulose column[2].

The effect of pepsin on the ester substrates was investigated at *p*H 2–4 at 37° C during 20–48 h; the concentrations of substrate and enzyme were $2{\cdot}7\times10^{-3}$ M and $2{\cdot}7\times10^{-5}$ M, respectively. Because of limited solubility of APPL and APPMe, all the experiments were performed in the presence of 10–15 per cent of ethanol.

The cleavage of the ester bond was estimated by the hydroxamate reaction, by the method of Hestrin[3]. After 20-min treatment by hydroxylamine, HCl (about 4 N) was added, followed, after incubation for 2 min, by a 10 per cent solution of $FeCl_3{\cdot}6H_2O$ in 0·1 N HCl. Optical density at 540 mμ (spectrophotometer *SF*–4) was immediately measured in filtrates of these samples.

Table 1. Effect of Pepsin on Some Esters

Substrates	ATEE		APPMe		PBE		APPL	
*p*H	2	4	2	4	2	4	2	4
Hydrolysis: (per cent)	0	0	0	0	0	0	30	0

The data presented in Table 1 show that after incubation of pepsin with the N-acetyl-L-tyrosine ethyl ester (ATEE) and N-acetyl-L-phenylalanyl-L-phenylalanine methyl ester (APPMe) no decrease in the quantity of esters could be detected although the cleavage of the peptide bond occurred in the course of incubation of APPMe with pepsin. No cleavage of the ester bond was noted in experiments with phenylalanine benzyl ester (PBE). But after the incubation of N-acetyl-L-phenylalanyl-L-phenyllactic acid (APPL) with pepsin at *p*H 2 a decrease in the quantity of ester was observed. Table 1 shows that cleavage of APPL at *p*H 2 during 48 h is about 30 per cent. In control samples, where APPL was incubated without the enzyme or in the presence of pepsin previously inactivated by alkali, no decrease in the quantity of ester was recorded. The hydrolysis of APPL under the influence of pepsin was confirmed by silica-gel thin-layer chromatography in a methanol : ethylacetate (1: 5 v/v) system, and iodine treatment. The outline taken from the chromatogram (Fig. 1) shows, in the control sample, a single spot of APPL. In the sample incubated with pepsin during 48 h at *p*H 2 a second spot

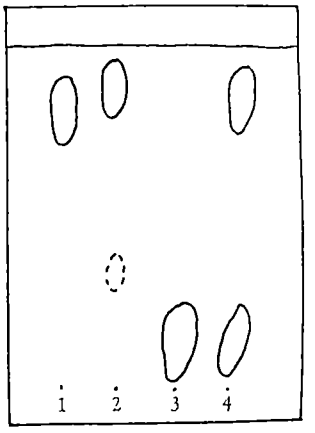


Fig. 1. Schema of silica-gel thin-layer chromatogram in methanol–ethylacetate (1 : 5, v/v) system. 1, N-acetyl-L-phenylalanine; 2, L-phenyllactic acid; 3, control sample, APPL after 48-h incubation at *p*H 2 without the enzyme; 4, experimental sample, APPL after 48-h incubation at *p*H 2 in presence of pepsin

was found. The position of this spot corresponds to N-acetyl-L-phenylalanine and L-phenyllactic acid. Neither of these compounds is resolved in the solvent system used.

Cleavage of APPL under the influence of pepsin occurs at *p*H of about 2. No hydrolysis of APPL by pepsin at *p*H > 3 could be detected (Table 1).

It could be supposed that APPL is hydrolysed by some other enzyme probably present as an impurity in the pepsin preparation used. To exclude this possibility, we have investigated the effect of various preparations of pepsin on APPL. Three preparations of pepsin purified on a DEAE-cellulose column were used. One of these preparations was free from admixtures of gelatinase. In one of the experiments, pepsinogen activated directly in the experimental samples was used. All the preparations of pepsin used hydrolysed APPL. The degree of hydrolysis of the ester was about 20–30 per cent. Hydrolysis of APPL by pepsin could also be demonstrated with several different preparations of APPL.

The data presented here show that pepsin, like many other proteolytic enzymes, possesses the ability to hydrolyse ester bonds. But the ester bond, to be hydrolysed by pepsin, has to be formed not only by a specific amino-acid residue but also by a definite alcoholic component.

The data obtained are of interest in relation to the mechanism of pepsin action. They suggest that CO-activation takes place during the action of pepsin. A similar conclusion may be drawn from the published data on the isotope exchange of oxygen in carboxyl groups of some N-substituted amino-acids occurring under the influence of pepsin in H_2O^{18} (refs. 4 and 5). On the other hand, it is known that pepsin catalyses transpeptidation of the 'amino transfer' type[6,7]. In view of these observations, it may be supposed that the cleavage of peptide bonds by pepsin includes a synchronous 'attack' on both CO- and NH-groups.

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Oxidation-reduction Potentials and Capillary Activities of Humic Acids

APART from the effects which humic acids have on the physical properties of the soil, such as its structure, permeability and water retention, and through which they influence the growth of plants, it has been shown that humic acids also have a directly stimulating effect on plant growth[1–3]. In an attempt to trace the cause of this stimulation, Flaig and Otto[4] investigated the influence of quinones and other oxidation-reduction systems on the growth of plant roots, and found in many instances a favourable effect. Flaig and Beutelspacher[5] found that in an acid environment and in the presence of nitrogen the rH value (redox potential) of humic acids decreased. Proper E_0 values of humic acids, however, have not as yet been published and it was therefore considered to be of interest to study the oxidation-reduction humic acid system in greater detail.

We found that humic acids could be reduced with hydrogen in the presence of colloidal palladium, prepared as described by Gatterman[6]. After reduction of the acids and subsequent removal of the reductant, the reduced acids were titrated in a phosphate buffer at pH 7·0 in a nitrogen atmosphere, with potassium ferricyanide as oxidant. The formal oxidation-reduction potential of the humic acid system was determined from the titration curve. From Fig. 1 it follows that the redox potentials at pH 7·0 of humic acids extracted from the core samples of a tropical sphagnum peat deposit 17 m deep (for further data see Visser[7]) decrease with progressive humification of the peat samples. The mean values of the normal potentials appear to lie between +0·32 and +0·38 V. In view of these oxidation-reduction properties it also seems possible that apart from their influence on plant growth, which can probably be attributed to an influence on plant respiration, the relatively stable humic acids are playing a part in the oxidation process of soil organic matter.

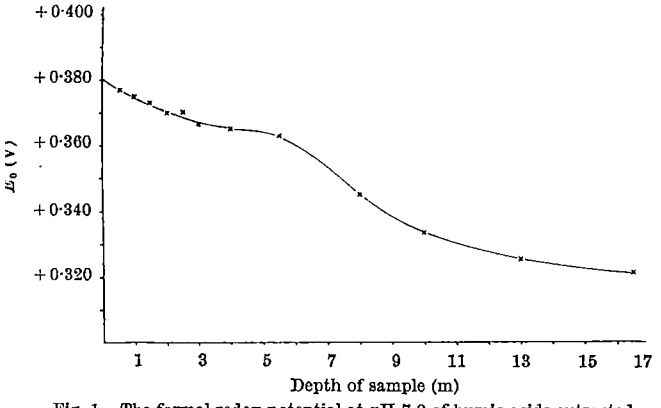


Fig. 1. The formal redox potential at pH 7·0 of humic acids extracted from a tropical sphagnum peat deposit

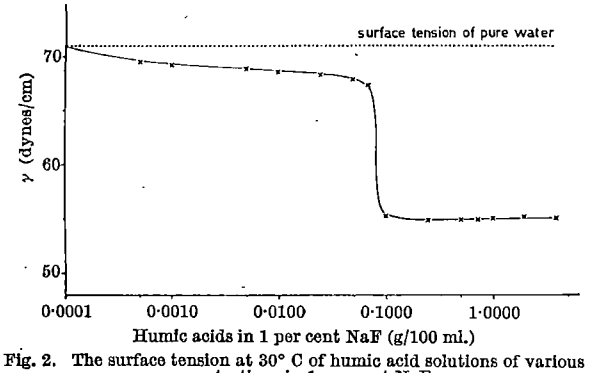


Fig. 2. The surface tension at 30° C of humic acid solutions of various concentrations in 1 per cent NaF

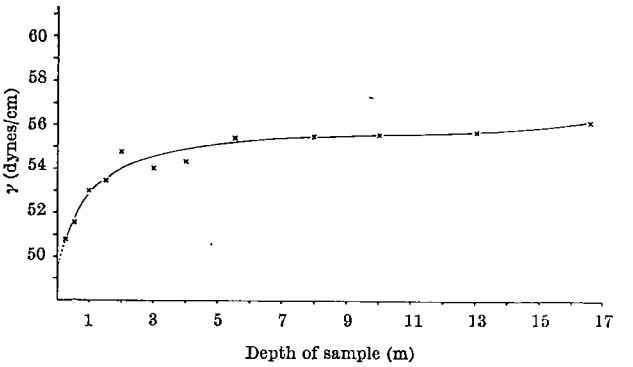


Fig. 3. The surface tension at 30° C of 0·2 per cent solutions in 1 per cent NaF of humic acids extracted from a tropical sphagnum peat deposit

Apart from their effects on plant growth, humic acids are probably also capable of raising the permeability of the cell-protoplasm, which may explain the increased ionic uptakes described by Blagowestschenski and Prosorowskoja[8] and by Chaminade and Blanchet[9]. For this reason the influence of these acids on the surface tension of water was more closely investigated.

For the measurement of the surface tension of humic acid solutions the acids were dissolved in 1 per cent neutral sodium fluoride. By using Morgan's apparatus[10] (falling drop method), kept in a thermostatically controlled bath at 30° ± 0·001° C, it was shown that humic acids behave like typical capillary active substances (Fig. 2). From Fig. 3 it can be deduced that the depression of the surface tension is markedly lower in samples nearer the deeper end of the core than in those nearer the top end, and the capillary activity of humic acids is thus shown to decrease with progressive humification.

Because of their surface-active properties, and of their abundance in soils and in some natural waters, it seems very probable that humic or pre-humic substances contribute to the foam often observed on swamp waters, and, because of their capillary properties, will contribute to the pF value of soils.

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PHYSIOLOGY

A Non-dialysable, Heat-stabile, Hypertensive Substance in the Serum of Rats

INCUBATION of rat serum at 37° C, without any digestion by proteolytic enzymes or change of the pH, effects the liberation of a highly pressure-active substance. This hypertensive effect appears after an incubation of 5 h and reaches its maximum after 15 h at 37° C (Fig. 1*A*). The blood pressure of rats nephrectomized 24–48 h before or after application of a ganglionic blocking agent or infusion of hypotonic glucose is controlled. 0·25 ml. of incubated serum given intravenously causes a rise of the blood pressure of about 35 mm mercury; this equals, in terms of pressor activity, 0·05 μg of 'Hypertensin' (CIBA). Infusion of a hypertonic sodium solution diminishes the

hypertensive effect both of the incubated serum and of synthetic angiotensin (hypertensin (CIBA)). Incubation of serum during a period longer than 40 h slowly reduces the hypertensive activity of incubated serum. The hypertensive substance is not dialysable against sodium chloride. Ultrafiltration across a cellulose membrane is not possible (Fig. 1*B*). The pressure-active principle is found at 40–60 per cent saturation with ammonium sulphate. Boiling of this fraction at *p*H 1 for 10 min is sustained without any reduction in hypertensive effect; at *p*H 12 the hypertensive principle is destroyed.

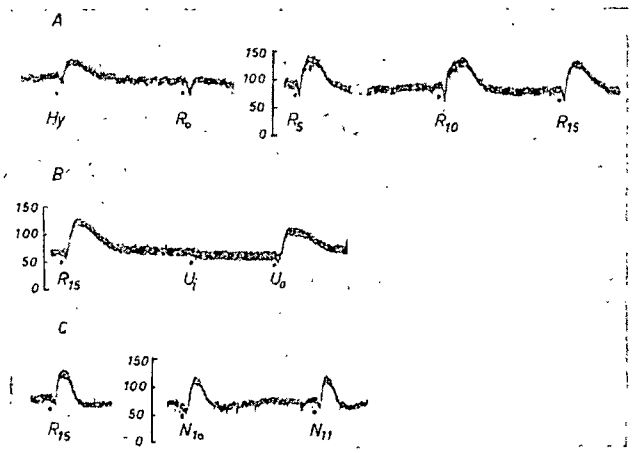


Fig. 1. *A*, Changes in arterial blood pressure. *Hy*, 0·05 μg of 'Hypertensin' (CIBA); R_0 native rat serum; R_5 rat serum, 5 h of incubation at 37° C; R_{10} rat serum, 10 h of incubation at 37° C; R_{15} rat serum, 15 h of incubation at 37° C. *B*, U_1 ultra-filtrated serum fraction of R_{15}; U_0 non-ultra-filtrable serum fraction of R_{15}. *C*, N_{10} serum of a rat 24 h after nephrectomy, incubation for 15 h at 37° C; N_{11} serum of a rat 24 h after nephrectomy, incubation for 15 h at 37° C. Volume of each single dose, 0·25 ml.; interval between injections, 5 min

In contrast to renin, which disappears from the serum within 1 h after nephrectomy[1], serum of nephrectomized rats shows the same hypertensive effect after incubation at 37° C (Fig. 1*C*).

Analysis of these results indicates that the pressure-raising activity of incubated rat serum may be caused by the action of two substances: (1) one similar to the known hypertensive polypeptides such as angiotensin, pepsitensin or anephrotensin linked to a protein or (2) a free, unknown hypertensive hormone.

We have excluded the possibility that the active principle produced by incubation at 37° C was bradykinin. This vasodepressor substance induces hypertensive reactions in certain circumstances[2], but acts as a depressor under the conditions described here.

Anephrotensin appears in rat serum after incubation at 38° C and *p*H 4 and is quickly destroyed by blood at normal *p*H. The hypertensive principle of incubated rat serum is not influenced by blood or native serum *in vitro*. Furthermore, the yield from nephrectomized rat serum is, in contrast to our results, three times greater than obtained from intact rat serum[2].

The mild treatment of native serum by incubation without any digestion or any change of the *p*H indicates that this pressure-active principle, which also exists in human and pig serum, may be involved in some physiologically important mechanism.

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Achromotricia in Iron-deficient Rats

In the course of an examination of iron deficiency in rats, we noticed that the deficient animals became grey. There was variation among individuals in the time of onset and the severity of greying; but it was apparent in all test animals compared to controls (Fig. 1). Usually a change in colour of fur could be seen about four weeks after weanling animals had been placed on the deficient diet, and by eight weeks greying was pronounced.

Male weanling rats of the Long–Evans strain from the departmental colony were used; they were either all black or black-hooded. Animals were housed in individual galvanized iron cages equipped with bottles of distilled water. The iron-poor diet, fed *ad lib.*, was whole dry milk to which was added 10 mg $CuSO_4.5H_2O$, 250 mg $MnSO_4$, 1 mg KIO_3 and 1·8 g choline bitartrate per kg. The control diet contained in addition 300 mg $FeSO_4.7H_2O$ per kg and was given to both pair-fed and *ad lib.* control groups. Analysis of the mixed food indicated that the deficient diet contained 6 p.p.m. iron, compared to 66 p.p.m. in the supplemented diet. Three times weekly, the animals were given 2 ml. of a water-soluble vitamin mixture (84 mg thiamine hydrochloride, 126 mg riboflavin, 945 mg nicotinamide, 630 mg calcium pantothenate, 147 mg pyridoxine·hydrochloride, 21 mg folic acid, 21 mg biotin, 0·63 mg vitamin B_{12} and 21 mg menadione dissolved in 1,500 ml. of 5 per cent ethanol) to which was added 3 drops of an oil-soluble vitamin mixture (2 g vitamin A distillate containing 500,000 I.U./g, 250 mg irradiated ergosterol containing 400,000 I.U./g, and 5 g α-tocopherol in 1 kg of cottonseed oil).

The hair of young deficient rats was softer than that of controls, but was matted and harsh to the touch in older animals; some members of the latter group had red scaly deposits on the skin, particularly the back. The deficient animals showed the classical signs of pearly white incisor teeth, compared to brown-tinged ones in controls, and their paws were white in contrast to the pink paws of the controls. After eight weeks on the deficient diet the animals had blood hæmoglobin levels of about 3 g/100 ml. compared with about 14 g/100 ml. in controls.

The metal commonly associated with achromotricia is copper rather than iron[1]; however, a so-called 'cotton fur' effect noted in mink fed a fish diet was afterwards attributed to lack of iron[2]. A depigmentation from red to white in feathers of iron-deficient chickens has been reported[3]. We have found no reports of greying in iron-deficient rats which received adequate copper. Perhaps this is due mainly to the fact that most investigators have used albino animals.

An explanation for the greying phenomenon is not obvious. The results of a very limited number of assays



Fig. 1. Greying of fur in an iron-deficient rat. Both animals had been on the experimental diet for seven weeks. Top, iron deficient; bottom, iron supplemented

showed that there was more iron in hair from deficient animals (64 μg/g, mixed sample from two animals) than in that from controls (34, 23 and 22 μg/g, individual samples).

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Ovarian Ascorbic Acid Depleting Activity in Human Brain Extracts

IT has been established that the hypothalamus controls the secretion of luteinizing hormone (LH) of the adenohypophysis through a luteinizing hormone releasing factor (LH–RF)[1,2]. This substance occurs only in the stalk median eminence of the hypothalamus[3,4] and, according to the literature, not in other brain tissue. Johnson[5] has shown that the whole brain, the hypothalamus excluded, also contains a substance displaying characteristics proper to LH–RF.

The lack of information on human material led us to carry out an investigation using extracts from different regions of human brains obtained in autopsies no more than 12 h after death. They were carefully dissected and cerebral cortex, habenular region and pineal glands were pooled apart in acetone or frozen at −10° C. Extracts were prepared by homogenizing the acetone-dried tissue with water at room temperature or with 0·25 per cent acetic acid and heating to boiling point. The soluble fraction was assayed in the ovarian ascorbic acid depletion test (OAAD) of Parlow[6,7] in normal and hypophysectomized rats. Pure LH, kindly supplied by Prof. C. H. Li, was used as a standard reference. Ascorbic acid depletion was obtained (Table 1) with most of the extracts, but great fluctuations in the yield of active material from different batches of tissues were observed.

No regional differences in activity were found.

The aqueous extracts were always more active than the acid ones or than those prepared according to Altschule[8] or obtained with glacial acetic acid and sulphuric ether precipitation.

The active material dialyses through 'Cellophane' and becomes rapidly inactive when kept in solution at ordinary temperature.

Table. 1. OVARIAN ASCORBIC ACID DEPLETION INDUCED BY DIFFERENT EXTRACTS IN PARLOW TEST

Treatment		% depletion (*S.E.*)	No. of rats	*P*
Saline		8·01 ± 4·77	17	
C. cortex–aq.ext.	(150 mg)	21·25 ± 2·96	9	< 0·02
C. cortex–aq.ext.	(8 mg)	21·67 ± 4·76	9	< 0·05
C. cortex–aq.ext.	(50 mg)	26·69 ± 1·90	5	< 0·01
C. cortex—alc.ext.	(75 mg)	21·31 ± 5·02	5	no sign
C. cortex–gl.acet.	(280 mg)	40·41 ± 2·12	4	< 0·01
Pineal–alc.ext.	(10 mg)	33·84 ± 4·80	5	< 0·01
Pineal–alc.ext.	(100 mg)	26·45 ± 3·27	5	< 0·01
Pineal–aq.ext.	(8 mg)	30·72 ± 4·12	10	< 0·01
Pineal–aq.ext.	(2 mg)	27·85 ± 4·21	9	< 0·01
Pineal–aq.ext.	(200 mg)	37·07 ± 5·75	5	< 0·01
Habenular–aq.ext.	(225 mg)	27·85 ± 4·21	9	< 0·01
Habenular–aq.ext.	(3·7 mg)	34·10 ± 3·89	5	< 0·01
Habenular–aq.ext.	(8 mg)	21·94 ± 2·91	10	< 0·02
Luteinizing hormone	0·5 μg	27·17 ± 4·16	8	< 0·01
	1·0 μg	34·75 ± 2·15	15	< 0·01
	2·0 μg	45·27 ± 4·53	19	< 0·01
	5·0 μg	55·06 ± 1·39	14	< 0·01
	10·0 μg	59·87 ± 1·06	4	< 0·01
Serotonin	10·0 μg	19·69 ± 2·31	9	< 0·02
	50·0 μg	25·27 ± 5·74	10	< 0·02
Melatonin (in propylene glycol)	10·0 μg	22·11 ± 9·02	10	no sign
	50·0 μg	17·48 ± 5·70	10	no sign
Propylene glycol	0·1 ml.	26·29 ± 6·36	10	< 0·02

Nos. in brackets, doses expressed in mg of dried tissue. Aq.ext., aqueous extract; alc.ext., alkaline extracts; gl.acet., extract prepared with glacial acetic acid and ether precipitation. *P*, related to control group treated with saline, with the exception of the group treated with 'Melatonin' which is compared to the depletion obtained with the solvent of this substance.

The same extracts were not active in hypophysectomized rats (Fig. 1) in a period when the sensitivity to LH was completely normal. This fact suggests the nature of a LH–RF and permits one to rule out the presence of LH.

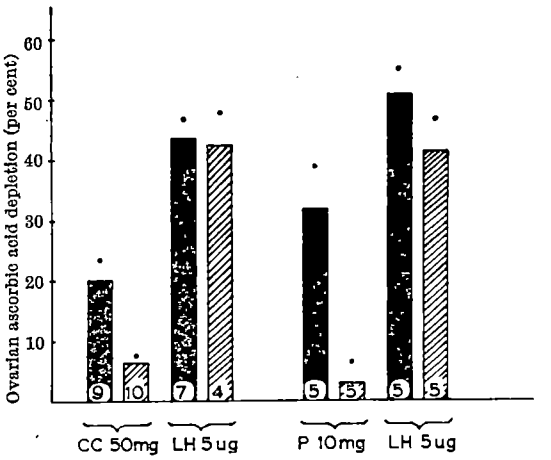


Fig. 1. Ovarian ascorbic acid depletion produced in normal and acutely hypophysectomized rats by administration of cerebral cortex and pineal extracts. The sensitivity of the rats to LH was assessed in each experiment with luteinizing hormone (LH). Dots represent the *S.E.* Numbers at the base of the bars represent the number of test animals. Black, normal; hatched, hypophysectomized; CC, cerebral cortex; LH, luteinizing hormone; P, pineal

The extracts increase the LH-like activity of the plasma of ovariectomized rats treated with œstradiol or œstradiol and progesterone[9] as shown in Fig. 2. The nature of this effect is not clear because it is not known if the intravenously injected extract remains in the blood when the plasma is taken out and assayed in the Parlow test, so the effect could be due to the injected substance and not to LH released from the animal's own pituitary.

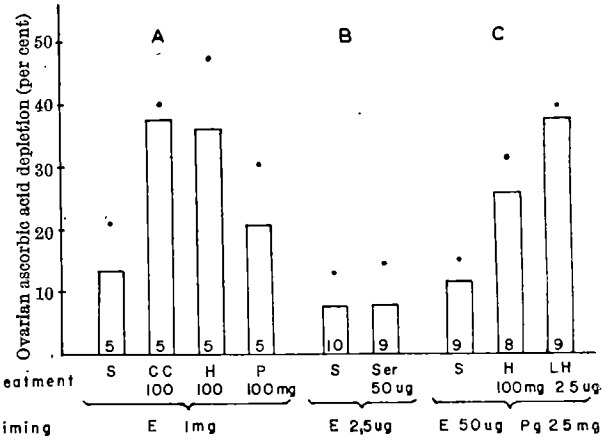


Fig. 2. Effect of different extracts, serotonin (Ser) and LH (luteinizing hormone) on plasma LH activity of ovariectomized adult rats primed with ovarian steroids. Plasma was obtained 15 min after the intravenous injection of a volume of 1 ml. of saline (S), cerebral cortex (CC), pineal (P), habenular (H) extract, serotonin or luteinizing hormone. The bars represent the ovarian ascorbic acid depletion with *S.E.*; number of recipient rats indicated at the base of each bar. The priming of the rats was with: *A*, 1 mg œstradiol benzoate (E); *B*, 2·5 μg E; *C*, 50 μg E, 25 mg progesterone (Pg) given subcutaneously 72 h previously

The daily subcutaneous injection of the extracts during 7–15 days to normal rats did not produce significant changes in the weight of the endocrine organs with doses equivalent up to 100 mg dry tissue per day. When the extracts were assayed in rats kept under constant illumination and displaying a state of constant œstrus, no change in the vaginal smear or weight of the endocrine organs was evidenced. Some experiments were performed

to establish the capacity of the extracts to induce ovulation in androgenized female rats[5] which have no spontaneous ovulation, and no effect was found with doses up to 400 mg dry tissue, which are rather low as compared with those used by Johnson. He induced ovulation with brain extracts in doses equivalent to 4–8 g of wet tissue.

The activity of the extracts on plain muscle was examined on the rat uterus *in vitro*, blood pressure of the anæsthetized rat and chicken cæcum rectale. No activity was found in these tests, so the presence of substance *P* may be discarded. Serotonin, the other substance present in brain tissue, is active in the Parlow test in high doses, which produce respiratory effects not evidenced with the active extracts (Fig. 2).

The reported results show that the whole human brain contains a substance producing depletion of the ovarian ascorbic acid, which acts through the pituitary. These results agree with those of Johnson on the brain of the sheep. The relation of this substance with that reported by McCann on the rat hypothalamus has not been examined.

This research was supported by the U.S. Air Force under grant *AF–AFOSR*–447–63, monitored by the Air Force Office of Scientific Research, Office of Aerospace Research.

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Luteinizing Hormone-like Activity in Human Median Eminence Extracts

Recently, several investigators have succeeded in obtaining evidence of the presence of an LH liberating factor (LRF) in extracts prepared from the median eminence (ME) of different mammals, such as rats[1–3], cattle[2,4], sheep[3,5], rabbits[4] and monkeys[4].

The fundamental implications of this finding led us to attempt to establish a similar activity in the ME of human beings. Experiments were designed to investigate the ability of human ME extracts to elicit in the rat some of the phenomena commonly attributed to LH action such as ovulation and depletion of ovarian ascorbic acid (OAAD), and to assess whether the active substance should meet the requirements for classification as an LRF.

After resecting the pituitary stalk as completely as possible the ME was carefully removed by dissection from fresh or frozen hypothalamus obtained from cadavers of both sexes more than 10 years of age. The tissue was homogenized in 0·5 per cent acetic acid in the proportion of 1 ml./8 ME (the average weight for ME tissue was 35 mg). The homogenate was centrifuged at 2,500 r.p.m. for 20 min and the supernatant neutralized with sodium bicarbonate. Cerebral cortex (CC) extracts were prepared in the same way.

Female rats from our colony, weighing between 150 and 250 g, and with regular œstrous cycles, were used to test the ovulation-inducing ability of these extracts. The animals were kept in a room illuminated artificially from 7 a.m. until 9 p.m. by automatic control. A daily vaginal smear was taken at 9 a.m., and the rats found in pro-œstrus were injected intraperitoneally at 10.30 a.m. with 5 mg of chlorpromazine ('Largactil (R)', kindly supplied by Establecimientos Chilenos Colliere, Ltd.) per 100 g body-wt., a procedure which impedes ovulation during the following night[6]. After this the animals were anæsthetized with ether or bromethol and the extracts injected intravenously. LH dissolved in saline was also injected to check the sensitivity of the ovary under the effect of the blocking agent used (kindly supplied by Prof. C. H. Li, Berkeley, California). On the following day the Fallopian tubes were removed and the content of the ampulla examined under low illumination on the stage of a compound microscope to ascertain whether or not ovulation had occurred. The results are shown in Table 1. Eight ME and 4 CC extracts were assayed.

Seven of the nine rats which did not ovulate after ME injections were injected with the minimal dose of the respective extracts; the other two were injected with a dose greater than the minimal. LH was given at four different levels: 1, 5, 10 and 20 μg; each one to six animals. Failure to ovulate was seen in two rats at the dose of 1 μg, in one rat at the dose of 5 μg and in two rats at the 10-μg level. There was no clear relation between the number of ova shed and the quantity of LH administered at the dose-levels used.

Median eminence extracts were then assayed in hypophysectomized rats. As hypophysectomy in the chlorpromazine-treated rat causes a very high mortality the assays were performed in rats in which ovulation was blocked by hypophysectomy itself. By performing the operation at different hours in the morning of the day of pro-œstrus it was determined that the ovulatory release of LH occurs after 11.45 a.m. in our rats under the illuminating conditions described.

Ovulation was obtained in all animals hypophysectomized before 11.45 a.m. and injected with 1 or 5 μg of LH afterwards. As the rat showed, under these conditions, high responsiveness to the ovulatory stimulus, ME extracts were injected in nine hypophysectomized animals, ovulation being obtained in all of them.

The OAAD test was performed using immature female rats prepared as described by Parlow[7]. The extracts as well as the LH standard and the control solution were injected in the femoral vein. ME extracts were given also to hypophysectomized rats operated on 9 h before. The results shown in Table 2 are in keeping with those of the ovulation-inducing experiments, and both of them reveal a significant LH-like activity in the hypophysectomized animal.

This finding strongly suggests that LH may be present in ME extracts prepared from human material. This location of the hormone could be explained by the fact that the pars tuberalis covers the surface of the ME although it is generally believed that this part of the anterior lobe is devoid of hormone activity, as it is com-

Table 1. Effect of LH, Human ME and CC Extracts on Chlorpromazine-blocked Pro-œstrous Rats

No. of animals	Extract	Dose	No. which ovulated
29	None		0
39	ME	16–456 mg	30
17	CC	284–2,000 mg	0
24	LH	1–20 μg	19

Table 2. Effect of LH, Saline, Human ME and CC Extracts on OAAD of Intact and Hypophysectomized Rats

Extract	Dose	No. of rats	% depletion ± S.E.
Intact			
ME	170 mg	11	39·36 ± 2·68
CC	170 mg	7	12·02 ± 1·91
Saline	0·2 ml.	32	10·02 ± 2·44
LH	0·5 μg	8	27·17 ± 4·16
LH	1·0 μg	15	34·75 ± 2·15
LH	2·0 μg	19	45·27 ± 4·53
Hypophysectomized			
ME	170 mg	9	31·59 ± 1·65

posed mainly of chromophobe cells[8]. Despite this, ovulatory activity in the chlorpromazine-treated rat was also obtained with extracts prepared from human hypothalamus in which all possible contamination with pituitary tissue had been completely avoided by carefully excluding ME. Experiments on hypophysectomized rats will demonstrate whether the substance involved has either LH- or LRF-like activity. Since the active substance in these extracts does not dialyse, the first possibility seems more likely. The presence of LH-like activity in extracts prepared from sheep hypothalamus and ME has been reported by Guillemin *et al.*[9].

No matter how these results may be interpreted, the existence of LRF cannot be dismissed, and its occurrence in the human hypothalamus is still under investigation.

This work was supported by the U.S. Air Force under grant *AF–AFOSR–447–63*, monitored by the Air Force Office of Scientific Research, Office of Aerospace Research.

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Development of Shock-inducing Factor in Canine Endotoxin Shock

DURING the course of our investigations on the mechanism of enhanced susceptibility to endotoxin after adrenalectomy in dogs, it was found that a transferable shock-inducing factor develops in the absence of glucocorticoids. The shock induced by endotoxins which have no direct cardiovascular action has been difficult to interpret. There has been debate on the indirect mechanisms[1], such as endotoxin-epinephrine synergism or histamine release. However, none could explain the effectiveness of glucocorticoids in preventing the endotoxin shock[2]. Concerning endotoxin susceptibility after adrenalectomy in rabbits, Fukuda[3,4] has pointed out that the diminution of hepatic glycogen which follows the accumulation of endotoxin in liver might have an intimate relation to the development of circulatory weakness. Prevention of the attendant hypoglycæmia and the lactacidæmic acidosis by infusions of a hypertonic glucose and sodium bicarbonate solution has been shown to be effective in the recovery of adrenalectomized rabbits even after intravenous administration of the lethal dose of endotoxin (200 μg/kg; LD_{100}).

However, in adrenalectomized dogs well maintained with DCA these procedures could have only slight ameliorating effect on the progress of profound shock, and the animals succumbed to even small doses of endotoxin such as 5 μg/kg.

As shown in Fig. 1, intravenous administration of endotoxin induced after about 30 min a progressive fall of arterial blood pressure. This type of shock could easily be prevented by glucocorticoid pretreatment. Intravenous administration of 'Decadron' (dexamethasone 21-phosphate, Merck) in a dose of 4 mg just before or within 15 min after endotoxin could also prevent the shock together with the development of hypoglycæmia due to liver glycogen depletion. Moreover, the shock was characterized by the rapid onset of intense fever accompanying shivering. As the fever induced by endotoxin has been shown to be due to the formation of endogenous pyrogen also in this species[5], it was tentatively supposed that massive formation of endogenous pyrogen in the absence of glucocorticoids might have some relation to the induction of shock.

Therefore, transfer of plasma or serum from the shocked adrenalectomized dog to another adrenalectomized dog maintained with cortisone, or to intact dogs, was tried. Thereby, an eventual combination of animals showing slight hæmagglutination with each other was avoided by cross-match blood tests. As shown in Fig. 2, a relatively small amount of serum (less than 10 c.c./kg) could induce an immediate fall in blood pressure, which was found to be due to an intense hepatosplanchnic blood pooling. Usually a prompt fever with shivering occurred. However, no correlation could be found between the fever induced and the degree of shock. Therefore, this transferable shock-inducing factor seemed to be a separate factor from the endogenous pyrogen and was shown to be heat-unstable (56° C, 30 min). From its mode of action it might act as a histamine-releaser on the liver. It developed in detectable amounts only in the blood of the shocked adrenalectomized animals. Glucocorticoids could prevent its development, but could not inhibit its action. This might well explain why glucocorticoids were not effective unless they were administered before or shortly after endotoxin.

The development of the endogenous shock-inducing factor in the absence of glucocorticoids might explain the extreme susceptibility to endotoxin in adrenalectomized dogs which could not be fully ameliorated by correcting the hypoglycæmia and acidosis. The intimate relation of its development to the almost complete depletion of liver glycogen (less than 100 mg per cent wet wt.) might suggest

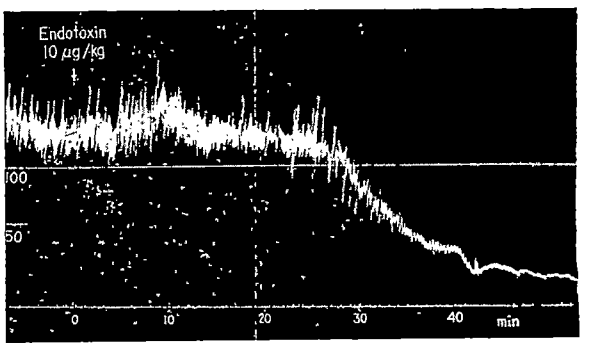


Fig. 1. Endotoxin shock in an adrenalectomized dog maintained with DCA. The blood pressure recording at femoral artery. The experiment was done 2 weeks after adrenalectomy without anæsthesia. Endotoxin dose of 10 times this amount had no effect on the blood pressure of unanæsthetized intact dogs

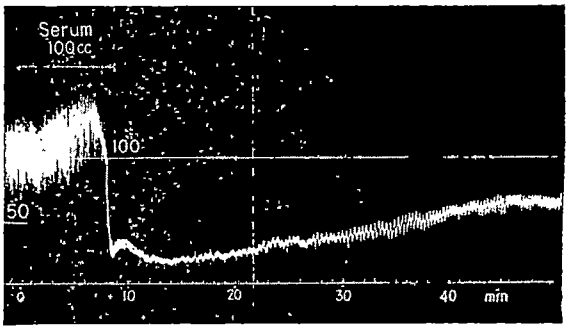


Fig. 2. Transfer of serum from an endotoxin-shocked animal to an adrenalectomized dog maintained with cortisone

its origin in the liver. Preliminary saline extraction of the factor was made by soaking liver slices (about 2 mm thick) of the shocked adrenalectomized dogs in a physiological saline solution of equal volume (4° C, 24 h). Saline washes cleared of cellular debris by centrifugation were tested, like the aforementioned serum. Only the saline extracts from the shocked animals induced an immediate fall in blood pressure, just as the serum from the shocked animals did. Thus the shock-inducing factor seemed to be liberated from the shocked liver. However, being heat-unstable it differed from VDM[6]. Thus the effectiveness of glucocorticoids in preventing endotoxin shock seems to depend on their ability to suppress the hepatic responses induced by endotoxin, such as glycogen depletion and liberation of the shock-inducing factor.

The shock in adrenalectomized dogs which could be induced even by small amounts of endotoxin differs in its mechanism from the well-documented immediate and transient falls in blood pressure observable only in this animal species with massive doses of endotoxin[7]. It seems to correspond to the late progressive shock which follows thereafter in intact dogs. It is really in this phase of shock that the effect of glucocorticoid pretreatment can be expected[2]. Whether the shock-inducing factor here demonstrated participates in endotoxin shock in other species in the absence of glucocorticoids remains to be elucidated. In rabbits, for the reasons mentioned here, the part played by this factor might be negligible. This might explain why susceptibility to endotoxin is not so much enhanced after adrenalectomy in this animal as in dogs.

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Action of Enzymatic Inhibitors (Potassium Cyanide and Sodium Fluoride) on Muscle Fibre Sensitivity to Potassium Ion

The experiments reported by Carey and Conway[1] and by Conway[2] on skeletal muscle, and by Hodgkin and Keynes[3] on nerve fibre, show that ionic exchanges, especially sodium and potassium transfer, are related to aerobic metabolism in the living tissue. Therefore, metabolic inhibitors such as cyanides modify these exchanges between cell and surrounding medium by decreasing Na^+ efflux and K^+ influx.

It has been suggested that a shift of ionic exchanges when inhibitors (cyanides or fluorides) are added to the bath could consequently modify some pharmacological effects such as potassium contracture of striated and smooth muscle.

Frog rectus abdominis. We have observed that contraction of the isolated rectus abdominis of the frog produced by potassium chloride is considerably increased by addition of very low doses of potassium cyanide to the Ringer solution in which the muscle is immersed.

The rate of increase of contractions depends on the respective doses of potassium chloride and of the inhibitor. The concentration of cyanide was maintained constant at 2×10^{-4} (3 mM) and potassium chloride concentration varied from 0·5 to 2·5 × 10^{-4} (6·7–33·7 mM). For the lower concentrations of potassium chloride, increased contraction by cyanide may reach 10–20 times the initial contraction height.

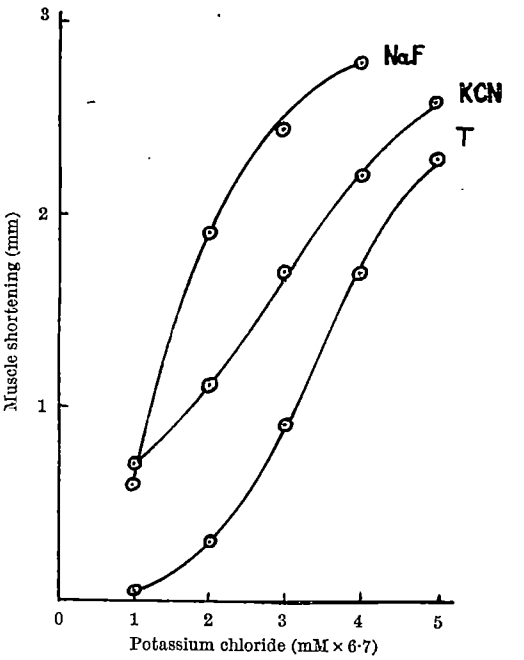


Fig. 1. Effects of sodium fluoride (4 mM) and potassium cyanide on the height of contractions produced by different doses of potassium chloride on frog rectus abdominis

Repeated experiments have shown that the degree of sensitization depends on the incubation period of potassium cyanide in the preparation. No effect has been observed for short periods of contact (15 sec), but sensitization takes place within 30 sec and reaches a maximum value for 60-sec contact. It is abolished after 5-min incubation.

Similar experiments were performed with sodium fluoride. At a concentration of 4 mM, very close to that used for potassium cyanide, sodium fluoride strongly potentiates potassium contraction of frog rectus abdominis; but the maximum potentiation can be obtained

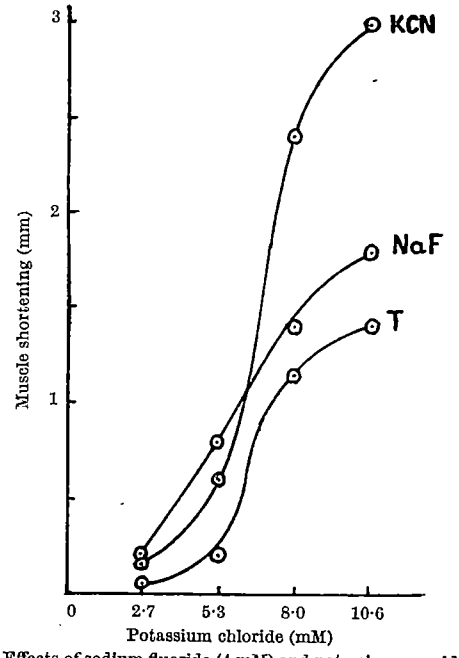


Fig. 2. Effects of sodium fluoride (4 mM) and potassium cyanide on the height of contractions produced by different doses of potassium chloride on guinea-pig ileum

only after an incubation period of 5 min. The potentiation is even stronger than that obtained with potassium cyanide. The contraction height is 3–6 times higher than the control instead of 1·9–3·7 times with cyanide, for potassium chloride concentrations ranging from 1 to 1·5 g per 1,000 (13·5–20 mM).

Fig. 1 curves describe muscle shortening (in millimetres) as a function of potassium chloride concentration in the bath, in normal conditions without any inhibitor, with cyanide and with fluoride.

Guinea-pig ileum. We observed that both enzymatic inhibitors previously used have the same influence on smooth muscle fibre potassium contracture as well as on skeletal muscle fibre.

Our experiments were performed on guinea-pig ileum, which is more sensitive than frog rectus abdominis: we obtained the same shortening with doses of potassium chloride 3–4 times weaker than in the previous experiments. Furthermore, the smooth muscle is more sensitive to the inhibitors, active doses of which did not exceed more than 1 per cent of those used on rectus abdominis. Also, it has been possible to reduce incubation period to 30 sec. So, in 0·03-mM potassium cyanide Tyrode, guinea-pig ileum shortening by potassium chloride is increased three-fold at a salt concentration less than 5·3 mM and two-fold at higher concentrations. At low potassium chloride concentrations the results are even more obvious with sodium fluoride, but at higher concentrations the potentiation is only 1·2–1·3 times.

We tried other enzymatic inhibitors with the same method: 2,4-dinitrophenol, sodium iodoacetate, sodium nitrite. No definite conclusion can be drawn from these later experiments because the results were irregular.

As a whole, the results reported here show that enzymatic inhibitors are able to sensitize smooth and striated muscle fibres to contracture produced by potassium chloride, by influencing either cellular oxidation phenomenon (cyanide) or glycolysis (fluoride).

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PHARMACOLOGY

A New Antiviral Agent: 4-Bromo-3-methylisothiazole-5-carboxaldehyde thiosemicarbazone, M and B 7714

STIMULATED by reports of the antiviral activity of some thiosemicarbazones[1], and particularly of the activity against neurovaccinia of isatin β-thiosemicarbazone[2,3], we sought to enhance the activity of these compounds by structural modification. Thus we synthesized *N*-ethylisatin β-thiosemicarbazone independently of, but somewhat later than, Bauer and Sadler[4]. At the same time, we had been making a systematic study of the chemistry and chemotherapeutic properties of derivatives of the new monocyclic ring system, 1,2-thiazole (isothiazole), and we observed that 3-methylisothiazole-5-carboxaldehyde thiosemicarbazone (M and B 7453) also protected mice infected intracerebrally with neurovaccinia. The relatively high toxicity of this compound (acute LD_{50} = 0·7 mg/g orally in mice) led us to examine related thiosemicarbazones, one of which, 4-bromo-3-methylisothiazole-5-carboxaldehyde thiosemicarbazone (M and B 7714), is considerably less toxic (acute LD_{50} = 4·3 mg/g orally in mice).

4-Bromo-3-methylisothiazole-5-carboxaldehyde thiosemicarbazone is a yellow crystalline solid, m.p. 228°–230° (decomp.)[5]. It is less than 0·1 per cent w/v soluble in water at 37°, but dissolves as a salt in alkaline solutions.

In mice infected intracerebrally with neurovaccinia (*IHD* strain) and dosed orally once daily for four days, a marked protection was observed (Table 1).

Table 1. THERAPEUTIC ACTIVITY OF M AND B ADMINISTERED ORALLY TO MICE INFECTED INTRACEREBRALLY WITH NEUROVACCINIA

	Daily dose mg/kg (× 4)	Mean survival time (days)	Animals surviving at 10 days
Controls	Nil	4·6	0/30
M and B 7714	500	9·4	22/30
M and B 7714	250	9·0	18/29
M and B 7714	120	8·0	12/29

Table 2. EFFECT OF DELAYED DOSAGE WITH M AND B 7714 ON THE SURVIVAL OF MICE INFECTED INTRACEREBRALLY WITH NEUROVACCINIA

	Dose mg/kg	Delay before treatment (days)	No. of doses given	Median survival times	Animals surviving at 14 days
Controls	Nil	–	–	4·8	0/10
M and B 7714	1,000	0	4	12·7	8/10
M and B 7714	1,000	1	3	13·2	9/10
M and B 7714	1,000	2	2	11·1	7/10
M and B 7714	1,000	3	1	8·4	4/10

Protection was still observed when treatment was delayed (Table 2). Surviving treated animals were immune to challenge from 10^5 LD_{50} infecting doses of virus. During treatment, the circulating virus titre was below that in the infected, untreated, controls. The degree of meningo-encephalitis was less after treatment.

The compound has been tested against variola major in baby mice and found to be active[6].

M and B 7714 also shows high activity in rabbits infected intranasally with rabbit pox (Utrecht strain), but is inactive against influenza, encephalomyocarditis (Columbia *SK*), and rift valley fever viruses.

Details of a fuller laboratory investigation, including toxicological studies, and the results of an extended therapeutic and prophylactic trial will be published in due course.

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Distribution of Homovanillic Acid in the Human Brain

HOMOVANILLIC acid (3-methoxy-4-hydroxyphenylacetic acid, HVA) is formed by the action of the enzymes monoamine oxidase and catechol-*O*-methyl-transferase as a final product of dopamine metabolism, and its occurrence in urine has been described[1–6]. From other investigations[7,8] it may be concluded that in experimental conditions the HVA level in the brain tissue reflects the dopamine turnover, although with a certain delay. It also seems likely that for the normal brain tissue a similar statement may be made. The actual HVA concentration in the normal brain tissue, therefore, might be a suitable index in the evaluation of the local dopamine turnover. Sharman[9] has recently demonstrated the presence of HVA in the caudate nucleus of animals of several species. This seems consistent with the high dopamine concentration in this brain area[10]. Detailed investigations have been performed on the local distribution of dopamine in the human brain[11–15]. This account describes the occurrence and local distribution of HVA in the human brain.

Human brains were selected in the autopsy room, from patients not having suffered from cerebral or mental

disease, 3–18 h after death. The brains were dissected immediately and the selected tissues were frozen and kept at −20° C until the extraction procedure took place, usually within two days. The HVA was extracted by Sharman's method[9] and the final extracts were subjected to a two-dimensional ascending paper chromatography on Whatman No. 1 paper in the following systems: isopropanol : water : concentrated ammonia solution in the proportions 40 : 9 : 1, and benzene : propionic acid : water in the proportions 100 : 70 : 4. Spots were detected by spraying the air-dried papers with freshly prepared alkaline solutions of diazotized sulphanilic acid or of diazotized *p*-nitroaniline[10]. The HVA was identified by its R_F-values and colour reactions, compared with reference spots of synthetic HVA. For the quantitative evaluation the sulphanilic acid spray only was used. The stained areas corresponding to HVA were cut out, put into test tubes, and eluted with 4 ml. of a mixture described by von Studnitz[17]. The optical density of the eluates was assayed at 500 mμ in a Beckman *DU* spectrophotometer. Standard curves were prepared from 2 to 16 μg of synthetic HVA, including the chromatographic procedure, and appropriate paper blanks were subtracted. Sufficient amounts of tissue were extracted to achieve distinct spots of HVA on the chromatogram. However, in some brain areas the tissue level of HVA was low and no colorimetric assay could be performed: in these areas the HVA concentration was estimated to be < 1·0 μg/g.

Table 1. Homovanillic Acid in Human Brain

Region	No. estimations	Homovanillic acid (μg/g tissue) Range	Mean
Caudate nucleus	3	1·3–2·4	1·8
Putamen	4	2·8–5·3	4·0
Globus pallidus	5	1·6–3·4	2·4
Substantia nigra	3	1·7–2·0	1·8
Hypothalamus	3	< 1·0	—
Thalamus	4	< 1·0	—
Nucleus amygdalae	1	< 1·0	—
Nucleus ruber	1	< 1·0	—
Floor of IVth ventricle (Formatio reticularis)	2	< 1·0	—
Cortex (Gyrus praecentralis)	2	< 1·0	—
White matter	2	< 1·0	—
Nucleus dentatus cerebelli	2	< 1·0	—

The results are summarized in Table 1. They show clearly that concentrations of HVA exceeding 1·0 μg/g were found in several regions of the human brain: the caudate nucleus (1·8 μg/g), the putamen (4·0 μg/g), the globus pallidus (2·4 μg/g) and the substantia nigra (1·8 μg/g). It is worth noting that all these areas belong to the extrapyramidal system. In the other areas investigated, namely, the thalamus, hypothalamus, nucleus ruber, nucleus amygdalae, floor of the IVth ventricle with the reticular formation, cortex (gyrus praecentralis), white matter and the dentate nucleus of the cerebellum, the HVA concentration was too low for accurate colorimetric assay and was considered to be < 1·0 μg/g.

It may be stated that in all brain areas where the HVA concentration is less than 1·0 μg/g the dopamine level is also low. Within the corpus striatum, on the other hand, the high HVA concentration (1·8 μg/g in the caudate nucleus and 4·0 μg/g in the putamen) seems to be related to the high dopamine concentration in this region. The concentration of HVA in the substantia nigra (1·8 μg/g) also seems to be consistent with a distinct dopamine accumulation in this area[15]. In contrast, no similar relation can be established for the globus pallidus, where a high HVA concentration (2·4 μg/g) was found, but where the dopamine concentration is low. This high concentration of HVA in the globus pallidus should not be disregarded, as it seems to indicate a high dopamine turnover in a place where little dopamine accumulation occurs. The significance of a high dopamine turnover in the globus pallidus cannot yet be explained, but it might be related to the fact that the major afferent fibres of the globus pallidus stem from the dopamine-rich corpus striatum.

I thank Prof. L. Haslhofer for permission to work on the autopsy material, and Mrs. Eva Kótai for assistance.

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Semi-automatic Extraction using a Rotary Extractor

A simple rotary extraction procedure has been developed for the extraction of urine with chloroform. It has been found to be efficient for the extraction of unconjugated aldosterone from urine. It virtually obviates emulsion formation and has been adopted for routine use in this laboratory; it appears to be suitable for wide application in liquid–liquid extraction systems.

Two parallel, horizontal, rubber-padded metal rods are rotated by a motor through a reduction gear. A glass bottle containing urine and chloroform is laid on the rods, and partition of solute between the two liquid layers occurs as the bottle rotates on the rods (Fig. 1). In these experiments the glass bottles were of approximately 5·5 in. diameter, the rollers were 4 in. apart, and the bottles revolved 32 times per min; 10-min rotation periods were used for each extraction. At this speed the chloroform layer remains below the aqueous layer during the period of rotation and the weight of the liquid maintains the bottle in position on the rods without external support. Several bottles can be used simultaneously. The recovery of added labelled aldosterone was measured in 23 urine specimens from patients with a variety of diseases—precisely those circumstances in which the frequent occurrence of emulsions had previously occurred using separating funnels for extraction. When the daily urine volume was two or more litres, 1 l. was taken for extraction; otherwise the urine was diluted to 2 l. with water and 1 l. was used. After adjustment of the *p*H to 6·5, the urine was extracted five times with 250 c.c. re-distilled chloroform (containing 1 per cent ethanol, v/v, as stabilizer) to remove free aldosterone. The chloroform layer was withdrawn from the bottle by aspiration each time and discarded. The aqueous phase was then adjusted to *p*H 1·0 with hydrochloric acid, radiochemically pure 1,2-^{3}H aldosterone (0·1–0·2 μC) was added, and the mixture was allowed to stand at room temperature for 24 h with occasional shaking; this procedure is known to release free aldosterone from its acid-hydrolysable conjugate. After 24 h the urine was re-extracted with chloroform and the total volume of 1·25 l. chloroform was washed with small volumes of dilute sodium carbonate and water, taken to dryness and made

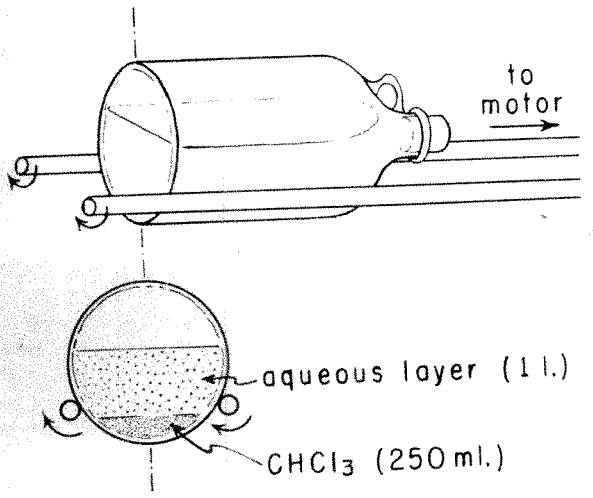


Fig. 1. Semi-automatic rotary extractor

up in methanol. A 5 per cent aliquot was taken for liquid scintillation counting. It was dried in a counting vial and 10 ml. of scintillation solution added. Each litre of scintillation fluid contained 4 g of 2,5-diphenyloxazole (PPO), 100 mg 1,4-*bis*-2-(4-methyl-5-phenyloxazolyl)-benzene (dimethyl-POPOP), 20 ml. absolute ethanol and 980 ml. of spectroquality reagent toluene (Matheson, Coleman and Bell). All values were corrected for quenching using the internal standard technique. The counting error was 1 per cent for most samples and less than 3 per cent in every case. The mean recovery of labelled aldosterone following acid hydrolysis was found to be 100 per cent with a standard deviation of ± 12 per cent.

In a few instances a very small amount of emulsion was formed, but disappeared during the washing procedure; centrifugation was not necessary. It should be emphasized that these urine samples were very 'dirty' and in several cases were from patients with serious disease, or during menstruation or significant proteinuria. Investigation of such variables as rotation-rate, volume of solvent, and duration of the extraction period is required to define the optimum conditions for extraction of aldosterone, but preliminary experience emphasizes the usefulness of this procedure, and justifies its consideration for other problems involving extraction of material contained in liquid phases.

This investigation was supported by Public Health Service research grant *AM*–06607 and training grant 2*A*–5230, National Institute of Arthritis and Metabolic Diseases, and by the Oregon Heart Association. The gift of ^{3}H-aldosterone from the Endocrinology Study Section, U.S. Public Health Service, is gratefully acknowledged. We thank Dr. W. R. Todd for advice.

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HÆMATOLOGY

Hæmatopoietic Stem Cells

Normal hæmatopoiesis is initiated and maintained by hæmatopoietic stem cells. These cells perform two functions: (1) they differentiate into mature hæmic elements, and (2) they maintain and renew the population of stem cells. It is these stem cells which seed, differentiate and reproduce, thus effecting successful hæmatopoietic transplants.

Over a long period, many theories have been proposed concerning the role of the stem cell in maintaining normal hæmatopoiesis. Some of these theories have incorporated imaginative mathematical models which have been solved by sophisticated electronic computers[1], and other theories have resulted from detailed histological examination of embryonic tissues[2]. So far, with the limited techniques available for examination of stem cell kinetics, it has not been possible to accept or reject any of these proposed theories.

In 1962 we introduced a sensitive bioassay for the transplantation potential of marrow, providing a means for quantitating either the renewal capacity or the differentiating capacity of hæmatopoietic repopulating cells[3–5]. This bioassay was based on the observation made by Till and McCulloch[6], that transplanted murine hæmatopoietic tissue seeds and grows as distinct colonies in the spleens of host mice. Recently, these investigators have shown that each colony develops from a single cell[7]. Using this bioassay, we have made significant observations on hæmatopoietic repopulating cells and can show they undergo renewal and differentiation, thus indicating they are stem cells.

The technique for producing these colonies has been described in detail[3–5]. Small numbers of hæmatopoietic cells are transplanted into heavily irradiated isologous hosts. On the 9th or 10th day the spleen is removed and fixed and the colonies studied. We find that a rather constant proportion of the injected colony-forming cells seed in the spleen and proliferate rapidly with a doubling time of 10–14 h. The number of colonies formed is a function of the dose of the inoculum and the size of the colony is related to the time post-inoculation. It is our experience that colonies do not increase in number from day 5 to day 11 but merely increase in size. By the 9th day they are easily visible to the naked eye, being approximately 1–2 mm in diameter.

Renewal of repopulating units was studied using a modification of this model. At post-injection times varying from 1 day to 11 days, spleens were taken from irradiated mice which had been transfused with 45,000 isologous marrow cells. These spleens were minced and reinjected into lethally irradiated hosts. Since a rather constant proportion of repopulating cells seed in the spleen, this technique allowed us to examine the multiplication or renewal of repopulating cells within the spleens of mice which had received 45,000 marrow cells. Nine days after the injection of this donor spleen, the spleen of the second recipient was removed and the colonies counted. The mean number of colonies formed on each spleen in a second host is shown in Table 1.

It is noted that there is no increase in the number of colony-forming cells from day 1 to day 4. This is particularly significant when it is remembered that on day 4 each colony contains 200–400 immature, rapidly proliferating cells. From their appearance, we had anticipated that these cells would function as stem cells, that is, they would

Table 1. Colony-producing Potential of Spleens from Lethally Irradiated Mice following Infusion of 45,000 Isologous Marrow Cells

Day injected*	1	2	3	4	5	6	7	8	9	10	11
Mean colonies formed†	0·8	1·6		0·4	4	16	12·2	47·2	> 50‡		> 100§

* Day on which the donor spleen was minced and injected into the tally host.

† Mean number of colonies formed on five tally spleens nine days after injection of minced donor spleen.

‡ One-half of each donor spleen was injected into one tally host, and on each of the 10 tally hosts the colonies were 'too numerous to count'. As fewer than 25 colonies per spleen can always be counted, we have considered that each tally spleen contained more than 25 colonies, thus the total colonies formed from each donor spleen were scored as greater than 50.

§ One-fourth of each donor spleen was injected into one tally host, and as each of the twenty tally spleens contained colonies 'too numerous to count' we have considered the total colonies formed from each donor spleen to be greater than 100.

be capable of initiating hæmatopoiesis and would develop in host's spleens as colonies of mature hæmic elements. It is evident that they did not do so. These studies indicate that these cells which appear immature and undifferentiated using available techniques of light microscopy do not reverse their directed differentiation and do not function as stem cells effecting colony production. On day 5, there was a sharp rise in the ability of the minced spleen to form colonies and by day 11 the resulting colonies were too numerous to count, even though only one-quarter of each colony bearing spleen had been injected into the tally hosts. Thus it is apparent that, following transplantation, repopulating cells first are directed to produce differentiated hæmic elements, and later begin to replenish their own population. These data confirm observations on stem cell renewal previously reported from our laboratory[6].

The differentiation capacity of these transplanted repopulating cells was studied. Four-μ sections were cut from 260 hæmatopoietic colonies on the 9th day post-injection, mounted serially and examined. 42 per cent of these colonies contained only recognizable developing erythrocytes, 21 per cent contained only granulocytes, 21 per cent contained only megakaryocytes, and 16 per cent contained mixtures of these cell types. Some of these mixed colonies appeared to represent two colonies of pure cell type which had grown in adjacent areas and overlapped. Thus, the colony forming cells that successfully seed in the spleen of irradiated hosts grow predominantly along one of three cell lines.

The foregoing results suggest that either repopulating cells may be unipotential in their capacity for differentiation, or they may be pluripotential but are directed at the time of seeding to differentiate along one cell line. In order to obtain information bearing on these possibilities, the following investigations were carried out: Ten-day-old colonies were excised and divided into two parts. The first half was fixed in formalin and sectioned serially in order to identify the cellular composition of the colony. The second half was minced and injected into an irradiated host. Nine days later, this host's spleen was removed, fixed and sectioned, and the cellular composition of the colonies studied. The results are shown in Table 2.

Table 2. Cellular Composition of Colonies resulting from the Injection of 1/2 of a 10-day-old Colony of Erythroid Precursors

Colony No.	Ery-throid precursors	Granu-locytes	Megakary-ocytes	RBC Mega.	WBC Mega.	RBC WBC	RBC WBC Mega.	Total
1		1	1		1			3
2		2	1		1	1		5
3								0
4	2		1					3
5								0
6	1	3	4	1	3		1	13
7			2	1	2			5
8								0
9	8							8
Total	11	6	9	2	7	1	1	37

It will be noted that the cellular composition of colonies formed from the injection of half a colony containing only developing erythrocytes is similar to the cellular composition of colonies formed from the injection of normal hæmatopoietic tissue. Thus, the daughter repopulating cells resulting from the renewal of repopulating cells in a 10-day colony of erythroid precursors possess the capacity to produce colonies of granulocytes and megakaryocytes as well as erythrocytes.

The foregoing experiments indicate that the class of cells called by us 'repopulating units' or 'repopulating cells' differentiate as well as undergo renewal of their own kind. Thus they satisfy the definition of stem cells. The term 'stem cell' refers to the functional capabilities of a cell and not to its morphological characteristics. As the repopulating cells of mice possess the functions attributed to stem cells, they must be considered as such.

In conclusion, evidence has been presented that: (1) hæmatopoietic colonies which develop in the spleens of irradiated mice injected with isologous hæmatopoietic tissue are formed from cells which satisfy the definition of stem cells; (2) following transplantation and seeding, these cells first begin to differentiate into mature hæmic elements; (3) following this directed differentiation into mature hæmic elements, these cells begin to renew or increase their own numbers, thus increasing the number of cells capable of initiating hæmatopoiesis; (4) up to the ninth post-irradiation day, more than 80 per cent of these stem cells differentiate along one cell line; (5) stem cells from colonies in which the only recognizable cells are red cell precursors differentiate in a manner similar to stem cells found in normal hæmatopoietic tissue.

From these results, it also seems that hæmatopoietic stem cells are pluripotential and are directed at the time of seeding to differentiate along one cell line and that daughter stem cells produced in parallel with differentiated hæmic cells retain their pluripotential capacity for differentiation.

This work was supported by research grant *C*–4144 from the National Cancer Institute, U.S. Public Health Service, and by a grant from the American Cancer Society, Inc., Illinois division.

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Erythrocytes and the Liquefying of Clotted Amphibian Blood *in vitro*

Samples of the whole blood of two amphibians (*Bufo marinus* and *Hyla caerulea*) which we have examined, after clotting *in vitro*, either naturally or by recalcification or on the addition of bovine thrombin, regularly become liquid again within a few hours. Investigation of this phenomenon, with human fibrinolysis in mind as a model, has revealed some interesting contrasts.

The clots of these amphibian bloods are jelly-like and have not the fibrous quality of mammalian fibrin. The blood contains an easily demonstrable prothrombin–thrombin conversion mechanism[1,2]. Ninhydrin-positive material, which is dialysable and is soluble in 7 per cent trichloroacetic acid (TCA), and which migrates towards the anode in acetate buffer at *p*H 4·15, is formed during clotting. Thus the thrombin–fibrinogen reaction appears to be fundamentally similar to that of a number of mammals[3].

We have been unable to show, by tyrosine assay, any further increase in TCA-soluble fragments in sera in which liquefaction of the amphibian clot has taken place, nor has electrophoresis revealed any characteristic products. This is in contrast to the human fibrinolytic system where some of the digested fibrin is eventually rendered TCA-soluble[4]. No acceleration of this amphibian clot liquefaction phenomenon by streptokinase has been demonstrable. It seems that a proteolytic plasmin-type of fibrinolysis is not involved.

The liquefaction does not occur in plasma gently centrifuged free of red cells; it returns when they are replaced in control preparations to at least one-third of their normal numbers in the whole blood. A curious finding has been that clots show little or no tendency to liquefy when prepared from whole blood which has been centrifuged briskly at speeds or for periods greater than the bare sedimentation of cells requires, and then merely resuspended. It is as though physical distortion or pressing of the cells in the centrifuge tube inhibits or counters their activity in the liquefaction of the clot. Adding tissue extracts or disrupted red cells to whole blood before clotting does not inhibit the subsequent liquefaction. Saturation of a blood sample with oxygen, or the addition of ascorbic acid or glutathione, does not alter the part played by red cells in the phenomenon. Red cell suspensions, concentrates, or extracts, placed on cell-free clotted toad plasma plates, do not affect them. It seems that natural incorporation of the cells in a clot is necessary for it to dissolve. White cells alone, both conventional phagocytes and 'thrombocytes', are not limiting factors in the process. These findings indicate that erythrocytes are involved in the resolution of blood clots in amphibians.

This work was supported by the National Heart Foundation of Australia.

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HISTOCHEMISTRY

Various Cell Types of the Adrenal Medulla

COUPLAND *et al.*[1] have reported that noradrenaline was synthesized in the so-called 'dark' cells, and adrenaline in the 'light' ones of the adrenal medulla. According to Coupland the 'light' or 'dark' character of these cell types depends on specific histochemical and electron-histochemical properties induced by glutaraldehyde and osmic acid treatment, enabling adrenaline- and noradrenaline-containing cells to be distinguished. Coupland's theory is supported by several other authors[2–5]; however, there are some observations, especially those of Yates, Wassermann and ourselves[6–9], which contradict it. These authors identified, by histochemical and electron-microscopical methods, cell-types containing noradrenaline (with granules of 1500–3000 Å) and adrenaline (with granules of 500–1000 Å) in the adrenal medulla of the golden hamster, and in the grass snake (*Natrix natrix*).

The purpose of this communication is to contribute, on the basis of Coupland's and our own observations, some new experimental results for the identification of adrenaline- and noradrenaline-containing cells.

Coupland[1] states that—after fixation with glutaraldehyde—the noradrenaline-containing 'dark' cells reduce osmic acid more intensively than the adrenaline-containing 'light' cells. Fig. 1 shows the reduction curve of adrenaline and noradrenaline to osmic acid obtained in *in vitro* experiments. It will be clearly seen that in *p*H 7·5 veronal buffer, adrenaline reduces osmic acid more intensively and rapidly than does noradrenaline. Even after 1 h, the extinction of osmic acid reduced by adrenaline was higher than that of osmic acid reduced by noradrenaline. It may be assumed that this is also true for the process in the tissues.

According to Coupland[1], after prefixation with formol or other chemicals, the dark cells cannot be observed. However, Fig. 2 shows that we succeeded in demonstrating the 'dark' and 'light' cells of the adrenal medulla of the rat, after prefixation with formol for 48 h. There is no essential difference between the granules of the two cell types. Otherwise the 'dark' and 'light' cell types were found in all animals examined by us—so far, the frog, grass snake, domestic fowl, rat, dog, hare, etc.

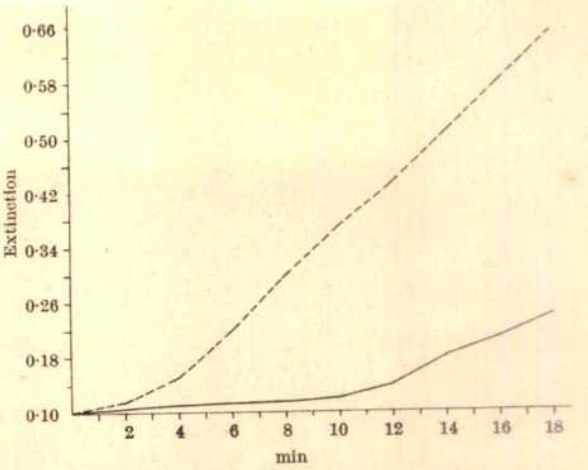


Fig. 1. Reduction curve of adrenaline (– – –) and noradrenaline (——) to osmic acid *in vitro*. In 1 ml. 1 per cent Palade's solution contained 100–100 μg adrenaline or noradrenaline

Fig. 3 shows the adrenal medulla of the domestic fowl, which is well known for its high noradrenaline level. Yet it contains 'dark' cells only here and there. The wide variety of size of some of the catecholamine granules within one and the same cell is striking. Wassermann[7]

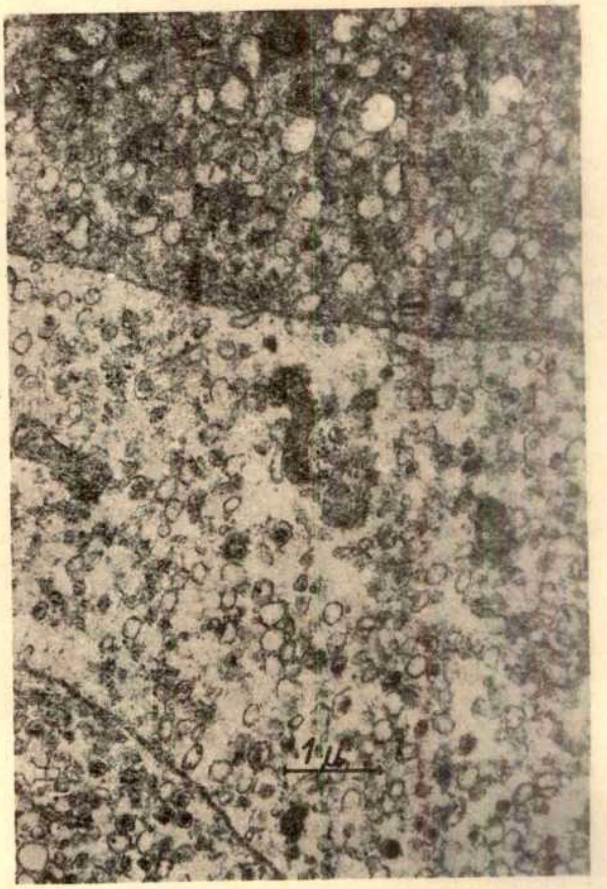


Fig. 2. 'Dark' and 'light' cells of the adrenal medulla of the rat, after prefixing with formol for 48 h (× *c.* 12,500)

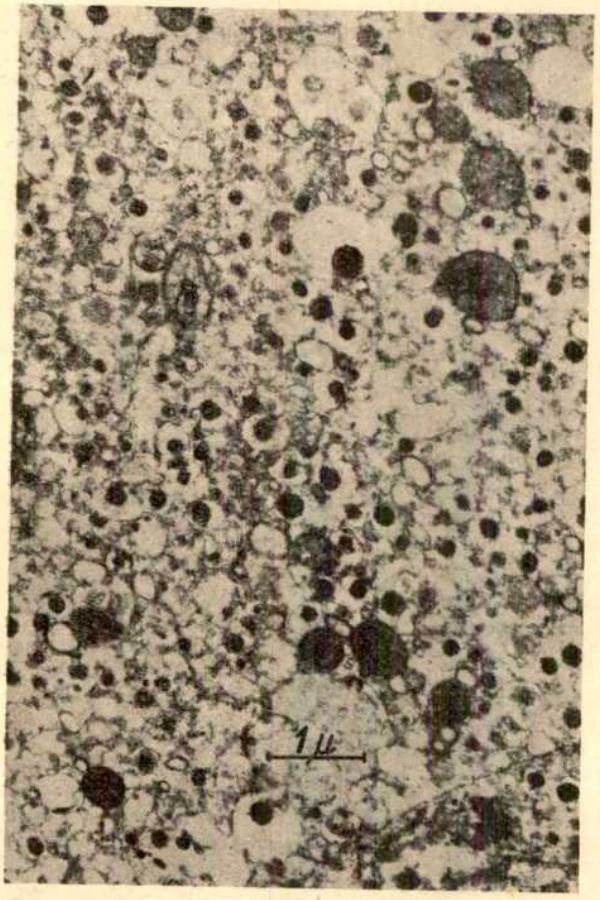


Fig. 3. 'Light' cells of the adrenal medulla of the domestic fowl. The wide variety of size of some of the catecholamine granules within one and the same cell is striking (×*c.* 12,500)

states this is characteristic of noradrenaline-containing cells.

After centrifugation of a homogenate of dog adrenal medulla at 3,000*g* the sediment was found to contain 80 per cent noradrenaline. But the fraction sedimented at 24,000*g* contained 64 per cent adrenaline. In the electron-microscopic photographs of the first fraction the catecholamine granules 2–3000 Å in size were dominant; but in the second fraction the presence of granules of 1000 Å was typical[10].

In the adrenal medulla of the grass snake we succeeded in demonstrating histochemically and electron-microscopically the presence of medullary cells containing these two types of granules[8].

On the basis of observations by Yates[6] and Wassermann[7], and all the facts described here, we are inclined to think that the 'dark' and 'light' cells which are present in the adrenal medulla of all animals (even in the rabbit, in which the noradrenaline content is very low) represent different functional and developmental stages. According to our investigations the 'dark' character of these cells is not a specific biochemical property caused only by glutaraldehyde pretreatment, for it was observed in numerous cases (dog, mouse, frog, etc.) even after prefixing with formol[9].

Our *in vitro* observation of the ability of adrenaline and noradrenaline to reduce osmic acid is at variance with the results of Coupland, and proves that the reducing capacity of adrenaline is stronger than that of noradrenaline.

Coupland's theory could not be proved even in the case of the domestic fowl, because in its adrenal medulla the 'light' cells predominate, although the adrenaline content is only 50 per cent of the noradrenaline content.

Fractionation of the granules with the ultracentrifuge, together with histochemical and electron-microscopical investigations, substantiated that noradrenaline-containing granules (2–3000 Å) and adrenaline-containing cells (1000 Å) could be found in the adrenal medulla[10].

These granules lie in separate cells (grass snake, frog) or in the same cells (dog, rat). The size of the granules mentioned here could be observed only in the 'light', 'storing' cells, while in the 'dark', 'transitional' cells, depending on the phase of synthesis, no differentiated granules with various sizes could be found.

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PATHOLOGY

Toxicity of Whole Tobacco Tar

An experiment was designed to investigate the mortality pattern produced by large doses of tobacco tar in an inbred strain of mice. The object was to find the maximum dose that could evoke a definite carcinogenic response, without causing high mortality. An attempt was also made to investigate the possibility of the development of tolerance to large doses of tobacco tar, by means of varied schedules of administration.

The animals used were 4-weeks-old female inbred albino mice of an *I.C.I.* strain. Solutions of whole tobacco tar in acetone–water (9 : 1), containing the appropriate doses in standard 0·25 ml. quantities, were painted on a standard area of shaved skin on the back. All mice, except a group of untreated controls, received 3 applications per week to a total of 159. The surviving mice were killed 10–12 days after the final application, and tissues which appeared abnormal at autopsy were examined histologically.

The mice were divided into 11 groups of 24, except the untreated control group 11, with 50 mice. Each mouse in groups 1–6 respectively received 5, 10, 20, 40, 80 and 160 mg of whole tobacco tar at each application. In group 7 the initial dose was 20 mg of tar at each application for 2 weeks, increased to 40 mg for the next 2 weeks, then to 80 mg for the remaining weeks. In group 8 the dose was 20 mg at each application for 4 weeks, followed by 40 mg for the next 4 weeks and 80 mg for the remainder. In group 9 the initial dose was 10 mg for 4 weeks, then 33 mg for another 4 weeks, then 80 mg in subsequent weeks. In group 10, the mice received 0·25 ml. of the vehicle alone (acetone–water) at each painting.

The mice in group 6, and to a lesser extent the mice in group 5, showed a high incidence of mortality in the early period of painting (see Table 1). In group 6, in spite of high initial mortality, 8 mice survived until the twelfth month, suggesting the development of some tolerance to the tar. The same effect was observed in group 5. Thus it appears that the toxicity was more closely related to the amount of tar per painting than to the cumulative

Table 1

Group No.	No. mice	Dose at each application (mg)	No. dead after: 1 month	4 months	8 months	12 months	No. tumours at site of painting: Papilloma	Carcinoma
1	24	5	0	0	2	2	1	0
2	24	10	0	4	6	7	0	0
3	24	20	0	0	2	2	0	0
4	24	40	0	0	4	6	0	0
5	24	80	0	1	9	10	1	0
6	24	160	10	12	16	17	0	1
7	24	20–80	0	0	6	8	0	2
8	24	20–80	0	3	7	9	0	0
9	24	10–80	0	0	4	6	0	0
10	24	Acetone-water	0	0	4	8	0	0
11	50	Control	0	1	6	9	0	0

amount of tar received over a longer period. The pattern of mortality in the other groups did not significantly differ from that in the control groups.

Histological examination of the tissues of the mice which died early revealed widespread hæmorrhage in a variety of tissues, the liver, spleen and lymph nodes, with atrophy of the lymphoid elements in the latter. There were benign and malignant tumours of the skin at the site of painting and there was an increase in the invasive character of these tumours as the total dose of tar at each application was increased.

The results therefore show that a dose of whole tobacco tar of up to 40 mg, though capable of inducing neoplastic response at the site of application, has no immediately toxic effect on this strain of mice. Toxic effect could be discerned at a dosage level of 80 mg per application and at 160 mg the mortality reached a level of about 50 per cent within the first months. The animals that survived the early months proved to be reasonably viable during the remainder of the experiment.

I thank the Tobacco Research Council for financial support and for the supply of tobacco tar, and Dr. T. Ghose for help with the histological work.

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Absence of Kynurenine in Hair of Patients with Scleroderma

ONE of the diseases in which there may be a defect of tryptophane metabolism is scleroderma. This belief is based on such experimental evidence as the increased excretion of the intermediate metabolites of tryptophane after oral administration of large doses of trytophane[1]. One of these metabolites, kynurenine, has been identified in water extracts of rat hair[2]. It seemed of interest, therefore, to determine whether or not kynurenine could be found in the hair of patients with scleroderma. Kynurenine has not been reported in previous analyses of normal human hair[3,4].

Hair clippings were obtained from five patients with scleroderma of the progressive systemic sclerosis type. Hair clippings were also obtained from three normal subjects. All the specimens were handled identically. The hair was pulverized in a jar mill with stainless steel balls, and the resulting powder was then defatted by refluxing with ether for five hours. The powder was then extracte with distilled water at 50° C overnight, evaporated *in cuo* to concentrate the extract, and then spotted on 'W atman No. 1' chromatograph paper.

De ending chromatograms were run, using commercially obtained kynurenine as well as rat hair extracts as controls. The solvent used consisted of *n*-butanol, methanol, benzene and water in proportions of 2 : 4 : 2 : 2 (ref. 2). Kynurenine was detected by fluorescence under long-wave ultra-violet light, by ultra-violet absorption spectra after elution from the paper, and by spectrophotofluorometry after elution.

No kynurenine could be detected in normal human hair or in the hair from patients with scleroderma, although it was found that amounts as low as 0·05 γ of pure kynurenine could be detected on chromatograms by visible fluorescence.

It appears, therefore, that patients with scleroderma, in spite of an apparent metabolic defect which may result in the presence of increased amounts of kynurenine in the urine, do not deposit this amino-acid in the hair.

This project was supported by U.S. National Institutes of Health research grant *RG* 6868 (*C*1).

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Cell Differentiation and the Primary Lesion in Mouse Mammary Carcinogenesis

IN high cancer strains of mice carrying the milk agent[1], the formation of hyperplastic alveolar nodules, the preneoplastic nature of which was demonstrated by De Ome *et al.*[2], is at present considered as the earliest sign of carcinogenesis. These nodules appear in adult mice, usually after one or several pregnancies, and therefore much later than the initial malignant transformation of the original mammary cell. Experimental data gathered from tissue culture tests now appear to locate this initial lesion in some of the alveolar cells which differentiate from the elementary duct system of the virgin mouse.

When mouse mammary fragments are explanted as organ cultures on a nutritive medium, the morphology and to some extent the functional activity of the glandular tissues can be maintained only in the presence of very specific hormones[3,4]. These hormones normally control mammary differentiation in the animal and their combination varies according to the stage of pregnancy[5]. In early pregnancy, the prevailing hormones are ovarian (œstradiol; progesterone) and hypophyseal (growth hormone; prolactin). In late pregnancy and lactation, prolactin and adreno-cortical hormones are principally involved. Whereas the first combination induces lobulo-alveolar differentiation and active cell replication, the second mostly stimulates the secretory function[6,7].

When attempts are made *in vitro* to reverse the physiology of the mammary cell, for example, by placing lactating explants in the presence of hormones which stimulate growth, extensive necrosis of the glandular tissues occurs. If, however, explants of early pregnancy are exposed to adrenal hormones and prolactin, cell multiplication stops and vesicular structures are formed[8]. In other words, the death of the mammary tissues which results from attempts to return the cells to an earlier stage of differentiation does not necessarily occur when the differentiation process is accelerated rather than reversed. These findings suggest a physiological irreversibility in the gradual differentiation of the mammary cell towards maturity and functional activity.

Evidence for this hypothesis of irreversibility was recently obtained from monolayer cultures of normal mouse mammary epithelium. As opposed to the organotypic cultures mentioned in the preceding paragraphs, where fragments of organs explanted *in vitro* as a whole grew in an organized fashion, the monolayer cultures were derived from a total enzymatic dissociation of the pooled glands from one mouse. The glands were minced in a solution of collagenase (1 mg/ml. of balanced salt solution) and dissociated at 37° C in about 45 min with moderate agitation. A mild centrifugation then eliminated most of

the adipose tissue, which remained with the supernatant. The epithelial and stromal cells collected in the pellet were re-suspended in the culture medium (Eagle's basal medium, 10 per cent calf serum and 0·5 per cent lact-albumin hydrolysate). A final suspension of 15–20 × 10^4 cells per ml. was then distributed in 10 ml. aliquots into *T*60 flasks. The free cells grew at random on the glass surface with seldom any evidence of tissue organization. Small, isolated colonies began to form after 24 h.

The results of 45 attempts to grow the mammary tissues from 3 agent-free strains of mice (*C57BL*, *ZBC*, *Ax*) and one high-cancer strain, *R*111, are summarized in Table 1.

Table 1. Comparative Cultivation Time of Mouse Mammary Tissues in Monolayer Cultures

Physiological stage	Agent-positive *R*111		*C*57		Agent-free *ZBC*		*Ax*	
	Weeks	No. exp.	Weeks	No. exp.	Weeks	No. exp.	Weeks	No. exp.
Virgin	—	—	10	3	4–5	2	10	3
Early pregnancy*	3	1	4–5	8	4–6	3	—	—
Late pregnancy	2	2	2–3	2	2–3	11	—	—
Lactation	1	1	1	1	1	6	—	—
Resting	—	—	—	—	5	2	—	—

* In early pregnancy the cultures underwent 2–4 transfers in a 1 : 2 ratio. Up to 6 transfers could be made with mammary tissues from virgin *C*57 and *Ax* mice.

It can be seen that when taken in lactation or at the end of pregnancy the mammary cells are extremely labile and cannot be maintained for periods longer than one week if lactating, or a maximum of two weeks if prelactating. They do not multiply, but necrose and autolyse. At these stages, the bulk of the explanted cells come from the alveolar epithelium; other cell types, although present, can scarcely be found in the cultures.

For cells from early pregnancy the maintenance period is extended to 4 or 5 weeks. Most of the epithelial cells are still of alveolar origin, but fibroblasts, hystiocytes and lymphoid cells account for roughly one-fourth of the total volume. All cells replicate actively and transfers are necessary. After the fourth transfer, however, the mitotic activity subsides; foci of necrosis appear and the same autolytic phenomena as observed in the former series occur. These observations are comparable in all the strains of mice observed.

The longest sub-cultivation (10 weeks) was obtained in the mammary cells from 3 month-old virgin mice. The cultures then present a mixed population of epithelial cells and fibroblasts, all replicating actively. The stroma cells are the most numerous, but the epithelium, easily detected as circular patches of cells, is carried throughout the successive passages.

These results, consistently reproducible, show that the mammary cell gradually loses its replicating properties while progressing towards functional activity and complete differentiation. Even though the culture medium used in the foregoing experiments is poor in specific hormones, it is unlikely that early autolysis of the most differentiated cells expresses a state of hormone dependency. In two instances, one with mammary tissues from early pregnancy, the other from late, adding the proper hormones to the medium did not increase the length of the cultivation period but rather shortened it by at least two days. Recently data have been compiled by Hayflick[9] to indicate that somatic cell strains in general have a limited life *in vitro*, unless they undergo substantial alteration. For human diploid strains from a variety of tissue sources the doubling potential *in vitro* appears to be finite, and to centre around 50. It might be expected that tissues like the mammary epithelium, which normally undergo a cyclic differentiation from stem cells, would exhibit a greatly shortened life-span and reduced doubling potential proportionate to the length of their cycle. This expectation appears to be borne out by the mouse mammary epithelium in our cultures, the *in vitro* life-span of which is even related to its position in the cycle at time of explantation.

It is now possible to interpret the results from earlier experiments with organ cultures: it has been observed that when mammary fragments from agent-positive *R*111 mice early in their first pregnancy are stimulated *in vitro* by ovarian and hypophyseal hormones, foci of alveolar hyperplasia eventually develop. Such foci have been found to maintain some mitotic activity even when exposed to the antagonistic action of adrenal hormones and have therefore acquired hormone independency[8]. They are also a site of viral particle (*B* type) production as evidenced by electron microscopy[10]. Alveolar hyperplasia and presence of particles are the basic characteristics of the preneoplastic lesion of De Ome. However, whereas the nodules are observed *in vivo* only in 4–5-month-old mice, the tissue culture technique shows that they really are initiated as early as the first pregnancy.

The only cells which in this first pregnancy can originate foci of hyperplasia are those which have acquired the MTA during the suckling period. Under the initial stimulation of the specific hormones they enter a phase of active replication most favourable to virus production. The agent-free cells normally proceed in their differentiation to functional activity; however, even though such cells can later become infected and be a site of virus multiplication, they will not originate a new centre of hyperplasia because they already have differentiated beyond their capacity to replicate. In conclusion, it is only in the early beginnings of pregnancy that the actively dividing mammary cells can be modified by the MTA and build up the preneoplastic lesion.

This work was supported by a U.S. Public Health Service grant *CA*–04588 from the National Cancer Institute.

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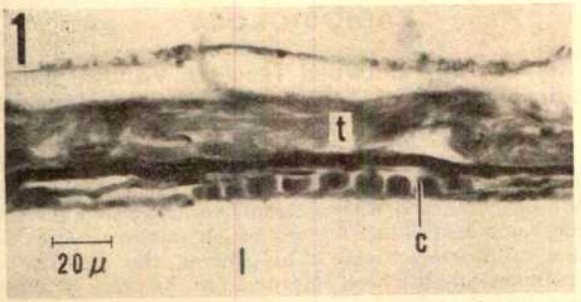
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ANATOMY

A Hadal Fish (*Bassogigas profundissimus*) with a Functional Swimbladder

During the Danish Galathea Expedition (1950–52) a specimen of the brotulid fish, *Bassogigas profundissimus* (Roule, 1913), was caught in the Sunda trench (Station No. 466, depth 7,160 m); it measured 157 mm in standard length. The gear used was a herring otter trawl, which was operated at the bottom for a period of 2 h. The bottom material consisted of bluish mud, and the bottom temperature was 1·5° C. The uphaul of the trawl took 4 h.

Unfortunately, it is not known for certain whether the present specimen was actually caught at the bottom or at a pelagic depth, as the gear could not be opened and closed again at any specific depth. Fifteen genera of fish, known to be pelagic, were also represented in the trawl. On the other hand, reasons have been put forward for considering the specimen benthic[1]. In summary: (1) The bottom material and benthic invertebrates in the trawl showed that the trawl did work at the bottom. (2) The stomach contained specimens of the Isopod genus *Eurycope*, of



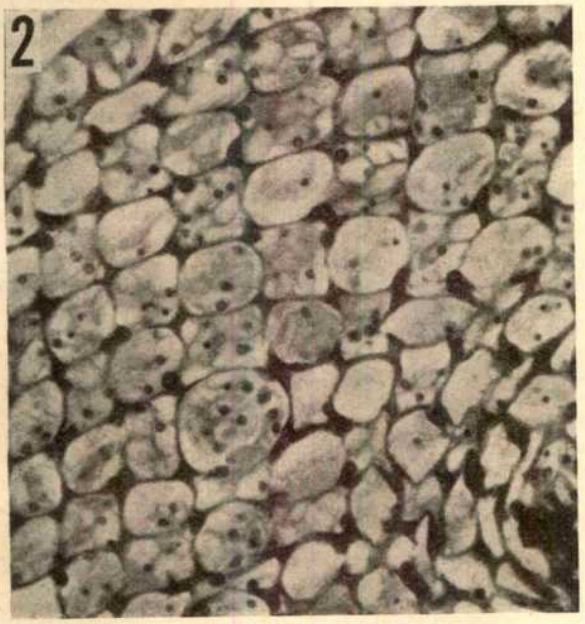


Fig. 1. Section (8μ) through resorbent area in swimbladder wall. *c*, resorbent capillary; *l*, lumen of swimbladder; *t*, tunica externa of swimbladder wall. (Hæmatoxylin and eosin, × *c.* 390)

Fig. 2. Section (8μ) through part of rete mirabile. (Hæmatoxylin and eosin, × *c.* 390)

which almost all species are benthic. (3) The present species is now known in four specimens, three from the Atlantic and one from the Indian Ocean, from stations varying in depth from 5,610 m to 7,160 m. On all four occasions it was proved that the trawl had reached the bottom. The fact that *B. profundissimus* was never caught in the many abysso-pelagic and hado-pelagic hauls also indicates that it lives on the bottom. It should perhaps be mentioned that from the Galathea Expedition alone, about 20 pelagic hauls were made at depths between 5,000 and 7,000 m.

On dissecting this specimen, it was found to contain a closed swimbladder, 15 mm long, and situated in the usual position. (It was not ruptured or forced out into the œsophagus and mouth, which is often the case with other fish.) In the ventral wall of the swimbladder was found the gas gland, the long winding rete mirabile, and the gas gland ganglion. The well-developed gas gland lies in the submucosa (terminology according to Fänge, 1953, ref. 2), in the central part of the ventral wall. It appears to consist of medium-sized cells[3] with intercellular capillaries, but owing to its brittle consistency it was badly damaged during cutting.

The gas gland ganglion is situated in the ventral wall, slightly behind the gas gland. The single unipolar rete is situated in the submucosa of the ventral wall; owing to its winding course, the length of the rete is approximately equal to that of the swimbladder. The capillaries of the rete form a typical chequer-board pattern[4] (Fig. 2). There are approximately 220 capillaries. An area with resorbent capillaries is found in the dorso-caudal part of the swimbladder wall (Fig. 1). There is no histological evidence of regression, and since the swimbladder appears perfectly normal as regards morphology it is, possibly, also functional.

In pelagic deep-sea fishes living below 1,000 m the swimbladder is either missing or regressed, but in at least half of the benthic deep-sea fishes living below a depth of 2,000 m, normal swimbladders with very long retia mirabilia are found[3]. The deepest-living species with a well-developed swimbladder known hitherto is the brotulid *Bassozetus taenia*, which has a known depth range of 4,575–5,610 m. The depth range of *Bassogigas profundissimus* may now be regarded as 5,610–7,160 m (ref. 1). The length of the rete mirabile in *Bassozetus taenia* is 25 mm, while that of the present species is 15 mm; this means that when the number of capillaries is about 220, the total length is 3·3 m.

The major constituent of the gas in the swimbladder of deep-sea fishes is oxygen, which forms 80–90 per cent of the volume[5–7]. At a depth of 5,000 m the density of oxygen is about 7/10ths that of sea-water, while at 7,200 m its density is equal to that of sea-water[3]. Thus, the swimbladder of *B. profundissimus* can scarcely be considered to have any significance as a hydrostatic organ. However, as the oxygen content decreases rapidly at the last few metres near the bottom[8], the swimbladder might function as an oxygen store, as proposed by Jones[9] and Fänge[2].

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IMMUNOLOGY

Rabbit Gamma-globulin Allotypes

ALLOTYPIC specificity of rabbit γ-globulins, differentiating individuals of the same species, was first described by Oudin[1]. This kind of specificity is defined by means of reactions between rabbit iso-precipitating sera and the serum of individuals of the same species. The iso-precipitating rabbit sera used for differentiating rabbit γ-globulins were obtained in two ways: (1) by injecting rabbits with immune complexes composed of heterologous antigen (human, equine, sheep and hen erythrocytes) and their corresponding rabbit immune antibodies; (2) by injecting rabbits with suspensions of rabbit globulins obtained by precipitation with aluminium potassium sulphate. In both cases it was necessary to take into account differences in γ-globulin characteristics between the rabbit donor of hetero-agglutinins or globulins and the rabbit donor of iso-precipitins.

By means of iso-precipitating sera obtained in this way, five allotypes of rabbit γ-globulins, designated *a*, *d*, *e*, *f*, *g*, were distinguished. Further investigation showed that these allotypes are hereditary, and that the types *a*, *f* and *g* form an independent genetic system controlled by three autosomal allelomorphic genes.

This conclusion was based on the examination of 1,250 rabbit sera with 15 iso-precipitating sera containing antibodies against the allotypic characteristics *a*, *d*, *e*, *f* and *g*, and statistical analysis of the frequency with which the

genes governing the appearance of these characteristics occurred in the rabbit populations concerned. Their hereditary transmission was also investigated.

The material consisted of sera from four rabbit populations, in which different frequencies of characteristics *a*, *f* and *g* were found, depending on the choice of animals used for breeding. The largest 'mixed' group, consisting of rabbits purchased randomly from different suppliers, was therefore considered to be the most representative. The frequency distribution of characteristics *a*, *f* and *g* within the four populations is shown in Table 1.

Table 1. Frequency of the Allotypic Characteristics *a*, *f* and *g*

Source	No. rabbits	Allotypic characteristics of γ-globulins *a*	*f*	*g*
Mixed	842	0·156	0·985	0·058
Albinos	210	0·094	0·994	—
Path. dept.	68	0·583	0·655	0·148
Own stock	130	0·422	0·883	0·015

As can be seen in Table 1, the albino population, which was composed of rabbits from three different farms, is more homogeneous than the remaining populations, in which the frequency of the characteristics *a*, *f* and *g* varies considerably.

On the basis of the frequencies of characteristics *a*, *f* and *g*, the expected frequency of allotypes in the *afg* system was calculated for the mixed rabbit population, in accordance with the theory of Bernstein. The observed and expected data are compared in Table 2.

Table 2. Frequency of Allotypes of the *afg* System in a Mixed Population of Rabbits compared with Expected Values

Values	Allotypes of the *afg* system *aa*	*af*	*ag*	*ff*	*fg*	*gg*
Expected	0·007	0·144	0·005	0·789	0·052	0·001
Observed	0·0067	0·146	0·0052	0·783	0·056	0·001

Assessed by means of the χ^2 criterion, the difference between the observed and expected frequencies of allotypes of the *afg* system is not statistically significant.

Hereditary transmission of the allotypes of the *afg* system was investigated in 130 young rabbits, offspring of 29 rabbit pairs with known allotypic characteristics. The results demonstrated the hereditary character of the rabbit allotypic γ-globulins and confirmed the existence of an independent *afg* genetic system controlled by three autosomal allelomorphic genes.

Comparison with earlier findings[2] indicates that characteristics *a*, *f* and *g* correspond to characteristics *A*4, *A*5 and *A*6 determined by three allelomorphic genes, described earlier[3].

In Table 3 the frequency of occurrence of rabbit γ-globulin allotypes is summarized, using both the present nomenclature and that proposed by others.

Table 3. Frequencies of Rabbit γ-Globulin Allotypes

Nomenclature according to: Oudin	Dray	Dubiski	Grodecka	Dray, Dubiski, Kelus, Lennox and Oudin
a − 0·97	II − 0·952	*F* − unknown	*f* − 0·985	*A*4
g − 0·10	I − 0·246	*A* − 0·111	*a* − 0·156	*A*5
f − 0·12	—	*C* − 0·087	*g* − 0·058	*A*6

These observations, which show that the differentiation of rabbit serum γ-globulins is genetically conditioned, may serve as the basis of a method for assessing the purity of breeds of experimental animals. Moreover, the results confirm the fact of the differentiation of serum proteins, hitherto only assumed as a possible cause of some cases of post-transfusion shock in human beings.

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RADIOBIOLOGY

Relationship between Nuclear Volume and Radiosensitivity of Different Cell Types in Gemmæ of *Marchantia polymorpha* L.

The radiosensitivity of various species of plants has been investigated for many years[1,2]. Much recent work has been concerned with attempts to discover if there is some common cellular feature which might serve as an index of radiosensitivity. In general, the nucleus (or its components) has been considered as the primary object determining radiosensitivity[3–7]. One method of investigation has been to irradiate a number of different species and then to correlate the responses of the irradiated plants with inherent particular nuclear parameters of apical meristem cells of unirradiated control plants. In general, plants with the smaller nuclei have been found to be more resistant to radiation than those plants with the larger nuclei. Indeed, where the species being compared differed in chromosome number, Sparrow *et al.*[8] have shown that the quotient of the nuclear volume divided by the chromosome number (the interphase chromosome volume) is a more accurate index of radiosensitivity than nuclear volume alone. Thus chromosome number is important in determining radiosensitivity where variation in numbers exists.

The gemmæ of *Marchantia polymorpha*, a bryophyte, are convenient well-defined organs with features which make it possible to examine the radiosensitivity of particular cells within the structure. Normally, one of the two apical cells at each end of the gemma produces the new thallus; but in the absence of a functional apex other cells in the gemma are capable of producing new thalli. Thus it is possible to examine the radiosensitivity of the particular cells of this multicellular vegetative structure by noting the position of the vegetative outgrowths produced by the gemmæ.

Gemmæ were collected from stock plants and prepared for aseptic culture according to the procedures previously published[9]. Some of the gemmæ were exposed in plastic containers with a wall thickness of 1 mm to various amounts of cobalt-60 γ-rays and all gemmæ were allowed to grow aseptically for two weeks on inorganic agar medium. The light intensity at the level of the Petri plates was 1,000 ± 250 ft.-candles, the photoperiod was 18 h, and the temperature was 78° ± 2° F. At the end of the first week of growth the original shapes of the gemmæ were still discernible and the gemmalings were scored for apical and/or non-apical survival. At the end of the second week the entire gemmæ were scored as 'survivors' or 'non-survivors' since a non-apical outgrowth is often not discernible until this time. The 0 per cent survival point was determined for the apical and vegetative cells from the exposures at which no survival of each type was observed. Nuclear volumes of 20 apical and 80 non-apical vegetative cells were obtained according to the previously published method[8]. The energies absorbed by the two types of nuclei at the lethal exposure were then calculated and compared (Table 1).

The average nuclear volume of the apical cells was 98·9 ± 4·9 μ^3; the average nuclear volume of the non-apical vegetative cells was 54·7 ± 3·3 μ^3. The 0 per cent survival

Table 1. Summary of Nuclear Volumes for Vegetative Cells of a Gemma and Calculated Amounts of Energy Absorption at the Lethal Exposure

Cell type	Nuclear volume (μ^3)	Lethal exposure (kr.)	Energy absorbed per nucleus at lethal exposure (× 10^6 eV)*
Apical	98·9 ± 4·9	29	164·9 ± 8·2
Non-apical†	54·7 ± 3·3	55	173·0 ± 14·8

* Based on an average value of 32·5 eV per ion pair and 1·77 ionizations per cubic micron of wet tissue per roentgen (Table 2, ref. 11) multiplied by the exposure in r.
† Rhizoid initial cells are excluded since they are not capable of producing a new thallus.

exposure was 29 kr. for the apical cells and 55 kr. for the vegetative cells. It is apparent that those cells with the larger nuclei are more sensitive to radiation[5,8]. In fact, the nuclei of the apical cells, which are about twice the volume of the nuclei of the vegetative cells, are about twice as sensitive as the vegetative cells to the radiation when the exposure is expressed in kr. The calculated energy absorbed at the 0 per cent survival point for each type of cells was $164{\cdot}9 \pm 8{\cdot}2 \times 10^6$ eV for the apical nuclei and $173{\cdot}0 \pm 14{\cdot}8 \times 10^6$ eV for the nuclei of the vegetative cells; there is no statistical difference between these two values. Thus the apparent difference in tolerance disappears when energy absorption in the nuclei at the 0 per cent survival exposure is used as a measure of radiosensitivity; this observation for the radiosensitivity of cells within a plant organ is consistent with previous findings for the comparative radiosensitivity of a number of different higher plants at the lethal exposure[8,10]. The fact that the energies absorbed at the lethal exposure by the apical and vegetative cells are not significantly different from each other is evidence for the idea that it is not the relative number of deleterious events which leads to the same end point but rather the absolute number[8]. The nature of these deleterious events remains to be determined, however. Further work on the radiosensitivity of gemmæ of *Marchantia* is in progress and will be reported in detail elsewhere.

This work was supported by the U.S. Atomic Energy Commission.

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Relationship between Chemical Structure and the Extent of Protection against Ionizing Radiation given by Alcohols to *Salmonella typhimurium LT2*

Burnett *et al.*[1] concluded that methanol, 2-propanol, 1-butanol, 2-methyl-1-propanol, 2-methyl-2-propanol, 1,2,3-propanetriol, 1,2-propanediol and 1,2-ethylenedioxydiol are about as effective as ethanol in affording protection against radiation. The results given here permit more definite conclusions to be made.

The radiation source was electrons from an 8-MeV linear accelerator in most of the experiments; but in some cases a Solus–Schall constant potential X-ray unit operating at 250 kV was used.

Pre-irradiation culture procedures. The bacteria were cultured overnight on a nutrient agar slope (Oxoid medium) at 37° C. A further culture was then made on a slope for approximately 2 h at 37° C prior to collecting, first in 0·1 M phosphate buffer *p*H 7·0, followed by one washing and re-suspension in *tris* buffer *p*H 8·0 (6 g of sodium hydroxide dissolved in 70 ml. of 1 M *tris*-hydroxymethylaminomethane, *p*H adjusted to 8·0 with conc. hydrochloric acid and made up to 1 l.). The density of the suspension was gauged by means of a nephelometer. A suitable aliquot was re-suspended in *tris* buffer to act as control, while another aliquot was re-suspended in *tris* buffer containing the alcohol under test at 0·8 M concentration.

Irradiation. The irradiation of the liquid suspension was carried out under fully oxygenated conditions in vessels permitting the passage of a generous stream of air. (For a description of these vessels, see Howard-Flanders and Alper[2].)

Post-irradiation culture procedures. Following irradiation, suitably dense suspensions were plated out on to nutrient agar (Difco medium) by means of a platinum tipped dropper delivering 0·002 ml. per drop, and cultured overnight at 37° C. The resulting colonies were then counted (three plates each for control and test at each dose level) giving counts of the order of 100 colonies per plate. Irradiation was carried out over six intervals covering the range 0–50 krads.

The alcohols tested were pentane-1,5-diol, butane-1,4-diol, butane-1,3-diol, glycerol, propane-1,2-diol, propane-1,3-diol, erythritol, ethanediol, methanol, ethanol, isopropanol, mannitol and *n*-propanol. In addition to these, primary, secondary and tertiary butanol, isobutanol, amyl and tertiary amyl alcohol, allyl alcohol and propargyl alcohol were investigated, but proved too toxic to be included in the radiation trials.

For control and test, respectively, the logarithm of the survival fraction was plotted against the radiation dose for each alcohol examined. The protection factor was then evaluated from these curves by comparing the radiation dose giving a specific survival fraction in the presence of the alcohol, with the dose giving numerically the same survival fraction in the control. This is essentially the method used by Kelner[3].

In the case of pentane-1,5-diol, erythritol and mannitol, which were tested on the same batch of organism, no control curves were available. The protection factor for these three compounds was obtained by using a control curve derived from the mean of 16 control curves obtained in the other experiments in this series.

Fig. 1 shows the results of plotting the protection factor found for each alcohol against carbon chain-length.

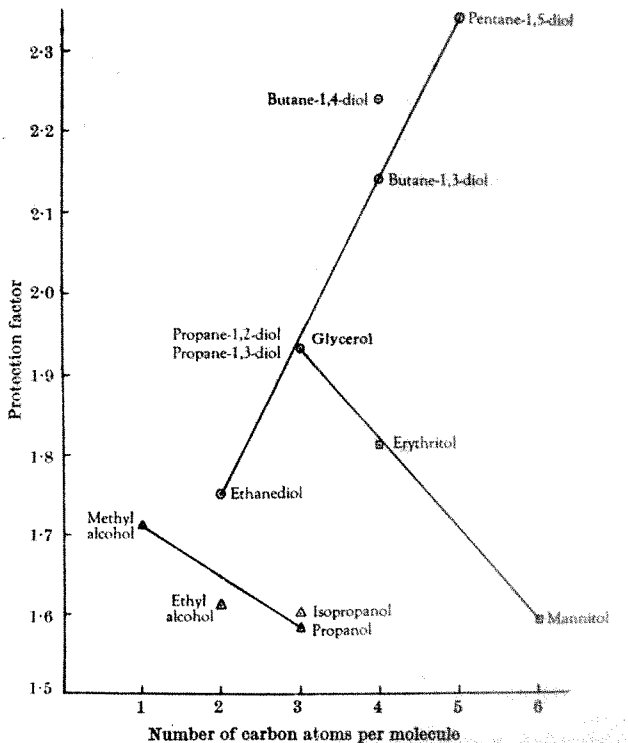


Fig. 1. The protection factor for each compound, plotted against the number of carbon atoms per molecule

The following points emerge from Fig. 1: (1) There is a regular increase of protection with increasing chain-length in the case of the diols. (2) With the polyhydric and monohydric alcohols, the tendency is for a decrease in protection with increasing chain-length. (3) The diols are a better class of protector than either the polyhydric or the monohydric alcohols.

It could be inferred from this that a balance must be struck between the hydrophilic and the lipophilic features of the molecule in order to achieve optimum protection, and that such a balance is achieved in the case of the diols. This in turn may be a reflexion of a favourable partitioning of these compounds between aqueous and lipoid systems in the organism whereby oxygen is excluded or reduced. This would lead to diminished oxygen-dependent radiosensitivity of the organisms.

Ebert and Hornsey[4], investigating the effect of a number of gases on the irradiation of bean root (*Vicia faba*), were able to show a parallel between lipid–water partition coefficients and the effectiveness of these gases in reducing radiosensitivity. In order to explain their observations, they offered the oxygen displacement principle referred to here.

The small range of values for the protection factors in the case of the monohydric alcohols accords with the results obtained by Burnett *et al.*[1].

This work was carried out at the Medical Research Council Experimental Radiopathology Research Unit, Hammersmith Hospital Postgraduate Medical School, London.

I thank my colleagues for their advice and support.

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Recovery from Sub-lethal X-ray Damage of Mammalian Cells during Inhibition of Synthesis of Deoxyribonucleic Acid

The ability of mammalian cells to recover from sub-lethal X-ray damage was first demonstrated by Elkind *et al.*[1], who found that the effect of two doses separated by an interval of time was less than the effect of the same total dose given as a single exposure. This recovery phenomenon has been confirmed *in vitro* and *in vivo* in a wide variety of mammalian cell types[2–5]. These investigations characterized the dependence of radiation sensitivity on the interval between divided doses as having an initial increase to a maximum followed by a minimum and a subsequent new maximum plateau. The nature of the recovery curve between the fractionated doses suggested that the curve may be the result of two opposing reactions and that one reaction at least may be dependent on the metabolic state of the cell. Hence, the recovery curve may be subject to alteration when a major metabolic process is inhibited. We recently reported that the repair processes were operative under conditions in which DNA thymine was partially replaced by 5-bromouracil[5]. Reported here are the results of experiments designed to test whether the recovery phenomenon requires uninterrupted DNA synthesis during the interval between fractionated doses. In these experiments 5-fluorodeoxyuridine (FUDR) was used as an inhibitor of DNA synthesis.

HeLa *S*–3 cells (obtained from Dr. P. I. Marcus, Albert Einstein Medical College, New York) were grown in monolayers on a flat glass surface in French square bottles using Eagle's medium supplemented with 10 per cent calf serum. Four–six-day stock cultures were trypsinized and plated in 60-mm plastic Petri dishes (Falcon Plastics) in 4 ml. of medium containing FUDR (4×10^{-7} M). After about 14 h of incubation, cultures were irradiated for inactivation studies. The dose rate was 170 rads per min (200 kVp., 15 m.amp, half-value layer 0·8 mm copper). All irradiations were performed at room temperature. FUDR was kept in the medium for 22 h and replaced with the medium containing thymidine[6] (4×10^{-5} M). Cell plating efficiencies were generally 0·6–0·7 for control cells (no FUDR) and 0·3–0·4 for cells exposed to FUDR. The extent of incorporation of deoxycytidine-^{3}H into DNA was considered a measure of DNA synthesis. The DNA was isolated for liquid scintillation counting by a modification of the procedure of McIntire and Sproull[7].

Exposure of bacterial and mammalian cells to FUDR inhibits the synthesis of DNA[6,8,9]. This inhibition was tested for HeLa *S*–3 cells under our cell culture conditions in two ways. As shown in Fig. 1, FUDR (4×10^{-7} M) inhibited rapidly and almost completely the utilization of tritiated deoxycytidine for DNA synthesis. Inhibition of DNA synthesis by FUDR was also demonstrated by the autoradiographic studies summarized in Fig. 2. The percentage of cells able to utilize tritiated thymidine (15 min pulse) for DNA synthesis increases with the time interval of exposure to FUDR (Fig. 2). The results shown in Fig. 2 were interpreted in terms of the mitotic cycle of the untreated or control cells. FUDR inhibited DNA synthesis in cells which were in the *S* period and prevented other cells from entering the *S* period. After the periods of *G*–2 plus *M* plus *G*–1, almost all the cells should be ready to enter the *S* period when thymidine is supplied. The time for this maximum labelling was about 14 h (Fig. 2).

In order to test whether or not the repair process requires uninterrupted DNA synthesis, the fraction of clones surviving a 558-rad dose administered in two equal doses was plotted as a function of the interval between doses. The recovery pattern for cells not exposed to

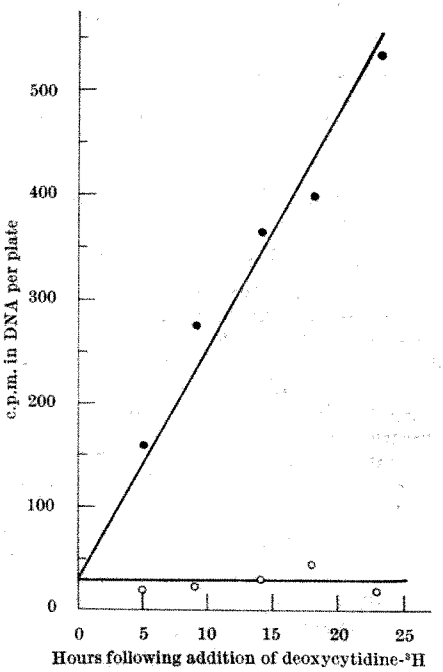


Fig. 1. Incorporation of tritiated deoxycytidine into DNA of HeLa *S*–3 cells. HeLa *S*–3 cells (approximately 5×10^5) were incubated in media containing and lacking FUDR (4×10^{-7} M) and supplemented with tritiated deoxycytidine (0·05 μc./ml.). After various intervals the DNA was isolated from the cells and its radioactivity determined. ●, Control cells; ○, cells treated with 5-fluorodeoxyuridine

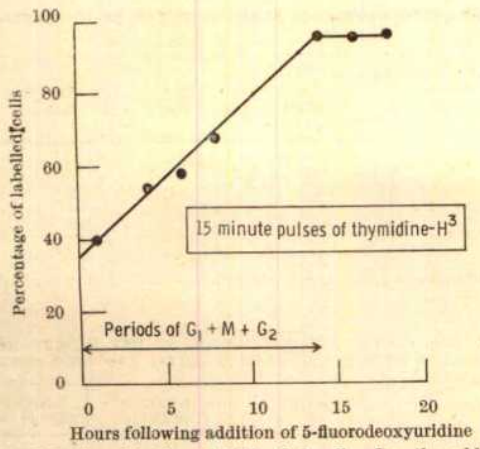


Fig. 2. Labelling kinetics of HeLa *S*-3 cells after the addition of FUDR (4×10^{-7} M). The number of labelled cells was determined at various times after the addition of FUDR by 15-min pulse labelling of tritiated thymidine (0·05 μc./ml.). Autoradiographs were made and the number of labelled cells scored

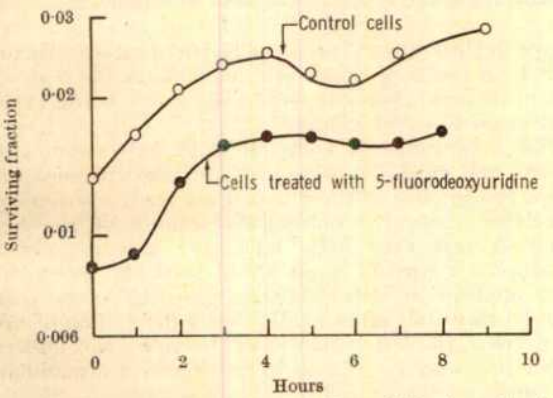


Fig. 3. Fraction of clones surviving a total dose of 558 rads as a function of time-interval between two equal doses.[1] Lower curve (solid circles) refers to the cells treated with FUDR during the fractionation studies. Cells were exposed to FUDR (4×10^{-7} M) for 14 h before the first radiation dose of 279 rads. The FUDR-containing medium was replaced by medium containing thymidine (4×10^{-5} M) 8 h after the first radiation dose

FUDR is shown in the upper curve (Fig. 3). The curve has a maximum at about 4 h, followed by a minimum at about 6 h. Below (filled circles) is the recovery curve for cells treated with FUDR for 14 h before X-irradiation. After this radiation exposure the cell population should be at the end of *G*–1 period or immediately before the *S* period with the exception of those cells which were in the *S* period at the start of exposure to FUDR. Thus, during the interval between doses about 60 per cent of the cell population was arrested at the end of *G*–1 or early part of the *S* period. A comparison of the curves in Fig. 3 demonstrates that the principal features of the recovery pattern are similar. Consequently, these experiments demonstrate that the mechanism of the repair process does not require uninterrupted DNA synthesis. The well-defined structure of the initial maximum in the recovery curve was demonstrated under conditions in which DNA synthesis was almost completely inhibited.

Cells exposed to FUDR under the conditions used in Fig. 3 appear to be more radiosensitive since the recovery curve for these cells lies below the control curve. This may suggest enhanced radiosensitivity in the late *G*–1 stage, and this point will be explored more thoroughly.

Alteration of the initial recovery peak by temperature changes and the use of actinomycin *D*[10,11] suggest a dependence of the recovery curve on the metabolic state of the cell. We are at present attempting to elucidate mechanisms underlying repair by experiments using inhibitors of nucleic acid and protein synthesis and studies using a population of synchronized cells.

We thank A. Perez and M. Steinglass for their assistance. This work was supported in part by grant *AT* (30–1)–910, from the U.S. Atomic Energy Commission.

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BIOLOGY

Boron Toxicity in *Hevea brasiliensis*

It has recently been found in Malaya that the rubber tree, *Hevea brasiliensis*, when growing on certain soils, can accumulate sufficient boron to cause the appearance of boron toxicity symptoms on the leaves. The soils on which evidence of boron toxicity has been found are the recently deposited alluvial clay soils on the west coast of Malaya, on which approximately 20 per cent of the rubber in Malaya is grown, and certain coarse sandy loams derived *in situ* from a very acid granite where only a shallow layer of top soil overlies the decomposing parent material. The main leaf symptoms of boron toxicity on rubber, common to many plants, are the development of necrotic spots in the interveinal areas, particularly around the leaf margin, and of a tip and marginal necrosis which may take the form of a narrow regular band or may result in a large area of the leaf, at the distal end, becoming necrotic (Fig. 1).

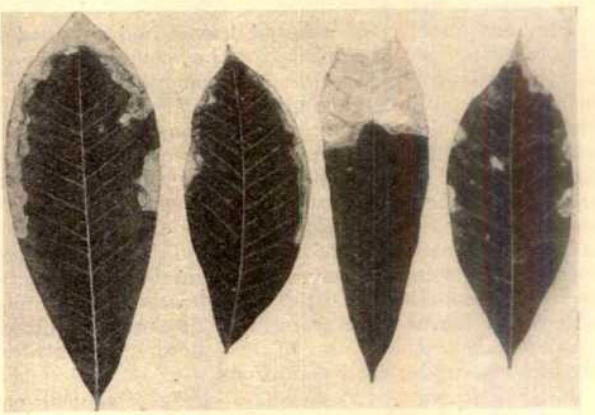


Fig. 1. Boron toxicity symptoms shown by two leaflets from clone *PB* 86 (left) and clone *G* 1 (right); trees growing on coastal alluvial clay soil

Sand culture experiments. A simple experiment involving the daily application of six levels of boron (applied as H_3BO_3) to established rubber seedlings, eight months old, was carried out to determine the effects of excess boron supply on leaf development and the leaf concentration of boron associated with toxicity symptoms. The first symptoms were observed three weeks after

Table 1. Effect of Different Levels of Boron, supplied as H_3BO_3, to Rubber Seedlings growing in Sand Culture on the Concentration of Boron in the Leaves

Boron treatment (p.p.m.)	Top whorl*		Second whorl*		Top whorl † entire lamina
	Green, central part of lamina	Necrotic edge of lamina	Green, central part of lamina	Necrotic edge of lamina	
0·36	41	No sample	65	No sample	20
0·72	142	152	217	241	36
1·80	253	216	337	477	70
3·61	290	459	491	609	104
7·21	385	365	329	924	137
14·43	560	1,416	711	1,251	221

Leaves were sampled from top and second whorls 31 weeks* after first application of boron, and top whorls again after 52 weeks†. Boron concentrations expressed as p.p.m. on a dry weight basis.

commencement of treatment with 14·4 p.p.m. boron; the concentrations of boron in the leaves are shown in Table 1. Leaves from the top and second whorl on the unbranched main stem were sampled separately after 31 weeks: at the second sampling after 52 weeks it was only possible to collect leaves from the top whorl since much defoliation of the older leaves, including the leaves on the second whorl, had taken place. Some branching from axiliary meristems on the main stem occurred particularly on plants treated with the highest level of boron; on these plants the apical meristem appeared to become dormant but was not killed.

Tip and marginal necrosis of the leaf occurred when the leaf boron concentration was approximately 140–150 p.p.m. The accumulation of boron in the necrotic extremities of the leaf which were analysed separately from the central portion of the lamina, and the greater accumulation in the older second whorl leaves, are in accord with previous findings[1].

In another experiment excessive accumulation of boron and development of marginal and tip necrosis in older leaves on the fourth whorl of approximately one-year-old seedlings were observed following the continuous daily application of 0·24 p.p.m. boron: concentrations of boron in the central green portion of the lamina of 40–60 p.p.m., and of 160–240 p.p.m. in the marginal necrotic portion of the leaves, were recorded.

Field studies: coastal clay soils. Leaf symptoms of tip and marginal necrosis, particularly on leaves which are older than approximately six months, are frequently observed on rubber growing on coastal alluvial clay soils reclaimed from mangrove[2]: these soils are poorly drained and the symptoms are generally thought to be associated with water-logged conditions.

Previous analysis of affected leaves had revealed no abnormalities with respect to the concentrations of nitrogen, phosphorus, potassium, magnesium, calcium, manganese, sodium and chloride in the leaves. Following identification of the symptoms of boron toxicity, affected leaves were collected from different locations situated on coastal alluvial clay soils on the west coast of Malaya and analysed for boron. Without exception all leaves contained abnormally high concentrations of boron, particularly in the necrotic leaf margin and tip. The boron concentrations in the leaves shown in Fig. 1 are given in Table 2, together with the concentrations in a limited selection of leaves taken from different clones of different ages, to show the range of leaf boron concentration encountered under field conditions.

It is reasonable to expect, from geochemical considerations, a high concentration of boron in the coastal alluvial deposits in Malaya, and work is in progress to determine the amounts of boron in soils derived from such deposits.

Boron toxicity on sedentary soil derived from granite. Symptoms similar to those illustrated in Fig. 1 have recently been found on rubber growing on eroded hill-tops on soils derived from an acid granite. The boron concentrations, averaged over seven samples, in the central portion of the lamina and in the necrotic leaf margin and tip were 86 p.p.m. and 257 p.p.m. respectively. As no

Table 2. Boron Concentrations (p.p.m. on Dry Weight Basis) in Leaves showing Symptoms of Marginal and Tip Scorch

Clone	Age of tree (yr.)	Green, central part of lamina	Necrotic edge of lamina
G 1*	15	107	265
PB 86*	11	108	257
PR 107	6	74	145
RRIM 526	3	168	274

* Leaves shown in Fig. 1.
Analysis carried out on bulk samples collected from thirty trees.

borax fertilizers have been applied it must be concluded that high levels of boron are available in the soil. The extent of such granite-derived soils where boron toxicity may occur is as yet unknown.

The importance of boron toxicity in restricting tree growth and yield is not known but in one instance where borax was applied at the rate of 1 oz. per tree to one-year-old trees, extensive marginal scorching of the leaves occurred and very little apical or axillary meristem development was observed for at least eighteen months after application. Rubber trees growing on the coastal alluvial clay soils often exhibit early defoliation of upper branches in the latter months of the year: this abnormal defoliation may be related to an excessive accumulation of boron.

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Isotopic Investigations of Nitrogen Fixation in Non-legume Root Nodules

Investigations involving the use of nitrogen-15 and previously reported have been continued as suitable plant material became available. It was shown earlier[1] that when 10 per cent of nitrogen is present in the supplied atmosphere (total pressure 1 atm.), fixation in detached nodules of *Alnus*, *Casuarina*, *Myrica* and *Hippophaë* is depressed if the proportion of oxygen exceeds 20 per cent (mean for the different genera). In a further experiment, with *Casuarina* nodules, the extent of this oxygen-inhibition in the presence of a higher level of gaseous nitrogen was examined, with the results shown in Fig. 1. In order to reduce the number of nitrogen-15 assays required, the digests from the triplicate samples of nodules at each gas mixture were pooled prior to assay; thus statistical treatment cannot be applied, but previous data[1] show that this nodule material replicates satisfactorily and indicate for the mean of three samples a standard deviation of the order of 10 per cent of the value of the mean. The results when 10 per cent of nitrogen was provided are similar to those previously reported. Some irregularity is shown in the results at 30 per cent nitrogen; but it is clear that the inhibitory effect of increasing oxygen tension is now considerably mitigated. This could be explained by a competition between oxygen

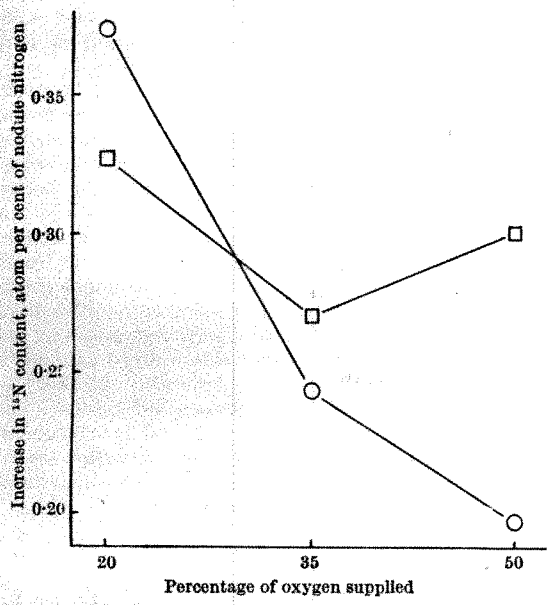


Fig. 1. Effect of increasing oxygen supply on fixation in *Casuarina* nodules at two levels of gaseous nitrogen. Samples of 1-g nodules from second-year plants of *C. cunninghamiana* were placed in 28-ml. containers charged with gas mixtures comprising the stated proportions of nitrogen (with 36 atom per cent excess nitrogen-15) and oxygen, and argon to 1 atm. Exposure was for 5·5 h. The experiment was carried out in August. ○, Ten per cent nitrogen; □, 30 per cent nitrogen

and nitrogen at some stage in the fixation process, evidence for which has been adduced by other authors in respect of *Azotobacter*[2] and legume nodules[3]. The suggestion in Fig. 1 that at 20 per cent oxygen fixation was greater with 10 per cent nitrogen than with 30 per cent could also be explained in this way.

Again in respect of nodules detached from the plant, it was reported earlier[1] that the excision of the rootlets which in *Myrica* and *Casuarina* grow from the nodule lobes resulted in a substantial reduction in the fixation shown by the nodules. This finding has been confirmed in further tests, though the explanation remains uncertain. No evidence of fixation was obtained when samples of the rootlets alone were exposed to labelled nitrogen.

Tests have been made to determine whether a capacity for fixation survives in homogenized nodules. Preparations made by grinding nodules of *Alnus*, *Myrica* and *Casuarina* in water (1–4 parts by weight) showed a scarcely significant maximum enrichment of 0·007 atom per cent of the whole nodule nitrogen after exposure for 4 h to a gas mixture comprising 10 per cent nitrogen (with 36 atom per cent nitrogen-15), 20 per cent oxygen and argon to 1 atm. In further tests with *Casuarina* nodules in which only the fraction of nodule nitrogen extracted by 3N hydrochloric acid was assayed, an enrichment of 0·012 was shown, and was not increased when sucrose or ketoglutarate was added initially. The fixation indicated, though far feebler than in the intact nodules, warrants further investigation.

A final aspect concerns the path of translocation of fixed nitrogen in the nodulated plant. Previously[4] it was shown that in *Alnus* the removal over a short zone at the base of the shoot of the tissues external to the xylem resulted in a marked reduction of fixation in the nodules, but did not prevent an upward movement into the shoot of such nitrogen as was fixed. It was concluded that this movement can, and probably does, normally occur through the xylem. The results of a similar test with *Hippophaë* are presented in Table 1, and permit the same conclusion.

Table 1. Effect of Stem-Ringing on Translocation of Fixed Nitrogen in *Hippophaë*

Plant number	Treatment	Enrichment shown in nitrogen-15, atom per cent of total nitrogen: Roots and nodules	Shoot
1	Not ringed	0·342	0·150
2	Not ringed	0·426	0·078
3	Not ringed	0·260	0·025
4	Ringed	0·045	0·020
5	Ringed	0·159	0·088

The root systems of first-year plants of *Hippophaë rhamnoides* were sealed into 100-ml. jars containing culture solution and a gas space charged with 15 per cent nitrogen (36 atom per cent excess nitrogen-15), 20 per cent oxygen and argon to 1 atm. The plants were left set up for 96 h under greenhouse conditions (in August).

I thank the staff of the National Institute for Medical Research, London, and Dr. R. H. Burris, University of Wisconsin, for the nitrogen-15 assays.

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Root Formation on Cuttings of *Camellia reticulata* var. 'Capt. Rawes'

Most camellias will form roots readily on cuttings, but the *C. reticulata* cultivar, 'Capt. Rawes', is a notable exception. Failure to induce roots in cuttings of this camellia, despite the use of modern techniques such as growth regulators, etc., has led to the belief that it is unable to form roots, and several reasons have been put forward to account for this. It may be due to anatomical features, such as fibre, creating mechanical resistance[1] or the presence of inhibitors which prevent root formation[2] or the absence of some internal factor which is essential for root formation. Went[3] first postulated the presence of such a factor which he termed 'rhizocaline', but the presence of a single substance of this nature has never been demonstrated. Hess[4] has shown, however, that certain substances which are present in the juvenile form of *Hedera helix*, but absent in the mature form, could account for the failure of the mature form to initiate roots on cuttings. He has described these substances as 'co-factors of rooting'.

Camellia reticulata, var. 'Capt. Rawes', has been compared with *Camellia japonica*, var. 'Emperor', which roots readily from cuttings, to determine which, if any, of these reasons could explain its failure to form roots. The cuttings were taken in November, after the new season's shoots had become firm. Microscopic examination of sections of the base of cuttings showed no anatomical differences between *C. reticulata* and *C. japonica* that could account for the failure of the former to form roots.

Leaves taken from the cuttings of each variety were examined for the presence of growth promoters or inhibitors, using the mung bean bioassay described by Hess[4]. The results are shown in Fig. 1. These show that

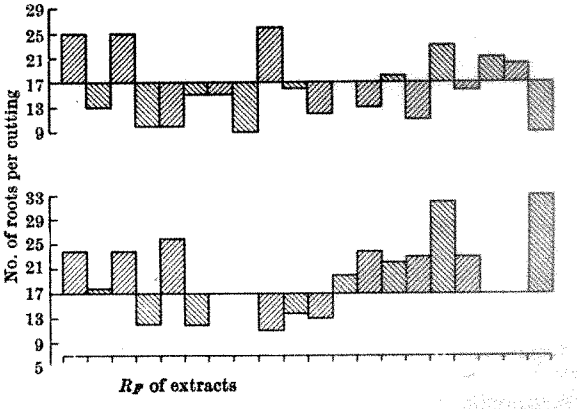


Fig. 1. Roots formed per mung bean cutting (average of 20 replicates) in the presence of extracts of *Camellia* leaves. Above, *C. reticulata*; below, *C. japonica*

Table 1. No. of Each Cutting/Scion Combination forming Roots

Combination	Cutting	Scion	No. forming roots
1	*C. japonica*	*C. japonica*	17
2	*C. japonica*	*C. reticulata*	18
3	*C. japonica*	—	17
4	*C. reticulata*	*C. reticulata*	0
5	*C. reticulata*	*C. japonica*	10
6	*C. reticulata*	—	0

C. reticulata did in fact contain a number of inhibitors of root formation which did not occur in *C. japonica*, whereas *C. japonica* contained a prominent group of growth-promoting substances which were absent in *C. reticulata*.

To elucidate this further, a cutting/grafting experiment was laid down. Cuttings of *C. reticulata* were grafted with either *C. reticulata* or *C. japonica* scions, and cuttings of *C. japonica* were grafted with *C. japonica* or *C. reticulata* scions. Ungrafted cuttings of each variety were also included. The cuttings were all treated with indolyl butyric acid, 0·8 per cent in talc, and planted in pumice in a propagating frame at 70° F. Twenty cuttings were used in each treatment. The results are shown in Table 1, and it is obvious that grafting a scion on to a cutting of the same *Camellia* species does not affect its potential to form roots. When *C. reticulata* scions are grafted on to *C. japonica* cuttings, any inhibitor in the scion is not effective in preventing root formation in the cutting, which casts doubt on whether the inhibitors present in *C. reticulata* are really effective in preventing root formation.

When *C. japonica* scions are grafted on to *C. reticulata* cuttings, however, there is a stimulation of root formation, from which one can postulate that some substance is conducted across the graft union, and is effective in promoting root formation. The nature of this substance has not yet been determined.

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Kinins in the Embryo and Endosperm of *Prunus persica*

It is increasingly evident that endogenous kinins are involved in cell division in plants. Van Overbeek[1] reviewed in 1962 their possible role in fruit development. Several investigators have demonstrated their presence in a number of fruits including the peach[2–6]. In studies of peach fruit development we have found that alcoholic extracts of endosperm and embryo tissues show pronounced kinin activity.

Endosperm was removed from ovules of developing fruits of peach (*Prunus persica* cv. 'Halehaven') on July 14, 1960, when endosperm dry weight was still increasing, and on July 28 after its dry weight had started to decline because of digestion by the embryo. Embryo samples were taken on July 21, just after the beginning of rapid embryo development, on August 4 when it was well under way, and on August 18 when embryo growth was still progressing, but nearing the end of the period of rapid development, as measured by dry-weight increases. The tissues were immediately frozen with dry ice, and later lyophilized. Extractions were made with 75 per cent methyl alcohol. The alcohol was evaporated and the residue partitioned between water and *n*-butyl alcohol to attempt some purification. Both the aqueous and alcohol fractions were then serially diluted and assayed by means of the tobacco pith explant test[7]. At the end of three months, when growth had almost stopped, the callus was cut from the explants. Both callus and explant were weighed and the callus yield calculated as a percentage of the explant weight.

Appreciable kinin activity, as measured by growth of the callus, was detected in all samples of both the aqueous and butyl alcohol fractions. There were no clear-cut differences in activity between the butyl alcohol and aqueous fractions of a particular sample, nor between similar samples taken on different dates. There was only a suggestion that the endosperm was a richer source of kinins than the embryo. The data for the aqueous fraction of the July 14 endosperm sample and the aqueous fraction of the July 21 embryo sample are summarized in Fig. 1. At all concentrations of the endosperm extract, and at all but the highest concentration of the embryo extract, the growth of the explants was considerably greater than could be accounted for by endogenous indolylacetic acid. In one case it was equal to that caused by the coconut milk. Further comparisons of kinin concentration between the various samples are not valid since the distribution of inhibitors of the bioassay is unknown.

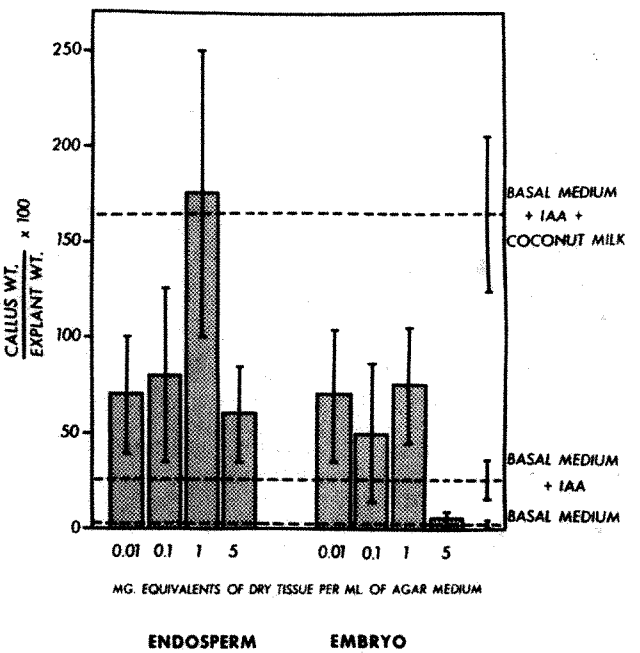


Fig. 1. Kinin activity in the aqueous fraction of extracts of endosperm (July 14) and embryo (July 21) from seeds of developing peach fruits. Tobacco pith explants were grown on 1·9 per cent agar containing basal medium (consisting of modified White's salts, sucrose, vitamins, and iron), indolylacetic acid (IAA), 2 p.p.m., and endosperm or embryo extract at the concentrations indicated. Control explants were grown on (1) basal medium only; (2) basal medium plus indolylacetic acid; and (3) basal medium plus indolylacetic acid plus 10 per cent coconut milk. Standard deviations from the mean are indicated

It has been suggested[1,8] that kinins may play a part in fruit development during the early stages of this process, since it is at this time that cell division in the pericarp may be occurring. In the peach pericarp cell division is said to continue for three to four weeks after fertilization[9]. Mitoses have been observed in an embryo 12 weeks after bloom (unpublished data), and are obviously required for the growth of the endosperm and embryo. The present data show that kinins exist in immature peach seeds for at least three months after fertilization. It seems likely that kinins are necessary for the normal development of the ovule, and thus must play a vital, if indirect, part in the middle as well as the early stages of peach fruit development, since the development of the pericarp is dependent on the ovule well into the middle period of fruit development of this species[10].

This work was performed while the senior author was a member of the Department of Pomology, New York State Agricultural Experiment Station, Cornell University, Geneva. We acknowledge the advice of Dr. F. G. Dennis in the performance of the bioassay, and the assistance of Mr. Bock Chan in securing the endosperm and embryo samples.

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Bimodality of Laying–Hatching Times in *Testudo elegans* Schoepff (Chelonia)

A FEMALE and three male Indian starred tortoises (*Testudo elegans* Schoepff)[1] have been kept since August 1962 in Bhubaneswar in a yard which is only partially paved. All the tortoises were obtained from dealers in Calcutta.

The eggs and young observed are listed in Table 1. 62I and 63I were resting on grass. 62I was put on damp blotting paper in a glass jar. It was discarded after it had cracked and become malodorous. The eight eggs 63I and 64I–VII were also put individually in glass jars and buried 6–8 cm deep in soil which was not moistened afterwards.

Table 1

No.	Laid	Where	Size (cm)	Hatched	Incubation period	Hatching weight (g)
62I	17/10/62	Surface	5·1 × 3·7			
63I	26/3/63	Surface		15/7/63	111	13·87
64V	18/4/64	Buried	4·25 × 3·5	4/6/64	47	16·13
II	18/4/64	Buried	4·35 × 3·55	4/6/64	47	18·94
VI	18/4/64	Buried	4·4 × 3·55	11/6/64	54	21·53
I	18/4/64	Buried	4·05 × 3·15	18/8/64	122	14·43
III	18/4/64	Buried	3·95 × 3·1	22/8/64	126	11·93
VII	18/4/64	Buried	4·4 × 3·3	29/8/64	133	13·77
IV	18/4/64	Buried	4·65 × 3·4	12/9/64	147	15·22
				Found		Weight when found
VIII				26/6/64		20·66
IX, X, XI		One at least buried with XII		3/7/64, 3/7/64, 3/7/64		19·14, 19·07, 20·74
XII		Buried		Died near term		

On April 18, 1964, at about 6 p.m., the female was found digging with her hind feet. About 1 h later, she was seen sitting quietly with her hind parts in the pit thus made. At midnight, she was more than 8 m away from this place, which was marked and excavated next morning. The soil was swarming with ants and had to be flooded before it could be handled. The eggs were buried 5–15 cm deep, each touching at least another. The numbers given to them unfortunately do not record the order in which they were taken from the pit. Though we extended this pit for at least 10 cm in all directions from this group of eggs, we found no more.

The female has not since had mud on her hind parts as she had after burying these eggs. However, four other baby tortoises have been found in the yard. The three young, 64IX–XI, were found virtually simultaneously and within a few metres of the previous egg pit. Very near the site of our previous excavation, we found a freshly opened cylindrical hole, about the diameter of a young tortoise, descending vertically. This contained fragments of egg-shell and an egg containing a putrid embryo very near term. As such a hole would have been quickly washed away in the rain then falling, and as the three young were so near to it, we have little doubt that we found them within a few minutes of their leaving the soil.

The laying to hatching periods of the 1964 eggs given in Table 1 show the young falling into two groups, the quick developers and the slow developers, and that the hatching weights of the former are greater than those of the latter. Their weights confirm that the four young found after hatching are plausibly quick developers, and that 63I is a slow developer.

It is known that the period between laying and hatching of reptile eggs is very variable within a species. This is attributed to their dependence on external temperatures, which vary from season to season and in different parts of the species range[2]. A range of laying–hatching times differing by a factor of 3 (which is, incidentally, larger than any listed by Goin and Goin[2]), together with its bimodality, suggests that the differences observed among the littermates 64I–VII kept in apparently identical conditions are genetically determined. The two groups may represent the sexes.

Here we are somewhat isolated from the literature and would be grateful to learn of any comparable variation in laying–hatching time in any other vertebrate. Like variation in time needed to reach sexual maturity, it may be characteristic of long-lived, and correspondingly infertile species. Wynne-Edwards[3] discusses the adaptive value of such patterns of development.

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'Metarchon': a New Term for a Class of Non-toxic Pest Control Agents

CONCERN over the contamination of the environment by pesticide residues is directing attention to the possibility of combating insect pests by means of chemical or other influences which, without being toxic, are able to affect an organism adversely by inducing deviations from the normal behaviour. Insect repellents and insect attractants (both chemical and optical) are familiar examples, but they do not exhaust the possibilities. For example, a sex-attractant scent could be distributed broadcast in order to confuse or attenuate the male response, and other olfactory signals might be used to induce a female to oviposit at the wrong time or place. Other possibilities no doubt remain to be discovered.

Since no single term denotes agents using this principle, I suggest that behaviour-modifying influences purposefully applied as a means of pest control be given a special name, 'metarchon', from the Greek meta, a prefix indicating change (as in metamorphosis), and archon, ruler or controller (as in monarchy or anarchy).

'Metarchons' are defined as external stimuli artificially introduced into the environment of an organism for the

purpose of modifying its behaviour, by eliciting an inappropriate response or inhibiting an appropriate one.

The term 'pheromone' has been coined to denote chemical signals which emanate from one individual and evoke a behavioural or developmental response in a second individual of the same species[1]. A pheromone might, therefore, be used as a metarchon, but the latter term also embraces food attractants and multi-component odours, as well as such physical agents as lights or sounds.

I thank Dr. Malcolm F. McGregor for his advice.

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Paralogy, a Concept Complementary to Homology and Analogy

In the application in practice of the concept of homology it is necessary to keep taxonomical considerations constantly in mind if false homologies are not to be drawn[1].

It would seem that there is also some need to consider the concepts of homology and of analogy and to delimit a third and complementary concept, which might be termed 'paralogy'[2].

For meaningful discussion to be possible it is essential that there should be strict uniformity of usage in regard to the important concepts of homology and analogy, but, as was pointed out by Boyden[3], strict usage has by no means always been the rule. When working some years ago in the Department of Anatomy of the University of Sydney this point was forcibly brought home to me. Indeed, even in books devoted to comparative anatomy, it is possible to find examples of looseness of usage of both the term 'homology' and the term 'analogy', or their related parts of speech.

For the meaning of these concepts we can do no better than to go back to Richard Owen, who first clearly defined them in the glossary to his *Lectures on the Comparative Anatomy and Physiology of the Invertebrate Animals*, delivered at the Royal College of Surgeons of England, and published in 1843: "Analogue. A part or organ in one animal which has the same function as another part or organ in a different animal" and "Homologue. The same organ in different animals under every variety of form and function."

While these definitions are, perhaps, not perfect as such, the nature of the concepts is clearly indicated. The confusion in the usage of terms relating to these concepts, and in their practical application, appears to have been in part engendered by the lack of explicit definition of the complementary concept of purely anatomical or structural resemblance, for which the term 'paralogy' is suggested. Following Owen, its meaning may be indicated thus: 'Paralogue'—a part or organ in one animal similar in anatomical or microanatomical structure to a part or organ in a different animal.

Paralogy, then, refers only to anatomical similarity and has no phylogenetic or functional implications. Although lacking the comparative anatomical importance of true morphological correspondence (homology) and the comparative physiological significance of functional resemblance (analogy), a term which may help in limiting the drawing of false homologies on the basis of superficial resemblance in structure appears to be amply justified.

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VIROLOGY

Transfer of Lily Curl Stripe by a Leaf Union Method Applicable to Monocotyledonous Plants

Curl-stripe disease has seriously affected certain plantings of *Lilium longiflorum* Thunb. in Oregon and California. Control approaches have been uncertain because occurrence patterns and symptomology were equally indicative of nutritional imbalance or virus ætiology and because we had been unable to transmit the disease[1]. Early-season symptoms, central striping of young leaves due to retardation of plastid formation, followed by curling and twisting of leaves, are striking. Such symptoms may continue or, more usually, later formed foliage may be normal. Bulbs from such plants may or may not produce visibly diseased plants the following year. Such symptom variability is atypical of virus infections of bulb plants.

Initially, the presence of a soil-borne virus, probably tobacco rattle, was suspected. Tests by us and by Dr. R. G. Grogan, of the University of California, proved that while rattle virus is occasionally present in Easter lilies, it is certainly not the cause of curl-stripe. Numerous inoculations over a 5-year period failed to isolate a probable virus from typically diseased plants or to effect a transfer from lily to lily. Probable virus ætiology was indicated, however, by electron microscopy and by the development of an antiserum which gave a positive Ouchterlony agar double-diffusion reaction to sap from typically diseased plants. Virus ætiology has now been verified by infecting 20 of 22 lilies by leaf union inoculation. The procedure should be applicable to similar problems in tropical diseases of coconut and maize.

The objective is to fasten the distal half of an infected leaf to the basal half of a leaf on a test plant in such fashion that the cut ends of the parallel veins will be joined and union will occur. The equipment needed is two razor blades, strips of paper masking tape approximately 20 mm × 40 mm and a spectacle-type magnifier which can be worn. Two operators are required so the union can be made and sealed in 30 sec or less. A leaf on the test plant is cut in two with a razor blade and a piece of tape attached to the lower surface so it extends out about 10 mm beyond the cut end of the leaf (Fig. 1). Then the distal half of the leaf cut from the infected plant is fitted to the basal half of the test plant leaf so the ends of the veins meet. Slight magnification is needed to ensure adequate joining of the veins. The sticky tape extending beyond the cut end of the test plant leaf enables one to hold both cut ends in position until an assistant can fit a second piece of tape above them to form a waterproof sandwich.

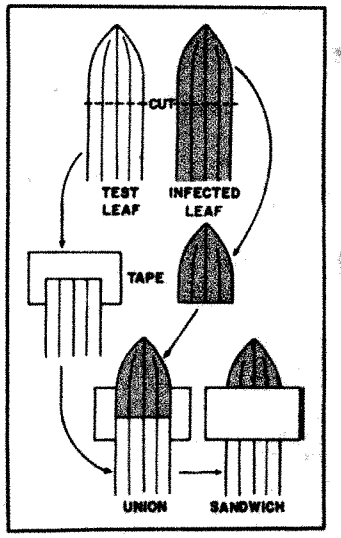


Fig. 1

The critical points in the technique are: (1) the grafting must be performed rapidly while the veins are still 'bleeding'; (2) a suitable easily handled paper tape must be used. The very sticky, easily curled 'Cellophane' tapes used for sealing purposes are unsuitable.

Initial trials of this technique on lilies growing outdoors, without recourse to humid chambers considered necessary for difficult grafts, showed that lily leaves so united usually remained green and turgid for at least 30 days; occasional leaves for more than two months. It is not claimed that a true graft union developed; but exchange of vascular fluids did occur as witnessed by long life of the attached leaf part and significant transfer of a hitherto 'non-transmissible virus'.

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GENETICS

Effect of Supernumerary Chromosomes on Sex Ratio in *Calligrapha philadelphica* L. (Coleoptera: Chrysomelidae)

Supernumerary chromosomes occur sporadically in plants and animals. In the animal kingdom, they have been observed in at least 50 insects and two turbellarians, and they may be present or absent in different populations of the same species[1]. They are generally considered to be inert as they are usually heterochromatic and their carriers are not distinguishable morphologically. The chromosomal polymorphism that they confer on some populations suggests that they play an adaptive part in the success of the population[1,2]. In plants supernumerary chromosomes have a harmful effect on vigour and fertility, and the effect becomes more pronounced as their number increases[3]. The work of Lewis[4] and Frost[5] has suggested that their presence may be an adaptation to arid regions[3].

This report concerns *Calligrapha philadelphica* L., one of 17 species of the genus that I have examined cytologically. Most of the species, including *C. philadelphica*, are bisexual, but six are obligatorily parthenogenetic. The adults hibernate in the ground in winter and emerge in the spring to mate (if bisexual) and lay eggs. There is a single generation each year. The bisexual species have a basic chromosome formula of $n = 11 + XO$ ($2n = 23$♂, 24♀), which in some species is modified by the presence or absence of supernumerary chromosomes in colonies from different localities. The parthenogenetic species are ameiotic tetraploids ($4n = 48$). Bisexual *C. philadelphica* was found in 1961 to contain 5–8 univalent supernumerary chromosomes per individual in a colony at Berthierville, Quebec, and none in a colony at Ottawa, Ontario. Accordingly, it was clear that a cross might provide some interesting hybrids.

In two years of collecting and rearing, the sex ratio from each colony was virtually 50 : 50. In 1963, chromosome samples indicated that the colonies had not changed with regard to the presence or absence of supernumerary chromosomes. Because of technical limitations only a one-way cross, that is, males from Berthierville with females from Ottawa, has been attempted this year. Two lots, *A* and *B*, containing two males and three females, and three males and two females, respectively, were bred and reared separately. Lot *A* yielded 18 adult progeny, lot *B* 14, from a total of 43 young larvæ. All the progeny were female. At the same time 39 Berthierville control females were allowed to mate in lots of two to five individuals, and these yielded 218 female and 199 male progeny, derived from 900 young larvæ. In other words, the number of females per female parent was 6·4 in the test cross and 5·5 in the control, so that the yield of female progeny in the two samples was not very different. However, the figure 5·5 actually represents 52·5 per cent of the total progeny whereas 6·4 represents 100 per cent, that is to say, the test cross gave only females. The survival-rate from young larva to adult was 46 per cent in the control and 74 per cent in the test cross, and as this certainly does not indicate an excessive mortality in the larval stage of the test group it is suggested that the male component was lost at fertilization or in the egg stage.

Thus the male sex in *C. philadelphica* is not represented in the adult progeny of a cross between females from the Ottawa colony and the males from Berthierville, and the most probable explanation is that this result depends on the addition of supernumerary chromosomes originating from the Berthierville colony. Further experiments with the surviving 32 female adults of the test cross as well as adults from laboratory-reared stock from both localities are in progress.

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PSYCHOLOGY

Effect of Pentobarbital Sodium on Sleep Latency and Length of Sleep in the Rat

The effects of repeated dosages of pentobarbital sodium on two sleep responses in the rat have been investigated. The responses observed were sleep latency (the time between drug injection and an observed sleep response) and sleep length (the time between the observed sleep response and arousal). The dosage used was purposely designed to obtain a significant delay between injection and the sleep response. The study was an outgrowth of an attempt to produce, in a minimally distracting environment, a low variability but delayed sleep latency which would be susceptible to systematically introduced environmental sleep distractors. The investigations are a part of a larger programme directed toward determining the environmental antecedents of sleep.

The experimental observations were carried out in a sound-deadened room maintained at 70° F and kept dimly lighted during the experiment.

Injections were given intraperitoneally in a dose of 20 mg pentobarbital sodium/100 g body-wt. A solution containing 16·7 mg/c.c. of pentobarbital sodium was freshly prepared each day.

All animals were adult males of the Long-Evans hooded variety. The four animals in the first experiment were 140 days old at the beginning of the experiment. The six animals of the second experiment were 190 days old at the beginning of the experiment. These animals had free access to food and water except for the period in which they were in experimental cages.

After injection, the animals were placed in individual observation cages which were 10 in. × 7 in. × 7 in. made of meshed galvanized wire. The time of sleep onset was measured to the nearest second. Sleep onset was determined by observational means. The animal was considered asleep when it lay down, body flaccid, respiration shallow, no gross movements occurring, and would not respond to an object waved in front of its eyes. Total length of sleep was measured to the nearest minute starting with the first appearance of the sleep response until arousal. The animal was considered awake when it responded to

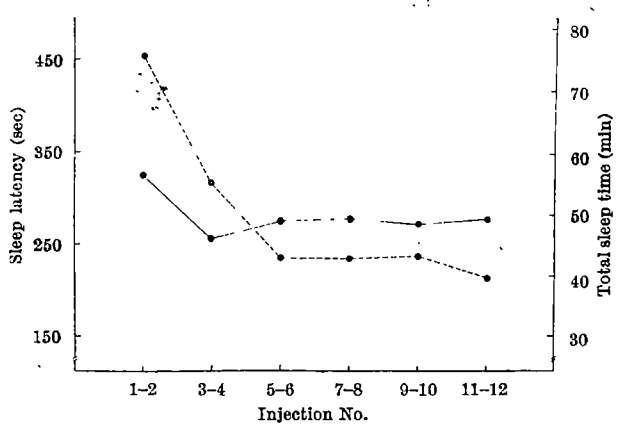


Fig. 1. ———, Sleep latency; - - - -, total sleep time

an object waved in front of its eyes and moved about the cage.

There was a 48-h interval between each drug administration.

In the first experiment four animals were given a series of 42 injections of pentobarbital sodium and sleep latency was determined. The average sleep latencies in seconds in blocks of six injections for the four animals were as follows: 404, 318, 383, 379, 405, and 411. An analysis of variance of these six unit blocks of *S*s was performed. The between-blocks variance (in this case tolerance effect) was not significant. There was a significant difference between *S*'s in the effect of pentobarbital sodium on sleep latency time ($P = 0{\cdot}01$). A 43rd placebo injection (distilled water) was given after 48 h. No animal slept within a 30-min observation period, indicating no conditioning of the sleep response with the delay latency obtained in this experimental design.

In a second experiment six animals were given a series of 12 injections of pentobarbital sodium and both sleep latency and sleep length were measured. The results are shown in Fig. 1. A highly significant tolerance effect was present in the case of length of sleep. This effect was demonstrated within the first three trials. Four injections produced more than 50 per cent of the reduction in time of sleep. The length of sleep variable appears to become asymptotic after 5 injections. An analysis of variance of the latency data using blocks of three trials yielded no significant tolerance, *S*, or interaction effect; an analysis of variance of the sleep length data yielded significant *S* and tolerance variances, but no interaction effect ($P = 0{\cdot}01$ and $0{\cdot}001$, respectively).

This work was supported by grant *NIH–MH*–03881–03 from the U.S. National Institutes of Health.

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Lateral Preferences in the Monkey

There is general agreement that the majority of monkeys show lateral preferences on a particular task[1–6], about as many animals showing a strong preference for the left hand as for the right. However, Ettlinger[5] reported that significantly more monkeys preferred to use the left hand than the right on their first discrimination task (irrespective of the task being visual or tactile). Seventeen of the 42 monkeys showed a strong preference for the left hand, six for the right and 19 animals showed weak or ambiguous preferences. Brookshire and Warren[6], as a result of their more comprehensive investigation of lateral preferences under a variety of test conditions, wrote: "Four monkeys were judged to have had a predominantly right-hand preference, nine were judged left-handed, and six were considered either ambidextrous or without definite preference". It is not yet known what factors are associated with the preferential use of a particular hand (left or right) on a given task[6]. If a significant asymmetry in the incidence of lateral preferences in favour of the left (or right) hand can be established it will be important to seek for neural or other correlates in a species which is generally considered to lack cerebral dominance.

Hand preferences have now been recorded for 27 unoperated immature rhesus monkeys, all receiving their first training on a simple tactile discrimination task. These animals were all housed and examined under constant conditions during the period 1962–64. Training, under standard conditions (40 daily trials, non-correction), was begun in the light but quickly (after 1–4 sessions) transferred to the dark. Two test-objects, a cylinder and a sphere, were made available on each trial. At first the animals were able to see these objects; afterwards they were only able to palpate them in the dark. Choice of the one object (cylinder), whether to left or right, was rewarded. The animals indicated their choice by pushing one test-object and thereby opening the lid of one food box. Following correct choice the animals were able to pick up a food reward (peanut) from the food box which was uncovered. Records were taken of the hand used to push the test-object (and, therefore, the lid of the box) on all trials; and also of the hand used to bring the food to the mouth on all rewarded trials. Responses made during initial trials in the light have been excluded from analysis. Separate tabulations were made for: (1) all the trials (totalling 3,585) in the dark during the stage of training before the animals reached a standard level of performance (90 per cent correct responses in 200 consecutive trials); (2) for trials 71–100 of the stage of training in the dark when the animals had reached the standard level of performance (a total of 810 trials). In addition, animals were individually graded as showing a left or right lateral preference if 90 per cent or more of their responses during either stage of training were made with a single hand.

Table 1. Number of trials on which response to the test-object (and also to the food reward on rewarded trials) was made with the left or right hand at two stages of training on a tactile discrimination task; and number of animals using the left or right hand for the test-object (and also the food reward on rewarded trials) in 90 per cent or more of trials, at two stages of training on the same task

Hand used:	Left	Right		Left	Right	
Trials	1,925	1,422	$\chi^2 = 75{\cdot}6$ $P < 0{\cdot}001$ Binomial	448	270	$\chi^2 = 44{\cdot}2$ $P < 0{\cdot}001$ Binomial
Animals	7	4	*N.S.*	12	7	*N.S.*
	During stage of training before performance is 90 per cent correct			During stage of training when performance is 90 per cent correct		

Trials on which animals used both hands to push the test-object, and rewarded trials on which different hands were used for the test-object and food reward, have been excluded from the table. Similarly, animals not using the same hand in 90 per cent of trials have been excluded from the analysis related to individual animals.

The results are shown in Table 1. At both stages of training there were significantly more trials on which the left hand was used in preference to the right hand. However, the number of animals showing left-hand preferences does not significantly exceed the number of right-handed animals. Nevertheless, it is noteworthy that a trend towards a greater incidence of left than right lateral preferences has been reported in two recent investigations[5,6] and also in this communication.

We thank Prof. G. D. Dawson and Prof. P. M. Daniel for facilities, and the Medical Research Council for financial support.

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, November 9

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Submarine Telephone Cables—Protection, Damage and Repairs" opened by Mr. R. J. Halsey and Mr. N. E. Holmblad.

PHARMACIA (GREAT BRITAIN), LTD. (at the Royal Society of Medicine, 1 Wimpole Street, London, W.1), at 6 p.m.—Dr. J. Killander (Sweden): "Fractionation of Serum Proteins by Gel Filtration". (To be repeated at the Molecular Biology Laboratories, Hills Road, Cambridge, on November 10 at 2.30 p.m.)

UNIVERSITY OF LONDON (at Birkbeck College, Malet Street, London, W.C.1), at 6 p.m.—Prof. G. Ekman (Stockholm): "Psychological Scaling. II: On the Psychological Function and Related Problems".*

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.15 p.m.—Capt. J. M. Loyd: "Across the Wahiba Sands".

Tuesday, November 10

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p.m.—Dr. Ursula Mittwoch: "Sex Chromosomes and Sex Chromatin".*

ZOOLOGICAL SOCIETY OF LONDON (at the Zoological Gardens, Regent's Park, London, N.W.1), at 5 p.m.—Scientific Papers.

UNIVERSITY OF LONDON (in the Assembly Hall, Institute of Education, Malet Street, London, W.C.1), at 5.15 p.m.—Mr. Victor Clark: "Local Education Authorities versus Bingo". (Fifth of a series of lectures on "Leisure and Education—Challenge and Response".)*

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, London, S.W.1), at 5.30 p.m.—Mr. W. J. Leaman and Mr. R. K. Postlethwaite: "Oil Jetties at the Port of Hull".

INSTITUTION OF ELECTRICAL ENGINEERS, SCIENCE AND GENERAL DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. J. A. Weaver: "Computer Aided Study of Character Recognition".

INSTITUTION OF THE RUBBER INDUSTRY, LONDON SECTION (at the Royal Society of Tropical Medicine, 26 Portland Place, London, W.1), at 5.30 p.m. —Mr. S. M. Ardley: "The Use of Resins in Rubber Reinforcement". 7 p.m. —Mr. G. J. Lake: "The Roles of Ozone and Flexing in the Failure of Rubber".

UNIVERSITY OF LONDON (at Birkbeck College, Malet Street, London, W.C.1), at 6 p.m.—Prof. G. Ekman (Stockholm): "Psychological Scaling. III: Applications of Scaling Techniques in the Study of Psychological Mechanisms".*

INSTITUTION OF MECHANICAL ENGINEERS, AUTOMOBILE DIVISION (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6.30 p.m.—Mr. G. L. Wilde and Mr. F. J. Allen: "Supercharging the 1½-litre V-16 B.R.M. Racing Engine".

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (at Norwood Technical College, Knight's Hill, West Norwood, London, S.E.27), at 7 p.m.—Film Show.

Wednesday, November 11

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Du Cane Road, London, W.12), at 2 p.m.—Prof. C. J. O. Morris: "Recent Advances in the Fractionation of Protein Mixtures".*

UNIVERSITY OF LONDON (at Bedford College, Regent's Park, London, N.W.1), at 5.15 p.m.—Prof. W. J. G. Beynon: "International Co-operation in Science".*

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, ELECTRO-ACOUSTICS GROUP (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Dr. M. Borner and Mr. A. Russen: "Electro-Mechanical Filters".

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Mr. A. H. Strickland: "Pest Control and Productivity in British Agriculture".

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP (at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Mr. A. T. S. Babb: "A Recording Instrument for the Rapid Evaluation of the Compressibility of Bakery Goods"; Dr. D. G. Land: "The Off-Flavour of Dehydrated Carrot Exposed to Oxygen"; Dr. H. Fore and Mr. R. Walker: "A Problem Associated with Food Colouring Matters".

Thursday, November 12

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 11 a.m.—Discussion Meeting on "International Indian Ocean Expedition. The Geology and Geophysics of the North West Indian Ocean" organized by Dr. M. N. Hill, F.R.S.

UNIVERSITY OF LONDON (in the Recital Room, Goldsmiths' College, New Cross, London, S.E.14), at 4.45 p.m.—Mr. T. K. Rees: "Modern Views on the Mechanism of Evolution".*

GLACIOLOGICAL SOCIETY (in the Geology Department, The University, Edgbaston, Birmingham), at 5 p.m.—Mr. Albert Bauer: "Antarctic Traverse".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. K. Preece, Mr. P. Lundberg, Mr. P. Selway and Dr. V. G. Tull: "Pressure Sensitive Effects in Semiconductors".

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Current Balance Earth-Leakage Circuit-Breakers—Design Features and Future Employment" opened by Mr. E. J. Sutton.

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. J. T. Eayrs: "Thyroid and Nervous System". (Seventh of fourteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)*

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. P. G. Foote: "The Saga of the Faroe Islanders" (Inaugural Lecture).*

ROYAL AERONAUTICAL SOCIETY (at 4 Hamilton Place, London, W.1), at 6 p.m.—Discussion Meeting on "Is the Aeronautical Industry Making the Best Use of Its Graduates?" opened by Mr. S. L. Bragg and Mr. R. L. Lickley.

OIL AND COLOUR CHEMISTS ASSOCIATION (at the Royal Society of Tropical Medicine and Hygiene, 26 Portland Place, London, W.1), at 7 p.m.—Mr. P. Whiteley: "Mould Resistance of Paints in the Tropics".

RADAR AND ELECTRONICS ASSOCIATION (at the Royal Society of Arts, John Adam Street, Adelphi, London, W.C.2), at 7 p.m.—Mr. L. F. Mathews: "Televising the Tokyo Olympics".

ROYAL INSTITUTE OF CHEMISTRY (at Croydon Technical College, Fairfield, Croydon, Surrey), at 7.30 p.m.—Mr. R. A. Hudson: "The Physical Chemistry of Detergency".

Friday, November 13

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Du Cane Road, London, W.12), at 4 p.m.—Dr. J. D. N. Nabarro: "Steroid Therapy".*

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. S. A. Nasar: "Electromagnetic Theory of Electrical Machines".

ROYAL AERONAUTICAL SOCIETY, TEST PILOTS GROUP (at 4 Hamilton Place, London, W.1), at 6 p.m.—Mr. E. B. Trubshaw: "Low Speed Handling with special reference to Super Stall".

OCCAM SOCIETY (at the Northampton College of Advanced Technology, St. John Street, London, E.C.1), at 6.15 p.m.—Mr. H. L. Jolley: "Causality".

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (joint meeting with the Cambridge University Chemical Society and the East Anglia Section, at the Cambridge University Chemical Laboratory, Lensfield Road, Cambridge), at 8.30 p.m.—Prof. John Yudkin: "Needs and Wants—The Dilemma of Civilization and the Role of the Chemist".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Mr. A. H. Ewen: "'The Friend of Mankind'—a Portrait of Count Rumford".

Saturday, November 14

GLACIOLOGICAL SOCIETY (joint meeting with the Scott Polar Research Institute, at the Scott Polar Research Institute, Cambridge), at 8.30 p.m.—Mr. Albert Bauer: "Antarctic Traverse".

Monday, November 16

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 5 p.m.—Lieut. Comm. J. B. Dixon, R.N.: "Hydrographic Surveying in the Antarctic".

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Dr. R. C. Brian: "Electrons and the Mode of Action of Certain Herbicides".

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. P. M. S. Blackett, F.R.S.: "Britain and India's Development Plans" (Rickman Godlee Lecture).*

Monday, November 16—Thursday, November 19

INSTITUTION OF MINING AND METALLURGY, in co-operation with the Institution of Mining Engineers and the Institute of Quarrying (at Caxton Hall, Westminster, London, S.W.1)—Symposium on "Opencast Mining, Quarrying and Alluvial Mining".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

UNIVERSITY LECTURER (with a special interest in genetics including microbial genetics) IN BOTANY—The Administrator, Department of Botany, The University, South Parks Road, Oxford (November 10).

ASSISTANT LECTURER OR LECTURER (preferably qualified in the fields of biophysics, biochemistry, ecology, genetics, microbiology or physiology) IN THE SCHOOL OF BIOLOGICAL SCIENCES—The Assistant Registrar (Establishment), University of Sussex, Stanmer House, Stanmer, Brighton, Sussex (November 20).

LECTURER OR ASSISTANT LECTURER IN THEORETICAL PHYSICS—The Assistant Registrar (Establishment), University of Sussex, Stanmer House, Stanmer, Brighton, Sussex (November 20).

RESEARCH FELLOW for mainly experimental work in the hovercraft field—The Secretary and Registrar, Room 9, The University, Highfield, Southampton (November 20).

TUTORIAL RESEARCH STUDENT IN MATHEMATICS (Pure or Applied)—The Principal, Royal Holloway College (University of London), Englefield Green, Surrey (November 20).

LECTURER and an ASSISTANT LECTURER IN PURE MATHEMATICS—The Secretary, The University, Aberdeen (November 21).

LECTURER (medically qualified or non-medically qualified) IN THE DEPARTMENT OF PHARMACOLOGY—The Registrar, The University, Liverpool, quoting Ref. CV/344 (November 21).

LECTURER OR ASSISTANT LECTURER IN APPLIED MATHEMATICS—The Registrar, The University, Sheffield (November 28).

CHAIR OF MECHANICAL ENGINEERING—The Registrar, University College of Swansea, Singleton Park, Swansea (November 30).

EXPERIMENTAL OFFICER (chemist or biochemist with at least H.N.C. or L.R.I.C., with some research experience) to assist with research into drug metabolism in the horse—Head of Forensic Laboratory, Equine Research Station, The Animal Health Trust, Newmarket, Suffolk (November 30).

JUNIOR LECTURER IN MATHEMATICS; and a LECTURER/SENIOR LECTURER IN PSYCHOLOGY at Massey University of Manawatu, Palmerston North, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, November 30).

JUNIOR LECTURER IN ZOOLOGY; and a JUNIOR LECTURER IN BOTANY at Massey University of Manawatu, Palmerston North, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, November 30).

LECTURER (with a science degree with special qualifications (and preferably postgraduate experience) in entomology or helminthology) IN ZOOLOGY—The Secretary, West of Scotland Agricultural College, 6 Blythswood Square, Glasgow, C.2 (December 1).

BIOCHEMIST, Basic Grade (with an honours degree in chemistry or biochemistry, or A.R.I.C.)—The Group Secretary, Southmead Hospital, Westbury on Trym, Bristol (December 5).

SENIOR LECTURER OR LECTURER IN PURE MATHEMATICS at the Australian National University—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, December 7).

SIR WILLIAM DUNN CHAIR OF PATHOLOGY at Guy's Hospital Medical School—The Academic Registrar, University of London, Senate House, London, W.C.1 (December 10).

CHAIR OF PHARMACOLOGY at the University of Hong Kong—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Hong Kong and London, December 15).

RESEARCH ASSISTANT (with a good honours degree in engineering or metallurgy) in a group working on the strength properties of materials under dynamic loading—Dr. J. D. Campbell, Engineering Laboratory, The University, Parks Road, Oxford (December 31).

SECOND CHAIR IN THE DEPARTMENT OF MATHEMATICS—The Registrar, The University of Essex, Wivenhoe Park, Colchester, Essex (January 5).

BIOCHEMIST OR MICROBIOLOGIST (with a Ph.D. degree) to work on factors controlling the biosynthesis of enzymes in bacteria—The Director, National Institute for Medical Research, Medical Research Council, Mill Hill, London, N.W.7.

BIOLOGIST (between 24 and 35 years of age, with an honours degree in zoology and postgraduate experience in animal ecology) in Tanganyika, to carry out investigations concerned with the management of wild life and to advise the Chief Game Warden—The Appointments Officer, Room 301, Ministry of Overseas Development, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 275/173/01.

LECTURER IN MATHEMATICS—The Principal, The Training College, Bognor Regis, Sussex.

MASTER TO TEACH CHEMISTRY (large share in sixth form work)—The Headmaster, Epsom College, Epsom, Surrey.

PHYSIOLOGIST or PATHOLOGIST (preferably with postdoctorate or other experience) to carry out a research programme concerned with the physiology and pathology of fish—Dr. D. R. Idler, Director, Fisheries Research Board of Canada, Technological Research Laboratory, P.O. Box 429, Halifax, Nova Scotia, Canada.

RESEARCH ASSISTANT (with a good honours degree in animal physiology or zoology or an equivalent qualification, and preferably an interest in biochemistry) IN THE PHYSIOLOGY DEPARTMENT, for investigation of blood levels of oxytocin in relation to reproduction and lactation—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, Berks, quoting Ref. 64/16.

RESEARCH ASSISTANT (with an honours degree in physics) IN THE DEPARTMENT OF PHYSICS, to undertake research work in nuclear physics—The Registrar, Bradford Institute of Technology, Bradford, 7.

SENIOR PHARMACIST (full- or part-time)—The Physician Superintendent, Oakwood Hospital, Maidstone, Kent.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

The Third Phase in Metallurgy. By Prof. A. V. Bradshaw. (Inaugural Lecture, 3 December 1963.) Pp. 131–153+11 plates. (London: Imperial College of Science and Technology, 1964.) 4*s*. 6*d*. [89

Department of Scientific and Industrial Research. Research for Industry 1963: a Report on Work Done by Industrial Research Associations in the Government Scheme. Pp. v+54+19 plates. (London: H.M. Stationery Office, 1964.) 5*s*. 6*d*. net. [89

Commonwealth Scholarship Plan. Fourth Annual Report for the period ending 31 March 1964. Prepared by the Association of Commonwealth Universities for the Commonwealth Education Liaison Committee. Pp. 76. (London: H.M. Stationery Office, 1964.) 5*s*. [89

Tenth Report from the Estimates Committee together with part of the Minutes of the Evidence Taken Before Sub-Committee F and Appendices, Session 1963–64—Department of Technical Co-operation. Pp. xxix+243. (London: H.M. Stationery Office, 1964.) 16*s*. 6*d*. net. [89

Ministry of Labour. Manpower Studies, No. 1: The Pattern of the Future. Pp. 52. (London: H.M. Stationery Office, 1964.) 4*s*. 6*d*. net. [89

Frank Mason and Co., Ltd. Directors' Report and Statement of Accounts for the year ended 31st March 1964. Pp. 8. (London: Frank Mason and Co., Ltd., 1964.) [89

The Institution of Civil Engineers. British Civil Engineering Achievements Today. Pp. 7. (London: The Institution of Civil Engineers, 1946.) [89

Agricultural Research Council. Annual Report of the Radiobiological Laboratory, 1963–64 (ARCRL 12). Pp. viii+92. (London: Agricultural Research Council, 1964. Obtainable from H.M. Stationery Office.) 7*s*. 6*d*. [610

Department of Scientific and Industrial Research. Ergonomics for Industry 6: Noise in Industry. By D. E. Broadbent. Pp. 16. (London: Information Division, Department of Scientific and Industrial Research, 1964.) [610

A Monograph on the Theory of the Intelligent Universe. By Dr. David Foster. Pp. 4. (Windlesham, Surrey: David Foster, White House, Sunninghill Road, 1964.) [610

The British Glass Industry Research Association. Ninth Annual Report, 1964. Pp. 40. (Sheffield: The British Glass Industry Research Association, 1964.) [610

The Nuffield Foundation. Report for the year ended 31 March 1964. (Nineteenth Report, 1963–64.) Pp. xvi+204+2 plates. (London: The Nuffield Foundation, 1964.) [610

Scientific Proceedings of the Royal Dublin Society. Series A, Vol. 2, No. 3: The Structure and Mineralisation of the Mountain Mine Area, Allihies, West Co. Cork, Ireland. By D. J. Sheridan. Pp. 21–27+plate 3. 4*s*. Series B, Vol. 1, No. 12: The Effect of Aureomycin Supplementation on Urea Utilization in the Rations of Growing-Fattening Lambs. By D. Cahill and Prof. D. M. McAleese. Pp. 123–130. 4*s*. (Dublin: The Royal Dublin Society, 1964.) [610

White Fish Authority. Preliminary Report on South African Hake. Pp. 6. (London: White Fish Authority, 1964.) 2*s*. 6*d*. [610

Civic Trust. Derelict Land: a Study of Industrial Dereliction and how it may be Redeemed. Pp. 72. (London: Civic Trust, 1964.) 7*s*. 6*d*. [610

British Antarctic Survey. Scientific Reports, No. 46: Geophysical Investigation of the Scotia Arc. By Dr. D. H. Griffiths, R. P. Riddihough, H. A. D. Cameron and P. Kennett. Pp. i+43. (London: British Antarctic Survey, 1964.) 18*s*. net. [610

Incandescent, Ltd. Leaflet No. V76: Link Belt Conveyor Furnaces. Pp. 4. (Smethwick: Incandescent, Ltd., 1964.) [610

Manchester Literary and Philosophical Society. *Memoirs and Proceedings*, Vol. 106, 1963–64. Pp. 131+iii+4 plates. (Manchester: Manchester Literary and Philosophical Society, 1964.) 31*s*. 6*d*. [610

The Overseas Development Institute. British Aid—4: Technical Assistance. Pp. 196. (London: The Overseas Development Institute, 1964.) 7*s*. 6*d*. [610

Ciba (A.R.L.), Ltd. Technical Notes, No. 260 (August, 1964): Explosion Forming with Araldite Dies. Pp. 6. (Duxford: Ciba (A.R.L.), Ltd., 1964.) [610

Other Countries

United States Department of the Interior: Geological Survey. Water-Supply Paper 1600: Geology and Ground-Water Conditions of Clark County, Washington, with a Description of a Major Alluvial Aquifer Along the Columbia River. By M. J. Mundorff. Pp. vi+268+plates 1–3. Water-Supply Paper 1673: Magnitude and Frequency of Floods in the United States. Part 2-A: South Atlantic Slope Basins, James River to Savannah River. By Paul R. Speer and Charles R. Gamble. Pp. x+329+plates 1–3. Water-Supply Paper 1724: Compilation of Records of Surface Waters of the United States, October 1950 to September 1960. Part 2-B: South Atlantic Slope and Eastern Gulf of Mexico Basins, Ogeechee River to Pearl River. Prepared under the direction of E. L. Hendricks. Pp. xii+458+plate 1. Water-Supply Paper 1725: Compilation of Records of Surface Waters of the United States, October 1950 to September 1960. Part 3-A: Ohio River Basin Except Cumberland and Tennessee River Basins. Prepared under the direction of E. L. Hendricks. Pp. xiii+560+plate 1. Water-Supply Paper 1732: Compilation of Records of Surface Waters of the United States, October 1950 to September 1960. Part 8: Western Gulf of Mexico Basins. Prepared under the direction of E. L. Hendricks. Pp. xiii+574+plate 1. (Washington, D.C.: Government Printing Office, 1964.) [89

Agricultural Research Institute (Ghana Academy of Sciences). Technical Report No. 62: Soils of the Seilo-Tuni Land Planning Area, Wala District, near Wa, Upper Region. By H. B. Obeng. Pp. 196+4 maps. (Kumasi: Agricultural Research Institute, 1963.) [89

Mental Health Book Review Index, Vol. 9. Compiled by the Editorial Committee and Contributing Librarians. Pp. xvii+71. Annual Subscription: 5 dollars. Published under the auspices of the Council on Research in Bibliography, Inc., c/o Research Center for Mental Health, New York University. (Flushing, New York: The Index, c/o Miss Lois Afferbach, Paul Klapper Library, Queen's College, 1964.) [89

Colony of Mauritius. Meteorological Observations and Climatological Summaries. July 1963. Pp. 28. August 1963. Pp. 35. September 1963. Pp. 35. (Port Louis: Government Printer, 1964.) [89

Republic of Cyprus: Ministry of Commerce and Industry. Annual Report of the Geological Survey Department for the year 1963. By Dr. L. M. Bear and Y. Hji Stavrinou. Pp. 85. (Nicosia: Geological Survey Department, 1964.) [89

United States Atomic Energy Commission: Division of Technical Information. Nuclear Science Abstracts, Semiannual Index, January–June 1964, Vol. 18, Abstracts 1–21741. Pp. 1444. (Washington, D.C.: Government Printing Office, 1964.) [89

United States Department of the Interior: Geological Survey. Bulletin 1107-H: Geology and Uranium Deposits of the Pumpkin Buttes Area of the Powder River Basin, Wyoming. By W. N. Sharp, E. J. McKay, F. A. McKeown and A. M. White. Pp. v+54+plates 10–17. Bulletin 1133-E: Subsurface Geology of the National Reactor Testing Station, Idaho. By Eugene H. Walker. Pp. iii+22+plates 1 and 2. Bulletin 1147-F: Geology and Uranium Deposits at Crooks Gap, Fremont County, Wyoming. By James G. Stephens. With a section on Gravity and Seismic Studies in the Crooks Gap Area by Don L. Healey. Pp. vi+82+plates 1–11. Bulletin 1153: Geology of the Georgetown Canyon-Snowdriff Mountain Area, Southeastern Idaho. By Earle R. Cressman. Pp. v+105+plates 1–7. Bulletin 1161-G: Bedrock Geology of the Penn Yan and Keuka Park Quadrangles, New York. By M. J. Bergin. Pp. iii+35+plates 1–3. Bulletin 1161-H: Geology of the Cosio Knob and Espinosa Canyon Quadrangles, Montery County, California. By David L. Durham. Pp. iv+29+plates 1–4. Bulletin 1181-C: Geology of the Platte Canyon Quadrangle, Colorado. By Warren L. Peterson. Pp. iv+23+plate 1. Bulletin 1181-I: Stratigraphic Reconnaissance of the Matanuska Formation in the Matanuska Valley, Alaska. By Arthur Grantz. Pp. iv+11–33. Bulletin 1182-B: Geology and Coal Resources of the Coal-Bearing Rocks of Alabama. By William C. Culbertson. Pp. v+79+plates 1–4. (Washington, D.C.: Government Printing Office, 1964.) [510

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Telephone Number: Whitehall 0081. Telegrams: Phusis London W.C.2

Annual subscription: Inland £13, Overseas £13 15*s*., payable in advance, postage paid to any part of the world.

Advertisements only should be addressed to
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Telephone Number: Holborn 4743

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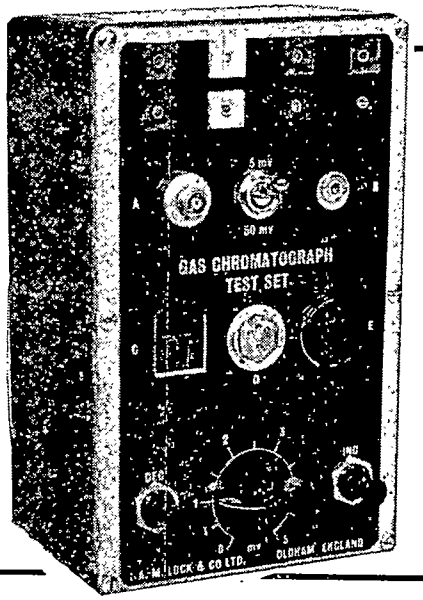
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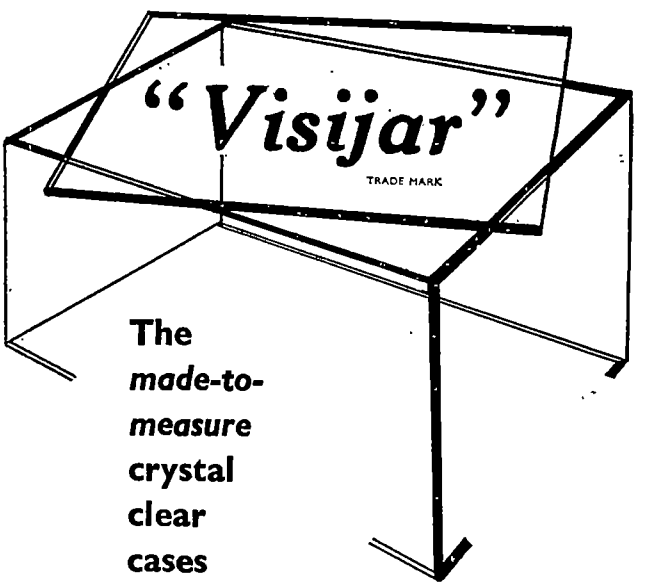
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SCIENTIFIC MAN-POWER AND THE NEXT TEN YEARS

THE science resources planning study, *Sustaining Academic Science*, 1965–75, prepared by W. V. Consolazio, of the National Science Foundation, deals primarily with the question of what resources the United States will require to train adequately all those who will seek higher education in science, mathematics and engineering during this decade. It does not discuss the related questions of the adequacy of the economy or the adaptability of the social and cultural *milieu* to utilize this man-power fully. Dr. Consolazio predicts that during this decade man-power in academic science and engineering will approximately double at both the trainer and the trainee level and that a full-time faculty of 173,000, including 93,000 with doctorates, will be needed for an output estimated in terms of 2,600,000 bachelor's degrees, 480,000 master's degrees and 130,000 doctorates.

The instructional costs in science and engineering at colleges and universities are estimated at some 28,000 million dollars, with a further 15,000 million dollars for research, 9,000–10,000 million dollars for plant, 7,000 million dollars for assistance to fellows and scholars, 2,500 million dollars for information services and a similar amount for improving the teaching of science and mathematics in the schools. Dr. Consolazio regards the total cost of 65,000 million dollars as a conservative figure, but, while he assumes that the nation possesses the necessary resources, only some 37,000 million dollars is expected to be available from non-Federal resources, and he is doubtful whether the Federal Government will, in fact, assume the responsibility for the difference of some 28,000 million dollars. He shows clearly enough what is likely to be involved if the United States attempts to provide the supply of scientific and technical man-power that he postulates, but he does not attempt to consider whether the provision of these resources is justified, still less to examine whether in fact such trained man-power would be wisely and effectively used.

Nevertheless, this paper, now reprinted from the Spring 1964 issue of *The Educational Record*, published by the American Council on Education, makes an interesting complement to Dr. C. F. Carter's address, "The Economics of Higher Education", before Section F (Education) at the British Association meeting in Southampton on August 27. Moreover, it complements the Manpower Study, *The Pattern of the Future**, issued by the Ministry of Labour at almost the same time, and the long-term building programme of £83 million for universities and colleges of advanced technology between 1966 and 1969 announced on September 2 by the then Secretary of State for Education and Science, Mr. Q. Hogg. Of this amount 3 million was proposed for the first year, but the figures of £25 million for each of the subsequent two years were provisional, pending more precise advice from the University Grants Committee on the capital position as it will be when the existing building schemes have been completed. They provide for the colleges of advanced technology and two Scottish central institutions, the Heriot Watt College, Edinburgh, and the Glasgow College of Commerce, which is to be combined with the University of Strathclyde. These figures were related only to building costs and did not include the cost of sites, professional fees or equipment.

Dr. Carter's paper went much nearer to asking the vital questions that should be considered before we determine the magnitude of the expansion in higher education that our resources allow, still more the means by which the desirable expansion should be achieved. He was critical of the Robbins Report on some points, and his paper was a stimulus to clear thinking. He was not content to show that the cheapest place to expand is probably an existing institution with access to a large cheap site, or to question whether any more new institutions are needed, in addition to the seven recently-founded universities, if the plans for resiting and expanding the present colleges of advanced technology are carried out. He provoked, deliberately, some realistic thinking about student life and halls of residence and the like.

As Dr. Carter rightly stated, before we ask for great additions to the substantial support which the State already gives to higher education, we owe the community some clear thinking about the results that we are trying to achieve, and the necessity of the expenditure proposed as a means of achieving those results. He quoted with approval questions asked by Prof. B. R. Williams in an article on "Capacity and Output of Universities", published in May 1963 in *The Manchester School*. Dr. Carter himself was manifestly prepared to consider the possibility of a longer teaching year if work is programmed to give members of staff uninterrupted periods of research. Quite apart from any possibilities which research into teaching methods may indicate, he was also open-minded on the length of the academic day and believes that there may be educational advantage in devising a teaching day in which formal teaching, private study, social contacts and recreation are well mixed, with no long periods allocated to any single activity.

Dr. Carter is too wise to overstrain the argument for economic efficiency but he and Prof. Williams believe that it is possible, without detriment to academic autonomy or quality, to give a university some direct incentive to think of ways of using its buildings and equipment most efficiently. Higher education cannot be considered in complete isolation from the economic life and position of the nation, and that in itself is sufficient reason for looking at university expansion in the light of the first of a series of investigations coming from the Ministry of Labour's Man-power Research Unit. This examination surveys Britain's future man-power resources, describing the expected changes in the size and structure of the working population, and then attempts to forecast future employ-

* Ministry of Labour, Manpower Studies No. 1: *The Pattern of the Future*. Pp. 52. (London: H.M.S.O., 1964.) 4*s*. 6*d*. net.

ment trends in the different groups of industry and the distribution of man-power between broad sectors of the economy, before finally considering the main implications for policy. It is essentially concerned with the national level and does not deal with regional employment problems, providing, however, the national setting within which examination of regional employment problems may be made.

First, the Study points out that, while between 1958 and 1963, in spite of unusually large numbers of school-leavers and immigrants, addition to the working population barely kept pace with the rising numbers of those outside it, in the next five years it is expected that they will fall short by a considerable margin, and after that the gap will widen. In the decade 1963–73 the estimated increase among those at work will be only one-third of the increase in the remaining population. Accordingly, the growing volume of goods and services needed to maintain and raise our general standard of living must come from relatively few extra people at work. This not only implies that productivity must increase at a faster rate even if it is to maintain the present rate of improvement in standards of living, but it implies also a two-sided challenge to all institutions of higher education, to teachers and students alike. To use man-power—or woman-power—efficiently is as imperative as to provide more efficient and highly trained man-power and woman-power in the first instance. The note of responsibility is unmistakable and unavoidable.

Of the detailed examinations of the manufacturing or services sectors of the economy set forth in the survey it should suffice here to note that employment in chemicals and in iron and steel, which has tended to rise over the past decade, may tend to level off. Moreover, in several other expanding sections—food, drink, tobacco, the large engineering groups and the paper and printing industries—the rate of growth may be slower. The decline in the textiles and clothing group is expected to moderate, and in many other industries no major changes of trend are foreseen. In services, the largest increases are expected in health and education services and in distribution, though with some retardation of the rate of growth for the last-mentioned. In financial services, the rapid growth of business is expected over the next five years to outweigh the effect of introducing computers and an increase is also expected in employment in public administration. One detail to be noted in the present context is that employment in educational services increased by 44 per cent, about half this being in the last four years of the decade, and in these services the proportionate increase for men was slightly larger than that for women.

The four remaining groups—agriculture, forestry and fishing; mining and quarrying; the construction industries; and the public utilities—have little in common and show widely different trends. For the first group a continuing decline in employment over the next few years is forecast, and a similar continuing decline is expected in the coal industry, as well as in the mining and quarrying group as a whole. In the construction industries, employment is expected to continue at least at its present level, but some increase in the labour force may well be required even allowing for substantial rises in productivity. Employment is expected to increase, but more slowly, in the electrical industry, but the gas industry is expected to meet increasing demand with a somewhat smaller labour force.

A feature of the forecast is the stability expected in the share of employment represented by manufacturing industry, which over the next five years is expected to stay at about the level of 1963, or just more than 9 million in 1968, when more than 11·5 million are forecast in the services sector and about 3 million in the rest of the economy. The increased percentage share of the services sector is likely to be mainly at the expense of the rest of the non-manufacturing sector. Since between 1963 and 1968 the working population is expected to be growing at a rather slower rate than hitherto, it is probable that, taking account of technological changes already operative or known to be in prospect, the needs of an expanding economy will at least provide the sufficient number of jobs for the foreseen increase in the number of workers.

Accordingly, the Study concludes that total man-power resources are unlikely to be more than adequate during the period and the efficient and flexible use of man-power will continue to be of the greatest importance. At times of peak demand labour shortages comparable with those of recent years may well occur unless active steps are taken to prevent them. Further, while opportunities of employment for women may expand slightly more rapidly then those for men, the distribution of the labour force as between the sexes is likely to remain unchanged. The only major alteration expected in its structure is a fall in the number of single women and girls, matched by a corresponding increase in the number of married women. Pressure on female labour resources may be particularly strong, and the increasing proportion of the labour force over 45 years of age will necessitate greater readiness to retain or engage older workers.

It was outside the scope of this Study to consider the implications for education and training of these changes or of the continuing rise in the proportion of 'white-collar' workers in manufacturing industry, but no responsible administrator in education at any level can disregard those implications, whether in respect of the courses and education to be given or the demand for services made on the economy. Efficient utilization of man-power, the report reiterates, will become even more essential if the task of maintaining and raising the general standard of living is to be achieved with a labour force which will be smaller in proportion to the total population. Not only will the development of automation and of any other means of increasing productivity assume increasing importance and, even be stimulated by the shortage of labour, but also other factors, such as the decrease in the number of young people entering the labour market, will call for adjustments, while simultaneously tending to make the labour force less adaptable and flexible. The continuing rise in the number of married women employed will enhance the need for mobility among the rest of the working population.

These are all factors or trends of which due account must be taken in the shaping of educational policy and in the allocation of resources for education, nor is this picture of tightly stretched man-power resources likely to change significantly after 1968. Indeed, up to 1973 it is more likely to be intensified, and although, admittedly, at this range forecasts of employment are hazardous, the very slow increase in the labour force during this period suggests that overall shortage is much more likely than a surplus, and man-power resources may well be more severely strained after than before 1968. For that reason alone the expansion of education should be planned with prudence and with regard not merely to the imperative

need to equip the rising generation more effectively to cope with the problem of living in a technological and rapidly changing world but also to the resources which can in practice be made available for that task.

This is not to deny the responsibility of the State for the education of its young—or older—citizens. It is to assert that that responsibility must be discharged realistically, with an imaginative understanding of what is required, as well as with a keen appreciation of the nation's resources for the task and of the opportunities which will be available for those it educates and trains at particular levels. It means that educational policy must provide a firm basis in the schools which equips the young citizen with some understanding of the world in which he lives, encourages responsibility and adaptability and provides an appropriate starting point for particular technical or professional training later. It must also ensure that the provision of training for technicians, craftsmen and others is not only appropriate but also on a scale sufficient to permit the most efficient use of the more highly trained technologists, scientists or other professional workers when they will be called on to assist, and equally that the facilities for such higher education are both adapted to their purpose and on a scale commensurate with the resources and the needs of society. The quantitative aspect should never be entirely disregarded in a realistic educational policy, for to do so is indeed a grave disservice to the interests of the individual himself. Both Mr. Consolazio and Dr. Carter have given timely warnings of the danger of an unlimited and idealistic expansion of higher education which pays insufficient attention either to the resources available, to the capacity of the student or to opportunities open to him or her when trained.

BRITISH BUSINESS SCHOOLS

THE report on *British Business Schools* made by Lord Franks* last November (see *Nature*, 201, 1252; 1964) has been followed by one from a Working Party†, set up towards the end of December 1963 with Lord Normanbrook as chairman, by the Federation of British Industries. The object was to establish the acceptance by the universities concerned, by the University Grants Committee, and by industry and commerce, of the principles of partnership recommended by Lord Franks. The Working Party was to work out a capital budget, as well as an operating budget, and, on this basis, to chart out the financial requirements for each school, and establish a recommended basis of sharing the provision of funds. Lord Franks's recommendation that each of the two business schools should provide a one-year course for 200 postgraduate students and two post-experience courses annually, each for 100 students, giving a maximum of 300 students at any one time at each school, was accepted by the Working Party, together with the assumption that these post-experience courses should last 20 weeks. Without challenging Lord Franks's assumption that the courses would be most valuable if wholly residential, it was concluded that it would be unrealistic to plan on the basis that all students would be in residence, and the Working Party has assumed instead that only 50 per cent would be residential. Further, while the School at Manchester would, from the outset, be an integral part of the University, in London certain constitutional complications might be avoided by establishing the School in the first instance as an independent institution, although it would be sponsored by the London School of Economics and the Imperial College of Science and Technology, and would draw on their resources.

The capital cost of establishing a business school is estimated at £935,000 apart from the site. In Manchester the total capital expenditure is unlikely to exceed £1 million, but in London the cost of acquiring and clearing a suitable site may bring that figure to between £1·18 and £1·43 million. The recurrent expenditure is estimated as rising from £118,500 in the first year to £300,000 when the school is at full capacity in the eighth year, by which time the income of £134,000 should bring the recurrent cost to £166,000. The Working Party did not consider the detailed curriculum, but discussions on the purpose of the instruction to be given and the general content of the curriculum showed no substantial differences of view, on either methods or purposes, between the two Universities and the business interests represented on the Working Party.

The Working Party is in full agreement with Lord Franks's general description of the purpose and function of the business schools, to promote business and industrial efficiency by ensuring a larger supply of managers and potential managers familiar with the new techniques and tools of management, and trained to foresee and decide well in swiftly changing situations. It suggests, however, that emphasis should be given in the curriculum to the importance of acquiring a thorough knowledge and understanding of financial controls in a business organization, and it also hopes that the courses will be designed to inculcate a strong sense of cost-consciousness, which in the Working Party's view is needed in non-profit-making as well as in profit-making organizations. Further, while endorsing the proposals that the courses should aim to attract students between 28 and 35 years of age, it hopes that the average will be nearer the younger than the older end of the range, and it is believed that a course of this kind would be of most value to a man who has spent about ten years in industry. The schools may well decide later to offer other kinds of courses for older and more experienced people, and the Working Party observes that the facilities available must be capable of adjustment to meet such changes in demand.

The Working Party has thus not accepted the assumptions of Lord Franks's report uncritically or without constructive suggestions. It should be noted also that, as Mr. Stephen Aris has pointed out, in describing an enquiry into the present state of management education in Britain which he recently broadcast in the B.B.C. Third Programme (see *The Listener*, 982; June 18, 1964), while both industry and Government have officially accepted Lord Franks's broad proposals, some of his detailed suggestions and even some of his basic principles are being strongly challenged among business men and

* British Institute of Management. *British Business Schools:* Report by the Rt. Hon. Lord Franks, P.C., G.C.M.G., K.C.B., C.B.E. Pp. 20. (London: British Institute of Management, 1964.) 5s.

† British Institute of Management. *British Business Schools—The Cost:* Report by a Working Party under the Rt. Hon. Lord Normanbrook, P.C., G.C.B. Pp. 10. (London: British Institute of Management, 1964.)

among management teachers at the universities and elsewhere. Further, while repeated emphasis is placed on the importance of efficient management from many points of view both in industry, for example, by Lord Bowden, and in Government, as in Prof. G. Devons's broadcast talk, "Economic Management in Whitehall", the subject is attracting attention in many different places. A sentence about university training in Mr. S. P. Chambers's Chuter Ede Lecture on education and industry (*Nature*, 203, 227; 1964) was widely quoted out of its context, and one of the most interesting passages in the fifth report of the Estimates Committee, *Treasury Control of Establishments* (*Nature*, 203, 797; 1964), is that containing Sir Ronald Holroyd's evidence on the recruitment and training of staff by Imperial Chemical Industries, Ltd. It is also not without significance that, in launching a new *Business Supplement* on September 27, the *Sunday Times* included a long article on recent changes in the organization and administration of that firm, the trend of which is strikingly in line with the emphasis of Lord Normanbrook's Working Party on financial control and cost-consciousness.

Mr. Aris points out that while it is to-day widely recognized that there is urgent need for the manager to acquire a professional approach to his job, most British managers have only a sketchy idea of the tools available to them, and as yet have made singularly little effort to find out. Nevertheless, there are still those in industry who stoutly maintain that management is unteachable, that it is an art, not a science. Obviously, the answer depends on what you mean by management, and the academic approach has its limitations, as Dr. R. W. Revans, professor of industrial administration at the Manchester College of Science and Technology, admits. When the techniques have been learned, the problem is to apply them in a factory. Dr. T. Lupton, of the Department of Industrial Administration, Birmingham College of Advanced Technology, thinks that management teachers try too hard to persuade business that they can solve its problems, whereas what they have to offer is something which business men can use in solving their problems themselves. Moreover, industry is entitled to be concerned over the close linking of the two business schools with universities unless its teachers have some degree of practical business experience.

The need is for teachers with both the necessary intellectual skills and the practical experience, and in Britain we are now paying for the long years of neglect in which quite unnecessary obstacles were created to the movement of men between industry and the universities and colleges of technology. Dr. Lupton does not regard the problem as insoluble though it will not be solved by the offer of high salaries alone. More may well depend on how far the universities are able to undertake research in this field, and the co-operation of industry is essential if teachers are to gain the necessary experience.

Apart from a reference to the desirability of efforts, parallel to the establishment of the two business schools, to establish management studies as part of the accepted structure of teaching and research in a number of universities, Lord Franks ignored this aspect of research, and Mr. Aris notes that it is primarily the members of the newer universities and the colleges of advanced technology who recognize that industry offers a legitimate field for intellectual enquiry. Some of them, however, have reservations as to the effect on their own institutions of an attempt at this juncture to build up two new schools almost from ground level, and would prefer to develop existing resources as the more economical policy. Here again it is a matter of man-power resources and of the limitations imposed by earlier neglect and lack of foresight.

Apart from these wider issues, it is clearly important for both industry and the universities, as both Mr. Aris and Dr. Lupton suggest, to consider even more closely the precise nature of the contribution which the schools can and should make. In addition to hard constructive thinking about the content of courses, industry must also take the development of management much more seriously. Some firms like Pilkington's, the glass manufacturers, already recognize that responsibility for training managers does not begin and end by selecting suitable men and sending them to a business school. Steps must be taken also to broaden the general outlook of the trainees, and here management courses at technical colleges and in the economics departments of universities and colleges of technology, as well as management consultants, may have something to offer. Part of the whole process is the selection and internal movement practised by Imperial Chemical Industries, Ltd., which Sir Ronald Holroyd described to the Estimates Committee.

With all this must go clear ideas as to the type of manager for which particular courses are designed, and it may well be necessary to take a fresh look at the functions of such an institution as the Administrative Staff College at Henley. Moreover, Mr. Aris made a sound point in emphasizing the need for an attempt to ensure that even the middle ranks of management are fully aware of the implications of new skills and developments, socially as well as technologically: much unrest in industry is the result of ignorance rather than of ill-will. Lord Bowden made rather a similar point in emphasizing the importance of research into the problems of society, though in his enthusiasm for vocational education he tended to suggest that an appropriate expansion of technological education is all that is needed. Certainly, Prof. Devons did not suggest that, whether in Government or in industry, techniques alone are sufficient, and, without being dogmatic as to the precise structure required, he was emphatic that much more attention should be paid in Whitehall to the selection and training of managers.

Mr. Chambers's lecture, "Education and Industry", is particularly significant against just this background, for he was concerned neither with academic education nor with vocational training but with the development of character, especially of all those elements which enable a man to work effectively and harmoniously with his colleagues. He recognized that the inculcation of moral values was also important, though he was not concerned specifically with this or with the equally important acquisition of knowledge, the development of the power of thinking and reasoning, or with specialized vocational training. He was concerned primarily with such characteristics as the capacity to evaluate factors and reach firm decisions; to control other men fairly and firmly and win their confidence; to delegate responsibility and to tolerate errors of judgment in subordinates within reason and without inquests; and to take firm action, however unpleasant, where the standard of performance has fallen below the tolerable limit.

Mr. Chambers pointed out that training in the principles of management at a later stage would not convert a timid man into a man of decision. It was in this context that he remarked that life at a university, with its intel-

lectual and inconclusive discussions at the postgraduate level was, on the whole, a bad training for the real world, and only men of very strong character could overcome this handicap. He was not attacking university education but directing attention to certain shortcomings which are often ignored—for example, in the 1963 Reith Lectures—and to which little attention is directed in planning the expansion of higher education. If, as Mr. Chambers reminds us, brilliant academic achievement is frequently linked with poor judgment, indecisiveness and incapacity to delegate work or control men, while the positive characteristics are frequently found in men with second- or third-class degrees, are we wise to attempt to channel so much talent into university institutions to the neglect of colleges of technology and other institutions of further education ?

Mr. Chambers's lecture is important for the questions which it raises and on which the Robbins Report scarcely touches, and these questions have a significance which is far from being limited to training for management. It has implications for the universities themselves, and although the Robbins Committee, like the University Grants Committee, recognized the importance of character training, little attempt is apparent in working out the implications. Certainly it would be hard to find in Dr. Sloman's Reith Lectures any satisfactory discussion of either the development of character or of the related problem of discipline.

Mr. Chambers's lecture was thoroughly realistic on both these issues and he showed just where we are heading if we fail to devote a good deal more attention to problems which are often highly unpopular. Besides this emphasis and its call for hard constructive thinking, as in his reference to the importance of education for management, his remarks on applied science deserve to be widely read. Mr. Chambers has no use for the arrogance which regards pure science as of a higher order of intelligence than applied science. The combination of powers of intellect and judgment required for applied scientific work by brilliant scientists struggling with a specific objective may not be possessed by the pure scientist who looks down on them, nor will that scientist understand how much harder is the task of working successfully in a team than the individual attack on abstract problems, just as he will also fail to appreciate the importance of problems of management or team-work generally.

This spirited defence of applied science is important and timely, and while it includes a plea for greater respect for efficient management in the context of management generally, the importance of Mr. Chambers's address lies in its reminder that if the new business schools are to be assured of a continuous supply of suitable students, we must see that the foundations are firmly laid in our educational system as a whole. Whatever changes in the staffing or methods of our universities may be desirable, we cannot wait until the student enters the university or the college of technology before coming to grips with the related problems of discipline and the development of character. A start must be made in the schools, which must share the responsibility with the universities and the other institutions of higher education. Unless, indeed, this responsibility for the training of character is fully and speedily recognized and appropriate measures taken with real determination, neither the institution of business schools nor the expansion of higher education is likely to provide Britain with the managers, the men or the sense of purpose that is needed.

EXPERIMENTAL AND THEORETICAL TWINNING OF CRYSTALS

Mechanical Twinning of Crystals

By M. V. Klassen-Neklyudova. Authorized translation from the Russian by Dr. J. E. S. Bradley. Pp. xiv+213. (New York: Consultants Bureau, 1964.) 19.50 dollars.

MECHANICAL Twinning of Crystals provides a good comprehensive review of experimental and theoretical work on the twinning of crystals. As with other publications of this kind, its particular advantage lies in its up-to-date survey of Russian work in this field.

The ground covered by the book is more extensive than the title at first suggests. In addition to a full treatment of the fundamentals of mechanical twinning there are interesting chapters dealing with martensitic transitions, recrystallization twins, and lattice re-orientation in inhomogeneous deformation (kink bands, deformation bands, etc.).

The book is divided into three parts. The first part deals with experimental data on mechanical twinning from the point of view of twin geometry and crystallography. A clear and detailed exposition of the geometrical laws and the methods for determining the twinning elements in the different crystal systems is given. Methods of producing mechanical twins in crystals are dealt with and there is an interesting account of elastic twinning in crystals. Short chapters deal with twinning without change of form in quartz and with the role of mechanical twinning in plastic deformation and fracture. The latter is very brief and confined mainly to Russian work on this subject. The second part of the monograph considers effects related to mechanical twinning such as recrystallization and martensitic changes. In this section, too, there is a good account of large-angle re-orientations accompanying inhomogeneous deformation. The final part of the book deals with the theory of twinning and has a chapter on twin growth considered in terms of the macroscopic theory of elasticity, and a chapter on atomic and dislocation models of twinning.

Appendixes contain tables of twin elements for metals, alloys and non-metallic crystals. There is also a short review prepared by V. I. Startsev for the English edition of selective dislocation-etch methods for examining the twinning mechanism in calcite.

The monograph is primarily a review and as such it is a good one. It is concise, possibly too concise in parts; on the other hand, the field dealt with is large and it is better in a work of this kind to provide references (there are more than 500) than to repeat the arguments in full. The book is intended for research people already working in the field. Someone new to the subject would benefit, however, from the introductory chapters dealing with the fundamentals of twinning.

The book is well produced and illustrated and the translation reads smoothly. Its publication in English is to be welcomed, but it is a pity that it should be so expensive.

J. H. BRUNTON

TUMOURS OF THE HUMAN ENDOCRINE GLANDS

Human Tumours Secreting Catecholamines

Clinical and Physiopathological Study of the Pheochromocytomas. By Prof. Henri Hermann and Prof. René Mornex. Translated by Dr. R. Crawford. (International Series of Monographs on Pure and Applied Biology, Vol. 23.) Pp. xiii+207. (London and New York: Pergamon Press, 1964.) 60*s*. net.

TUMOURS of the human endocrine glands that are not easily detectable by clinical means usually secrete abnormal quantities of their characteristic hormone, and

are thus open to detection by chemical or biological tests. Interest in these tumours is consequently shared by physiologists and clinicians. *Human Tumours Secreting Catecholamines* deals with the phæochromocytomas; the adrenaline and noradrenaline secreted in more than normal amounts produce effects in the human body which are familiar to the physiologist from his animal experiments, and which assist the clinician in diagnosis. It is thus appropriate that this investigation should be the result of collaboration of a physiologist and a physician. It represents about 30 years of investigation of the subject in the School of Medicine at Lyons. The authors have collected details of more than 500 published cases, and the various aspects discussed are illustrated by reference to appropriate patients.

The most obvious manifestations of these tumours are cardiovascular, with paroxysmal or sustained hypertension, as might be expected from the hypertensive action of adrenaline and noradrenaline. Hypertension is such a common symptom that the difficulty is to detect the relatively small number of cases in which it is due to a tumour of chromaffin cells. There are excellent chapters dealing with the various manifestations, cardiovascular, metabolic, and endocrine; and unusual clinical forms, for example, asymptomatic cases in which the adrenomedullary tumour may remain latent throughout life, only to be discovered at operation or autopsy. These tumours of chromaffin tissue may be single or multiple, confined to the adrenal medulla or ectopic (developing from embryonal rests); all these are well described in the chapter on anatomical forms. The most important chapter is that on diagnosis, an aspect which is often difficult because of the diverse symptoms produced by the tumour.

Profs. Hermann and Mornex stress the importance of interrogation and clinical examination. But from their experience of a large number of urinary catechol estimations, they are struck by the number of cases in which the clinical diagnosis of a tumour has to be rejected on the laboratory evidence. This is a general experience of those carrying out tests. The authors point out that no single technique is sufficient for complete investigation; they favour the estimation of urinary catecholamines, first by a biological method using dogs, followed by chemical methods for vanillyl mandelic acid or other methoxylated derivatives, and for the most accurate estimations the fluorimetric (trihydroxyindole) method.

Pathological anatomy, biological investigations, and physiopathology are dealt with in the concluding chapters. One would like to have seen more mention of the neuroblastomas, the other human tumours that secrete catecholamines. The 500 cases are listed in a table, with references, and all statements are supported by references to the literature contained in a bibliography of 800 papers. By contrast, the subject index is so brief as to be of little value. The translation reads well. This book is a most excellent and comprehensive treatment of the subject.

A. B. Anderson

VITAMINS AND COENZYMES

Vitamins and Coenzymes

By Dr. Arthur F. Wagner and Dr. Karl Folkers. Pp. xvi+532. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, 1964.) 132*s*.

THIS volume, which derives from the extension of a chapter on vitamins in the second edition of *Medicinal Chemistry* (Burger, 1960), is introduced by a discussion of the evolution of the vitamin theory—appropriately headed by Sir Frederick Gowland Hopkins's comment: "No general or widespread belief in the view that an adequate diet must contain indispensable constituents other than adequate calories, a minimum of protein, and a proper mineral supply, could be said to exist until the years 1911–12". After the historical introduction, the known activities and the nomenclature of vitamins are outlined before the main thesis of coenzyme connexions is explored. The earliest recognition of coenzymes related to vitamins was in the investigation of thiamine metabolism and of cocarboxylase. The consideration of coenzyme activity is preceded in the chapter on thiamine, as in succeeding chapters, by an account of the isolation, chemical identification and synthesis or biosynthesis of the vitamin. Nutritional and therapeutic investigations in animals and man are included as a final section for every chapter.

Within the framework thus designed, a useful and comprehensive account is given of many highly diverse biological systems. The vitamins of the B complex participate in many enzyme groupings and work in the field of B vitamins has been the main source of the expanding knowledge of coenzyme structures and activities. The original naming of diphosphopyridine nucleotide and triphosphopyridine nucleotide as coenzyme I and coenzyme II is an example of the basic importance of B vitamins in the coenzyme field. With the recognition of the role of pantothenic acid as an integral part of the universally important coenzyme A, a further wide field of metabolic investigation was opened in physiology, medicine, nutrition and microbiology. With pyridoxine and codecarboxylase, another step was taken towards the elucidation of the biochemical mechanisms essential to life from the cellular level up to the higher organisms. Biotin, which for many years was mainly investigated in relation to so-called "egg white injury" and then as a bacterial growth factor, has now emerged as an essential part of the "activated carbon dioxide" in the acetyl coenzyme A carboxylase system.

Folic acid and its array of derived pteroylglutamic compounds is another important field and so is cyanocobalamine with cobamide coenzymes related to vitamin B_{12}. The chapter on lipoic acid is very useful since it is not easy to find a full and satisfactory account of this recently discovered factor elsewhere in the literature.

For the fat soluble vitamins there were few clear coenzyme connexions known until the discovery of coenzyme Q or ubiquinone related to vitamin A. The review of the ubiquinone group provides a good final chapter to round off a valuable contribution to the biochemistry bookshelf. Though the emphasis of the material is essentially biochemical, the nutritional and therapeutic roles of vitamins and coenzymes are discussed so that *Vitamins and Coenzymes* serves as a reference source for many disciplines.

The way in which chemical and structural formulæ are presented is very good. The book can be recommended for advanced biochemical teaching though it is probably too chemical for medical students. For biochemical and nutritional research it is invaluable, since it covers most recent literature and presents a balanced review of the vitamin field. As the authors state in their preface, omission of material inevitably occurs, deliberately or inadvertently, but the whole picture is undoubtedly well conveyed.

A. M. Copping

THEORY AND PRACTICAL IMPLICATIONS OF QUANTITATIVE INHERITANCE

Quantitative Genetik

Von Gunnar Eilert Hiorth. Pp. viii+467. (Berlin: Springer-Verlag, 1963.) 86 D.M.

QUANTITATIVE Genetik covers the theory and practical implications of quantitative inheritance, and students with a moderate knowledge of German will

find that it is a useful introductory text in this field. The greater part of the illustrative material used in the book is taken from research work on crop plants and, unlike most other modern books on the subject, the author's approach is non-mathematical.

The treatment is a description of the biological basis and practical utilization of quantitative variation rather than a statistical interpretation of the genetical basis of quantitative inheritance. Indeed, anyone interested in the latter approach would find the volume particularly disappointing. For example, the absence of a serious discussion of Mather's contributions to biometrical genetics and of his theory of polygenic inheritance is to be regretted in a book of this kind. Furthermore, the lack of treatment in depth of the basic tools and concepts of quantitative genetics, such as variance analysis, heritability, the variance analysis of diallel crosses and the coefficient of inbreeding, is an unfortunate omission in a specialist text-book on quantitative genetics. Advanced students of genetics will therefore find the treatment incomplete, but for non-specialists in biology and agriculture, the numerous examples and detailed description of methods supply a thorough basic understanding of the characteristics of quantitatively inherited variation.

The first three chapters are devoted to a historical introduction, to elementary statistics and to the basis and general nature of quantitative variation. Chapter 4 is a valuable assembly of detailed summaries of the behaviour of some of the best-known examples of quantitatively inherited characters in plants. The eight chapters which follow present the genetic basis of quantitative variation including: gene conversion, cytoplasmic and environmental effects, gene interaction, heterozygosis, allelic frequencies, gene mutation, correlated characters, the effect of competition on quantitative characters in populations of inbreeding and outbreeding species, the effects of inbreeding and the genetic basis of inbreeding depression. Regrettably, however, neither additive gene action nor directional dominance is discussed in connexion with the latter. Three chapters are devoted to selection where the emphasis is on description of methods of selection, particularly those used in plant breeding. There is also some discussion here of the principles underlying selection of quantitative characters such as heritability, the response to selection and correlated response (*nebeneffekte der Selektion*), but, in general, the treatment is rather brief and sketchy.

Heterosis is discussed at length in three chapters, one of which describes the methods used for the isolation and testing of inbred lines for superior combining ability in hybrids. Chapter 19 is a miscellany of breeding methods additional to those covered in previous chapters, including gamete selection, polycross testing, synthetic varieties and the utilization of male sterility. Finally, a very short chapter is devoted to "The System of Variability", and a concluding chapter recapitulates some of the main themes. There is a useful bibliography appended to each chapter, and the text is well supported by numerous tables and figures, and by author and subject indexes.

WATKIN WILLIAMS

DEVELOPMENT OF A BIOLOGICAL THEORY

Heat and Life

The Development of the Theory of Animal Heat. By Everett Mendelsohn. Pp. x+208. (Cambridge, Mass.: Harvard University Press; London: Oxford University Press, 1964.) 36*s*.

AT the present time, when the volume of scientific literature is increasing at an overwhelming rate, less and less attention is paid to material and ideas published before the turn of the century. It is therefore very refreshing to trace the history of an important biological problem to that point in time when most modern developments take root. The choice of animal heat as a subject has been made carefully in order to provide a biological problem which has occupied the minds of philosophers and scientists since the earliest times and also one where the development of theory has been linked with advances in physics and chemistry. The problem of the origin of body heat is ideal for this purpose and, using it, the author sets out to investigate how far the reduction of a biological phenomenon to a physical analogue has been useful in biology.

The history begins with the Ancient Greeks who believed in a vital heat which burned in the heart. Even at this early stage, long before the experimental approach was used, a connexion between body heat and true fire was postulated, the breath serving both to cool the fire and fan the flame while food provided the fuel. No attempt was made, however, to put forward a physical or mechanical explanation for the warmth of the body, innate heat being regarded as mysterious and in some way akin to the heat of the Sun.

These, and similar views, were held until the seventeenth century and the beginnings of modern science. At this time Harvey, while still keeping the idea of an innate heat, suggested that the blood served to transport this heat about the body. In his later works the concept of innate heat lost for Harvey a little of its mystery and he tended to regard it simply as a property of blood. Descartes also held ideas about animal heat and he advanced a theory of the heart's motion based on the expansion of the blood as it entered the heart which was, he believed, extremely hot. In these notions Mr. Mendelsohn sees the first willingness on the part of man to explain the action of the body in mechanical terms, and he traces the development of theory through Willis, van Helmont, Hook and Boyle. As he points out, the changes in biological theory at this time were related not so much to new information as to a new approach to the problem.

The next phase of development, in the later seventeenth century, consisted in further attempts to relate combustion and animal heat, and in the beginnings of the experimental approach. During this period Borelli was at least able to show that there was no burning heat in the heart. The emphasis then shifted in the early eighteenth century towards a mechanical explanation based on the heat of friction generated as the blood passed through the blood vessels. In spite of several attempts at measurement and calculation this period produced a great confusion of thought which, in Mr. Mendelsohn's opinion, stemmed partly from the lack of instruments and techniques but mainly from the primitive state of chemical and physical theory. The principal difficulty lay in the confusion of temperature with quantity of heat.

The step which was needed to form the basis of modern theory was made by such men as Black, Crawford and Lavoisier, who finally established the connexion between combustion and metabolism and recognized the importance of blood in the transport of oxygen and carbon dioxide. In the latter part of the book the author directs attention to the circumstances which made Lavoisier's ideas acceptable while similar theories had failed in the past. He concludes that progress in the theory of animal heat depended simply on progress in physics and chemistry, and suggests that further development was made because scientists were able to deal with the body in parts, regarding the blood, for example, simply as a liquid. The advances made since the time of Lavoisier are not mentioned, being the subject of a further investigation by Mr. Mendelsohn.

Throughout the book there are numerous footnotes and references to writers of every period, and it is evident that the text is based on an extensive reading of the literature. The book would be useful reading for any student of biology.

D. L. INGRAM

ARCHITECTURE, TECHNOLOGY AND SOUTH KENSINGTON*

By Sir WILLIAM HOLFORD
Professor of Town Planning, University College, London

THE first nucleus of the present Imperial College of Science and Technology, London, came into existence in 1845 as the Royal College of Chemistry, and during that century despite the coming together of a number of scientific and teaching institutions—such as that which led to the establishment of this federated College in 1907—there have been continuous demarcations of subject and of professional practice.

One of the technologies which has taken a forked path has been that of construction; and now that the two directions taken by civil engineering and architecture are becoming once again parallel, or actually converging a little, it is interesting and revealing to trace some of the major changes of attitude that have occurred on the way from *A* to *B*.

I need not go back to the mid-seventeenth century when that experimental philosopher, Christopher Wren, having graduated bachelor of arts at the University of Oxford and carried out applied research in physiology and physics, became Gresham and then Savilian professor of astronomy before he became Surveyor of Works and what we now call an architect. Yet it is interesting to note that Wren, in drafting the preamble of the Charter granted by Charles II to the Royal Society of London, stressed the social value of the arts and sciences, both in theory and application.

Let me start instead at the early nineteenth century, when modern technology begins and when professions were emerging, other than medicine and the law, concerned for the first time with machines and with the application of scientific techniques to industry and commerce. Above all things at that time the new professional associations desired to achieve respectability and integrity. I stress the professional association because this has been powerful in architectural education from the very beginning and still gives a special flavour to the Architectural Association School in Bedford Square. In 1820, the embryonic Institution of Civil Engineers wrote from Gilham's Coffee House to Thomas Telford, Esq., Fellow of the Royal Society of Edinburgh, an invitation to "... patronize the Institution by taking on himself the office of President to the same". They declared its objects as being "... to facilitate the acquirement of knowledge in engineering; to *circumscribe the profession*; to establish in it the respectability which it merits; and to increase the indispensable public confidence". Telford accepted, and in his inaugural address the following year he shrewdly observed that talents and respectability are preferable to numbers. No doubt he recognized that the public, grown somewhat suspicious of lawyers and physicians, were not inclined to favour further professional associations. "From too easy and promiscuous admission", he said, "unavoidable and not infrequently incurable inconveniences perplex most societies."

Certainly there were a number of inconveniences perplexing the architects at this time, and perhaps they helped to lead them away from Telford and Brunel and into a deep cutting of their own. During the previous century they had achieved recognition as designers of buildings and landscapes. But all their clients—and they were then a limited class—possessed what was termed a 'gentlemanly knowledge' on the same subject. More than a gentlemanly knowledge, as Lord Chesterfield said, would have been lowering in the eyes of the fashionable world. On the other hand, the execution of designs was undertaken by builders and craftsmen, who needed only the minimum of drawings, and could at a pinch produce, reproduce or adapt these themselves. So the architect's special function was most apparent when he worked as an original artist. When it came to professional association the architect, like the civil engineer, sought respectability, integrity and public recognition, but his activities were less easy to circumscribe. On one hand, he had to know how to enclose space for an infinite number of human purposes; he must be aware of the capacity of the crafts, the limitations and possibilities of building materials and structural methods. Above all, he must heed his client's wishes but meet his real needs, and the two did not always coincide. On the other hand, the architect had to maintain his autonomy as an artist, because this was the very quality that gave him professional distinction. Yet, unlike the painter or the musician, he could not put his ideas into execution without a client and a builder. He had to spend other people's money in order to express himself.

So, although it is rare in practice to find the skills combined in any one individual, the professional image of the typical architect had to become that of a man who was competent simultaneously as a business adviser, technician and artist. Thus, in 1834, the Virtuosi and the Dilettanti, the Incorporated and the Free Societies of Artists, gave way to the Institute of British Architects whose professed aims were very similar to those announced by the Engineers sixteen years earlier. They were "... to facilitate the acquirement of architectural knowledge. the promotion of the different branches of science connected therewith, and to establish an uniformity and respectability of practice in the profession".

Again the emphasis on respectability, but each profession pursuing it in its own way. At one end of the widening scale the engineer-contractor concerned himself with machines and with constructions for industry and commerce, communications and utilities; while at the other end the artist-architect became more and more interested in the individual and social uses of buildings, and the style and setting appropriate to them.

The year 1834 was remarkable for another turning point. A hundred and thirty years ago, last week, the Old Palace at Westminster was burnt down. Soon afterwards a parliamentary committee announced a competition for a new design "in the Gothic or Elizabethan style". This proved to be the central building of the Gothic Revival, a movement which you will see, from the announcement of another parliamentary committee earlier this year, has not yet spent itself. Yet the Houses of Parliament—"a triumph of the Picturesque", as Sir Kenneth Clark called them—made no contribution to technology, although they did a great deal to stimulate craftsmanship.

Meanwhile, the engineers were developing continuous beam theory, inventing 'tarmac' for roads, hydraulic power tools and Bessemer steel, constructing canals and docks, the Menai and the Saltash Bridges. In the decade after 1840 they built nearly 5,000 miles of railroad in Great Britain, which is more than can be equalled in motorway mileage to-day: and when in 1842 Chadwick published his famous report on "The Sanitary Conditions of the Labouring Classes", it was the engineers who became professionally and statutorily responsible for under-

* Substance of an address delivered before the Imperial College of Science and Technology on Commemoration Day, October 22.

ground sewerage, water supply and other municipal services. During the nineteenth century and until the First World War all the major technological advances were made by engineers and scientists. The products of the first machine age were assimilated into the architects' world mainly as services and pieces of equipment, which at first scarcely altered their approach to design. Looking back, for example, to the Great Exhibition of 1851—with which so much in South Kensington was connected—one may well think of it, in terms of architecture, as grotesque: and in its worst manifestations it was. Paxton's enormous, utilitarian and yet magnificent 'greenhouse'—the Crystal Palace—was in itself the precursor of a new movement ; but we can still be amazed at its contents—the objects illustrated in the catalogue, or found surviving (because of their sturdy craftsmanship) in ancient hotels. We can still be amused at the character that Dickens invented —Mr. Pecksniff. "Of his architectural doings", wrote Dickens, "nothing was clearly known, except that he had never designed or built anything ; but it was generally understood that his knowledge of the science was almost awful in its profundity."

At the other end of the scale, the true architects were striving to keep alight a fire to which the technologists had little time to attend. They were concerned to distinguish the human and social purposes of building, and having found them, to extend them. They were trying to adapt their art, and the patronage under which it had grown up, to industrialization on one hand, and a fast-growing population on the other. They were passionate about architectural scholarship, about symbolism, about ecclesiology, about restorations.

At this point I want to say something about the origins of the Architectural Association and its School, which wove itself into the pattern of events in the 1840's. Sir John Summerson's centenary history of the Association, 1847–1947, gives a fascinating account of the profession seen, as it were, from the gallery, or through a minifying glass. The Association was formed from a group of architectural draughtsmen stirred into action by certain young architects disgruntled at their lack of education under the apprenticeship system. In other words, it was formed by students. The students started a School of their own. "What is to prevent such", said one of the young founders, aged eighteen, "if a number of us put our shoulder to the wheel . . . ?" It is interesting to note that at the first meeting of the Association (October 8, 1847) one of the speakers was Prof. Donaldson, described by Summerson as "that versatile, kindly character who had created the role of Professor of Architecture in the University of London". So there was at the start a sympathy between the academic establishment of the day and the irregular, independent and unconventional School designed by architects, for architects, and supported by architects for the next 120 years. This is a remarkable record. If its future is to be linked with that of the Imperial College of Science and Technology* it is not to be expected that it should abandon its training methods or the independence which has given it such vitality in the past.

Moreover, it is likely to share with the new universities the characteristic of being student-based. Throughout its history the Architectural Association has never allowed its teachers to go to sleep in their chairs. The school has been a nursery of the profession and the Association has been and remains attached to it as a special—indeed unique—form of convocation.

Now, looking back, one can see that although the two main constructional professions had started out in the nineteenth century with the same public aims, their pre-occupations have long been dissimilar. I have often thought that divergences in their approach to design, by an architect and an engineer, date from this period. The engineer tends to simplify and reduce his terms of reference, so as to bring his problem down to essentials and make it soluble by calculation. The architect tends to enlarge his terms, relating his structure to its social or emblematic purposes, its setting among other buildings, its whole environment. He must, therefore, complicate his answer to a building problem with a number of value judgments ; although this does not mean that the process is undisciplined ; simply that many disciplines are involved. This is a generalization, of course, and, like all generalizations, it can be proved wrong in certain cases. Yet it is broadly true to say that in the nineteenth century the technologists were extending their knowledge in depth ; the architects in breadth. Sir Edwin Lutyens, who was the last of our great architects to design convincingly in the grammatical, renaissance tradition, could say as late as the 1930's that " . . . architecture with its love and passion begins where function is achieved".

In the context in which Lutyens used these words, I believe them to be profoundly true. The question is whether they are true any longer. Science, too, can be pursued with passion ; and it can study emotion scientifically; but it does not introduce it into the process of scientific enquiry. Architecture, on the other hand, must include emotional response as one of the conditions of its brief ; and it can only become significant at this level by means of solving a functional problem. At what point can we say, then, that function has been achieved ? This phrase of Lutyens's, to a modern architect, would be the equivalent of saying that you cannot have cake until you have had the bread and butter; that the two are separate commodities—as Marie-Antoinette is said to have remarked in a totally different context. Whereas the contemporary approach is an integrated one ; our aims are to make the bread so good that it is—in effect—cake !

Now if one thinks this is a mere play of words, let me say that for the first time since the eighteenth century architects and their clients are now beginning to use common standards of performance and of criticism. Let me also quote a very distinguished modern architect, Mies van der Rohe, who has worked and taught for many years in institutes of technology. In an address to the Illinois Institute: "Technology", he said, "is far more than a method, it is a world in itself, something that has a meaning and a powerful form—so powerful, in fact, that it is not easy to name it. Is that still technology or is it architecture ? And that may be the reason that some people are convinced that architecture will be outmoded and replaced by technology. Such a conviction is not based on clear thinking. The opposite happens. Wherever technology reaches its real fulfilment, *it transcends into architecture*. That is the reason why technology and architecture are so closely related. Our real hope is that they grow together, that some day the one will be the expression of the other".

Well, I have now told you, in the words of Mies van der Rohe, what to expect if architecture is taken in among the scientists and technologists. Your technologists, when they surpass themselves, will be adopted as architects. We have already done so in the case of a number of men of world repute, such as Pier Luigi Nervi of Italy ; and I hope this will be seen as the gesture of recognition that it is, and not as a piece of professional gamesmanship.

The advantages of a real *rapprochement* rest on more than philosophical points. There is the whole strategy of coming to terms with our environment, not only the external environment in which we live and work and take our leisure, but the mental environment in which we create ideas and exchange them. It has often been said —and Lord Brain, the physician, said it again this year in his presidential address to the British Association for the Advancement of Science—that " . . . the potentialities of science and technology for the benefit of mankind . . . are almost inconceivably great, but the preparations which we are making for their use and development are

* It is planned that the School of the Architectural Association becomes the fourth constituent College of the Imperial College of Science and Technology.

pitiably small". All of us can see that there have been conspicuous failures in population and traffic forecasting, in the planning of resources, in conservation, in the control of land-use and land-development and in charting the many ways in which the human being shapes his environment and is shaped by it. Perhaps this is due partly to the fact that we apply old unscientific habits of action to new scientific concepts, or that we read the wrong lessons from history, or that we fail to distinguish between public demand—particularly in its statistical or commercial forms—and social need. I do not know. But I am sure that a technological university must interest itself increasingly in human behaviour and in social control mechanisms, at the same time as it makes advances in the mechanical and natural sciences.

The results of skill and invention can, on the other hand, prove inimical to human health and happiness. The nuclear deterrent is an obvious example. Not only the scientists but also all those who think at all into the future know very well that its use has to be rigorously and comprehensively controlled and the social and political checks have to be considered along with the technical ones. In the same way, but on a different scale, the legitimate desire of individuals, families and firms to own and use a means of mechanical transport—in other words a private car—has been a stimulus to automobile technology and productivity, but has caught us unprepared to control the environment which the automobile has invaded. It was a car designer (Alec Issigonis) who said, "The car is going to contribute very largely to wrecking our civilization". In this field Imperial College has been percipient. It recognized that Prof. Colin Buchanan had done useful research into the nature of traffic and its effect on towns; and by appointing him professor of transport the College is enabling him to do more. It is precisely this extension of technology for the purpose of studying related problems of use, control, development and design, that could also make the architectural school, with its large and active professional association behind it, of great potential value to Imperial College.

NEWTON, HOOKE, AND 'BOYLE'S LAW'

(DISCOVERED BY POWER AND TOWNELEY)

By Prof. I. BERNARD COHEN

Chairman of the Committee on the History of Science, Harvard University, Cambridge, Mass.

A CASE can be made out for at least six claimants to the law relating the pressure to the volume of a confined gas at constant pressure: Robert Boyle, Viscount Brouncker, Robert Hooke, Edme Mariotte, Henry Power and Richard Towneley. Called in Great Britain and in the United States 'Boyle's law', and on the Continent 'la loi de Mariotte' or 'das Gesetz von Boyle und Mariotte' (with all possible variations), the law was first published by Boyle in 1662.

The first edition of Boyle's *New Experiments physico-mechanicall, touching the Spring of the Air* (Oxford, 1660) did not contain a statement of the law. It appeared first in the second edition, *Whereunto is added a Defence of the Authors Explication of the Experiments, against the Objections of Franciscus Linus, and, Thomas Hobbes* (Oxford, 1662)[1].

The claims of Boyle and Mariotte have often been discussed. Mariotte's claim was substantially dismissed by P. G. Tait some eighty years ago[2], on the grounds that Mariotte's account of the law (1676) follows Boyle's prior statement by fourteen (or, perhaps, seventeen) years, that Mariotte used apparatus almost identical to Boyle's, and that the comparison of compressed air and squeezed sponges must have been taken from Boyle by Mariotte. Tait concentrated on evidence that Mariotte may have known Boyle's book and be thus disqualified from a claim to the law. But this procedure ignores the possible grounds for attributing (or, for not attributing) the law to Boyle. Douglas McKie[3] presents convincing evidence that the traditionally assigned date of 1676 for the publication of Mariotte's essay "may have crept in by some misprint" and that "no date earlier than 1679 can safely be assigned to Mariotte's essay at present".

As to Richard Towneley, C. Webster has recently restated his claim[4]. Basically, Webster points out that Boyle was acquainted with the results of experiments on air at different pressures carried out by Towneley and by Henry Power in 1660–61. Although Webster makes out a very good case for the priority and influence of Richard Towneley and Henry Power, he does not give any evidence to support the claim (occurring both in the text and in the title of the article) that Towneley rather than Power, or rather than both together, deserves the credit for the law. From these experiments Power and Towneley concluded: "So that here is now four Proportionals, and by any three given, you may strike out the fourth". Hence it was possible to "prognosticate the effects, which follow in all *Mercurial* Experiments, and predemonstrate them, by calculation, before the senses give an Experimental [eviction] thereof". Rewriting this statement from Power's book in the language of proportionality, either as $PV = P'V'$ or as $P : V :: P' : V'$, we may see at once that any three of these quantities determine the fourth. In 1663—a year after Boyle had received the data and the generalization based on them of Towneley and Power and had published the law—Power published his own book on *Experimental Philosophy*[5], in which any reader could learn for himself the nature of the pre-Boyle stage of 'Boyle's law'.

The Towneley–Boyle relation was studied carefully in 1909 by Prof. E. Gerland[6], who concluded that, despite the suggestion made by Towneley, the credit for the law should be assigned to Boyle (and surely not to Mariotte). But Webster has added new evidence from the unpublished correspondence of William Croone to show that: (1) Towneley and Power actually sent their data and conclusion to Croone, who forwarded these to Boyle; (2) although Boyle mentions only Towneley's name (as do those who have based their accounts on Boyle's statement), the suggestion of the constancy of the product of pressure and volume should apparently be divided between Power and Towneley.

In his book on the *Spring of the Air*, this is what Boyle himself had to say on the subject:

> . . . I shall readily acknowledge that I had not reduc'd the tryals I had made about measuring the Expansion of the Air to any certain *Hypothesis*, when that ingenious Gentleman Mr. Richard *Towneley* was pleased to inform me, that having by the perusal of my *Physico-Mechanical* Experiments been satisfied that the Spring of the Air was the cause of it, he had endeavoured (and I wish in such attempts other ingenious men would follow his example) to supply what I had omitted concerning the reducing to a precise estimate how much Air dilated of it self loses of its Elastical force, according to the measures of its Dilatation. He added, that he had begun to

set down what occurred to him to this purpose in a short Discourse, whereof he afterwards did me the favour to shew me the beginning, which gives me a just Curiosity to see it perfected. But, because I neither know, nor (by reason of the great distance betwixt our places of residence) have at present the opportunity to enquire, whether he will think fit to annex his Discourse to our *Appendix*, or to publish it by it self, or at all; and because he hath not yet, for aught I know, met with fit Glasses to make an any-thing-accurate Table of the Decrement of the force of dilated Air; our present design invites us to present the Reader with that which follows, wherein I had the assistance of the same person that I took notice of in the former Chapter, as having written something about Rarefaction: whom I the rather make mention of on this occasion, because when he first heard me speak of Mr. *Townley's* suppositions about the proportion wherein Air loses of its Spring by Dilatation, he told me he had the year before (and not long after the publication of my *Pneumatical* Treatise) made Observations to the same purpose, which he acknowledged to agree well enough with Mr. *Townley's* Theory: and so did (as their Author was pleased to tell me) some Tryals made about the same time by that Noble *Virtuoso* and eminent Mathematician the Lord *Brouncker*, from whose further Enquiries into this matter, if his occasions will allow him to make them, the Curious may well hope for something very accurate.[7]

There are three comments to be made on this statement:

(1) Boyle mentions Towneley but not Power. This is explained by Webster on the basis of the title of the transcription made by Power and forwarded to Croone: "Additional experiments made at Townley Hall, in the years 1660 and 1661, by the advice and assistance of that Heroick and Worthy Gentleman, Richard Townley, Esqr. and those Ingenious Gentlemen Mr. John, and Mr. Charles Townley, and Mr. George Kemp". Clearly, as Webster points out, "Although the manuscript emanated from Power, it is quite possible that Boyle overlooked Power's part in the work, which would not be easily guessed from the title"[8].

(2) As to Lord Brouncker, Boyle hoped for "further enquiries", but actually none was forthcoming. Nevertheless, "some trials made at the same time" as those made by Boyle's assistant and collaborator, Robert Hooke, also agreed "with Mr. Townley's theory". Boyle gives no evidence to enable us to guess whether Brouncker's experimental results merely confirmed the relation discovered by Towneley and by Power and given in the equation $PV = P'V'$, or whether he had found the relation for himself. (There is no biography of Brouncker nor, so far as I know, any published examination of his scientific work except that of J. F. Scott and Sir Harold Hartley[9].)

(3) Boyle refers explicitly to Hooke's claim that when he had first heard Boyle speak of the "proportion" supposed by Towneley, he had stated unequivocally that he himself "had the year before (and not long after the publication of my pneumatical treatise) made observations to the same purpose". Hence Hooke allegedly obtained results which proved to agree with the Power–Towneley relation or 'Boyle's law' late in 1660 or 1661, and undertook further experiments to verify "Mr. Townley's theory" even before Boyle did so himself.

Hooke's experiments, and his conclusion "that the Elater of the Air is reciprocal to its extension, or at least very neer", are to be found in his celebrated *Micrographia*. The detailed description begins as follows:

> I had several other Tables of my Observations, and Calculations, which I then made; but it being above a twelve month since I made them; and by that means having forgot many circumstances and particulars, I was resolved to make them over once again, which I did *August* the second 1661. With the very same Tube which I used the year before, when I first made the Experiment (for it being a very good one, I had carefully preserv'd it:) And after having tryed it over and over again; and being not well satisfied of some particulars, I, at last, having put all things in very good order, and being as attentive, and observant, as possibly I could, of every circumstance requisite to be taken notice of, did register my several Observations in this following Table. In the making of which, I did not exactly follow the method that I had used at first; but, having lately heard of Mr. *Townly's Hypothesis*, I shap'd my course in such sort, as would be most convenient for the examination of that *Hypothesis*; the event of which you have in the latter part of the last Table.
>
> The other Experiment was, to find what degrees of force were requisite to compress, or condense, the Air into such or such a bulk.
>
> The manner of proceeding therein was this: I took a Tube about five foot long, one of whose ends was sealed up, and bended in the form of a *siphon*, much like that represented in the fourth Figure of the 37. *Scheme*, one side whereof AD. that was open at A, was about fifty inches long, the other side BC, shut at B, was not much above seven inches long; then placing it exactly perpendicular, I pour'd in a little Quicksilver, and found that the Air BC was 7/8 inches, or very near to seven; then pouring in Quicksilver at the longer Tube, I continued filling of it till the Air in the shorter part of it was contracted into half the former dimensions, and found the height exactly nine and twenty inches; and by making several other tryals, in several other degrees of condensation of the Air, I found them exactly answer the former *Hypothesis*[10].

Taking Hooke at his own word, we may conclude that by the summer of 1660 he had completed a series of experimental measurements on the volume and pressure of a confined mass of air, that on August 2, 1661, he "was resolved to make them over once again" and put his results in a form which enabled him to test "Mr. Townly's Hypothesis", of which he had "lately heard". These experiments had been made to ascertain the relations between pressure and volume when air expands (that is, at pressures less than one atmosphere), using a very original form of apparatus. But he also made experiments "to find what degrees of force were requisite to compress, or condense, the Air into such or such a bulk". The apparatus he devised was a J-tube of glass, of which the short end was sealed, into the long (open) end of which Mercury was poured. Having made several trials, he "found them exactly [to] answer the former *Hypothesis*".

It should be clear then that whether or not Hooke's early experimental results (1660) agreed with what was to become known as 'Boyle's law', Hooke himself did not claim to have discovered the law, only to have verified it. (W. S. James[11]: The Discovery of the Gas Laws, *Science Progress*, **23**, 263 (1928), discusses Hooke's experiments (pp. 267), analysing Hooke's procedures and comparing them with Boyle's. I see no compelling reason to support James's claim that "certain doubt must be attached to Hooke's statement of 1664 that he verified the hypothesis [of Townsley and Power] on August 2, 1661". This point will be discussed further in the next paragraph.) Presumably it was from Boyle that Hooke first heard of the Power–Towneley relation, and the question of interest therefore is whether indeed Hooke verified the "hypothesis of Towneley" (and Power) before, and independently of, Boyle. It seems evident that Boyle was the source of information, since Hooke, like Boyle, refers only to Towneley and not to Power. If all this be so, then Hooke should be given credit for the law rather than Boyle, having had priority in verifying the 'hypothesis'.

Two cogent arguments for giving Hooke credit for 'Boyle's law' are made by Prof. Andrade in his celebrated Wilkins Lecture on Hooke in 1949[12]. The first is "that Hooke, who always expressed the greatest veneration for Boyle, would never have published this [statement] in 1665 [in the *Micrographia*] if it was likely to give pain to, or be disputed by, Boyle". The second is Boyle's own statement in 1662 that when Hooke first heard him "speak of Mr. Townley's suppositions about the proportion wherein Air loses its Spring by Dilation", Hooke told him "he had the year before . . . made observations to the same purpose, which he acknowledged to agree well with Mr. Townley's Theory". Prof. Andrade also cites a third argument raised by Louis Tronchard More in his life of Boyle in favour of Hooke's importance

in the formulation of this law: that after Hooke ceased to work with Boyle, Boyle never did any further quantitative scientific work[13].

To the foregoing arguments I now add a fourth. It is well known that Isaac Newton was generally reluctant to give credit to Hooke for any significant contribution to science. Hooke had challenged the Newtonian theory of light and colours in the 1660's, and in the 1670's he had made public a private letter of Newton's containing a scientific blunder in the dynamics of bodies falling on a moving Earth[14]. In 1686 Hooke's insistent demand for credit in the *Principia* almost resulted in Newton's suppression of Book 3, in which the abstract principles developed in the first two books of the *Principia* are applied to the "system of the world"[15]. Happily, Halley's tact and persuasion prevented this proposed disfigurement of Newton's masterpiece. It is amusing to note that in the MS. sent to the printer, and from which the printer composed the *Principia*, the text reads at one point: "Wrennus, Halleus et Hookius". But in the printed version this was altered (presumably by Halley) to "Wrennus, Hookius & Halleus". (Newton's MS., chiefly in the hand of Humphry Newton, with corrections by Isaac Newton and by Edmund Halley, is in the Library of the Royal Society. This MS. has been collated *verbatim et literatim* with the printed first edition for our forthcoming critical and variorum edition of the *Principia*.)

These things being so, any statement of Newton's in the years 1686–87, giving credit to Hooke for any discovery, is to be taken very seriously. In *De systemate mundi*, the first (and unused) version of Book 3 of the *Principia*, Newton discussed a "ratio, [by which] we may compute the rarity of the air in all places whatsoever (by the help of Cor., Prop. XXII, Book II), supposing the expansion thereof to be inversely proportional to its compression"[16]. He then stated that "this proportion has been proved by the experiments of *Hooke* and others". In an earlier composition, *De Ære et Æthere*, dating from the late 1660's or early 1670's[17], Newton also gives Hooke the credit rather than Boyle: "In just the same remarkable manner air rarefies and is condensed according to the degree of pressure. The whole weight of the incumbent atmosphere by which the air here close to the Earth is compressed is known to philosophers from the Torricellian experiment, and Hooke proved by experiment that the double or treble weight compresses air into the half or third of its space, and conversely . . .". (This important document was first published by Profs. A. R. and M. B. Hall.)

In some juvenile notes on Hooke's *Micrographia*, Newton wrote:

> 226. Mr Townlys Hypothesis is ye dimension (or expansion) of ye aire is reciprocall proportional to its spring (or force required to compresse it). By Mr Hookes experience . . .
>
> [*Then follows a table from the* Micrographia *giving in successive columns*: The dimension of ye aire; The perpendicular height of a Mercuriall cilinder compressing it; The Mercuriall cilinder counterpoised by ye Atmosphaere; The whole incumbent weight pressing ye aire; *and, finally, for a check on the theory*, By Mr Townlys Hypothesis ye incumbent weight should bee, . . .][18].

Shall we conclude that Newton was ignorant of Boyle's own statement and knew of the pressure–volume relation only through Hooke's *Micrographia*? Is this why Newton mentioned Hooke's name in connexion with the law rather than Boyle's? The catalogue of Newton's library lists "Boyle's Spring of the Air & its Effects, 8vo. 1660" but not the second edition of 1662 containing the law[19]. Such evidence is, however, far from conclusive, since Newton could have read this book in a copy other than one in his library, or he could have owned the book at one time and yet have had it no longer at the time of his death, or he could have heard about the law from someone by word of mouth—even from Boyle himself, whom he knew well and greatly admired. But it would be hard to deny the importance of the fact that Newton twice mentioned Hooke, whom he apparently detested, in relation to the law and never mentioned Boyle, for whom he had a great and sincere esteem. Hence we may also understand why Newton, in Prop. XXIII (and Scholium) to Book II of the *Principia*, mentions no name at all when discussing the law of gases—perhaps preferring to print nobody's name to having to link so important a discovery with Hooke.

The law relating the pressure and volume of a confined gas at constant temperature was discovered through experiments arising from Boyle's book on *The Spring of the Air*. Richard Towneley and Henry Power (with some "advice and assistance" of John and Charles Towneley and George Kemp) found data which they reduced to the proportion $PV = P'V'$, although they did not express this result in the most unambiguous clarity conceivable. From correspondence between Henry Power and William Croone (July 20, 1661; September 14, 1661; November 27, 1661; January 9, 1661 (1661/62)) we learn that Boyle was in possession of this material when he wrote his "continuation" (or "defence") for the second edition of the *Spring of the Air* (1662). Prof. McKie, *loc. cit.* (note 3 *supra*), has found MS. evidence that Boyle made a report to the Royal Society on September 11 and again on September 18, 1661, on "the experiment made by him of the compression of air with the quicksilver tube". The table of data (reproduced as Fig. 3 by Prof. McKie) presents experimental results "identical" with those in Boyle's publication of 1662. From this and other evidence it has been stated:

> Boyle's role in the stablishment of the gas law remains important; his experimental results were accurate and extensive; the apparatus which he devised has become the standard one for illustrating the gas law; most significantly he gave the weight of his great reputation to the law, and published it in a highly influential work. He became the publicist of the gas law, which would have remained unnoticed in Power's work and Towneley's correspondence with Boyle.[20]

Boyle, however, extended the Power–Towneley relation to pressures greater than one atmosphere. According to Boyle's statement of 1662 quoted above, Towneley had been concerned only with "the Decrement of the force of the dilated Air", whereas Boyle extended the relation to compression as well as rarefaction, that is, to pressures greater than one atmosphere (The question of whether such an extension be significant or not depends on whether the limitation in the first instance to pressures less than one atmosphere resulted from a failure to devise an instrument to make a test of this additional range or from a psychological inability to recognize that a law for the rarefaction of gases might equally be a law for the compression of gases. This and other questions concerning 'Boyle's law' are discussed by Prof. Joseph Agassi, of the University of Illinois, in a work in progress.)

If this be the basis for Boyle's name to be associated with the law, surely Hooke has an equal claim. He, too, obtained accurate experimental data to support the conclusion of Towneley and Power who discovered the law, at least for rarefaction, and whose statement of it was known to him as well as to Boyle. (Like C. Webster, I dismiss Messrs. John and Charles Towneley and Mr. George Kemp as having made contributions of a minor character.) Hooke also put his reputation behind the law and published it. It would seem that Hooke employed the *J*-tube before Boyle, and used it to show that the Power–Towneley relation holds for compression before Boyle did. The context of Hooke's experiments in the *Micrographia* is so entirely different from that of Boyle's in the *Defence* that Hooke's claim to have done this work independently is perfectly plausible. Considering this difference, and the fact that the quantitative law may be more Hookian than Boylian, is it not also plausible that Hooke contributed—in addition to the quantitative point of view—the apparatus used in experimenting, namely,

the *J*-tube? Furthermore, it was through Hooke, and not through Boyle as publicist, that Newton at least (and perhaps others) learned of the law. Hence, Hooke would appear to have an equal claim to Boyle's, and perhaps a greater one, likely anticipating Boyle in designing the apparatus, obtaining experimental confirmation, and in extending the law to compressed gases. Boyle's sole claim would then be priority in publication. We may dismiss Brouncker's claim, since his results apparently were not very conclusive. As to Mariotte, he probably made no original contribution to the law (but, then, neither did Boyle, really), but he too put his reputation behind it and published it in a highly influential work. Boyle was a prolix writer at best, and it may even be the case that Mariotte proved to be a far better publicist than Boyle. To answer this question fully, we need to examine the writings of men of science in the seventeenth century, in order to discover who was their source of information concerning the gas law—Boyle or Mariotte? I have not been able to learn who first called this law after either Boyle or Mariotte. So far as I know, we have only one documented example of a seventeenth-century scientist learning the gas law: that is, Newton, who learned of it through reading Hooke and who—in discussing it—mentioned only Hooke by name. Hence this is a law discovered by Power and Towneley, accurately verified by Hooke, accurately verified again by Boyle (aided in some degree by Hooke), first published by Boyle, but chiefly publicized by Mariotte, in short, the "law of Power and Towneley, and of Hooke and Boyle, and—to some degree—of Mariotte". This example certainly shows the complexities in making precise the individual contributions in the common multiply-discovered scientific law and thus argues the folly of eponymy in science.

I thank the U.S. National Science Foundation for its support.

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[16] For some account of the editions and translations of Newton's *System of the World*, and the question of its being an authentic composition of Newton's, see Cohen, I. B., *Pemberton's translation of Newton's* Principia, *with notes on Motte's translation, Isis*, **54**, 319 (1936).
[17] Printed from MS. Add. 3970, fols. 652–3 in Cambridge University Library by Hall, A. Rupert, and Hall, Marie Boas, in their edition of *Unpublished Scientific Papers of Isaac Newton* (Cambridge, 1962), text pp. 214–220, translation pp. 221–228.
[18] See Keynes, Geoffrey, *A Bibliography of Dr. Robert Hooke* (Oxford, 1960). Appendix IV, "Sir Isaac Newton's Notes on *Micrographia*"; these notes are printed here from the Halls' volume (see note 17 *supra*), pp. 400–413. Indeed, Hall, A. R., and Hall, M. B. (p. 399) were the first to call attention to Newton's citation of Hooke in relation to Boyle's law.
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INTERNATIONAL YEARS OF THE QUIET SUN, 1964–65*

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In its need for organized international co-operation, geophysics is, in a sense, in a different category from many other branches of scientific investigation. It is true that long-term measurements at one place of the atmospheric temperature or the pressure, the strength of the Earth's magnetic field or the height of ionized layers of the atmosphere have a certain intrinsic value, but ultimately such data only find their proper and useful place in relation to similar measurements made in other places. Geophysical investigations call for collaboration between the workers concerned; and to obtain a comprehensive understanding of natural phenomena, whether these be weather conditions, thunderstorm activity, magnetic or ionospheric storms, etc., demands international co-operation.

This essential need of geophysical research was clearly recognized in some of the earliest meetings of the British Association. Thus in 1838 the British Association, meeting at Newcastle, recommended that the network of magnetic observatories which had been established in Germany by Weber and Gauss during the period 1828–40 should be extended to British territories overseas and, with the co-operation of the President and Council of the Royal Society, sites were selected in both hemispheres, in the tropics, on continents and islands—a detailed statement of the objects of the investigation was drafted and, for the guidance of observers, an instruction manual prepared. It was planned to carry out careful magnetic observations for a period of three years, but not unexpectedly they found that most of the three years went in preparation and the enterprise had to be extended for an additional three years. Six years later the future of the project came up for review at an International Magnetic Congress organized by the British Association during the 1845 annual meeting at Cambridge. It was resolved "that the cordial co-operation which has hitherto prevailed between the British and foreign magnetic and meteorological observatories, having produced the most important results and being considered as absolutely essential to the success of the great system of combined observation which has been undertaken, it is earnestly recommended that the same spirit of co-operation should continue to prevail". Some of those magnetic stations established around 1840 are still—a century and a quarter later—contributing their data to the common store of knowledge on the terrestrial magnetic field.

The Bristol meeting of the Association in 1898 was distinguished by the fact that on that occasion the meetings of Section A were held in association with an International Conference on Terrestrial Magnetism and Atmospheric Electricity. The president of the conference, Prof. (later Sir Arthur) Rücker—then secretary of the Royal Society and later a president of the Association—underlined the need for international co-operation in

* Summary of Evening Discourse delivered on August 28 at the Southampton Meeting of the British Association.

studies of the terrestrial magnetic field in the following terms: "There are special and cogent reasons why the science of terrestrial magnetism should be cosmopolitan. The advance of some sciences is most easily achieved by the methods of guerilla warfare. In a hundred different laboratories widely separated workers plan independent attacks on Nature. In different universities and colleges little groups are devising stratagems and arranging ambuscades in the hope of wresting from our great opponent some of the treasures which she yields only to the violent who take them by force. But for those who would unravel the causes of the mysterious movements of the compass needle concerted action is essential".

Those brief extracts from British Association meetings 66 and 126 years ago indicate the noble part which the Association has played in fostering international scientific co-operation. It is indeed a happy thought that this spirit still prevails. Eight years ago, at the Sheffield meeting, Prof. Sydney Chapman, president of the International Geophysical Year Committee, delivered an evening discourse on the International Geophysical Year, and at this Southampton meeting I welcome the opportunity to outline the further world-wide project now under way with the somewhat romantic title of the "International Years of the Quiet Sun"—the 'IQSY'.

To understand the significance of the present project it is necessary to make brief reference to its immediate predecessor, the IGY. There can be little doubt that international co-operation in geophysical science reached a historic peak in 1957–58 with the International Geophysical Year. The underlying objective of the IGY was once summarized in the single phrase "the common study of our planet by all nations for the benefit of all", and it is certain that the enterprise successfully achieved that objective in full measure.

The IGY was not the first, but the third, international co-operative enterprise of this sort. The two earlier ventures were called "Polar Years" and were, of course, much more limited in scope. The first International Polar Year was organized in 1882–83 and the second 50 years later. In both these enterprises investigations were carried out mainly in polar latitudes over a period of one year. In the 1882–83 project the studies concerned were in meteorology, geomagnetism and aurora. These three disciplines also formed the main core of the studies in the second Polar Year of 1932–33, but on this occasion they were supplemented by a limited but important programme of radio investigations of the then newly discovered ionosphere. The IGY followed the second Polar Year after an interval of 25 rather than 50 years and there were two main reasons for this reduced interval. In the first place the period 1930–50 had witnessed an extremely rapid advance in scientific techniques and especially so in the field of radio—a science central to so many geophysical investigations. It was felt that the time was ripe for an intensive application of these advanced techniques to geophysical problems. The second reason for holding the IGY in 1957–58 was the fact that the second Polar Year of 1932–33 had occurred near a period of solar minimum activity and 1957–58 would be a time of maximum solar activity. As it happened, the choice of 1957–58 was excellent in that it turned out to be a period of most intense activity and the sunspot numbers during the IGY reached an all-time record—they were, in fact, larger than any previously recorded in the two centuries for which reliable figures are available.

Even in the early planning stages of the IGY it became clear that some observations should be continued at near the IGY-level through the waning portion of the solar cycle, and in certain disciplines there were sound scientific reasons for an enterprise on the scale of the IGY at the next period of minimum solar activity. It was clear that the full fruits of the IGY would only be reaped if data, comparable in quality and quantity, were available for a period of low activity—for a "quiet sun". Furthermore, such an enterprise could make full use of the powerful new space-research techniques developed since the IGY, and that is why the "International Years of the Quiet Sun" has been organized for the period January 1, 1964–December 31, 1965. Seventy nations—including all those which took part in the IGY—are taking part in it.

During the IQSY, investigations are being made in those branches of geophysics which are affected to a greater or lesser degree by solar activity—in meteorology, geomagnetism, auroræ and airglow, ionospheric physics, cosmic rays. There is also a very extensive programme of rocket and satellite studies, and throughout the two-year period a continuous watch is being maintained on the Sun with ground-based and satellite-borne instruments.

The following is a brief outline of the IQSY programme in the different disciplines.

Meteorology

The IQSY meteorological programme, like that in many other disciplines, is designed to complement and extend the programme carried out during the IGY. During the IGY the target in meteorology was the study of the atmospheric conditions below about 30 km. During the IQSY these studies are being extended to 100 or 120 km with particular attention to the 25–60-km height-range. The IQSY meteorological programme thus calls for a world-wide effort with high-flying balloons, with rockets and with satellites. At least 10 countries are carrying out regular rocket flights for meteorological studies, and the total number fired in the two-year period will be counted in many thousands.

Apart from its own intrinsic importance, the meteorological programme during IQSY is also required by workers in other disciplines. Thus, cosmic ray investigations call for some high-level aerological observations and data on thunderstorms and lightning flashes are required for the study of the propagation of atmospherics by the ionosphere.

Geomagnetism

It has long been known that disturbances in the Earth's magnetic field closely follow the variations in sunspot number and there is, of course, an intimate connexion between the Earth's magnetic field and many other geophysical phenomena. Investigations of the magnetic field must then inevitably occupy a prominent place in any programme of research concerning solar terrestrial relationships. The geomagnetic programme for the IQSY is principally directed towards a study of solar and lunar variations in this field, to a study of the geomagnetic effects of solar eclipses under the favourable conditions which prevail in the IQSY period and to a study of magnetic storm phenomena. Magnetic storms were, of course, a common occurrence during the IGY, but frequently these occurred in close succession and it proved difficult to sort out the phenomena arising from one storm from that of an earlier or later one. The quiet period of the IQSY should provide a good opportunity for the study of individual magnetic storms in comparative isolation. Studies of some of the large geomagnetic anomalies are being made using satellite-borne magnetometers co-ordinated with ground-based measurements. Further rocket studies of the equatorial electrojet are also being carried out during IQSY.

A new World Magnetic Survey was first discussed during the IGY and at the present time a number of geomagnetic surveys are in hand. The intensive geomagnetic studies of the IQSY will, in themselves, form an invaluable contribution to this longer-term overall study of the field.

Aurora

It has long been known that the frequency of occurrence of auroræ also follows the sunspot cycle. Recording the number of occasions on which an aurora is seen is not such

a straightforward task as recording disturbances in the Earth's magnetic field, since auroral observations are liable to be upset by the incidence of cloud cover on the one hand, or by moonlight on the other. Furthermore, until comparatively recently, we have had to rely on reports of visual observations from a very small number of voluntary observers. Hence the data on the occurrence of auroræ have not been all that complete or reliable. Nevertheless, 80 years ago, at the British Association meeting of 1884, Schuster presented a diagram to show that the incidence of auroræ showed changes which correlate with the solar cycle.

To-day we know that both magnetic storms and auroræ arise from the streams of charged particles—mainly protons which are ejected from the Sun, especially at times of high sunspot activity. These streams impinge on the Earth's atmosphere at high latitudes, having been deflected towards these high latitudes under the influence of the terrestrial magnetic field. The energetic incoming particles disturb the polar ionosphere, causing the atoms and molecules to emit radiation in the visible part of the spectrum (the aurora) and at the same time the particles stimulate great electric currents in the ionosphere and these currents, in turn, disturb the magnetic field. The streams of electrically charged particles from the Sun are most frequent and most intense at times of high solar activity, and hence the frequency of both magnetic storms and auroræ shows a close correspondence with solar activity and with sunspots.

Although visual observations still form a very important source of auroral data, increasing emphasis is now being placed on photographic recordings, and during IQSY new and improved 'all-sky' cameras are being used to study the aurora. These cameras record on one photograph the entire sky from horizon to horizon as it might be seen by an observer lying on his back and having a full 180° field of view in all directions.

Airglow

The phenomena of the aurora are a striking and impressive consequence of the interaction of solar particle radiation with the Earth's atmosphere in polar latitudes. But there is also a much weaker and less variable radiation of light from the high atmosphere which goes on all the time and at all places. This is the airglow or "light of the night sky" as it used to be called. The examination of these weak radiations also provides invaluable information on conditions in the high atmosphere and this for locations all over the Earth, not merely at the high latitudes at which auroræ are visible. In the presence of strong auroral radiations the airglow cannot be detected but, during the present quiet solar conditions, when the auroræ have receded to higher latitudes, studies of the airglow can be carried out at much higher latitudes than was possible in the IGY.

Ionosphere

Perhaps our most important source of information on conditions in the high atmosphere of the Earth comes from investigations of the ionized regions which exist above a level of about 60 km.

Like the magnetic field, the ionosphere plays a central part in many geophysical phenomena, and ionospheric studies inevitably form a very important part of the present enterprise. Thirty years ago, at the time of the second Polar Year, regular soundings of the ionosphere were just becoming established, and such work, although only carried out to a very limited extent at a few stations, was, nevertheless, a new and important part of the programme of that Polar Year. In the 25 years following the second Polar Year a great network of sounding stations grew up, and in the IGY there were about 150 of these ground-based stations with observations made regularly at ½-h or even 5-min intervals. For the IQSY this network has been still further strengthened and, in addition, a few stations have been added in which a new and powerful radio-sounding technique is being used. Conventional radio soundings of the ionosphere cannot yield information above a height of about 250–400 km. This arises from the fact that when radio signals are sent upwards to the ionosphere echo signals are received only if the frequency of the radio waves is less than a certain critical value which is determined by the prevailing ionospheric conditions—determined, in fact, by the maximum electron density in the ionosphere at the time. In general, the maximum electron density occurs at a height which may be anything between 250 and 400 km, and hence it is that conventional radio sounders cannot yield information on the ionosphere above this level. A limited amount of information on conditions in the upper side of the ionosphere can be obtained from moon echoes and from studies of signals from radio stars, but until recently we knew comparatively little about conditions in the 'top side' of the ionosphere. In recent years this situation has been dramatically changed and within the past two or three years it has been possible to study the top-side ionosphere in great detail. Many of these top-side data are coming from certain satellite experiments, but a great deal is also now emerging from a new ground-based technique, which is being used during IQSY at some locations. This 'incoherent scatter' technique which sounds the ionosphere not to a few hundred but to a few thousand km employs a very high-power, high-frequency, radar-type equipment. This yields weak scattered reflexions from *all* levels in the ionosphere and enables the electron density to be measured out to several thousands of kilometres.

However, the most complete information on the top side has come from a remarkably successful satellite experiment in which a complete radio-sounding equipment: transmitter, receiver and all auxiliary gear—in fact, a replica in miniature of that used at ground-based stations—has been carried in a satellite. This miniature 'ionosonde' was built by Canadian scientists and launched in an American satellite on September 29, 1962. Known as *Alouette*, it is still in orbit and has already provided hundreds of thousands of top-side ionospheric records of the highest quality.

At very great altitudes—from, say, 800 to 100,000 km above the Earth—the movement of the electrons and ions is influenced very considerably by the magnetic field, and the outermost parts of the ionosphere—in which these magnetic field effects dominate—has come to be called the 'magnetosphere'. One of the most fruitful sources of information on the magnetosphere has been the examination of strange naturally occurring radio signals. That these signals are strange can be inferred from the names which radio scientists have given to them: 'whistling atmospherics', 'dawn chorus' and 'hiss'. In recent years, and particularly during and since the IGY, it has been established that these radio noises have travelled very great distances and carry invaluable information about conditions in the atmosphere hundreds and thousands of miles above the Earth's surface. 'Atmospherics' are groups of radio waves of very long wave-length—tens of miles long—produced by lightning flashes. Sometimes these long radio waves from lightning flashes can penetrate the ionosphere and travel upwards into the high ionosphere along paths which follow the direction of the magnetic field lines. Thus an atmospheric, generated by a lightning flash somewhere in the northern hemisphere, can travel up to the ionosphere, there to be guided along by the magnetic field and gradually bent over and so down to the other end of the field line in the southern hemisphere. There it can be reflected at the ground and go back along the magnetic field again to the northern hemisphere. Under suitable conditions it may travel back and forth between the two hemispheres many times. At the radio receiver these atmospherics produce a characteristic whistling note of descending pitch, hence the term 'whistling atmospherics'.

At the highest point of their paths the whistling atmospherics may well be travelling through the atmosphere at altitudes of a few thousand miles, and they bring back with them a great deal of valuable information about the 'magnetosphere' at these great heights.

The second collection of strange radio noises—'dawn chorus'—was so named because of its resemblance to the familiar warbling of birds at dawn. Recent research indicates that both the 'dawn chorus' and the 'hiss' are radiations generated on the outer fringe of the Earth's atmosphere in the magnetosphere—by incoming streams of charged particles from the Sun. These phenomena are being closely examined during IQSY as part of an intensive investigation of the Earth's outermost ionosphere under quiet solar conditions.

Cosmic Rays

One of the primary objectives of cosmic ray studies during IQSY is an investigation of the emission of energetic particles by the Sun at the time of minimum activity, and the examination of their mode of propagation through the interplanetary magnetic field. The existence of quiet solar conditions is also being used to advantage in a search for intensity variations over the sidereal day, to determine the effect of meteorological factors on the intensity of the secondary radiation, and to investigate the composition, energy spectra, spatial and temporal variations of the geomagnetically trapped radiations during solar minimum.

Neutron monitors at nearly 70 stations distributed at all latitudes from the Arctic to the Antarctic have been supplemented at some stations by a number of super monitors with counting rates of 10^6/h. Special investigations include studies of rapid fluctuations in meson intensity, of the relations between cosmic ray intensity and the geomagnetic field, and measurements of the neutron albedo flux outside the Earth's atmosphere. It is hoped to carry out special experiments at both ground stations and in balloons and sounding rockets, in the vicinity of the South American/South African anomaly with the object of obtaining further information on the precipitation of particles from the inner radiation belt.

Rockets and Satellites : Aeronomical Studies

In the past five or six years our understanding of many solar and terrestrial phenomena has proceeded at a vastly quickened pace, and this has been due partly to the enormous stimulus given to geophysical research the world over by the International Geophysical Year of 1957–58 and partly to the advent of rockets and satellites.

The first artificial satellite was that launched by the Russians on October 4, 1957—during the IGY. From that historic day to the end of the IGY a total of seven satellites were put into orbit—three by the Russians and four by the United States. Some idea of the rate of progress since the IGY period can be gained from the fact that, according to the catalogue regularly published by the tracking stations, there are at this moment at least 24 active instrumental satellites in orbit and the number of bits and pieces (rocket casings, nose cones, etc.) orbiting round the Earth is about 280. Not only has the number of orbiting vehicles leapt up, but so, too, has the degree of sophistication of the scientific experiments and the complexity of the equipment carried in the vehicles.

In the IGY, rocket and satellite experiments formed only a small part—but nevertheless an exceedingly valuable part—of the whole programme. Seven years later, in the IQSY, the balance has changed tremendously and research with space vehicles forms a very major part of the IQSY programme.

Mention has already been made of the top-side ionospheric sounding satellite *Alouette*, which in its 23 months life has already produced vast numbers of high-quality records on the top side of the ionosphere. At least one other top-side sounding satellite (christened, appropriately enough, *Topsi*) has just been launched by American workers.

In a second IQSY satellite experiment the day-to-day flux of ultra-violet and X-radiation from the Sun is being measured. For a century and more the only direct means of quantitatively assessing activity on the Sun has been to count the spots or other obvious areas of activity to be seen on its surface. Several years ago it was found that the intensity of solar radiation in the decimetre wavelength band showed a very clear solar cycle variation and such measurements of the flux of solar radiation on 10 cm has provided another index of solar activity. It is to be emphasized that the sunspot number has proved a quite remarkable index of solar activity (at least when judged by the very striking way in which it correlates with so many geophysical phenomena) but, of course, it is necessarily only a qualitative and somewhat imprecise index of certain basic physical changes on the Sun. Long-term ionospheric and magnetic investigations have made it clear that the intensity of solar ultra-violet and X-radiations must undergo a very marked change over the sunspot cycle, and during IQSY, for the first time ever, these changes are being directly studied by the American solar radiation monitoring satellite. Launched in January 1964, this satellite is providing continuous data on the intensity of solar Lyman-α radiation and on intensities in three X-ray wave-length bands. Other satellites of interest to the IQSY programme which are either already in orbit or which will be launched during IQSY include the following:

Satellites *Electron 1* and *Electron 2* were launched simultaneously in the first month of IQSY by the U.S.S.R. These have the same perigee height (about 400 km) but vastly different apogees (about 7,000 and 70,000 km respectively). Observations are being made of the inner and outer radiation belts, magnetic fields, solar ultra-violet and X-radiation, cosmic rays, ion composition, etc. Earlier this year the U.S.S.R. also launched some additional satellites in its Cosmos series.

Explorer XVIII, launched by the United States in November last, is the first of four U.S. interplanetary monitoring satellites. It is making continuous observations out to 200,000 km on cosmic rays, on the spectrum of low-energy protons, the characteristics of the plasma and magnetic field.

The first Orbiting Solar Observatory (OSO) was launched in 1963 and it is planned to launch a second in 1965. The U.K.–U.S. satellite, *Ariel 1*, continues in operation and is concerned mainly with ionospheric, cosmic ray and aeronomic observations. *Ariel 2*—a second U.K.–U.S. effort—was launched earlier this year and is designed to measure galactic radio noise, the vertical distribution of ozone and the flux of micrometeorites.

In addition, a very substantial programme of IQSY rocket soundings is also under way. Wind motions and temperature in the ionosphere are being measured with rocket-borne instruments by groups of workers in 10 countries. About a score of rocket flights are being made to determine molecular and atmospheric densities by measuring the attenuation of solar radiation with height. Electron density and electron temperature rocket flights are being made at 15 different sites between latitudes 30° S. and 80° N., and other rocket firings will investigate features of the vast ionosphere current systems which are responsible for many of the variations in the magnetic field. There is also a very substantial programme of meteorological rocket soundings, at least 10 countries having plans for such firings.

All the information obtained during the IQSY is being exchanged through the World Data Centres which are now a well-established feature of the geophysical scene.

Much of the success of the IGY depended on organizing special days and periods for intensive schedules of observation and the organization of a world-wide alert system to warn workers everywhere of the imminence or start of

some solar or geophysical event of special interest. A similar scheme is being used in the IQSY with some special features appropriate to the solar minimum period. An IQSY geophysical calendar has been issued and the alerts will give warning not only of impending storm conditions but also of solar and magnetic calm.

The International Committee responsible for planning and organizing the IQSY is a special committee established by the International Council of Scientific Unions, and it is supported financially by that Council, by Unesco and by contributions from participating nations. It consists of representatives of the four scientific unions which are directly concerned with the IQSY together with representatives of other interested international scientific bodies such as the World Meteorological Organization, the Committee for Antarctic Research and the International Committee for Space Research (COSPAR). The Space Research Committee has no fewer than four representatives on the IQSY Committee—this being an indication of the importance which we have chosen to place on space research in the programme. The IQSY Secretariat has been set up in London—in accommodation kindly provided by the Royal Society.

One of the difficult features of establishing an international scientific committee is that the actual elected membership of the committee is often distributed very unevenly over the globe. In setting up the IQSY Committee we have tried to meet this problem to some degree by having four additional members elected on a regional basis.

An international project of this sort depends for its success on the goodwill of scientific academies, of national governments and their research institutions, and above all it depends on the willing co-operation of many individual scientific workers scattered throughout the world. The IQSY Committee has received warm co-operation on all sides, and it is good to report that no fewer than 70 nations are actively participating, including all those which co-operated in the IGY.

The IQSY is the fourth international co-operative effort in geophysics since 1882 and the second world-wide effort within a period of seven years. When the enterprise ends next year a vast store of geophysical results covering periods of both maximum and minimum solar activity will have been assembled and made readily available for further extended study. We may hope that some time in the future these data will yield the answers to some of the outstanding problems of the Sun and the Earth and thus, to quote again Sir Arthur Rücker at the British Association meeting of 1898, "crown the efforts not merely of those on whom the first glimpse of the truth may flash, but also the international co-operation which has, by way of preparation, made 'the crooked straight and the rough places plain'."

OBITUARIES

Dr. K. H. Barnard

Dr. K. H. Barnard died on September 22 at the age of seventy-seven, after fifty-three years working at the South African Museum in Cape Town. He was the director from 1946 until his retirement in 1956. With his death South Africa has lost one of her greatest zoologists.

Dr. Barnard was born in London on March 31, 1887, educated at a private school in England and at the Realgymnasium in Mannheim, Germany, where he went in order to learn German to help him with his studies. He went on to Christ's College, Cambridge, in 1905, where he took the Natural Science Tripos in botany, geology and zoology, and obtained his B.A. three years later. He also attended the new and then rather unusual courses on anthropology, ethnology and geography. After that he proceeded to study law, and was called to the Bar (Middle Temple) in 1911. He obtained his M.A. (Cantab.) *in absentia* in 1913. Science interested him more than law, however, and he returned to zoology. After a short time as honorary naturalist at the Marine Biological Laboratory in Plymouth he joined the staff of the South African Museum in 1911, where he continued to work until his death. He obtained the degree of D.Sc. from the University of Cape Town in 1924 for his work on the distribution of Crustacea, and in 1956 the University of Stellenbosch conferred on him the degree of D.Sc. *honoris causa*. During his life he produced more than two hundred articles, scientific papers and books.

At the South African Museum he took charge of the Marine collections, including the large collection amassed by the Cape Government trawler S.S. *Pieter Faure*. Based largely on an investigation of the collections in the South African Museum, he published many taxonomic works, including a series of papers on amphipods and isopods (1914–25), a monograph of South African marine fishes (1925–27), a monograph of South African crabs, prawns and mantis-shrimps (1950), and contributions to the South African marine mollusca (1958–64). He also made investigations of the Amphipoda from expeditions such as the *Discovery* and the *Terra Nova*, the *Great Barrier Reef*, the *John Murray*, the *Investigator* and the *Australasian Antarctic*, and contributed important papers in their reports. He also published reports for the British Museum, the Indian Museum, Transvaal Museum, Natal Museum and Durban Museum. His work has greatly increased our knowledge of the fauna of the seas around South Africa and farther afield. He published three popular books on fishes (1947), shells (1951) and shore life (1954).

His keen interest in mountaineering and the discovery of *Phreatoicus*, an Australasian genus of primitive Crustacea on Table Mountain, led to an intensive investigation of the mountain fauna of the south-west Cape ranges. Evidence of a relict fauna was investigated and studied in relation to the geology and topography. His presidential address to the South African Geographical Society in 1936 summarized some of the more important results of these investigations and the conclusions drawn from them concerning the former topography of the mountain ranges and the river courses. This work involved numerous collecting trips in the mountains of the South-west Cape. He made extensive collecting expeditions to the coast of Portuguese East Africa in 1912, the Natal coast in 1913, the Natal interior in 1917, Ovamboland and the Kaokoveld and north to the Kunene River in 1921, 1923 and 1926 (Zoological Survey of South West Africa) and Gordonia and the Orange River to the Aughrabies Falls in 1925. Some of these early trips were made by ox-wagon. He published many important papers on the fauna of the mountains and rivers of South Africa, based largely on his own collections.

He served as local secretary for the compilation of the international catalogue of scientific literature until the London secretariat took over the whole compilation, including records of papers published in South Africa. He helped in the formation of the South African Geographical Society in 1917, served on the Council for several years, was president for the session 1935–36 and the Society's representative at the International Geographical Congress at Cambridge in 1928. After his retirement he concentrated his research work on South African marine mollusca, his research work and publications being aided

by a senior bursary from the South African Council for Scientific and Industrial Research.

Dr. Barnard was awarded many honours during his life-time, including being elected a Fellow of the Royal Society of South Africa in 1920, and a Fellow of the Linnean Society of London in 1921. He received the Gold Badge of the Mountain Club of South Africa in 1924, the King George V Silver Jubilee Medal in 1935, the Senior Captain Scott Medal of the South African Biological Society in 1936, the Medal and grant of the South African Association for the Advancement of Science in 1945, the Queen Elizabeth II Coronation Medal in 1953. He was made an Honorary Fellow of the Royal Society of South Africa in 1956 and president of the Conchological Society of Southern Africa in 1962.

Dr. Barnard was recognized as a world authority in several fields, and it was often with astonishment that specialists in one of these subjects would discover his accomplishments in other fields. Dr. Barnard's world-wide reputation was built up on his unceasing and prodigious work over a long and active life. He was always willing to help his scientific colleagues or casual enquirers in his quiet, unassuming way. Despite his achievements, he remained a shy and modest man.

Our sympathy is extended to his wife and two children who survive him. JOHN R. GRINDLEY

Prof. F. H. Garner, O.B.E.

FREDERIC H. GARNER, emeritus professor of chemical engineering in the University of Birmingham, died suddenly at the age of seventy-one at his home at Wymeswold, Leicestershire, on September 19. Born in 1893, he attended the Market Bosworth Grammar School, going on to study chemistry at the University of Birmingham, and then proceeding to the Mellon Institute, Pittsburgh, for postgraduate studies.

After his return to England he was actively associated with the petroleum industry for many years, rising to become director of research of the Esso European Laboratories from 1935 until 1942.

He will be remembered particularly, however, for the enthusiasm he brought to bear in building up the Department of Chemical Engineering in the University of Birmingham. When in 1942 he was appointed to succeed A. W. Nash as professor and head of the Oil Mining Department, the latter was small and rather static; the undergraduate intake was only five students that year, and even fewer men were able to come as the Second World War progressed.

In 1946 Garner changed the name to the Department of Chemical Engineering, and began the programme of diversification and expansion that, in a little over a decade, transformed a small Department of Oil Mining into one of the largest Chemical Engineering Departments in the world. The academic staff increased from 3 to 28 and undergraduate admissions from 3 or 4 a year to about 80; postgraduate lecture courses were established both in chemical engineering and also (with remarkable foresight) in biological chemical engineering. The research school, too, increased in size and prestige, to an intake of about 23 new students each year. It was inevitable that the Department that he built up with such enthusiasm should become a dominant factor in his life. Inevitable also, perhaps, was Garner's role as the watchful, sometimes strict, father to his staff.

In his own research, he was particularly interested in later years in the surface phenomena associated with mass-transfer, and he was one of the pioneers in investigations of the circulation within falling drops and in the visualization of interfacial turbulence. Even after his retirement in 1960 he used to come into the Department to continue his experiments in these fields.

His death marks the end of an era—an era not only of the establishment of chemical engineering in British universities, but also of the benevolent-dictator type of professor who could, by his endeavours and by the impact of his personality, build up a large Department as his personal creation. Until the moment of his retirement it was *his* department in a very real sense, and he will long be remembered for his devotion to its interests and to those of his many students. J. T. DAVIES

NEWS and VIEWS

The Royal Society of Edinburgh: Elections to Council

AT the annual statutory meeting of the Royal Society of Edinburgh, held on October 26, the following were elected to the Council of the Society for the 182nd session: *President*, Prof. J. N. Davidson; *Vice-Presidents*, Dr. H. R. Fletcher, Prof. G. L. Montgomery, Prof. C. F. Davidson, Dr. Mowbray Ritchie, Prof. J. Allen and Dr. G. H. Mitchell; *General Secretary*, Prof. N. Feather; *Secretaries to Ordinary Meetings*, Prof. A. E. Ritchie and Dr. A. F. Brown; *Treasurer*, Dr. J. R. Peddie; *Curator of Library and Museum*, Dr. R. Schlapp; *Councillors*, Prof. R. B. Fisher, The Rev. A. M. Gillespie, Prof. A. Robertson, Prof. P. A. Sweet, The Rt. Hon. Lord Balerno of Currie, Dr. J. M. Jackson, Prof. I. N. Sneddon, Prof. P. E. Weatherley, Dr. N. Campbell, Prof. P. L. Pauson, Prof. J. R. Raeburn and Prof. J. D. Robertson.

Director of Smithsonian's Museum of History and Technology: Mr. John C. Ewers

MR. JOHN C. EWERS has been appointed director of the new Smithsonian Museum of History and Technology in succession to Mr. Frank A. Taylor, who has been serving as both acting director of the Museum and director of the U.S. National Museum. Mr. Taylor will continue in the latter post. A native of Cleveland, Ohio, Mr. Ewers began his service with the Smithsonian Institution in 1946. For the past six years he has been assistant director of the Museum of History and Technology, serving as planning officer for the new building. He was responsible for the design, construction, and installation of exhibits, which made it possible to open the building to the public in January 1964, a few months after its completion. Before joining the Smithsonian Institution, Mr. Ewers was a member of the Bureau of Indian Affairs, serving as the first curator of the Museum of the Plains Indian at Browning, Montana. He also has acted as museum planning consultant to the Historical Society of Montana, the Bureau of Indian Affairs, and the National Park Service. Recently he was consultant for the Canadian National Museum in planning its new museum of history and anthropology. A graduate of both Dartmouth College and Yale University, Mr. Ewers is a member of several professional societies, including the Washington Academy of Sciences, American Association of Museums, American Anthropological Association, Sigma *Xi*, Western History Association, and the American Indian Ethnohistoric Conference. He is also past-president of the Westerners, a Washington group interested in the history of the American West.

Environmental Design and Engineering at University College, London: Prof. R. G. Hopkinson

DR. R. G. HOPKINSON has been appointed to the new chair of environmental design and engineering in the Bartlett School of Architecture, at University College, London. The establishment of this chair has been made

possible by generous financial support from Messrs. G. N. Haden and Sons and Messrs. Pilkington Bros. Ltd. Dr. Hopkinson's aim will be to educate architects, engineers and scientists in ways of designing buildings that are pleasant and stimulating to live in, and at the same time functionally efficient and economical. He will be concerned with lighting, air conditioning, heating and the problems of noise. He will promote greater co-operation between engineers and architects, and encourage scientific research into the problems of improving human environment. Courses of study in which engineers, architects and research workers will work together will be set up at University College. Dr. Hopkinson is at present in charge of research on the lighting of buildings at the Building Research Station of the Department of Scientific and Industrial Research. His pioneer work on human adaptability and research into the needs of the human being in his environment has brought him into contact with architects and research workers throughout the world, and has resulted in many papers and publications, of which perhaps the most significant is his book *Architectural Physics: Lighting* published by H.M.S.O. in 1963. Dr. Hopkinson has lectured on environmental design in many universities in Britain and overseas and has acted as the University's 'opponent' in a 'doctorate disputation' in Stockholm, and he has directed a research programme in the Department of Electrical Engineering at Cornell University, where he spent a term as a visiting professor. He will take up his new post early in 1965.

The British Joint Corrosion Group

THE Councils of the Iron and Steel Institute and the Institute of Metals, together with the Council of the Society of Chemical Industry, have considered how best to meet the needs of corrosion specialists, metal producers, and metal users with regard to information concerning corrosion and its prevention. The excellent work of the Corrosion Group of the Society of Chemical Industry is fully acknowledged, but it has been recognized for some time that the publications of, and the discussions arranged by, the two institutes have not adequately catered for the interests of those of their members particularly concerned with the metallurgical aspects of corrosion, nor with the interests of those members who are corrosion specialists. The councils of the three societies have now agreed to form a joint group to be known as the British Joint Corrosion Group, with the view of: (*a*) providing a forum for discussion of papers on corrosion and protection of metals; (*b*) overcoming the present dispersion of papers dealing with corrosion throughout many publications, and strengthening the facilities for publishing such papers; (*c*) improving the arrangements for publication and discussion of the metallurgical aspects of corrosion; (*d*) representing United Kingdom corrosion interests internationally. The Institute of Metal Finishing was invited to join the Group and has accepted the invitation. The functions of the existing Corrosion Group of the Society of Chemical Industry will be incorporated into the new organization, and the co-operation of other bodies concerned with this field is also being sought.

Members of the four societies are entitled to register as members of the Group and, on so doing, to receive notices of Group meetings, and to attend such meetings without further payment. The Group will be administered by an organizing committee composed of representatives of the societies. A new publication to be called *The British Corrosion Journal* will be launched, sponsored by the Group, and controlled by a Publication Committee. The first issue of the *Journal*, which will be published in alternate months, will be circulated in July 1965. The Society of Chemical Industry will service the new Group, and provide editorial facilities for the Group's *Journal*. Manuscripts of papers submitted to the new *Journal* should be sent to the Editor, *The British Corrosion Journal*, 14 Belgrave Square, London, S.W.1. Members of the four co-operating societies are invited to register now as members of the British Joint Corrosion Group. Further information, and application forms for subscription to the *Journal*, can be obtained from the Secretary, the British Joint Corrosion Group, 14 Belgrave Square, London, S.W.1.

Interferon Information Exchange Group

AN information exchange group for research on interferon has been formed. The objectives and operations of an earlier established information exchange group have already been described in *Science* (143, 308; 1964). The new exchange will make it possible for a scientist working on interferon to communicate research findings or other scientific information quickly to all others in the same field throughout the world. Under the new scheme, the exchange member will send his communication to the exchange 'centre' where duplicate copies will be printed (by photo-offset) and distributed. Communications will include manuscripts (which will also have been sent to journals), discussions, criticisms, bibliographic omissions, memoranda of unpublished research findings, technical suggestions, unofficial notes of meetings or any other original communications. No editorial prerogatives will be exercised. Active investigators of interferon who did not receive the announcement letter are invited to apply for membership. The chairman is Dr. Alick Isaacs, National Institute for Medical Research, London, N.W.7, and the co-chairman, Dr. Samuel Baron, Laboratory of Biology of Viruses, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20014. All correspondence should be sent to the co-chairman.

Advanced Educational Courses

A *Bulletin* issued by the London and Home Counties Regional Advisory Council for Technological Education describes the special advanced courses held in the London and Home Counties Region, which do not regularly appear in college calendars or prospectuses as part of a grouped course or as subjects offered for endorsement on Higher National Certificates (*Bulletin of Special Courses in Higher Technology, Management Studies and Commerce*, 1964–65. Part 1: Autumn Term. Pp. 126. London: London and Home Counties Regional Advisory Council for Technological Education, 1964. 5*s*.). As a rule they are part-time courses (usually evening), but full-time courses, which are specially arranged and are not longer than three months duration, are also included. Sessional evening postgraduate courses leading to higher degrees in science (including mathematics) and engineering are not included. Among the wide variety of courses are: cost engineering in the process industries (Borough Polytechnic); varactor diodes (Brunel College); silicon gate-controlled switches (Enfield College of Technology); high fidelity sound reproduction (Hendon College of Technology); predetermined motion time standards (Kingston College of Technology); management for the clothing trade (London College of Fashion); geotechnical processes and soil stabilization (Northampton College of Advanced Technology); tall buildings (The Polytechnic); trading blocs (Slough College); and autocode programming (Wimbledon Technical College).

Royal Society Films for Hire

IN 1961 the Royal Society mounted an expedition to Sabah, North Borneo. The purpose of the expedition was to study the undisturbed forest life and soil variation at different altitudes of Mount Kinabalu and the Pinosuk Plateau. A film was prepared by members of the expedition showing some aspects of their work, and has now been made available by the Royal Society for hire by schools, educational institutions, film societies, etc. The film is of approximately 35 min duration and is in colour with a dialogue. The charge for hiring is 25*s*. Other films available from the Royal Society are: "The Volcanic

Eruption on Tristan da Cunha, 1961–62" (20 min, colour and sound; hire 25*s*.), which was prepared largely by members of the Society's expedition to the Island during October 1961–March 1962; "South from Chiloé" (40 min, colour and sound; hire 42*s*.); which was prepared by the Society's expedition to Southern Chile during October 1958–February 1959; "Halley Bay" (20 min, colour and sound; hire 25*s*.). All films are available from the Executive Secretary, Royal Society, Burlington House, Piccadilly, London, W.1.

Marshes and Wetlands of Europe

In recent years, improved machinery has made draining and reclamation of marshland and wetland a practicable proposition, and these areas are rapidly dwindling in Europe. According to the International Union for Conservation of Nature and the International Wildfowl Research Bureau, not enough thought has yet been given to the loss and damage which such drainage policies incur. The case for marshes and wetlands is set out in a booklet entitled *Liquid Assets* (Pp. 16. London: International Union for Conservation of Nature, 19 Belgrave Square, 1964). It is considered that there is an urgent need to preserve these areas, which, it is emphasized, are in many cases natural assets. Land drainage, undertaken at public expense, is a form of farm subsidy, and admissible only if it compares favourably with other means of stimulating food production. The 'worthwhileness' of a project must be assessed not merely on expected cost and profit, but on long-term advantages and disadvantages. Wetlands provide an unlimited field for biological research, and marshes, especially those on the coast, are among the most naturally fertile areas in the world. The booklet considers that if such areas are destroyed great opportunities will be lost for discovering and possibly exploiting new sources of food. Besides their amenities value, such areas are invaluable in teaching and demonstrating the elements of zoology and botany—especially ecology. Governments throughout the world would do well to take stock of their marshes and wetlands in the light of this publication and ensure that their dwindling 'liquid assets' are not only protected but also conserved.

The Wildlife Youth Service

The Wildlife Youth Service has developed into a nation-wide organization with nearly 18,000 individual members since it was launched in May 1963. A further 10,800 members are represented through the 700 registered school groups—and applications are still steadily flowing in at the Headquarters Office, 37 Hertford Street, London, W.1. The launching of the Service was planned to take place in three phases: the first included the National Nature Week, followed by four months recruiting of individual members. The second phase—the School Group Scheme—was opened during the first week in October. This has already produced encouraging results with 700 school groups registered and more to come. These groups, many of which exceed a membership of 200 children, are being organized with the aid of interested teaching staff. Most of the groups have entered into one of the 'wildlife study' projects and activities suggested by the Director. The third phase is scheduled to take place in a few months time, when every effort will be made to set up permanent evening centres for Wildlife Ranger and Panda members throughout Britain. So far, the Wildlife Youth Service has arranged seven major film programmes for schoolchildren at cinemas in England, Scotland and Wales; these have been attended by a total of 8,200 children. The Director has given talks at 127 schools to a total of more than 10,650 children. The Information Service has dealt with more than 5,000 questions on wildlife and pet care, and the Wildlife Youth Service has sent out a total of more than 61,000 letters in thirty weeks since it was first launched (*News for Naturalists*, 5, No. 2; 1964).

National Heron Census

Ten years have elapsed since the last national heron census was carried out, and now the British Trust for Ornithology has announced that a further attempt is to be made to count all the herons' nests in Britain. In addition to the usual factors which have always affected the heron population, the position is now being complicated by the effects of toxic chemicals, and hence it is important to make this census as accurate as possible. Ornithologists in England and Wales wishing to help in the new census should contact Mr. J. Stafford, Westering, Moor Lane, Brighstone, Isle of Wight; those in Scotland should contact Mr. C. P. Rawcliffe, 35 Comely Bank Road, Edinburgh, 4; and those in the Irish Republic, Mr. O. J. Merne, c/o National Bank Ltd., Rathkeale, Co. Limerick, Eire.

New Words in Biology and Related Subjects

Dr. N. W. Pirie, head of the Biochemistry Department at the Rothamsted Experimental Station, has prepared a preliminary list of words for inclusion in the proposed *Biological Council Dictionary* of new words in biology and related subjects (*Institute of Biology Journal*, 2, No. 3; August 1964). Of the 96 words submitted, none is included simply because it is rare: a word is excluded if it appears, in the sense with which biologists are concerned, in the *Oxford English Dictionary* or the eighth edition of Henderson's *Dictionary of Biological Terms* (1963). The rule is interpreted flexibly when the spelling has altered. Words that do not seem to have been used by anyone except the coiner are not included unless it seems likely that they will catch on. On the other hand, inclusion or exclusion are not intended as comments on the usefulness of the words; there will be time enough for comment after the first definite list has appeared. The names of individual organisms, diseases and substances are excluded because they are easily found in the abstract journals; only names of categories are recorded. Among the words are:

Actinophage—an anti-actinomycete phage.

Air spora—the population of air-borne particles of plant or animal origin, including pollen.

Aposymbiotic—removed from symbiosis or separated from the symbiotic partner.

Cryptobiosis—the state of an organism when it shows no life-like activities while still being capable of resuscitation.

Darwin—the rate of evolution of a quantitative character, the natural logarithm of which changes by unity in a million years.

Epontic—growing on any surface, plant, animal or mineral. A broader term than epilithic or epiphytic.

Euphenics—the improvement of individual people by chemical or surgical means. Analogy with eugenics in the sense that phenotype is opposed to genotype.

Gangliospore—a fungal spore developed from the swollen tip of a hypha.

Kronism—the killing (and sometimes eating) of eggs and nestlings by the parent bird.

Lomasome—a sponge-like structure in fungi contiguous with the hyphal wall as revealed by the electron microscope.

Poxvirus—a group of viruses of complex structure, 200–300 nm in diameter, usually causing lesions involving skin in birds and mammals (includes vaccinia, variola and various animal poxes).

Tachyphylaxis—the diminishing response to successive doses of any material active in biological systems.

Teleonomic—a neutral word implying that an adaptation is useful for achieving a certain result without the implication that design of purpose was involved.

Trophogenic—the surface layer of a body of water in which photosynthesis can occur. Also used for an effect produced by feeding.

Bakery Planning

No baker intending to build new premises, or to expand or modernize his existing bakery, has had cause for complaint since June 1964. For since then there has been available for the would-be planner a comprehensive guide

published by the British Baking Industries Research Association at the modest price of 10*s*. (Report No. 72: *Some General Principles of Bakery Planning*. By Mr. D. E. Chapman and Miss E. M. Laing. Pp. ii+82. Chorleywood, Rickmansworth: British Baking Industries Research Association, 1964. 10*s*.). It may be added that the British Baking Industries Research Association reports are not normally available to the public, but an exception has been made with this one, as it was felt to be one of general interest. The report attempts to answer in advance and in order the questions which an intelligent baker would ask in these circumstances. Thus, after some preliminary advice to the baker confronted with the dilemma of whether to adapt his existing bakery or build an entirely new one, the authors guide him through the intricacies of site-choosing, and introduce him to the complexities of lay-out and planning. The foreword makes it clear, however, that, except for small projects, he will need the services of an architect; and the report is intended to help in the liaison with the latter rather than to displace him. The design of individual parts of the bakery is discussed in detail, and includes ancillary services such as the despatch area, test-laboratory and boiler room: even the refuse store is not forgotten. Special attention is directed throughout to considerations of safety, to comfortable working conditions, and to the reduction of unnecessary labour: reference is made in an appendix to appropriate legislation.

Hydatid Diseases in New Zealand

Hydatid disease in New Zealand is as old as the sheep industry. A circular from Dunedin in 1887 to medical practitioners revealed that nearly all, even at that time, had wide experience of the disease. Such a well-entrenched disease will not readily be overcome, and reports of past efforts at eradication are conflicting. It was with this knowledge that the National Hydatids Council asked Dr. F. S. Maclean to write a history of the fight against hydatid disease in New Zealand (*Hydatid Disease in New Zealand: an Account of the Events leading to the Establishment of the National Hydatids Council, and the Methods adopted to Eradicate the Disease*. By F. S. Maclean. Pp. 48. Wellington, N.Z.: National Hydatids Council, 1964). The lessons that emerge from his report are striking, and they are useful in fields far wider than hydatid prevention alone. First, there has been no sudden or dramatic advance in medical knowledge. The methods advocated in the 1880's—never to allow dogs to feed on raw offal—are just the same as those advocated to-day. Secondly, the problem is one of people rather than dogs and other animals. For nearly a hundred years many efforts have been made to bring knowledge of hydatid disease and the methods of prevention to all New Zealanders, and many thousands of pounds have been spent. On two occasions at least, regulations have been framed and passed to stop dogs eating raw offal. As a result, many thousands of New Zealanders knew the truth about hydatid disease but took no action to check it. Some felt that any action taken by the individual was made useless by the carelessness of others. Some had a quite unrealistic belief that dosing with arecoline would, in some miraculous way, relieve them from the responsibility of preventing their dogs from eating raw offal.

In spite of every effort by the Government, the various Government departments, the medical schools, the research units and the medical profession, hydatid disease continued to take its yearly toll of health and life. Apathy and hopelessness fed on confused thinking, stories of failure and avoidance of personal responsibility. As indicated by Dr. Maclean, the atmosphere in 1957 suddenly changed. There was a dramatic upsurge of interest. For the first time farmers worked individually to eliminate the disease from their own farms, they co-operated with their neighbours to form local anti-hydatid committees, they elected members to fight the disease at a provincial level and, in conjunction with the Federation of Young Farmers Clubs, they finally prevailed on the Government of the day to form a national body to campaign against hydatid disease in all parts of the country. The change in attitude was due to participation. Farmers were told of a disease which primarily affected them and were told of methods of prevention which only they could apply. They were given responsibility for action, were asked to make their own decisions, plan their own policies and become involved themselves in a campaign they could call their own. Organizations and individual farmers devised methods of disposing of or treating offal, destroying carcases, controlling dosing, and feeding their dogs. Local committees took the message to every local dog owner. District committees defined boundaries and co-ordinated the work of local committees. Within two years approximately 500 local committees were set up in rural areas throughout New Zealand. Later, a National Hydatids Council was formed: its success is described in the booklet.

Protein Requirements of Man

In 1963 the World Health Organization and the Food and Agriculture Organization requested the views of experts on various aspects of protein metabolism. Following on this request a working party was set up, under the chairmanship of Prof. F. G. Young, to investigate the human requirements of protein with special reference to the dietary background of the United Kingdom; the report of this working party has recently been published (*Reports on Public Health and Medical Subjects*, No. 111: *Requirements of Man for Protein*. Pp. vi+90. London: H.M.S.O., 1964. 5*s*. 6*d*. net). Physiological variations which can occur in protein metabolism and regulation are dealt with in the report, which then discusses the nutritive value of proteins both in relation to the composition of the proteins and the calorific value of the diet. The relationship between different forms of biological assay of protein quality is discussed and the importance of 'nitrogen balance' methods is emphasized. Factors which affect the nutritive value of protein foods are considered and human factors which affect the requirements are discussed at length, while the effects of disease and injury are reviewed briefly. The mean lower and upper limits of dietary protein intake per day for all age-groups are summarized and suggestions are made as to the use of such data in the recommendation of protein allowances. The report concludes with a number of appendixes dealing with specialized aspects of protein supply and requirements.

The Ramsay Memorial Fellowships Trust: Fellowships for Advanced Students of Chemistry

Applications for two Ramsay Memorial fellowships for advanced students of chemistry will be considered in February 1965. One of the fellowships will be limited to candidates educated in Glasgow, but they can apply to be considered for both fellowships. The value of each fellowship will be £750 per annum, to which may be added a grant for expenses of research not exceeding £150 per annum, and will normally be tenable for two years.

Travel Grants

Applications for three Ramsay travel grants for the year 1965–66 will also be considered in February 1964. No travel grant will exceed £500. Two of the travel grants will be limited to junior academic chemistry staff of universities and colleges of technology or advanced technology in England, Wales and Northern Ireland. One travel grant will be limited to junior academic chemistry staff of universities in Scotland.

Further information concerning fellowships and grants can be obtained from the Joint Honorary Secretaries,

Ramsay Memorial Fellowships Trust, University College London, Gower Street, London, W.C.1. Applications must reach them not later than January 13, 1965.

The Night Sky in December

NEW moon occurs on Dec. 4d 01h 19m U.T. and full moon on Dec. 19d 02h 42m. The following conjunctions with the Moon occur: Dec. 1d 04h, Venus 2° S.; Dec. 10d 12h, Saturn 3° N.; Dec. 16d 09h, Jupiter 2° N.; Dec. 24d 19h, Mars 3° S. Venus is in conjunction with Antares on Dec. 26d 00h, Venus being 6° N. There is a partial eclipse of the Sun on December 3–4, invisible at Greenwich. There is a total eclipse of the Moon on December 19, visible at Greenwich. Moon enters penumbra 00h 01m, Moon enters umbra 00h 59m, total eclipse begins 02h 07m, mid-eclipse 02h 37m, total eclipse ends 03h 07m, Moon leaves umbra 04h 15m, Moon leaves penumbra 05h 14m. Magnitude of eclipse 1·18. Mercury is too close to the Sun for observation. Venus rises at 4h 45m, 5h 25m and 6h 15m on December 1, 15 and 31, respectively. Its stellar magnitude is −3·4; its distance from the Earth increases during the month from 125 to 139 million miles and the visible portion of the apparent disk from 0·848 to 0·913. Mars rises at 23h 50m, 23h 10m and 22h 35m at the beginning, middle and end of the month, respectively. Mars is in Leo, its stellar magnitude is +0·8, and its distance from the Earth on December 15 is 116 million miles. Jupiter sets at 6h 00m, 4h 55m and 3h 55m on December 1, 15 and 31, respectively. Its motion is retrograde among the stars in Aries, its stellar magnitude is −2·3 and its distance from the Earth on December 15 is 388 million miles. Saturn is in Aquarius, setting at 22h 15m, 21h 30m and 20h 40m at the beginning, middle and end of the month, respectively. Its stellar magnitude is +1·1 and its distance from the Earth on December 15 is 938 million miles. Occultations of stars brighter than magnitude 6 are as follows, observations being made at Greenwich: Dec. 12d 20h 23·5m, 30 Psc. (*D*); Dec. 12d 22h 14·8m, 33 Psc. (*D*); *D* refers to disappearance. The Geminid meteors are active during December 9–14, the maximum being on December 13; the radiant is near R.A. 7h 28m, Dec. +32°, and conditions for observation are favourable. The Ursid meteors are active during December 20–22, conditions for observation being unfavourable. The winter solstice occurs on Dec. 21d 19h 50m.

University News:

Birmingham

THE following appointments have been announced: *Senior Lecturer*, E. G. Knox (social medicine); *Lecturers*, Dr. J. E. Bowcock (mathematical physics); K. R. Chapman (physics); A. T. Clementson (engineering production); F. A. Garner (mining and minerals engineering); Dr. T. A. Hamor (chemistry); Dr. D. M. Hirst (chemistry); B. W. Jenney (engineering production); Dr. D. W. Jones (materials science); Dr. G. A. Parker (mechanical engineering); R. H. Saxton (civil engineering); R. M. Baddeley (surgery); Dr. D. A. Lewis (physiological chemistry); *Research Fellows*, M. J. Copsey (electron physics); Dr. W. P. Dodd (physics); Dr. A. Erndt (mining and minerals engineering); Dr. W. J. Feast (chemistry); Dr. U. Isaak (physics); R. J. Lumby (industrial metallurgy); Dr. T. F. Tuan (mathematical physics); A. Whittingham (chemistry); W. Young (mathematical physics); D. J. P. Barker (social medicine); Dr. T. Chojnacki (experimental neuropharmacology); Dr. R. Neisser (psychiatry); *Kenward Memorial Fellow*, J. A. Buzacott (engineering production).

London

DR. HILDE T. HIMMELWEIT, reader in psychology at the London School of Economics and Political Science, has been appointed to the chair of social psychology tenable at that School. Dr. G. L. Asherson of the Department of Immunology, National Institute for Medical Research, has been appointed to the readership in immunology tenable at the London Hospital Medical College. Dr. A. P. Willmore, lecturer at University College, has been appointed to a readership in physics tenable at that College. The title of professor of experimental pharmacology has been conferred on Dr. Franz Hobbiger in respect of the post held by him at Middlesex Hospital Medical School. The title of reader in embryology has been conferred on Dr. M. Ruth Bellairs in respect of the post held by her at University College.

Pennsylvania

DR. J. H. WILKINSON, reader in chemical pathology, of Westminster Hospital Medical School, University of London, has been appointed professor of clinical chemistry in medicine in the University of Pennsylvania, and expects to take up his new post in Philadelphia on April 1, 1965.

Strathclyde

MR. R. D. LORD, senior lecturer in mathematics in the University, has been appointed reader in mathematics. Dr. J. B. Helliwell, senior lecturer in mathematics in the University, has been appointed reader in gas dynamics. Dr. S. C. Hunter of the Royal Armament Research and Development Establishment, Ministry of Defence, has been appointed reader in solid mechanics. The three appointments became effective on October 1.

Announcements

DR. P. H. FOWLER, reader in physics in the University of Bristol, has been appointed to the Royal Society research professorship at the University of Bristol. Dr. Fowler is distinguished for his research work in high-energy physics and cosmic radiation, and for his contributions to the development of the emulsion technique. This is the sixth research professorship to be made by the Royal Society.

THE ninth congress on "Theoretical and Applied Mechanics" and symposium on "High-Speed Computation Methods and Machines" will be held at the Indian Institute of Technology, Kanpur, during December 20–24. Further information can be obtained from the Secretary, Indian Society of Theoretical and Applied Mechanics, Indian Institute of Technology, Kharagpur.

A SYMPOSIUM on "Hazards and Doses to the Whole Population from all uses of Ionizing Radiations and Radioactive Materials", arranged by the Hospital Physicists' Association, will be held in London on May 13. Further information can be obtained from the Secretary, Hospital Physicists' Association, 6 Paddington Street, Marylebone High Street, London, W.1.

THE one hundred and thirty-first meeting of the American Association for the Advancement of Science will be held in Montreal during December 26–31. The programme will include symposia on: primate behaviour; possible meteoric or lunar influences on meteorological phenomena; medical geology and geography; biochemical differentiation; science and the public mind—past and present; the sociology and ethics of science. Further information can be obtained from the Information Officer, American Association for the Advancement of Science, 1515 Massachusetts Avenue, N.W., Washington, D.C., 20005.

CORRIGENDUM. The top of Fig. 2 of the paper entitled "Limits of Human Vision" (*Nature*, 191, 471, 1961) by Dr. R. A. Weale contains an energy scale, inadvertently reduced by 1·477 log units. The bracket indicating an energy range thermally dangerous for the human eye will therefore correspond to a photometric reading of approximately 8·6 log scot. trol-sec.

EDUCATION AND RESEARCH IN ORAL SCIENCE IN THE MASSACHUSETTS INSTITUTE OF TECHNOLOGY

THE W. K. Kellogg Foundation of Battle Creek, Michigan, has made a grant of 440,000 dollars to the Massachusetts Institute of Technology for the further development of the Oral Science Program of graduate teaching and research in the Department of Nutrition and Food Science. The Oral Science Program in the Institute, directed by Prof. Robert S. Harris, professor of nutritional biochemistry, was established a year ago under a four-year grant in excess of 500,000 dollars from the National Institute of Dental Research, a division of the National Institutes of Health at Bethesda, Maryland.

The Kellogg grant, which will be used over a five-year period, represents collaboration among the private Foundation, the Massachusetts Institute of Technology and the National Institutes of Health in development of a broad programme of science-oriented graduate training for dental educators and research workers.

The Oral Science Program was initiated in response to the increasing need for the application of fundamental biological and physical sciences to the solution of problems in oral and dental disease and the growing demand for dental school faculty members, both dentists and life scientists, who possess a depth of understanding in the biological and physical sciences which underlie their professions.

Thus, the principal objectives of the Program embrace both the areas of professional education and fundamental research in the oral sciences. The Department of Nutrition and Food Science in the Massachusetts Institute of Technology was selected as the location for the Program, even though the Institute does not operate a dental school, because of the growing recognition of the importance of food and nutrition to dental health and because of the Institute's broad base in the underlying biological and physical sciences.

Several investigations are being carried out under the twin grants in the area of professional education, and foremost among these is a programme of graduate training in nutrition and oral science leading to a Ph.D. degree for selected graduate dentists and bioscientists of outstanding merit who will follow careers in dental education and research. This programme will normally require four years of study. Two post-doctoral students entered the programme a year ago; three more began recently, and eventually the number will increase to 15–20. The grant of the National Institutes of Health is being used to support students and their research. A substantial portion of the Kellogg grant will be used to provide specialized laboratories and clinical facilities.

A prime aim of the Ph.D. programme in oral science is to help broaden the scientific basis of America's dental school faculties and curricula by serving as a training centre for individuals who may become leaders in dental education and research. It is hoped that one result will be the eventual scientific broadening of the professional education provided to the nation's dental practitioners.

According to Prof. Harris, the research strides being made in understanding and preventing oral diseases, especially dental decay, will have the long-range effect of bringing about fundamental changes in the character of dental practice. Practitioners, in order to meet the demands of these changes, will need a broadened training in basic science in the course of their professional dental education.

Other activities being undertaken in this Oral Science Program to contribute to professional education include short summer courses for dentists and dental faculty members in nutrition and related biosciences, special seminars and conferences, educational television programmes directed to dental practitioners, and the publication and dissemination of research findings and critical reviews. There will also be activities intended to inform the lay public how nutrition and oral hygiene affect the teeth and gums.

Research under the Oral Science Program is focused on the biochemical, microbial and nutritional aspects of oral pathology. Much fundamental work remains to be done before a clear understanding can be obtained of the relationships between human nutrition, food-borne microbial agents, and human biochemical systems, and of their combined effects on oral and dental disease and health. [See also *Nature*, 204, 526; 1964.]

THE NUFFIELD FOUNDATION

THE nineteenth annual report of the Nuffield Foundation* covers the year ended March 31, 1964. It includes a just and dignified tribute to Lord Nuffield's interest in healing and research and civic welfare throughout the Commonwealth, as well as an appreciation of Mr. L. Farrer-Brown's services as director, from which office he retired at the end of the year. During the period covered allocations were made totalling £1,785,963, of which £240,493 was for biological research, £40,000 for research in rheumatism, £353,600 for other medical research, £322,300 for social research and experiment, £418,103 for education, and £407,535 for grants for the Commonwealth overseas. While the allocations followed the pattern of previous years, emphasis was placed on two grants for medical research, one of which, up to £350,000 to establish a unit for medical genetics in the University of Liverpool, is the largest single grant yet made by the Foundation for medical research. The other grant is one of £50,000 to the Royal College of Surgeons for a building at its Buckston Browne Farm to set up a colony of monkeys, including a section devoted to breeding, with the primary purpose of enabling the Department of Dental Science to make experimental investigations into dental caries. In directing attention to this grant the trustees comment that since 1946 the Foundation's support for dental research has been largely limited to its scheme of dental fellowships and scholarships and that the paucity of research grants reflects the lack of applications rather than any decline in interest by the Foundation.

With the last-mentioned observation should be set the later, more general, comment that, although the number of research applications submitted to the trustees increased during the year, there was not the overwhelming flood of applications that might have been expected from reports during the year of widespread lack of funds for research. In medicine and biology the Foundation made slightly fewer grants than in 1962–63, and the increase in the allocation reflects an increase in the average size of the grants. In the social sciences both the number of grants and their average size increased. The trustees do not

* The Nuffield Foundation. Report for the year ended March 31, 1964. (Nineteenth Report, 1963–64.) Pp. xvi+204+2 plates. (London: The Nuffield Foundation, 1964.)

anticipate any long-term decrease in the requests of the universities for Foundation support as a result of any Government action on the Robbins Report. On the contrary, the expected general increase in academic research may itself generate new demands, and perhaps also enable the Foundation in its choice of projects to adhere more closely to those areas most consonant with its policies and commission. Private foundations generally may well expect that their opportunities for useful action will always outrun their resources.

The £40,000 for research into rheumatism already noted includes a grant of £34,740 to the Middlesex Hospital Medical School to establish an Experimental Immunological Unit for the purpose of carrying out investigations into auto-immune phenomena in rheumatoid disease. Other grants for medical research include £52,800 over 5 years to the Department of Social and Occupational Medicine of the Welsh National School of Medicine for an investigation of bronchitis among steelworkers at Ebbw Vale and Port Talbot, and a further grant of £33,350 to the Unit of Biometry, University of Oxford, towards the cost of a small Elliot 803 computer, but the report records the failure, for lack of industrial support, of the promising occupational hygiene service at Slough. A grant of £15,000 over 5 years has been made in support of Dr. Stephen Black's investigation, by electroencephalogram studies and psychophysiological techniques, of the brain's responses to stimuli in different sensory modalities, and one of £11,000 to support studies of exploratory behaviour in children at the Park Hospital for Children, Oxford, for a period of 5 years.

Among the grants for biological and other scientific research may be noted one of £29,000 over 5 years to the Department of Botany, University of Cambridge, for an investigation into the mechanism governing the uptake and loss of ions by plant cells, and the distribution of ions within the tissue of the whole plant, while one of £42,150 over 5 years to the School of Biological Sciences, University of East Anglia, is to permit the building up of a team to study ion and water uptake by the roots of higher plants. A grant of £71,681 was made to provide accommodation for research at the Department of Botany, University of Glasgow, more particularly for research on the physiology of parasites, while a capital grant of £46,500 to the Strangeways Research Laboratory, Cambridge, now the largest centre for cell biology in Britain, is for equipment and increased accommodation for the Biophysics Section, particularly for investigation of the fibrous proteins of connective tissue and the constituents of the ground substance at different levels of structure. A grant of £11,250 over 3 years was made to the Department of Organic Chemistry, University of Manchester, for research into the transport of organic molecules across cell membranes, and £12,155 to the Department of Zoology, University of Sheffield, in support of biophysical work on the interaction of steroid hormones with macromolecular structures in the cell.

A new grant of £35,000 over 5 years was made to the Department of Metallurgy, Imperial College of Science and Technology, London, to enable a research group to be built up in process engineering metallurgy, while the Foundation is also assisting the Advanced School of Automobile Engineering, Cranfield, to develop its research activities with a grant of £15,000 over 5 years for an investigation of the effects of vibration on comfort and human performance. The Foundation has provided up to £250,000 to the Royal Society towards half the cost of the necessary reconstruction of part of Carlton House Terrace, on the understanding that the Government will secure the premises to the Society without charge for at least 99 years.

Of the grants for social research and experiment, the trustees direct particular attention to five: first, one of £70,000 over 5 years was made to the Institute of Race Relations for a survey of relations between the coloured communities in Britain and their host community; secondly, £45,000 over 4 years went to Prof. C. Buchanan of the Department of Civil Engineering, Imperial College of Science and Technology, for transport studies; thirdly, £42,000 over 4 years to the Institute for Operational Research for research into the planning processes of a local government authority; fourthly, £15,000 over a year and a half to the National Institute for Economic and Social Research for a study of the costs of bringing all the urban parts of Britain up to satisfactory standards in housing and other urban facilities; and, fifthly, £30,000 over 3 years to the Institute of Community Studies for the development of its plans for research through social experiments. A grant of £9,000 over 3 years was also made to the Institute of Social and Economic Research, University of York, for a study of the social and economic problems of urban redevelopment in the West Riding, while a similar grant was made to the Department of Social and Economic Research, University of Glasgow, for a comparative study of European regional economic problems. A further grant of £10,000 over 6 years was made to the Institute of Strategic Studies for its library, and a grant of £20,000 to Political and Economic Planning was to help with the removal to new premises; while one of £3,000 over 2 years went to the School of Architecture, University of Cambridge, for research into the design of science lecture-rooms.

Of the grants for education, one of £60,000 to the London School of Economics to finance a Unit for Economic and Statistical Studies on Higher Education is specially emphasized by the trustees. In the first place, the Unit will examine the relation between higher education, the structure of the labour force and economic growth, and will assess the demand from school-leavers for entry into different kinds of education beyond the compulsory school age and the relation of this demand to social, economic and educational factors. The Foundation is also particularly interested in investigations of language development and in fundamental work on intellectual development based on present-day biological research. A further £175,000 was allocated for the Science Teaching Programme, £75,000 was set aside for a project in science for 5–13-year-olds, and allocations of £6,000 were made for each of three groups concerned with identifying the main strategy and objectives in planning teaching material for physics, chemistry and biology at the Advanced Level of the General Certificate of Education. Of the £100,000 set aside for the Language Teaching Programme, £50,000 has been allocated to the Language Teaching Materials Project, and a grant of £22,000 over three years was made to the Communication Research Centre, University College, London, for research into the English language designed to contribute to the teaching of English in schools; £20,500 over five years to the Department of Psychology, University of Cambridge, for further investigation of the effect of personality factors on intellectual development; £10,500 over three years to the Department of Psychology, University of Hull, for research into learning transfer in young children; and £6,600 over three years to the Department of Statistics, University College, London, for research into university selection and performance.

Grants for the Commonwealth overseas include up to £4,800 for the first two years of a scheme of scholarships for economists from overseas to be run by the Overseas Development Institute; a further grant of £100,000 over five years to the Centre for Educational Television Overseas; and a final grant of £20,000 over three years to the Overseas Visual Aid Centre, conditional on satisfactory financial support from Government sources. A grant of £16,000 was made to the University of Ibadan to provide a nuclear magnetic resonance spectroscope for research on natural products in the Department of Chemistry; £26,050 over the next four years to the Nuffield Unit of Tropical Animal Ecology at Mweyz in Queen Elizabeth

National Park, Uganda; up to £12,000 for the production of a film illustrating the work on teacher training in the Kilimanjaro district of Northern Tanganyika; a further £7,500 for investigations in terrestrial ecology jointly by the University College of Rhodesia and Nyasaland and the University of Witwatersrand; and £3,800 over three years for research into the vegetation of swamps by Makerere College, University of East Africa. A grant of £80,000 was made to the Walter and Eliza Hall Institute of Medical Research, Melbourne, for extensions for immunological research, a similar grant to enable the Sydney Church of England Homes for Aged Persons to obtain a site and erect the Nuffield Garden Village, and £2,640 over three years for investigations by the Department of Biochemistry, University of Adelaide, into the association of deoxyribonucleic acid and the yeast cytochrome b_2 from which it is isolated and of the way in which deoxyribonucleic acid controls the behaviour of yeast. The University of Hong Kong received a grant of £26,650 over three years towards research in the Department of Zoology into the level of adrenocorticosteroids at cellular level, and one of £15,000 was made to the British Solomon Islands Protectorate for training college accommodation for women students.

As an experiment, the Foundation offered a special award for a medical fellowship to be held in Ibadan, Makerere, Vellore or the University of the West Indies, but although there was keen competition for the ordinary fellowships, of which four were awarded, only one candidate applied for the special fellowship, and as he did not wish to follow an academic career in medicine no award was made. One medical research fellowship was awarded, four fellowships, three assistantships for general practitioners, and 24 scholarships in tropical medicine, enabling senior students to spend three months attached to a Commonwealth medical school in the tropics. One research fellowship, five fellowships and three scholarships in dentistry were awarded, and the first six awards were made under the new scheme for food science. Two scholarships were awarded under the new scheme for sociological awards, and awards under the schemes for the Dominions and Colonies included 20 Dominion fellowships (six in medicine, eight in science, six in the humanities and social sciences) for graduates from Australia, Ceylon, India, Pakistan and New Zealand; three travelling fellowships for Canadian public officials and two for trade unionists; and nine short-term travel grants for senior Canadian scholars (one in medicine, five in science, three in the humanities and the social sciences). Two fellowships were awarded to Civil Servants from Pakistan; and under the scheme for training Africans in agriculture, a scholarship was awarded to a farmer from Kenya. The Foundation's grant to the joint scheme with the Royal Society for Commonwealth Bursaries was renewed for a further, final period of five years at £5,000 a year, and 24 awards were made.

COMBUSTION

THE tenth International Symposium on Combustion was held in the Department of Physical Chemistry, University of Cambridge, during August 16–21, 1964. Nearly 700 chemists, physicists and engineers from 25 countries attended. Among the overseas visitors were Dr. Bernard Lewis (President of the Combustion Institute) and Profs. Kistiakowsky, Kondratiev, Laffitte, Jost and Von Elbe.

The Plenary Lecture, entitled "The Study of Combustion by Photochemical Methods", was delivered by Prof. R. G. W. Norrish (Cambridge), who gave a broad survey of the development of understanding of the basic processes of combustion over the past forty years, with particular reference to the contributions made by kinetic spectroscopy and flash photolysis. The lecture was preceded by a short inaugural meeting and the presentation of the Combustion Institute Awards: the Sir Alfred Egerton Medal to Prof. P. Laffitte (Paris), the Bernard Lewis Medal to Prof. R. G. W. Norrish, and the Silver Combustion Medal, for the best paper presented at the ninth Symposium, to N. M. Howe, jun., C. W. Shipman and A. Vranos.

The papers in the discussion under the title "Elementary Combustion Reactions" fell into two groups—sixteen concerned with neutral species and nine with charged species. The review paper in the first group, "Elementary Combustion Reactions", was given by Prof. Ashmore (Manchester). The papers which followed centred round several topics. F. J. Wright (Esso Research) spoke on the nature of initial attack by oxygen atoms; C. F. Cullis, A. Fish and J. F. Gibson (Imperial College of Science and Technology) on the mechanism of spontaneous ignition of hydrocarbon mixtures; R. V. Blundell *et al.* (Dundee) on methane oxidation; G. B. Kistiakowsky and colleagues (Harvard) on methane and acetylene oxidation in shock waves; R. R. Baldwin and colleagues (Hull) on the use of the H_2—O_2 reaction for investigating the reactions between H and O atoms and OH and HO_2 radicals and hydrocarbons. G. Dixon-Lewis, M. M. Sutton and A. Williams (Leeds) reported measurements made in H_2—O_2 flames on the reaction rates of H atoms with O_2, D_2O and CO_2. P. Gray (Leeds) and J. C. J. Thynne (Edinburgh) discussed the kinetics of H atom abstraction by methyl radicals from substances containing the N—H bond, and L. Phillips and R. Shaw (Explosives Research and Development Establishment) discussed the reactions of methyl and methoxy radicals with NO and NO_2. The effect of SO_2 on H_2—O_2 explosion limits was considered by P. Webster and A. D. Walsh (Dundee). Several authors discussed atom and radical recombination: F. S. Larkin and B. A. Thrush (Cambridge), H atoms; K. L. Wray (Avco-Everett Research Laboratories), O atoms; C. P. Fenimore and G. W. Jones (General Electric Research Laboratories), radical recombination and heat release. J. R. Airey, J. C. Polanyi and D. R. Snelling (Toronto) considered hydrogen–halogen chain reactions. Carbon formation in premixed hydrocarbon–oxygen flames at reduced pressure was the subject of a paper by U. Bonne, K. H. Homann and H. Gg. Wagner (Göttingen), in which evidence was presented suggesting a correlation between the formation of poly-acetylenes in the reaction zone and the appearance and growth of soot particles. (Soot formation was also considered by K. S. Narasimhan and P. J. Foster in the General Session.) An interesting paper by A. A. Westenberg and R. M. Fristrom (Johns Hopkins) discussed a promising method of measuring free radical concentrations in flames by electron spin resonance. With probe sampling of flame gases combined with standard electron spin resonance techniques and using O_2 as a reference gas, they obtained absolute O and H atom profiles in hydrocarbon–oxygen flames at reduced pressure.

The second group of papers, concerned with ions, was preceded by a review, "Charged Species", by Dr. T. M. Sugden ('Shell' Research). Several papers in this section discussed the mechanism of chemionization in flames. In contrast to the ninth Symposium, where there appeared to be general agreement about the $CH + O \rightarrow CHO^+$ mechanism, there was no such agreement here. Prof. Kistiakowsky and colleagues (Harvard), on the basis of shock tube experiments, revived an earlier suggestion of Knewstubb and Sugden (later dismissed by them) that

$C_3H_3^+$ was the primary ion, formed by the reaction $CH^* + C_2H_2 \rightarrow C_3H_3^+$, CHO^+ having no importance; while A. Fontijn, W. J. Miller and J. M. Hogan, and H. F. Calcote, S. C. Kurzius and W. J. Miller (Aerochem. Research Lab., Inc.), from flow system and atomic flame experiments, and from a study of low-pressure flames, respectively, suggested that both CHO^+ and $C_3H_3^+$ were primary ions, which could predominate under different conditions.

J. L. Franklin and M. S. B. Munson (Humble Oil Co.) produced data on positive ions in crossed-beam experiments with methane and acetylene and oxygen. K. Schofield and T. M. Sugden (Cambridge) discussed observations on the ionization of alkali and alkaline earth metals and their compounds in hydrogen flames and proposed mechanisms for the formation of the positive ions known to be present. Two papers, by H. F. Calcote, S. C. Kurzius and W. J. Miller (Aerochem. Research Lab. Inc.) and by A. Feugier and A. Van Tiggelen (Louvain), provided data on negative ions in flames, but there appeared to be little agreement between them on the relative importance of the species observed. A. V. Phelps and J. L. Pack (Westinghouse Research Lab.) and M. A. Biondi and co-workers (Pittsburgh) reported results obtained with relatively simple plasmas, which might contribute to the understanding of more complicated flame systems. The former dealt with electron attachment and detachment in O_2 and in O_2—CO_2 and O_2—H_2O mixtures, and the latter with positive ion (Ne_2^+, N_2^+, O_2^+ and NO^+)-electron recombinations.

The discussion "Electrical Properties of Flames" centred round twelve papers. Three were concerned with the measurement of ionization. A. J. Borgers (Utrecht) described the application of the high-frequency resonance method to the measurement of electron concentrations in flames. B. E. L. Travers and H. Williams (Rocket Propulsion Establishment) discussed the use of probes for the measurement of positive ion densities in flames and suggested that double-probe measurements are more reliable, particularly when electron temperatures are required, and G. Wortberg (Aachen) reported some ion concentration measurements on flat, atmospheric pressure flames. G. N. Spokes and B. E. Evans (Stanford Research Institute) considered various factors which may influence the results when ions are sampled from chemical plasmas in mass-spectrometric work. U. W. Balwanz (U.S. Naval Research Lab.) reported on ionization in rocket exhausts. D. L. Turcotte and W. Friedman (Cornell) discussed the interaction between seeded combustion products and cold electrodes, and S. L. Soo and R. C. Dimick (Illinois) that between an ionized gas and solid particles. F. J. Weinberg and co-workers (Imperial College of Science and Technology) read papers on flames augmented with electric discharges and on the effect of electric fields on droplets and particles in an ionized gas. R. M. Davies (Gas Council) described heat transfer measurements in electrically boosted flames. N. I. Yushchenkova and S. I. Kosterin (U.S.S.R.) dealt with ionization in seeded air on supersonic expension; R. Friedman (Atlantic Research Corporation) described a method of producing electron-rich gases from a solid fuel mixture burning to CO, N_2 and Cs.

Twelve papers were grouped under the heading "Flame Chemistry". Several of these were devoted to various problems connected with the experimental determination of the composition of flame gases: K. N. Bascombe (Explosives Research and Development Establishment) described the measurement of H-atom concentrations in fuel-rich, highly diluted H_2—O_2 flames; W. G. Agnew and J. T. Agnew (General Motors), the mass spectrometric determination of composition profiles for two-stage flat diethyl ether–air flames; N. Bradley *et al.* (Liverpool), a mass-spectrometric examination of cool flames of aldehydes. The behaviour of cool flames of hydrocarbons was investigated by B. H. Bonner and C. F. H. Tipper (Liverpool); monopropyl pentaborane flames by W. G. Berl and co-workers (Johns Hopkins); hydrogen azide decomposition flame by P. Laffitte, I. Hajal and J. Combourieu (Paris); the kinetics of H_2—Cl_2 flames by R. Corbeels and K. Scheller (U.S.A.F. Aerospace Research Lab.); the inhibition of CH_4—O_2 flames by CH_3Br by W. E. Wilson, jun. (Johns Hopkins); and the reactions of alkali metals in fuel-lean H_2—O_2—N_2 flames by W. E. Kaskan (General Electric). Two papers, by P. H. Kydd and W. I. Foss (General Electric) and H. C. Hottel and colleagues (Massachusetts Institute of Technology), were concerned with the processes occurring in stirred reactors (the more practical aspects of stirred reactors were considered in the discussion on aerodynamics in combustion), and two with non-thermal emission from flames—H. G. Wolfhard (Institute for Defense Analyses) and collaborators (Thiokol Chem. Corp.) OH radicals in low-pressure flames, and P. J. Th. Zeegers (Utrecht) OH radicals and K atoms.

Of the twelve papers under the heading "Reaction Kinetics", five dealt with various aspects of the H_2—O_2 reaction. T. Asaba, W. C. Gardiner, jun., and R. F. Stubbeman (Texas), and V. V. Voevodsky and R. L. Soloukhin (U.S.S.R.), described shock wave investigations. Problems associated with the induction period were discussed by F. E. Belles and M. R. Lauver (N.A.S.A., Lewis Research Center); ignition kinetics by R. S. Brokaw (N.A.S.A., Lewis Research Center); inhibition of the second limit by HBr and HCl by D. R. Blackmore, G. O'Donnell and R. L. Simmons (Manchester). M. A. A. Clyne (Cambridge) discussed the reactions of the HNO molecule; V. N. Kondratiev (U.S.S.R.), thermal cracking of methane; A. Martinengo, J. Melczer and E. Schlimme (Göttingen), the analysis of stable reaction products from the adiabatic compression of hydrocarbon–air mixtures; J. Hay, J. H. Knox and J. M. C. Turner (Edinburgh), homogeneous and heterogeneous processes in the gas-phase oxidation of isobutane and isobutene. Three contributions in this section were devoted to hydrazine: pyrolysis kinetics, on the basis of a shock tube investigation, were considered by E. T. McHale, B. E. Knox and H. B. Palmer (Pennsylvania State); pyrolysis and oxidation by K. W. Michel and H. Gg. Wagner (Göttingen); and decomposition of hydrazine and its methyl derivatives by I. J. Eberstein and I. Glassman (Princeton).

The "Flame Spectroscopy" section comprised seven papers; two of these were concerned with the determination of the properties of hot gases by spectroscopic means: C. C. Ferriso, C. B. Ludwig and F. P. Boynton (General Dynamics) described an infra-red method of determining the CO_2/H_2O ratio and the flame temperature, and G. J. Penzias (Warner and Swasey Co.) discussed the CO_2 concentration. U. P. Oppenheim and A. Goldman (Israel Institute of Technology) presented data on the infra-red emissivity of water vapour, and S. H. Bauer (Cornell) and J. H. Jaffé (Weizmann Institute, Israel) on the collision broadening of infra-red emission lines of OH. S. Tsuchiya and K. Kuratani (Tokyo) considered the excitation and quenching of Na atoms behind a shock wave. Results of investigations on molecular radiative transfer in non-isothermal gases (such as a rocket exhaust plume) were presented by F. S. Simmons (Michigan). Problems connected with mass spectrometric sampling of flame gases at atmospheric pressure were discussed by T. A. Milne and F. T. Greene (Midwest Research Unit).

The seven papers in the "General" group contained no obvious unifying factor and appeared to be an 'overflow' from the Flame Chemistry and Reactions Kinetics sections.

The thermal ignition theory in autocatalytic reaction was considered by P. G. Ashmore and T. A. B. Wesley (Manchester), ignition of gases at a hot surface under non-steady state conditions by G. Adomeit (Aachen), the explosive decomposition of azomethane by N. Y. Gerri and F. Kaufman (Ballistic Research Laboratories). R. H.

Atalla and K. W. Wohl (Delaware) dealt with the effect of added N_2 on the light emission from butane–oxygen flames. Radiation processes connected with high-pressure H_2—O_2 combustion were considered by M. C. Barrows (NASA, Lewis Research Center), and supersonic firings into H_2—O_2 and H_2–air mixtures by H. Behrens, W. Struth and F. Wecken (Inst. Franco-Allemand). Results on soot formation on introducing CH_4 into burnt flame gases were presented by K. S. Narasimhan and P. J. Foster (Sheffield).

There were twenty-two papers grouped in the discussion "Aerodynamics in Combustion"; eight of these were placed under the heading "Free Burning Fires". The fundamental problems of free-burning fires were discussed by H. W. Emmons (Harvard). The influence of various factors on such fires was considered by a number of authors; the effect of irradiation, humidity and air velocity on the spreading of fire over controlled fuel beds by H. C. Hottel, G. C. Williams and F. Steward (Massachusetts Institute of Technology) and by H. E. Anderson and R. C. Rothermel (U.S. Forest Service), the effect of cross-winds on turbulent diffusion flames of gas by A. A. Putnam (Battelle Memorial Inst.). Some theoretical aspects of buoyant fire plumes were discussed by H. J. Nielson and L. N. Tao and by B. R. Morton (Illinois Institute of Technology); measurements on buoyant diffusion flames were reported by P. H. Thomas, R. Baldwin and A. J. M. Heselden (Fire Research Station, U.K.). C. Sandches Tarifa, P. Perez del Notario and F. Garcia Moreno (Madrid) presented results on the flight paths and lifetimes of burning wood particles.

Related topics were considered in a group of five contributed papers under the heading "Fire Research". Four of these, by S. Martin (U.S. Naval Radiological Defense Lab.), by W. D. Weatherford, jun., and D. M. Sheppard (Southwest-Research Inst.), by P. L. Blackshear, jun., and K. A. Murty (Minnesota) and by E. R. Tinney (Washington State), were concerned with the ignition and burning of cellulosic materials. D. Gross and A. F. Robertson (National Bureau of Standards) considered enclosed fires, and K. Akita and T. Yumoto (Fire Research Inst. of Japan) the combustion of liquid methanol.

The remaining fourteen papers in the discussion on aerodynamics in combustion were largely concerned with engineering problems connected with combustion. Of the four papers on rocket combustion instability, two, by R. W. Hart and F. T. McClure (Johns Hopkins) and by L. Crocco (Princeton), dealt with the theoretical aspects of solid and liquid rocket propellant combustion instability, respectively. The other two, by E. W. Price (U.S. Naval Ordnance Test Station) and by R. S. Levine (Rocketdyne), discussed the experimental data on instability.

The ten papers on low- and high-speed combustion processes were concerned with the problems associated with different types of combustors of industrial interest. A. M. Brown and M. W. Tring (Sheffield) discussed the application of pressure-jet burners to marine boilers, and F. Mauss, E. Perthius and B. Sale (Inst. Français du Petrole) low-frequency oscillations in fuel-oil boilers. Efficiency problems of combustors were investigated by J. M. Beer and K. B. Lee (International Flame Research Foundation), using suitable models. Results of theoretical and experimental examinations of high intensity combustors were presented by W. E. Francis (Gas Council). Oscillatory effect, in tunnel burners were considered by J. K. Kilham, E. G. Jackson and T. J. B. Smith (Leeds). Practical problems connected with gas turbine combustion were discussed by A. H. Lefebvre (College of Aeronautics, U.K.). Combustion rates in confined turbulent flames were investigated by N. M. Howe, jun., and C. W. Shipman (Worcester Polytechnic Inst.), and the burning of propane in a spherical combustor by A. E. Clarke *et al.* (Lucas Gas Turbine Equipment, Ltd.). F. S. Billig (Johns Hopkins) presented results on the supersonic combustion of aluminium alkyl fuels, and M. M. Gibson (Northern Research and Engineering Corp. International) data on flames in supersonic premixed combustible gas streams.

The "Detonation" session consisted of twelve papers. Six were concerned with condensed phase. Various aspects of the subject were considered—detonation limits in composite explosives by W. E. Gordon (Combustion and Explosive Research, Inc.), critical diameter by A. N. Dremin and V. S. Trofimov (U.S.S.R.), pressure measurements during shock initiation by V. M. Boyle, R. L. Jameson and F. E. Allison (Ballistic Research Labs.), detonation front structure by B. G. Craig (Los Alamos), the application of a shock wave approximation to detonation initiation by D. C. Pack and F. J. Warner (Royal College of Science and Technology, Glasgow). B. Hayes (Los Alamos) reported the results of electrical conductivity measurements on reaction zones of explosives and revived the long discarded (at least for flames with no visible soot formation) solid carbon theory to account for his observations. Six papers dealt with gaseous detonations: R. W. Getzinger *et al.* (Berkeley) discussed steady detonations in ozone; D. R. White and G. E. Moore (General Electric), induction zones in H_2—O_2 and CO—O_2 mixtures; P. A. Urtiew, A. J. Laderman and A. R. Oppenheim (Berkeley), the generation of pressure waves by accelerating flames; E. K. Dabora, J. A. Nicholls and R. B. Morrison (Michigan), the influence of a compressible boundary on detonation propagation; J. H. Lee, B. H. K. Lee and T. Shanfield (McGill, Montreal), two-dimensional unconfined detonation waves; M. L. Wilkins, B. Squier and B. Halperin (California University, Livermore), equations of state for selected explosives.

The contributed papers in the "Combustion and Flow" section centred round topics similar to those in the discussion on aerodynamics in combustion, and mainly related to flame instability and turbulence. Stability and oscillations of laminar jet flames were considered by I. Kimura (Tokyo), mechanisms of acoustic effects associated with flames by Tau-Yi Toong *et al.* (Massachusetts Institute of Technology); high-frequency oscillations in a rocket motor by H. Tsuji and T. Takeno (Tokyo), the combustion of droplets in a gas stream undergoing acoustic oscillations by W. C. Strahle (Princeton). Turbulence problems were dealt with by numerous authors: turbulent boundary layers, by G. A. Marxman (United Technology Center) and by C. E. Wooldridge and R. J. Muzzy (United Technology Center); concentration intermittency in jets by H. A. Beckers, H. C. Hottel and G. C. Williams (Massachusetts Institute of Technology); turbulent exchange behind flameholders by G. Winterfeld (D.V.L. Inst. für Luftstrahlentriebe); turbulent flashback by L. N. Khitrin *et al.* (U.S.S.R.). N. H. Pratt and J. E. C. Topps (National Gas Turbine Establishment) described a new experimental method for the investigation of heat release in expanding combustion products, and H. Edmondson and J. E. Garside (Leeds) the lifting of diffusion flames; H. Phillips (Safety in Mines Research Establishment), experiments on buoyant methane flames.

The "Solid Propellant Combustion Fundamentals" section comprised six papers. Three of these discussed perchlorates: G. A. McD. Cummings and R. A. Hall (Rocket Propulsion Establishment), premixed flames of perchloric acid with CH_4, C_2H_6 and CH_3OH; J. Powling and W. A. W. Smith (Explosives Research and Development Establishment), the surface temperature of burning ammonium perchlorate; M. Barrère and L. Nadaud (ONERA, France), the combustion of ammonium perchlorate spheres in a stream of gas. Chemical kinetics of cordite explosions were considered by G. Sotter (Sheffield); emittance of solid metal oxide particles in propellant combustion products by D. J. Carlson (Philco Research Labs.); regression rates of solid fuels and polymer degradation by B. Rabinovitch (United Technology Center).

The Symposium received support from U.S. Army Ballistic Research Laboratories, National Aeronautics and Space Administration, National Science Foundation, National Academy of Sciences—National Research Council Committee on Fire Research, the British Section of the Combustion Institute, and from many industrial firms in Britain, the United States and Canada.

The next symposium will be held in 1966 at Berkeley, California.

E. M. Bulewicz

THERMAL CONDUCTIVITY

THE expanding field of new materials and their use in all branches of technology, particularly those involving transfer of heat, has increased the scope of work on thermal conductivity during the past two decades and created a revival of interest in the subject. The problems are international, and this was apparent during July 15–17 when 150 invited delegates, representing 11 countries, met at the National Physical Laboratory to discuss their work in this subject. For many years the Laboratory has been involved in work on the thermal properties of materials and it was, therefore, a most appropriate venue for such a gathering.

The comprehensive programme of 50 papers contained a majority which were devoted to methods of measurement, supplemented by others which included results for some particularly important materials in solid, liquid or gaseous state. In some of the cases where metals or semiconductors were reported on, the results were accompanied by an analysis which was of value in relating the behaviour of real materials to the theory of solids.

Much attention was directed to the difficulties encountered in the measurement of thermal conductivity over the very wide range of temperature now considered of practical importance. It was also realized that poor experimental work cannot be explained with elaborate hypotheses; measurements to high accuracy are required before any attempt is made to assess materials or investigate theories. Significant advances have been made in methods for all conductivity ranges both for fundamental purposes and for the less accurate but rapid practical requirements.

Some particularly important developments have been made to the original unmatched guard apparatus first proposed by M. J. Laubitz (National Research Council, Ottawa). He has dispensed with the guard tube and now undertakes the measurement of temperature profile along the axis of his two-sectioned centrally heated rod by means of a tightly fitting thermocouple sliding inside an axial hole. These accurate measurements coupled with a reasonably simple mathematical analysis of the system seem capable of yielding results very quickly, since equilibrium conditions are attained rapidly, at temperatures up to 1,000° C. For basic studies of materials at these temperatures this is an important step forward and the possibilities of extending its temperature range by using optical pyrometry were seen immediately.

H. E. Robinson (U.S. National Bureau of Standards) discussed forms of line heat sources that could be used as heaters in apparatus for measurements at lower temperatures on insulating materials in disk and slab form. These new configurations lend themselves more readily to mathematical analysis, they are more simple to use and would appear to be able to yield more accurate results.

R. Berman (Clarendon Laboratory, Oxford), during a session devoted to low temperatures, described a significant new measurement technique whereby thermal conductivities can now be undertaken between 1° and 300° K using a single method involving thermocouples for temperature measurements. These couples are a combination of gold alloyed with 0·02–0·03 per cent iron together with chromel or a semi-conductor as the opposite element. The thermocouple sensitivity over the whole range is never less than 10 μV/°C and temperature differences of 0·001° C can be measured to a few per cent. By this means it has been possible to study solid helium in the body-centred cubic phase which exists only over a temperature range of a few hundredths of a degree. The method is also being applied to measurements on lithium fluoride using different concentrations of the ^{6}Li and ^{7}Li isotopes, in order to obtain a better understanding of the effects of phonon scattering.

In the two sessions devoted to fluids it soon became apparent that there was a division of interests between precise and practical requirements. B. Vodar (Centre National de la Recherche Scientifique, Bellevue) and his collaborators satisfied those delegates interested in the former by describing several new methods together with their results estimated to be accurate to 1 per cent for a series of gases and liquids over a 600° C temperature range and pressures up to 1,000 bars. There is, however, a very great interest in quick and reliable methods capable of yielding the more practical accuracies of 5–10 per cent. The thermal comparator method developed by R. W. Powell (National Physical Laboratory) and others, and the hot-wire method as suggested by D. T. Jamieson (National Engineering Laboratory), are two which should fulfil these needs. The whole subject of thermal conductivity of liquids is overshadowed by the very large discrepancies in the results that are available for a number of liquids in general use. H. Poltz (Physikalisch-Technische Bundesanstalt, Braunschweig), in discussing the effects of radiation in liquids, has found that the effective conductivity is dependent on the optical thickness of a liquid layer. There are measurable contributions due to this mode of heat transfer even at room temperature. It is possible that this radiation effect, if studied further, may well be a significant contribution towards resolving the existing divergencies which cannot as yet be fully explained.

The design and efficient operation of nuclear reactors are greatly dependent on a knowledge of the thermal properties of the materials used. These facts were emphasized in three contributions devoted to materials at present in use and two dealing with those for advanced reactors. Results for post-irradiated uranium, UO_2 during neutron irradiation and *Dragon* fuel cartridge materials were presented by D. Shaw, D. J. Clough and J. Milne (U.K. Atomic Energy Authority), respectively, and were supplemented by McElroy's (Oak Ridge National Laboratory) two reports, one on graphite materials to 1,000° C and the other dealing with UO_2 to 400° C. The borides and nitrides of Ti, Zr, Hf and Nb are now being considered as high-temperature materials of the future, and recent work has shown that they have valuable practical physical properties coupled with unusual electronic and lattice conductivity behaviour which have yet to be fully explained. R. Mendez-Penalosa (University of Madrid) discussed his work on zirconium nitride, and M. Hoch (Cincinnati University) presented results for a collection of these materials up to 1,800° C obtained using an inductive heating method. The evaluation of thermal conductivity required an accurate knowledge of emissivity and specific heat, which in this case were independently measured. This last-mentioned factor served to highlight the controversy that existed during several sessions whereby the relative merits of the measurement of thermal conductivity, as opposed to its evaluation from thermal diffusivity determinations, were elaborated. G. C. Danielson (Iowa State University) has indicated that

there is no doubt that thermal diffusivity can be measured both accurately and quickly. However, the derivation of thermal conductivity from this measured quantity is still dependent on an accurate knowledge of specific heat and density.

Since 1961 there have been three informal conferences restricted mainly to workers concentrated on the North American continent and devoted almost entirely to solid materials. The growth of the work has been sufficient to justify an international meeting which covered the subject more comprehensively. General benefit has resulted from the exchange of information concerning the problems and the anticipation of future requirements. However, it would appear that there are two important matters which workers in the field should settle without delay. The first point relates to the standardization of units and symbols, a case where physicists, engineers and chemists must co-operate before the number of units exceeds the number of methods. Secondly, there is an international requirement for the production and property evaluation of reliable and reproducible reference materials for a very wide range of thermal conductivity and temperature.

Abstracts of all the papers presented are available on application to the Basic Physics Division, National Physical Laboratory.

R. P. Tye

SPACED-SITE OBSERVATIONS OF JUPITER IN 1963

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JUPITER has been monitored at 18 Mc/s for 16 h daily during 133 days in 1963. This was achieved by combining observations from three stations in the United States, in Great Britain and in South Africa. Correlation coefficients between Jupiter activity and various indicators of solar activity have been computed, but these do not indicate any statistically significant cross correlation. The occurrence probability of the Jupiter radiation was found to differ considerably at each site.

It has been suggested by several investigators that the radiation from Jupiter is in some manner related to solar activity, in that periods of Jupiter activity frequently follow periods of solar activity within a few days. This effect was first reported by Warwick[1] and has also been observed by Carr *et al.*[2], by Douglas[3], and by Barrow[4]. There is, however, no general agreement as to an average delay time between periods of solar activity and possible related periods of Jupiter activity, estimates of from two to ten days having been proposed by the various investigators.

Roberts[5], using data obtained in 1960 by Carr *et al.*[2], computed the correlation coefficient between Jupiter activity and geomagnetic activity index A_p. A peak corresponding to a delay of eight days was indicated, but at the same time another peak of comparable significance indicated a negative delay of eight days, that is, for geomagnetic activity after Jupiter activity. Roberts mentions similarly inconclusive unpublished results obtained by Higgins and concludes that all the apparent correlations observed so far may be merely statistical fluctuations.

If the phenomena are in fact related, there are two possible explanations of these different results. Either the statistics are unsound, due to the fact that insufficient Jupiter observing time was available daily for comparison with solar activity, or else the relationship between the two phenomena is too complex to be expressed by a simple cause-and-effect explanation. A preliminary investigation of the former possibility was conducted during 1963 by Florida State University Radio Observatory in collaboration with observatories in Great Britain and in South Africa.

The experiment was simply to record the radiation from Jupiter for as long as possible each day and to compare the periods of Jupiter activity with periods of solar activity as indicated by flares, 10-cm solar radiation, sunspots, and geomagnetic activity. The Sun can be observed from somewhere on the Earth almost 24 h daily, whereas most Jupiter observers are limited to some 4–8 h according to their aerial systems. The daily period of observation of Jupiter was, therefore, increased to some extent by combining observations made at two additional sites, in Great Britain and in South Africa, with results obtained at Tallahassee. The locations of the sites, the corresponding altitudes of Jupiter at meridian transit and the aerials used are shown in Table 1. The combined observations from the three sites operating in tandem amounted to some 16 h continuous monitoring each night, a period corresponding to about 1½ rotations of Jupiter.

Table 1. Locations of the Three Stations monitoring Jupiter during the 1963 Apparition

Site	Latitude	Longitude	Altitude of Jupiter at transit	Aerial
Tallahassee	30° 27′ N.	84° 18′ W.	64° S.	Square corner reflector
St. Osyth	51° 47′ N.	01° 02′ E.	42° S.	Square corner reflector
Grahamstown	33° 19′ S.	26° 26′ E.	53° N.	Single dipole and eight dipole array

The aerial-receiver-recorder systems were identical at Tallahassee and at St. Osyth. Each consisted of a square corner reflector containing a single folded half-wave dipole fed to a commercial short-wave communications receiver. The bandwidth of the receiver was about 5 kc/s and the recorder constant was 0·25 sec. All the observations at these two sites were monitored following the procedure given recently by Barrow[6].

At Rhodes University a single folded dipole was used in conjunction with a broadside array of eight dipoles at another site some 100 miles away. The receiver bandwidth was 8 kc/s and the recorder constant was 0·8 sec. Some of the observations were monitored, but identification was also based on a comparison of the records taken at the two sites. The total observing time amounted to about 70 per cent of that at Tallahassee or at St. Osyth.

The first co-ordinated period of observation by all three sites was July 17–November 25, 1963. The combined observations are shown in a histogram in Fig. 1. The histogram has been compiled in terms of 'numbers of events' rather than 'occurrence probability', as the occurrence probability was found to be different at each site.

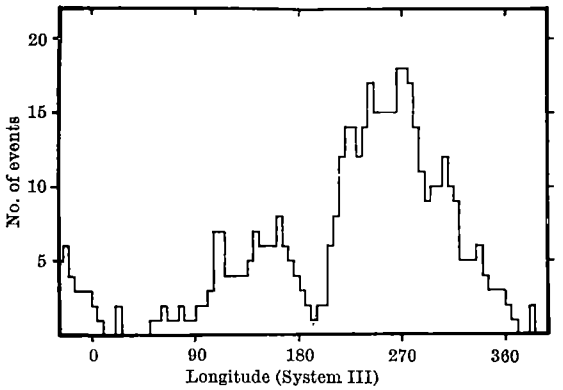


Fig. 1. Histogram showing the total number of Jupiter events recorded at 18 Mc/s by the three stations at Tallahassee, St. Osyth, and Grahamstown during 1963

Following Roberts[5] a preliminary examination of the data has been made by computing cross-correlation coefficients between the days on which Jupiter was active and the days on which the Sun was active, as evidenced by the following quantities: geomagnetic activity index A_p; Zurich relative sunspot number R_z; 10-cm solar radiation flux; solar flare activity, *HAO* index. The computed correlation coefficients are shown in Fig. 2. Although one or two peaks occur slightly above the 1 per cent significance level these can scarcely be regarded as any more than statistical fluctuation. Thus it appears that increased daily observation of Jupiter does not, by itself, produce any significant improvement in a search for simple cross correlation. This result does not, however, exclude the possibility of any relationship at all between Jupiter activity and solar activity as there are various factors which might obscure simple cross correlation. For example, if Jupiter activity is initiated by solar corpuscles, as Carr *et al.*[2] have suggested, both the magnitude and the direction of the velocity of the corpuscles would have a bearing on the situation. Particles travelling with different velocities on different days would give rise to different delay times and a direct search for cross correlation would be useless.

An interesting feature of the experiment is the pronounced difference in Jupiter activity recorded at each site. The day-by-day frequency of occurrence was found to be considerably higher at Tallahassee than at St. Osyth and considerably higher at Grahamstown than at Tallahassee. Such differences have also been reported by Carr[7] for observations made at Gainesville, Florida, at Santiago, Chile, and at Sydney, Australia. Effects such as these, as well as the spaced-site burst studies of A. G. Smith *et al.*[8] and Douglas and H. J. Smith[9], further emphasize the need for a comprehensive study of the effects of the Earth's ionosphere and magnetic field on the Jupiter radiation. Towards this end an experiment is to be conducted by Florida State University Radio Observatory during the 1964 apparition. Standard 18-Mc/s systems have been

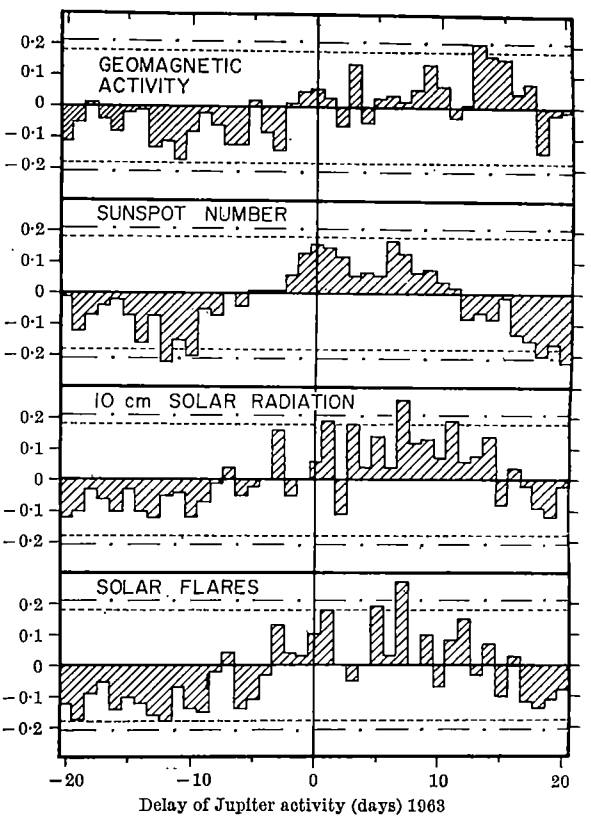


Fig. 2. Computed correlation coefficients between Jupiter activity and geomagnetic activity, sunspot number, 10 cm solar radiation, and solar flares. The significance levels (not allowing for serial correlation in the data) are 0·18 and 0·21 for 5 and 1 per cent respectively

installed in Nigeria, Spain, England and Norway along a rough north–south line through St. Osyth. Collaboration with the Rhodes University group is to continue and simultaneous observations will be made from all these sites. The occurrence probability and burst structure at each site will be compared.

This work was supported by U.S. National Aeronautics and Space Administration grant *NSG*–224–61, and, in South Africa, by the Council for Scientific and Industrial Research.

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ISOTOPIC AGES OF ASSYNT DYKES

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THE so-called 'Scourie' dykes which cut the Precambrian gneisses of the north-west Highlands of Scotland have assumed considerable importance in recent years on account of their usefulness in distinguishing between the effects of the early ('Scourian') and late ('Laxfordian') Lewisian metamorphisms in that area[1]. The initial concept of Sutton and Watson[1], that the abundant dolerite dykes were intruded during a single phase of uplift of the basement rocks between the two metamorphisms, has been modified to a varying degree

according to the more detailed work and interpretations of later workers. Thus O'Hara[2,3] and Tarney[4] have argued that the dykes were intruded at considerable depth into hot country rock. Bowes[5], Park[6], and Wright[7] have maintained that the dykes are not all of the same age, and that some may be contemporaneous with, or later than, the Laxfordian metamorphic event. In the Outer Hebrides, Dearnley[8] has suggested that a second granulite-facies event affected the dykes before the Laxfordian, while Evans[9–11] and Tarney[4,12] have cited evidence that in the Assynt district amphibolite-facies metamorphism occurred before and pene-contemporaneously with the intrusion of the dykes. Evans[10] has proposed the name 'Inverian' for this metamorphic event. In addition, O'Hara[3] and Tarney[4] have emphasized that while the typical 'Scourie' dolerites (tholeiites) are the dominant dyke type in the Lewisian, a variety of other dyke-types also occur.

It is largely the importance of the dykes in the elucidation of the metamorphic and structural history of the Lewisian, and the controversy surrounding their relations to the proposed metamorphic events, that have initiated this study of their isotopic ages.

Giletti *et al.*[13] made considerable progress towards an absolute chronology of Lewisian events when they established that almost 800 m.y. separated the two series of pegmatites (at about 2,400 m.y. and about 1,600 m.y.) assigned respectively to the Scourian and Laxfordian metamorphisms by Sutton and Watson[1]. They were unable at that time to date the dykes. Evans[9], summarizing his results from the Lochinver area, showed that the Scourian gneisses were older than 2,600 m.y., while the biotite pegmatites previously thought to be Scourian did in fact give ages about 350 m.y. later at about 2,250 m.y. He also established that amphibolite-facies gneisses which had been affected by major folding on west-north-west—east-south-east axes, and which were cut by the dykes, gave ages within the range 2,200–1,900 m.y.

The apparent potassium/argon and rubidium/strontium ages of the dyke samples studied are given in Table 1. An attempt has been made to date most of the dyke types listed by O'Hara[3] and Tarney[4] in order to establish, if possible, the period over which the dykes were intruded, and in particular to decide whether or not any of the fresh dykes are Laxfordian in age.

Potassium was determined using an EEL flame photometer coupled to an automatic strip recorder. An addition–dilution method[10] was used to correct for suppression of the potassium flame by other ions. A series of gravimetrically prepared standards were used in such a way that the difference between the unknown solution and the two standard solutions was always less than 1 p.p.m., thus avoiding the difficulty of non-linear response. Argon was determined by isotope dilution. After baking the sample overnight in vacuum an argon-38 tracer was added and the sample melted with a radio-frequency induction heater. The ratio of radiogenic argon-40 to tracer argon-38 was measured in a high vacuum mass spectrometer. The experimental error in measuring the potassium/argon ratio was small enough to give an error of less than ±50 m.y. on the ages quoted. An exception was 39*D* biotite with an error of ±88 m.y. The decay constants for potassium-40 were taken as $\lambda_e = 0{\cdot}584 \times 10^{-10}\text{yr.}^{-1}$, and $\lambda_\beta = 4{\cdot}72 \times 10^{-10}\text{yr.}^{-1}$.

The analytical techniques used for rubidium and strontium determinations were essentially those used by

Table 1. Isotopic Ages of Dykes from the Assynt and Scourie Areas, Sutherland

Potassium-Argon Apparent Ages

Spec. No.	Grid ref.	Locality	Sample	State of sample	Country gneiss	K^+(%)	^{40}Ar (c.c./g.) ($\times 10^4$)	Age (m.y.)
'Scourie' Dolerites and Epidiorites								
E. 520	*NC*085200	S.E. of Badnaban	W.R.	Fresh	Mainly pyx-gn.	0·327	1·660	3,860
E. 520	085200		W.R.	Fresh	Mainly pyx-gn.	0·327	1·611	3,800
L. 282	147259	N.W. of Little Assynt	W.R.	Fresh	Hornb-gneiss	0·289	0·713	2,740
L. 613	152254	N.W. of Little Assynt	W.R.	Fresh	Hornb-gneiss	0·400	0·483	1,800
L. 327	165255	N.E. of Little Assynt	W.R.	Amphibolized	Hornb-gneiss	0·361	0·295	1,390
E. 39D	087228	N.W. of Lochinver	Imp. biot.	Amphibolized	Hornb-gneiss	3·45	3·59	1,640
E. 49D	096232	N. of Lochinver	W.R. (−200)	Amphibolized	Hornb-gneiss	0·351	0·331	1,530
E. 49D	096232		W.R. (+200)			0·393	0·357	1,490
L. 630	146419	W. of Badcall	W.R.	Fresh	Mainly pyx-gn.	0·257	0·465	2,300
L. 631	146419	W. of Badcall	W.R.	Amphibolized	Mainly pyx-gn.	0·259	0·288	1,460
L. 635	150456	N.W. of Scourie	W.R.	Fresh	Mainly pyx-gn.	0·789	0·755	1,545
Bronzite-picrite Dyke								
E. 605	121238	Brackloch Farm	W.R.	Fairly fresh	Hornb-gneiss	0·396	0·642	2,160
E. 605			Biotite			7·32	8·76	1,790
Olivine-gabbro Dykes								
L. 543	136147	Loch Sionascaig	W.R.	Fresh	Pyrox-gneiss	0·565	0·881	2,110
E. 496	081206	Badnaban	W.R.	Fairly fresh	Mainly pyx-gn.	0·346	0·388	1,720
Two-pyroxene Dolerites (or Norites[3])								
L. 639	232229	W. of Inchnadamph	W.R.	Fresh centre	Hornb-gneiss	0·613	1·018	2,200
L. 640	232229	W. of Inchnadamph	W.R.	Fresh margin	Hornb-gneiss	0·610	0·845	1,970
L. 629	146418	W. of Badcall	W.R.	Fresh centre	Mainly pyx-gn.	0·601	0·720	1,860
L. 622	147417	W. of Badcall	W.R.	Amphb. centre	Mainly pyx-gn.	0·629	0·547	1,440
Amphibolite Dyke, Badcall[3]								
L. 623	147417	W. of Badcall	W.R.	Amphibolic	Mainly pyx-gn.	0·206	0·313	2,080
Thin Tholeiitic Dyke, Loch Assynt								
L. 604	152253	N.W. of Little Assynt	W.R.	Fresh centre	Hornb-gneiss	0·532	0·724	1,950
Thin Alkali-basalt Dyke, Loch Assynt								
L. 605	166259	L. Torr an Lochain	W.R.	Fresh centre	Hornb-gneiss	0·719	1·055	2,030
L. 258	166259	L. Torr an Lochain	W.R.	Fresh margin	Hornb-gneiss	0·849	1·431	2,200
Basic Gneiss near Alkali-basalt Dyke								
L. 257	166259	L. Torr an Lochain	W.R.	Ga-Hb-gneiss		0·408	0·678	2,200

Rubidium-Strontium Ages

Spec. No.	Grid Ref.	Sample	Rb (p.p.m.)	Sr (p.p.m.)	$\left(\frac{^{87}Sr}{^{86}Sr}\right)$ * now	$\left(\frac{^{87}Sr}{^{86}Sr}\right)$ initial	Age (m.y.)
Bronzite-picrite Dyke, Brackloch Farm							
E. 605	*NC*121238	Biotite	345	21·9	2·196	0·709	2,190 ± 50
E. 605		W.R.	15·7	50·9	0·739	0·709	2,190 ± 200

This gives a biotite/whole-rock intersection age of 2,190 ± 50 m.y.

* Normalized to an $^{86}Sr/^{88}Sr$ ratio of 0·1194.

Giletti *et al.*[13], but differed in two respects: (1) a pre-column separation of rubidium and strontium was carried out with ethyl acetate; (2) an additional pre-spectrometer clean-up of strontium samples was accomplished by mounting the filaments in specially prepared vacuum systems and passing a current of about 2 amp through them for about 2–3 h prior to mounting in the mass spectrometer. This procedure ensured complete freedom from rubidium and also enhanced strontium emission. The half-life of rubidium-87 was taken as $4{\cdot}7 \times 10^{10}$yr.

The concordant rubidium/strontium and potassium/argon ages for the bronzite-picrite dyke at Brackloch Farm, near Lochinver, constitute a convincing datum at 2,200 m.y. It is interesting to note that the margins of this dyke are rather badly amphibolized and that much of the olivine in the sample is serpentinized. The potassium/argon Laxfordian age given by the biotite indicates expulsion of the accumulated radiogenic argon at this time, although the rock as a whole remained a closed system. The serpentinized nature of the olivine and saussuritized character of the plagioclase suggests that this radiogenic argon has probably been retained in the pyroxenes.

The abundant thick and coarse-grained dolerites and metadolerites ('epidiorites'), those usually referred to as the 'Scourie' dolerites, were intruded before the picrites and later than the 2,250 m.y. pegmatites; their true age must also be about 2,200 m.y. However, the spread of whole rock potassium/argon apparent ages for the freshest representatives of these dykes, between 3,860 and 1,550 m.y., is clearly well outside the limits of experimental or sampling error, and indicates on one hand accumulation of an excess of argon in these dykes in addition to that produced since their intrusion by normal decay of potassium-40, and on the other hand expulsion of radiogenic argon from apparently fresh rock by the reheating or tectonic activity of the Laxfordian.

The presence of excess argon in these ancient dyke rocks is an interesting feature which requires consideration. Damon and Kulp[14] have noted that excess argon (and helium) may occur in beryl, tourmaline, etc., in pegmatites formed early in the Precambrian, and the problem has also been discussed by Hart[15] and by Kulp[16]. In the present instance, two hypotheses can be invoked to account for it: (1) A fundamental assumption of the potassium/argon dating method applied to igneous rocks is that any argon originally present in the magma along with other dissolved gases is expelled at the time of intrusion. This assumption may not be valid under conditions of intrusion at considerable depth with impervious wall rocks. Hence the excess argon-40 present in these dykes may have originated in the mantle, and may have been incorporated in the pyroxenes, etc., during crystallization. (2) If the dykes were intruded under waning metamorphic conditions as suggested by Tarney[4], or indeed by any reasonable interpretation of the age data, then, being intruded into an area of high argon flux, it is not unlikely that radiogenic argon being expelled from the gneisses could find its way into the fresh dyke centres, and may now perhaps be located in the dyke pyroxenes. Certainly it would be no more difficult for argon to diffuse into the dykes than to migrate through several kilometres of country rock.

The relative merits of these two hypotheses will be outlined in a later contribution. Sufficient to state here that on either hypothesis it is necessary to invoke dyke intrusion at considerable depth. Two other interesting suggestions can be made on the basis of these age data, however. All the dykes found to contain excess argon have, like others, substantial proportions of green hornblende and bear a plagioclase having a strong greyish clouding. The hornblende in these dykes has been attributed to the initial stages of Laxfordian metamorphism by Watson[1], and to autometamorphism by O'Hara[2], while Dearnley[8] has argued that the clouding has resulted from the effects of Early Laxfordian granulite-facies metamorphism. The dating clearly favours an interpretation regarding both hornblende and clouding either as primary features of crystallization, or as resulting from metamorphism very shortly afterwards.

Those dolerite samples which are completely amphibolized came from areas affected by Laxfordian movements. The spread of ages, if real, indicates continuing Laxfordian activity over the period 1,640–1,390 m.y., though it is apparent that movements continued even later in the Torridon and Gairloch areas[13,17].

The other dykes can be dealt with more briefly. The olivine-gabbro dykes are probably related to the picrites, and seem to be, within experimental error, of roughly the same age. That at Badnaban (the 'biotite-diorite') has been affected by Laxfordian shearing along its northern margin and has been up-dated accordingly.

It is difficult from the data available to give a complete assessment of the ages of the amphibolite and norite dykes at Badcall, near Scourie[3]. Allowing for the strong, but localized, Laxfordian shearing in this locality, it is not unlikely that both dykes have in fact been slightly up-dated by these shears. The norite is mineralogically and chemically similar to the two-pyroxene dolerite from the south side of Loch Assynt[4], dated at 2,200 m.y.

There is little direct field evidence in Assynt that any of the dykes were intruded after the Laxfordian shearing movements, though it has been the experience of both authors that shear structures simulating those of the Laxfordian were in existence in Assynt before dykes now known to be 2,200 m.y. old. However, thin basic veins are seen to cut the picrites and early dolerites in the area west of Loch Assynt. In consequence the two thin dykes noted by Tarney[4] just west of Loch Assynt, which are noteworthy in being extremely fresh compared with other dykes in their vicinity, have been sampled as dykes more likely than most to be Laxfordian in age. Both are intruded into amphibolite-facies gneisses, those gneisses in the vicinity of the dated alkali basalt sample giving an age of 2,200 m.y. The apparent ages of 2,030 m.y. for the alkali basalt dyke and 1,950 m.y. for the tholeiitic dyke may well be real considering the fresh state of the samples, though the fresh chilled margin of the alkali basalt gives a 2,200 m.y. age. Clearly they are not Laxfordian in age.

Treated as a whole (Fig. 1) the dyke ages show a distinct concentration at 2,200 m.y., with a steady fall-off towards the Laxfordian. The ages older than 2,200 m.y. are distinctly wild.

To conclude, the results suggest that (in Assynt) the majority of the 'Scourie' dolerites were intruded shortly before 2,190 m.y. ago, while emplacement of other dyke

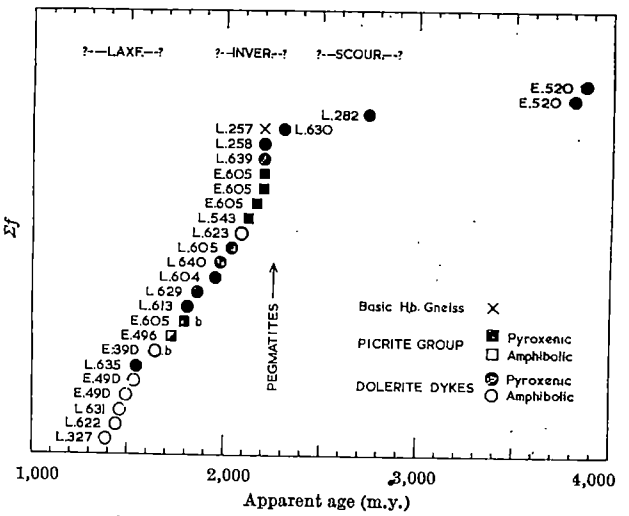


Fig. 1. Frequency distribution of dyke ages

types followed over a period which may have extended to as late as 1,950 m.y. No dykes of Laxfordian age appear to be present, though dykes of this age have been recognized in the Canadian Shield[18,19]. The close association in time between pegmatites, dykes, and amphibolization of the gneisses indicated by the dating, and the presence of excess argon in the earliest dykes, suggestive of intrusion of the dykes of considerable depth, and into a metamorphic environment. The problem of excess argon is an important one which may well occur, without realization, in other metamorphic terrains. There is clearly scope for further investigation here. The data on the early dolerites alone show how a single age determination in such an area may well be in error by as much as 1,000 m.y., and that even with a large number of determinations knowledge of the field relations is still of paramount importance in their interpretation.

One of us (C. R. E.) acknowledges an Imperial Oil Graduate research fellowship; the other (J. T.) a Department of Scientific and Industrial Research maintenance award while at the University of Newcastle.

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EFFECT OF CALCIUM ON INTRACELLULAR SODIUM AND POTASSIUM CONCENTRATIONS IN PLANT AND ANIMAL CELLS

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IT has been reported that mammalian brain[1,2], liver[3–5] and muscle[6,7] require calcium in the medium for maintenance of high intracellular potassium concentrations when examined under *in vitro* conditions. This is also true of red blood cells in lower vertebrates[8,9] and for human red blood cells under special conditions[10]; however, a calcium requirement for maintenance of high intracellular potassium has been looked for but not observed in frog and chicken red blood cells[8]. This article presents evidence that calcium is essential in maintaining both high intracellular potassium and low intracellular sodium concentrations in several different plant and animal systems, and suggests an explanation as to why this calcium requirement has not been demonstrated in all cell systems previously investigated.

The intracellular sodium and potassium concentrations of mouse pancreas, *R. pipiens* oocytes, HeLa cells, ascites tumour cells, yeast and *E. coli* were compared after incubation in medium containing calcium and in calcium-free EDTA medium. The systems were prepared as follows and incubated under the conditions outlined in Table 1.

Mouse pancreas was excised and each pancreas incubated in 3 ml. of Krebs III medium at 37° as described previously[11].

R. pipiens oocytes were excised from the ovary of a pithed animal and the epithelial membranes were removed with fine forceps. Fifty oocytes were pooled for each sample and incubated in amphibian Ringer's solution at 25°.

HeLa cells, strain *S*-3, were maintained as monodisperse cultures in Eagle's spinner medium[12] supplemented with 6 per cent fœtal calf serum and with equimolar NaCl substituted for the prescribed Na_2HPO_4. Cell density was kept at approximately 2×10^5 cells per ml.

Table 1. INCUBATION CONDITIONS FOR PLANT AND ANIMAL CELLS

Cell or tissue	Incubation medium*	*p*H	Time (min)	°C
Pancreas	Krebs III medium	7·0	15	37
Oocytes (frog)	Amphibian Ringer's	7·4	15	25
HeLa	Modified salt soln.†	7·0	15	37
Ascites	Modified salt soln.†	7·5	15	37
Yeast (baker's)	Salt solution‡	7·0	15	37
E. coli	Grey and Tatum's	7·0	15	37

* Magnesium ion omitted from all media.
† Modified salt solution contained per litre: NaCl, 144 mM; KCl, 5 mM; Na_2HPO_4, 0·08 mM; *tris* buffer, 30 mM; glucose, 5·6 mM.
‡ Salt solution for yeast contained per litre: NaCl, 40 mM; KCl, 40 mM; *tris* buffer, 50 mM; glucose, 5·6 mM.

Ascites tumour cells were obtained by aspiration from the peritoneal cavities of Swiss mice carrying cell populations which ranged in age from 4 to 8 days. The cell suspension was washed several times in a modified salt solution (Table 1).

E. coli were grown at 37° in Grey and Tatum's minimal medium[13] to the end of the log phase (10^8–10^9 cells/ml.).

Fresh baker's yeast (*Saccharomyces cerevisiae*) was thoroughly washed with distilled water and resuspended in the saline medium described in Table 1. Aeration and mixing were obtained by bubbling air through the cell suspension.

The calcium concentrations used in the incubation media for ascites, HeLa, oocytes and pancreas were those normally contained in the media. The calcium concentrations in the yeast and *E. coli* incubation media were arbitrarily adjusted to 1·0 mM/l. All calcium-free media contained 1·0 mM EDTA/l. Unless otherwise noted, magnesium ion was omitted from all media.

The monodisperse cell systems were studied in calcium-containing and calcium-free EDTA medium as follows: cells were collected by centrifugation and the supernatant fluid was discarded. One to two hundred milligrams of cells were taken for each sample. The cell sample was rinsed with the incubation medium, resuspended in 50 ml. of the same medium, and incubated at 37°. After 15 min the cells were again collected by centrifugation, transferred with about 0·7 ml. of the medium to hæmatocrit tubes, and packed at high centrifugal force. The supernatant fluid was removed, and the wet and dry weight of the cells determined. Ascites and HeLa cells were collected at 800*g* for 2 min and packed in hæmatocrit tubes at 6,000*g* for 1 min. Yeast and *E. coli* were collected at 20,000*g* for 5 min and packed in hæmatocrit tubes at 30,000*g* for 3 min. The volume of trapped extracellular fluid was measured by adding ^{14}C-inulin at the end of the incubation period. The packed cells were digested with 2–3 volumes of fuming nitric acid for 3 h at room temperature. The digests were diluted 1 : 100 with ion-free water and aliquots were either analysed for sodium and potassium or plated and counted in a windowless gas flow counter. Sodium and potassium were determined by internal standard flame photometry (Baird-Atomic *DB*-4).

Table 2. Effect of Extracellular Calcium on Sodium and Potassium Distribution between the Cell and its Environment

Cell or tissue	$[Ca]_o$	$[Na]_i$*	$[Na]_o$	$\frac{[Na]_o}{[Na]_i}$	$[K]_i$*	$[K]_o$	$\frac{[K]_i}{[K]_o}$	Sum $[Na]_i+[K]_i$
Pancreas	2·5	71	140	2·0	108	6	18·0	179
	0	149	140	0·94	60	6	10·0	209
Oocytes (frog)	1·1	69	114	1·6	108	2	54·0	177
	0	94	114	1·2	87	2	44·0	181
HeLa	0·65	31	144	4·6	138	5	28·1	169
	0	81	144	1·8	99	5	20·0	180
Ascites	0·65	25	144	5·8	137	5	27·4	162
	0	64	144	2·2	97	5	19·0	161
Yeast (baker's)	1·0	<1	40	> 40·0	407	40	10·2	407
	0	28	40	1·4	213	40	5·4	241
E. coli	1·0	33	27	0·8	226	36	6·3	259
	0	47	27	0·6	110	36	3·0	157

*Average values for 4 or more experiments; standard error ±4 mM or less for $[Na]_i$, ±5 mM or less for $[K]_i$.

The sodium and potassium analyses were corrected for trapped extracellular fluid and expressed in mM/l. cell water.

The intracellular sodium and potassium concentrations found in each of the six cell systems after an incubation for 15 min in calcium-containing and calcium-free EDTA medium are given in Table 2. The results show that in the presence of extracellular calcium all cell systems maintain a potassium gradient. The values for the ratio of $[K]_i$ to $[K]_o$ shown in col. 8 ranged from 6·3 for *E. coli* to more than 50 for *R. pipiens* oocytes. It has been shown by Abelson and Duryee[14], and more recently by Naora *et al.*[15], that 85 per cent of the intracellular sodium of the *R. pipiens* oocyte does not exchange with sodium in the medium. Correcting the intracellular sodium values shown in Table 2 for non-exchangeable or bound sodium, one obtains an exchangeable sodium concentration of about 10 mM/l. cell water in the oocyte. With the exception of *E. coli*, the intracellular sodium concentrations were lower than that of the extracellular fluid. The ratio of $[Na]_o$ to $[Na]_i$ given for *E. coli* in col. 5 is 0·8. This implies either: (1) a concentrating mechanism for both sodium and potassium, or (2) as in oocytes, that part of the intracellular sodium in *E. coli* does not readily exchange with the medium. One cell system, yeast, had no measureable intracellular sodium when incubated in a sodium and potassium salt solution containing 1·0 mM of calcium/l.

In calcium-free EDTA medium all cell systems gained sodium and lost potassium. These changes in cell sodium and potassium occurred within 5 min and the new values were relatively stable for at least 1 h. The one cell system, *E. coli*, in which initial intracellular sodium was higher than extracellular sodium in calcium-containing medium, further increased intracellular sodium in calcium-free medium. This suggests the exchange of sodium for calcium on anionic sites in the bacterial cell.

Under the conditions shown in Table 2, the gain in intracellular sodium and loss of intracellular potassium were readily reversed by the transfer of the cells to calcium-containing medium. Magnesium ion did not prevent the loss of potassium and gain of sodium when substituted for calcium in equimolar quantities.

In the two plant systems, yeast and *E. coli*, the sum $[Na]_i+[K]_i$ decreased by one-half on incubation in calcium-free medium. In the four vertebrate cell systems the sum $[Na]_i+[K]_i$ either increased slightly (pancreas and HeLa) or did not change (oocytes and ascites). The difference was due mainly to a greater percentage loss of total potassium in the plant cells, and may indicate that the potassium concentrating mechanism is less efficient in plant cells in general.

These investigations imply that extracellular calcium ion is of universal importance in maintaining low intracellular sodium and high intracellular potassium in living systems. It thus becomes necessary to explain reports where this role has not been observed. It may be that these results were due to uncontrolled conditions of *p*H, since we have found that when cells are incubated in calcium-free medium intracellular sodium and potassium vary with changes in extracellular *p*H. In HeLa cells, for example, intracellular sodium was low and potassium high at *p*H 5·0 in calcium-free medium, but sodium increased and potassium decreased with increasing *p*H and approached the extra values at *p*H 9·0. This *p*H effect is being examined and compared in the different cell systems.

This work was supported by U.S. Public Health Service grants *GM*–10757 and *GM*–11558 from the National Institute of General Medical Sciences. One of us (H. R. K.) was a post-doctoral trainee on physiology training grant 5T1–*GM*–395 from the National Institute of General Medical Sciences.

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INFLUENCE OF CHEMICAL COMBINATION ON ALUMINIUM *K* DIAGRAM AND NON-DIAGRAM LINES

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RECENTLY it was shown that the wave-length of aluminium $K\alpha$ is slightly influenced by the co-ordination number (number of nearest neighbours) of the site occupied by aluminium[1]. It has also been noted that the aluminium *K* spectrum is markedly affected by a change in valence of the aluminium, especially in the α satellite lines and the $K\beta$ band[2,3]. In that work[2,3], results were shown only for aluminium and aluminium oxide. This communication reports on the aluminium *K* spectrum from a number of aluminium compounds including intermetallic conductors, semiconductors, and insulators.

The vacuum spectrometer and associated equipment were specially designed and built by Picker X-Ray Corporation[4]. X-rays are produced by bombarding the

sample with electrons from a tungsten filament electron gun which is controlled by a Picker generator modified to operate at low voltages. The voltage was held at 6 kV and currents of 1–2 m.amp were used for aluminium K excitation. The analysing crystal was ethylenediamine ditartrate having a $2d$ spacing of 8·8030 Å. This crystal was chosen because it allows measurements to be made fairly far back in the back reflexion region (129·41° 2θ for $K\beta$ to 142·64° 2θ for $K\alpha_{1,2}$ using aluminium metal). The detector was a thin window flow proportional counter of the Siemens type using an argon methane (P–10) flow gas. Recording electronics including a pulse height analyser were standard Picker equipment except for a low noise pre-amplifier by Tennelec. A sample chamber containing four samples was used so that samples could be compared under the same conditions without breaking vacuum between samples. Usual operating vacuum was 1×10^{-5} torr. As a check for oxidation, the satellite line ratio α_4/α_3 was measured immediately on each sample. This ratio was then remeasured at the conclusion of all measurements. If the ratio changed, then earlier measurements were not used. As noted by Nordfors[2], this method is a very sensitive test for oxidation in aluminium. Intermetallic compounds were prepared from metal powders of commercial purity using induction heating.

The lines of the aluminium K spectrum studied in this work were the diagram lines $\alpha_{1,2}$ and β and the non-diagram or satellite lines α', α_3, α_4, α_5 and α_6. The $K\alpha$ doublet is the line least influenced by chemical combination and change in valence. However, since it is the most intense line in the spectrum, previous workers[1,5] have used this line with secondary excitation in determining effects of change of co-ordination number. Our results using primary excitation do not agree well with the referenced work, but the disagreement in $K\alpha$ can probably be attributed to two things. One is that because of low intensities in the secondary excitation technique, a fairly low resolution system was used which perhaps allowed α', α_3 and α_4 to influence the peak position of the parent $K\alpha$ doublet. Since these lines shift more in going from the metal to the oxide, for instance, than $K\alpha$ they would cause the $K\alpha$ line to appear to have shifted more than it actually had. The other obvious reason is that where co-ordination depends on water, as in many minerals, the electron beam probably causes dehydration and therefore a change in co-ordination. This effect is reflected in this present work by kaolinite the spectrum of which is very similar to Al_2O_3.

The satellite lines $K\alpha_3$ and $K\alpha_4$ are greatly affected by the chemical combination of aluminium. The lines shift two to three times as much as the parent doublet,

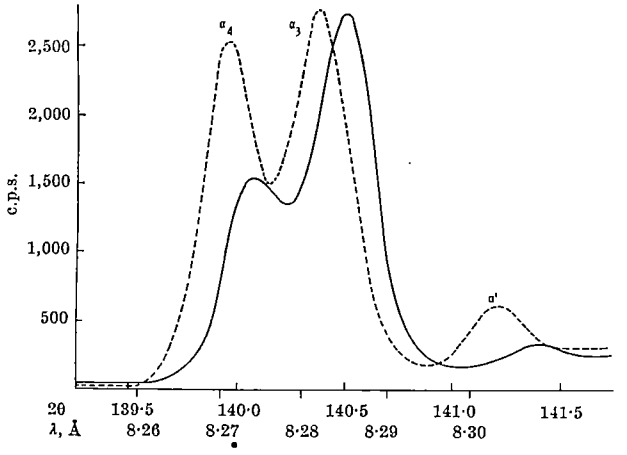


Fig. 1. Aluminium K satellite lines α', α_3 and α_4 for aluminium metal and Al_2O_3 (anodized coating), EDDT analysing crystal
——, Metal; – – –, oxide

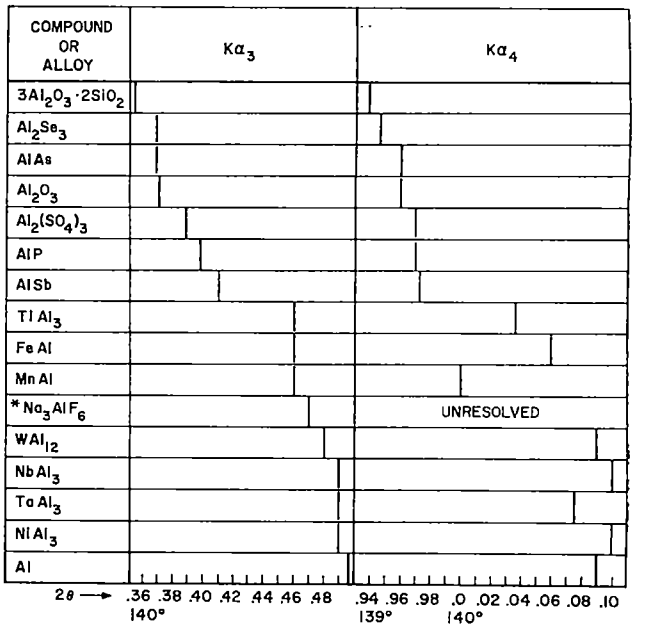


Fig. 2. 2θ positions of aluminium $K\alpha_3$ and $K\alpha_4$ using an EDDT analysing crystal

and the α_4/α_3 ratio changes from 0·50 in the metal to 0·94 in the oxide and 1·00 in mullite. Fig. 1 shows traces of $K\alpha'$, $K\alpha_3$ and $K\alpha_4$ for aluminium and Al_2O_3. The intensities shown in these traces are not relative between oxide and metal. They have been normalized to show α_3 at the same count rate for better comparison of spectra.

The 2θ positions of $K\alpha_3$ and $K\alpha_4$ using EDDT are shown in Fig. 2 for a number of conductors, semiconductors, and insulators. As can be seen, both lines shift to shorter wave-length in going from metallic species to insulator species. Cryolite (Na_3AlF_6), for which only $K\alpha_3$ is shown, apparently is decomposed in the beam, and appears among the metallic species, rather than with the insulators. No correlation can be made here between line position and co-ordination number. Exactly the same relative position is not occupied by each compound in Fig. 2 for both α_3 and α_4. This points up the complex changes that take place in α_3 and α_4. It has been predicted that α_3 and α_4 are not simple lines but are made up of α_3, α_3', α_3'' and α_4 where α_4 has two components[6]. The change of intensity of α_3 and α_4 is perhaps more significant than the wave-length change. Fig. 3 shows a plot of the intensity ratio α_4/α_3 for the compounds shown in the last figure. Here there is a rather gradual increase in the α_4/α_3 ratio in going from the metal to insulator species. Intermetallic compounds cluster about midway between metal and insulators, and compounds having the same structure such as $TaAl_3$ and $NbAl_3$ have essentially the same α_4/α_3 ratio. The III–V compounds appear together between the intermetallic compounds and the insulators, with AlSb having an energy band gap of 1·52 eV, appearing nearest the more metallic species. Again, cryolite does not seem to fit here, adding further proof to possible decomposition in the beam. Using secondary excitation, α_4/α_3 is unity for cryolite and the lines are much better resolved.

$K\alpha_5$ and $K\alpha_6$ are weak lines whose intensities are much less affected by chemical combination. Wave-length shifts are about the same or slightly greater than α_3 and α_4 in going from the metal to the oxide. The same sort of clustering of insulators at short wave-length and conductors at longer wave-length was observed for these lines.

The diagram line $K\beta$ is very weak in the aluminium K spectrum, but shows the greatest shift due to chemical

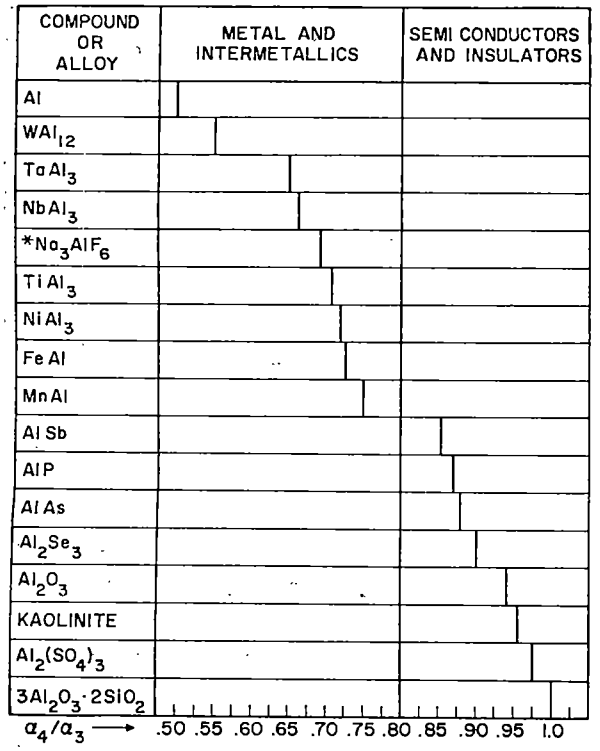


Fig. 3. Intensity ratio α_4/α_3 for aluminium compounds
* Decomposes in beam. $\alpha_4/\alpha_3 = 1$ using secondary excitation

combination of aluminium. In Fig. 4 the 2θ position of $K\beta$ is plotted for the compounds shown previously. The shift of nearly three-quarters of a degree going from metal to oxide is in the opposite direction compared to the shift of $K\alpha$ and $K\alpha$ satellites. As in all the other lines there is a clustering of compounds having similar properties except, again, cryolite which gives a doubled $K\beta$, one component near the metal and the other near the oxide. The true β position for cryolite (obtained with secondary excitation) is the one shown at the bottom of the figure, at 130·15°2θ. In addition to the large shifts, the shape of $K\beta$ is quite indicative of the electrical characteristics of aluminium compounds. In metallic compounds the $K\beta$ band is very asymmetrical, rising rapidly on the short wave-length side and falling off slowly on the long wave-length side. In the oxide and poorly conducting compounds, the $K\beta$ band is very symmetrical and has a slightly narrower half-width.

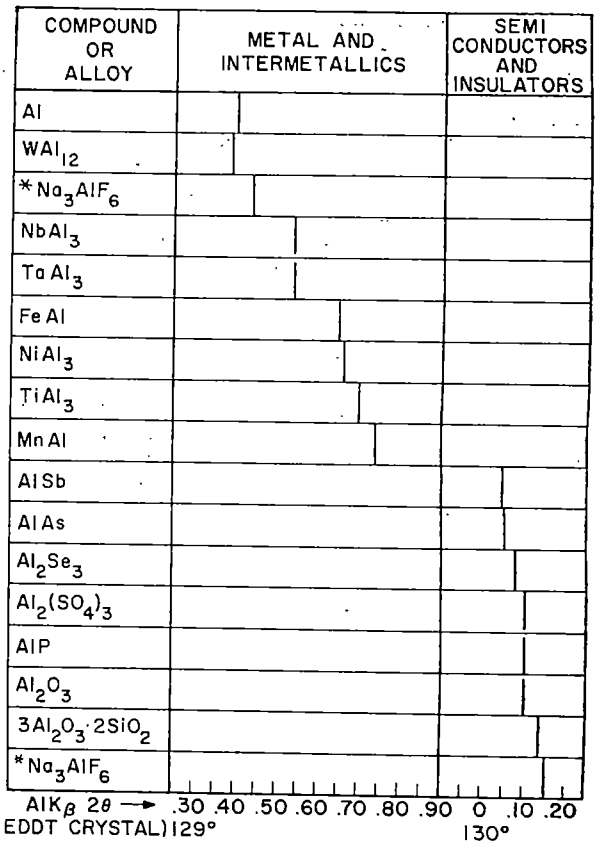


Fig. 4. 2θ position of aluminium $K\beta$ using an EDDT analysing crystal
* Decomposes in beam

It is possible to correlate physical properties of aluminium compounds with line position, and, in the case of satellite lines, with changes in line intensities. No direct correlation has been observed with changes in co-ordination number in intermetallic compounds. Probably co-ordination number may be determined reliably only where aluminium is co-ordinated by the same element.

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CRYSTAL STRUCTURE AND ELECTRICAL PROPERTIES OF SOME COBALT-GROUP CHALCOGENIDES

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In a previous paper[1] I have discussed the relations between the electrical properties of cobaltite-type compounds and the electronic configuration of their cations. The cobaltite family consists of two distinct groups formed by CoAsS (cobaltite) and NiAsS (gersdorffite) and their chemical analogues. In the former the trivalent cation acquires an electron configuration $d\varepsilon^6$, that is, its $d\varepsilon$ sub-shell is completely filled and, hence, non-metallic properties result. In the latter, however, the additional d electron occupies the incompletely filled $d\gamma$ band and, therefore, all representatives of the gersdorffite family show metallic behaviour. The cobalt-group dichalcogenides have the same number of valence electrons as the gersdorffite phases so that one would expect a close analogy to exist between their respective crystal structures and physical properties. Actually the analogy is restricted to a few cases only, a fact which makes these compounds all the more interesting.

Complete analogy to the cobaltite phases is exhibited only by the pyrite modifications of the rhodium chalcogenides, the properties of which are closely similar to those of the ternary palladium compounds (PdAsSe, etc.).

Table 1. Some Preliminary Electrical and Magnetic Data of Rhodium and Iridium Chalcogenides

The values for ΔE (el.) are calculated assuming $\varrho \sim \exp(\Delta E/2kT)$

Compound	Structure type	ΔE (eV)	$\alpha(\mu V/^\circ C)$	$10^6\chi_{mol}$ in c.g.s. units 295° K	85° K
$Co_{1/2}Rh_{1/2}S_2$	C2	Metallic			
RhSSe	$IrSe_2$	~0·7 (opt.)	~200 (*p*)		
$RhSe_2$ (H.T.)	C2 (ref. 2)	Metallic (ref. 2)	~8 (*p*)	+52	+65
(L.T.)	$IrSe_2$	~0·6 (opt.)	~80 (*n*)	−29	−29
RhSeTe	C2 (ref. 21)	Metallic (ref. 21)		+7	+12
$RhTe_2$ (H.T.)	C6 (ref. 3)	Metallic (refs. 2 and 3)			
(L.T.)	C2 (refs. 2 and 3)	Metallic (ref. 2)		−3	+2
$Rh_{1/2}Ir_{1/2}Se_2$	$IrSe_2$	~0·9 (opt.)	~350 (*p*)	−34	−24
IrS_2	$IrSe_2$	~0·9 (opt.)	~250 (*p*)	−35	
IrSSe	$IrSe_2$	~1·2 (opt.)	~350 (*p*)		
$IrSe_2$	$IrSe_2$ (ref. 17)	~1·0 (opt.)	~300 (*p*)	−50	−25
$IrSe_{3/2}Te_{1/2}$	$IrSe_2$	≧0·2 (el.)	~180 (*p*)		
$IrTe_2$	C6 (ref. 13)	Metallic			
$Co_{1/2}Rh_{1/2}S_{\sim 3}$	~C2	>0·2 (el.)	~40 (*n*)		
$RhS_{\sim 3}$	~C2 (ref. 18)	>1·5 (opt.)	~400 (*n*)	−45	−26
$RhSe_{\sim 3}$	~C2 (ref 18)	~0·7 (opt.)	~30 (*n*)		
$IrS_{\sim 3}$	~C2 (ref. 18)	~2 (opt.)		−70	−30
$IrSe_{\sim 3}$	~C2 (ref. 18)	≧0·45 (el.)	~150 (*n*)	−100	−65
$IrTe_{\sim 3}$	~C2 (ref. 13)	Metallic?	~20 (*n*)	~110	−95
Rh_2S_3	Rh_2S_3	0·8 (el.)	~300 (*n*)		

As evidenced by the magnetic data (see Table 1) the excess *d* electron responsible for the metallic character of these compounds is completely delocalized, thus allowing for the occurrence of superconductivity in $RhSe_2$ (ref. 2) and $RhTe_2$ (ref. 3). Among the cobalt compounds only the sulphide and the selenide are isomorphous with their ternary nickel analogues. The sulphide CoS_2 has particularly interesting properties. In the paramagnetic temperature-range its susceptibility follows a Curie-Weiss law with $\theta_p = 161^\circ$ K (ref. 4) or 150° K (ref. 5) and $n_p = 1{\cdot}85$ (ref. 4) or $1{\cdot}99\mu_{Bohr}$ (ref. 5). Below 110° K CoS_2 is ferromagnetic. From its saturation magnetization at low temperatures a magneton number $n_f = 0{\cdot}84\mu_{Bohr}$ (ref. 4) was determined.

It is somewhat surprising that the magneton number as measured in the paramagnetic temperature range is compatible with a localized $d\gamma$ electron whereas in the gersdorffite phases the magnetic moments are appreciably lower. Moreover, the observed S–S distance of 2·124 Å (ref. 6) corresponds to a single bond and thus agrees well with the ionic formula $Co^{2+}(S_2)^{2-}$. However, the deviation from the 'localized-spin value' of $1\mu_{Bohr}$ in the ferromagnetic temperature range indicates a certain overlapping of the $d\gamma$-level (or band) with an empty band. This would then account for the observed metallic behaviour[7,8]. In the paramagnetic pyrite modification of $CoSe_2$, on the other hand, a considerable deviation of the magnetic moment from the localized-spin value is found. The susceptibility of cubic $CoSe_2$ is reported to obey a Curie-Weiss law with $n_p = 2{\cdot}56\mu_{Bohr}$ and $\theta_p = -440^\circ$ K (ref. 9). These results, together with an Se–Se distance of 2·53 Å (single bond distance, 2·34 Å), agree well with the observed metallic conduction[7,8]. Synthetic, as well as natural, $CoSe_2$ normally occurs in the pyrite modification (mineral trogtalite), but the mineral hastite was reported to be a marcasite modification of $CoSe_2$ (ref. 10). No physical data other than the lattice constants are known of this modification, but I expect it to be metallic.

Among the remaining cobalt-group dichalcogenides no other pyrite phase is met. I found CoSeTe (high-temperature modification?) to have a CdI_2 structure with $a = 3{\cdot}70$ Å, $c = 5{\cdot}09$ Å, $c/a = 1{\cdot}38$. This compound, as well as the isostructural $CoTe_2$, is metallic. This is not surprising since the CdI_2-type phases are not polycompounds, so that tetravalent cobalt would be needed to make them semiconducting. But Co^{4+} is rarely obtained even in combination with strongly electronegative anions. On this point I disagree with Regel[11], who claims that $CoTe_2$ is a semiconductor. Not even in the low-temperature modification α-$CoTe_2$ (which might be the stoichiometric 1 : 2 compound[5] whereas the C6-type compound seems to have an anion deficiency) do we expect semiconducting properties, because the localized unpaired $d\gamma$ electron of hypothetical $Co^{2+}(Te_2)^{2-}$ would require a low symmetry of the cation co-ordination. The symmetry of cobalt in α-$CoTe_2$, however, is the same as that of iron in the semiconducting $d\varepsilon^6$ marcasite FeS_2, since the axial ratios of both compounds are very similar[12]. This is possible only if uncompletely filled *d*-levels form a metallic band. The electronic structure of α-$CoTe_2$, therefore, might be similar to that of cubic (and orthorhombic) $CoSe_2$ and this is confirmed by its magnetic behaviour: the susceptibility of α-$CoTe_2$ obeys more or less a Curie-Weiss law[5] leading to a magneton number $n_p = 2{\cdot}81\mu_{Bohr}$ and a Curie-Weiss constant $\theta_p = -540^\circ$ K (ref. 5). No magnetic measurements have been reported in the literature on β-$RhTe_2$ (ref. 3) and $IrTe_2$ (ref. 13) which are again metallic CdI_2-type phases; but we expect smaller and less temperature-dependent values for their magnetic susceptibilities.

It is interesting to note that rather wide ranges of miscibility exist between corresponding cobalt, rhodium and iridium dichalcogenides crystallizing in the pyrite structure. Complete miscibility was observed in the (Co, Rh) Se_2 system. Though one might expect solid solutions of $Co_xRh_{1-x}Te_2$ to crystallize in a CdI_2 structure, pyrite-type mixed crystals are formed up to about $x = \frac{1}{2}$. On a sample of nominal composition $x = \frac{1}{2}$, we determined a lattice constant $a = 6{\cdot}386$ Å, which seems to indicate that the actual cobalt content was somewhat lower. Even $Co_{1/2}Ir_{1/2}Te_2$ ($a = 6{\cdot}388$ Å) was found to have the pyrite structure though no C2 modifications are as yet known of the binary compounds. From X-ray patterns of two-phase material we conclude that the pyrite lattice is stable in $Co_{1-x}Ir_2S_2$ up to $x \approx \frac{1}{4}$ and in $Co_{1-x}Ir_xSe_2$ up to $x \approx \frac{1}{2}$. A broad homogeneity range was detected in the system (Co, Rh) S_2. However, pure RhS_2 does not seem to exist. All attempts to prepare this compound resulted in Rh_2S_3, RhS_3 or other phases. In $Co_{1/2}Rh_{1/2}S_2$ and in $Co_{\sim 0{\cdot}3}Rh_{\sim 0{\cdot}7}S_2$ which both crystallize in the pyrite structure we obtained $a = 5{\cdot}639 \pm 3$ Å and $5{\cdot}667 \pm 4$ Å, respectively. Extrapolation from these values leads to a lattice constant $a \approx 5{\cdot}73$ Å for hypothetical RhS_2. Moreover, on replacing cobalt by rhodium in a pyrite structure the lattice constant is increased by about 0·2 Å (ref. 15), which, with $a = 5{\cdot}535$ Å (ref. 6) for CoS_2, confirms the extrapolated value. The lattice constant $a = 5{\cdot}574$ Å reported by Thomassen[14] therefore seems much too small for RhS_2 but is very similar to the value which we obtained for $RhS_{\sim 3}$ ($a \approx 5{\cdot}58$ Å, neglecting a small splitting of the lines). Furthermore, Juza *et al.*[16] also found no evidence for RhS_2 from a tensimetric analysis of the Rh–S system.

In the structures discussed so far it is impossible for the cation to acquire the electron configuration $d\varepsilon^6$ which would give rise to semiconducting properties. The remaining compounds illustrate how a cation valency favourable for non-metallic properties can be achieved in the 4*d* and 5*d* compounds by a slight modification of the crystal structure. Orthorhombic $IrSe_2$ represents a first example of this behaviour. Its unit cell[17] contains eight formula units and in parts is built similarly to the marcasite structure. Each iridium atom is located at the centre of a distorted octahedron with three Se neighbours at a distance of 2·44 Å and three others at 2·52 Å. One half of the selenium atoms are tetrahedrally surrounded by three iridium atoms and one selenium atom, the Se–Se distance being 2·57 Å. The other half of the selenium atoms have a similar neighbourhood, but adjacent selenium atoms are spaced at 3·27 Å, so that there is no longer an anion–anion bond. The corresponding chemical formula is $Ir_2^{3+}Se_2^{2-}(Se_2)^{2-}$. The observed diamagnetism (Table 1) confirms the postulated $d\varepsilon^6$ configuration (neglecting the small splitting of the $d\varepsilon$ levels due to the deviation from exact octahedral symmetry, which does

Table 2. Lattice Constants of the New $IrSe_2$-type Compounds

Compound	a (Å)	b (Å)	c (Å)
RhSSe	20·48 ± 5	5·749 ± 9	3·629 ± 6
$RhSe_2$	20·91 ± 3	5·951 ± 6	3·709 ± 4
IrS_2	19·78 ± 3	5·624 ± 4	3·565 ± 4
IrSSe	20·43 ± 3	5·756 ± 4	3·649 ± 3
$IrSe_{3/2}Te_{1/2}$	21·43 ± 5	6·027 ± 7	3·813 ± 5

not alter their occupation). The expected non-metallic behaviour has been substantiated by electrical measurements.

The $IrSe_2$ type of structure was also found in IrS_2 and IrSSe. IrSTe does not seem to exist but, as evidenced by X-ray patterns, a small fraction of sulphur in IrS_2 can be replaced by tellurium. In $IrSe_2$ about one-quarter of the selenium can be substituted by tellurium without changing the structure. IrSeTe, however, was not obtained in a pure form. The $IrSe_2$ structure also occurs in RhSSe and in a low-temperature modification of $RhSe_2$. Complete miscibility was observed in the system (Rh, Ir) Se_2, whereas under the same conditions $Co_{1/4}Rh_{3/4}Se_2$ was obtained only in the pyrite structure. All these compounds have been prepared by sintering the powdered components at 700°–850° C. The $IrSe_2$-type phases are all diamagnetic and semiconducting. Lattice constants are listed in Table 2.

A second possibility to achieve non-metallic properties is shown by the compounds $MX_{\sim 3}$ (with M = Rh or Ir and X = S, Se or Te) which crystallize in a cation-deficient pyrite lattice[18]. The actual composition of these compounds appears to deviate somewhat from the ideal value. By sintering pressed powders at 600°–700° C I obtained samples of composition $MX_{2\cdot8-2\cdot9}$. Biltz *et al.*[19] gave the stoichiometry of the iridium sulphide as Ir_3S_8. Hockings and White[13] also proposed a 3 : 8 ratio for iridium telluride, Ir_3Te_8. This assumption seems to be justified from a crystallographic point of view, since in this case one of the four cation sites in the pyrite lattice remains empty, whereas in $IrTe_3$ one-third of all cation sites would have to be unoccupied. However, the fact that I found $RhS_{\sim 3}$, $RhSe_{\sim 3}$, $IrS_{\sim 3}$ and $IrSe_{\sim 3}$ to be diamagnetic semiconductors favours the formula MX_3 or $M_{2/3}(X_2)^{2-}$, which then shows the close analogy to the diamagnetic pyrite-type semiconductors RuS_2, $RuSe_2$, etc.[20]. It is not surprising that the lattice of the compounds with the highest cation deficiency remains no longer exactly cubic. A trigonal splitting of the pyrite lines was observed for $RhS_{\sim 2\cdot9}$, $RhSe_{\sim 2\cdot9}$ and $IrSe_{\sim 2\cdot9}$. For $RhSe_{\sim 2\cdot9}$ the lattice constants are:

$$a = 5{\cdot}962 \pm 2\ \text{Å}$$
$$\alpha = 90°\ 44 \pm 2'$$

Those for $IrSe_{\sim 2\cdot9}$ are almost identical.

It is interesting to note that by filling the cation holes the semiconductor $RhSe_{\sim 3}$ is converted into a superconductor. At the composition $RhSe_2$ the transition temperature lies already above 1° K (ref. 2) (T_c = 4·43° K, determined on a sample with a lattice constant a = 6·092 Å (ref. 21)) and reaches its maximum of about 6° K at $RhSe_{\sim 1\cdot8}$ (refs. 2 and 3). The pyrite structure is reported as far as $RhSe_{1\cdot78}$ (ref. 22). At the composition $RhSe_{1\cdot5}$, however, the character of the compound has definitely changed. In my attempts to prepare RhS_2 I obtained a semiconducting phase which turned out to be Rh_2S_3. Rh_2Se_3 and Ir_2S_3 were also synthesized and proved to be isostructural. My X-ray patterns are, however, different from that given by Rudnev and Malofeeva[23]. The non-metallic properties of these compounds lead to the conclusion that rhodium and iridium are in octahedral co-ordination displaying the diamagnetic configuration $d\varepsilon^6$. For Rh_2Se_3 we occasionally obtained somewhat diffuse X-ray patterns in which the main lines could be indexed by assuming a hexagonal unit cell with $a \approx 5{\cdot}4$, $c \approx 3{\cdot}6$, $c/a \approx 1{\cdot}5$. The lattice constants seem to vary with the Rh : Se ratio. We believe that these patterns can be attributed to a high-temperature modification of the Cr_2S_3 type. However, attempts to synthesize the mixture $CrRhSe_3$ failed.

I thank Dr. P. Giesecke, Cyanamid Research Laboratories, Stamford, who carried out diffuse-reflectance measurements on many of my samples, Mr. H. U. Boelsterli and Miss Elfriede Rittershaus for their assistance in sample preparation and X-ray work, and Dr. E. Mooser for his advice.

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INTERMEDIARY REACTIONS IN THE WATER-SPLITTING PART OF PHOTOSYNTHESIS

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A SIMPLIFIED scheme of the electron transport system in photosynthesis is depicted in Fig. 1. Electrons are transferred from water to TPN (coenzyme 2) through several intermediates. The electron flow is driven by the energy of two light absorptions $h\nu_I$ and $h\nu_{II}$. The development which leads to this scheme especially by the technique of flash photometry[1] and recently also by periodic relaxation[2] is published in refs. 3–18. Nothing is known so far about the mechanism of water splitting and the intermediate-reactions between water and Chl-a_{II} because this part of the reaction is not accompanied by measurable changes of absorption. First details, however (depicted in Figs. 1 and 3), have now been obtained, using the following technique.

Thirty years ago, Emerson and Arnold[16] tried to estimate the turn-over rate of photosynthesis. They measured the oxygen yield on *Chlorella* excited with periodic flashes of light. Short flashes were used with a duration of $\sim 10^{-5}$

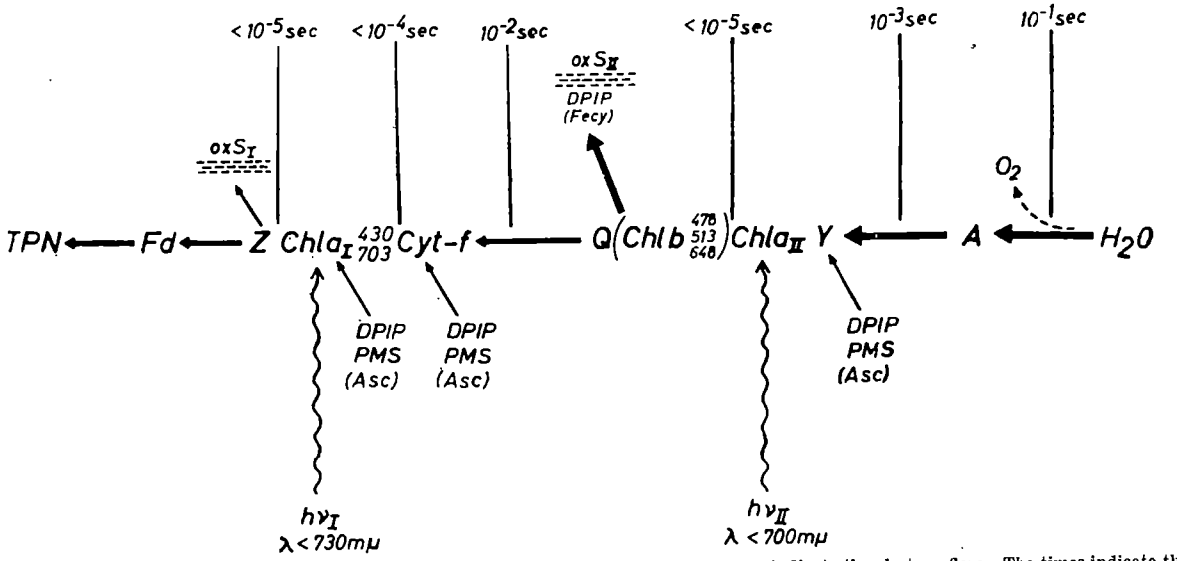


Fig. 1. Simplified scheme of electron transport in photosynthesis of green plants. The arrows indicate the electron flow. The times indicate the duration of the electron transfer. The back reactions[15,18] are not included in this scheme. TPN, triphosphopyridinenucleotide; Fd, ferredoxine; Cyt-*f*, cytochrome-*f*; Q, plastoquinone; Chl-a_I, special chlorophyll, absorption at 430 and 703 mμ; Chl-a_{II}, special chlorophyll-*a*; Chl-*b* (formerly called *X*), special chlorophyll-*b* with absorption at 478 and 648 mμ[16]. The reduced Chl-*b* has an absorption at 513 mμ (the brackets indicate that the position of Chl-*b* is yet not known exactly). *Z*, *Y* and *A*, detected but not identified substances. Chl-a_I can be excited with $\lambda < 730$ mμ and Chl-a_{II} with $\lambda < 700$ mμ. *Ox.S*I and *ox.S*II, artificial electron acceptors. DPIP, dichlorophenole-indophenole; *Fecy*, ferricyanide; PMS, phenazine-methosulphate; *Asc*, ascorbate

sec. In these experiments the oxygen-yield per flash is a function of the time t_d between the flashes. The time giving half the maximum yield of oxygen per flash was interpreted as the reaction time of the rate-determining step of photosynthesis. (As we shall see here, this interpretation cannot be sustained any longer.) This half-time was evaluated as $t_{d1/2} \approx 2 \times 10^{-2}$ sec (20° C). A similar value was found for chloroplasts[20].

The method of Emerson was used by us with two essential variations. (1) In chloroplasts the reaction system of Chl-a_I was blocked by trapping the electrons already at the position indicated in Fig. 1. This was attained by addition of oxidized dichlorophenole-indophenole DPIP (with ferricyanide in excess). The concentration of DPIP was kept at such a value that the trapping was fast (re-oxidation of Chl-*b*⁻ in $\tau_{1/2} \approx 5 \times 10^{-4}$ sec) compared with the normal reaction of Chl-*b* (ferricyanide without DPIP, re-oxidation of Chl-*b*⁻ in $\tau_{1/2} = 10^{-2}$ sec). Measurements of the oxygen yield per flash on such a separated system (arrows in Fig. 1) were expected to give information on the rate limiting step within the unknown reaction path between water and Chl-a_{II}. (2) The oxygen yield was measured on chloroplasts excited by periodic flashes (duration 5×10^{-5} sec) and also by flash groups (two flashes per group and long constant dark times between the groups; flash duration 5×10^{-5} sec).

Exciting the separated reaction system (arrows in Fig. 1) with periodic flashes (open circles in Fig. 2), half the oxygen-yield is obtained by $t_{d1/2} = 3 \times 10^{-2}$ sec (20° C). This value is nearly as large as that which was found by Emerson *et al.*[19] on the whole system.

If the half-time $t_{d1/2} = 2 \times 10^{-2}$ sec could be interpreted as the half-life of the rate determining reaction, the same result would be expected to excite the separated reaction system by flash groups. The result with flash groups, however, is completely different. The oxygen-yield per flash as function of t_d is bi-phasic. The first phase is characterized by $t_{d1/2} \approx 7 \times 10^{-4}$ sec and the second phase by $t_{d1/2} \approx 7 \times 10^{-2}$ sec (solid circles in Fig. 2). Owing to this discrepancy it is impossible to interpret the dark time for half oxygen-yield in periodic flashes ($t_{d1/2} \approx 3 \times 10^{-2}$ sec) as the half-life of the rate determining reaction.

Excitation by flash groups. The results in flash groups (solid circles in Fig. 2) can be interpreted in terms of two intermediates *A* and *Y* between water and Chl-a_{II}. These intermediates are characterized by the following data (Figs. 1 and 3):

(*a*) *Y* and *A* form a complex. The total amount of *Y* is equal to that of *A*: $Y_0 = A_0$. Therefore, the reaction between both is of first order.

(*b*) The value of the rate constant between *Y* and *A* is $k_1 = 10^3$ sec⁻¹, and that one between *A* and water $k_2 = 10$ sec⁻¹, $k'_2 = 4{\cdot}3$ sec⁻¹.

(*c*) The equilibrium constant *K* for $A^+ + \frac{1}{2}$ water $\underset{k'_2}{\overset{k_2}{\rightleftharpoons}}$ $A + H^+ + \frac{1}{4}$ oxygen is $K = 2{\cdot}3 = \frac{k_2}{k'_2}$. In equilibrium $0{\cdot}7\ A_0$ is in the reduced and $0{\cdot}3\ A_0$ in the oxidized state.

During the first flash *YA* (70 per cent) is oxidized to Y^+A, and YA^+ (30 per cent) to Y^+A^+ (Fig. 3). In the dark Y^+ is reduced by *A*, and A^+ is reduced by $\frac{1}{2}$ water (splitting in H^+ and $\frac{1}{4}$ oxygen). The yield of oxygen produced by the second flash at the time t_d after the first flash is given by the amount of *Y* which is reduced in the dark time t_d:

$$Y(t_d) = Y_0(1 - c_1 e^{-k_1 t_d} - c_2 e^{-k_2 t_d} \qquad (1)$$

$c_1 + c_2 = 1$, $c_1 = 0{\cdot}7$. Thus, the relative amount $Y(t_d)/Y_0$ must be equal to the relative oxygen-yield of the second flash at t_d:

$$\frac{Y'(t_d)}{Y_0} \text{ (solid line, Fig. 2)} = \frac{O'_2(t_d)}{O_2(t_d)} = \alpha' \text{ (solid circles, Fig 2).}$$

(It was proved that oxygen$(t_d \to \infty) \approx$ oxygen$(t_d =$ 250 msec) (see Fig. 2).)

Periodic excitation by short flashes. The extremely different results which are obtained by periodic flash excitation and, on the other hand, by flash groups (open and solid circles, Fig. 2) are both in complete accordance with the reaction scheme (Fig. 3). The exact calculation based on the reaction scheme (Fig. 3) gives namely for the relative oxygen-yield $\alpha''(t_d)$ in periodic flashes:

$$\alpha''(t_d) = \alpha'(t_d) - c_1 \frac{(e^{-k_2 t_d} - e^{-k_1 t_d})^2}{1 - e^{-k_1 t_d}},\ c_1 = 0{\cdot}7 \qquad (2)$$

$\alpha''_{max} = \alpha'_{max} = 1$. The theoretical curve (dotted line, Fig. 2) is in good agreement with the result of the oxygen measurements (open circles, Fig. 2).

Periodic excitation by long flashes. Tamiya and Chiba[21] also reported about oxygen-yield measurements in periodic flashes. Unlike Emerson and Arnold[19], flashes of long durations (0·6–8 msec), however, were used by these

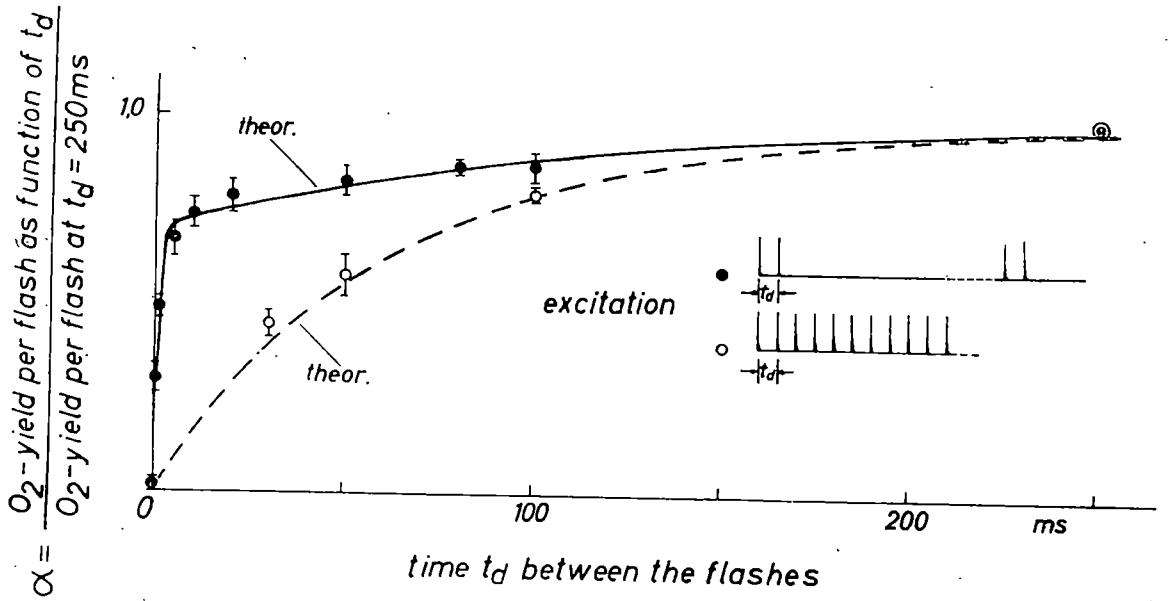


Fig. 2. Relative oxygen-yield per flash on chloroplasts as function of the dark time t_d between the flashes. Addition: 10^{-4} mole/l. dichlorophenole-indophenole (ferricyanide in excess, 10^{-3} mole/l.); flash duration 5×10^{-6} sec, saturating light intensity. *p*H 7·2; temperature 20° C. Chlorophyll contents, 0·5 in 2 ml. Solid circles: experimental results by flash groups; solid curve calculated according to the reaction scheme in Fig. 3 (see equation 1). Open circles, experimental results by periodic flashes; dotted curve, calculated according to the reaction scheme in Fig. 3 (see equation 2)

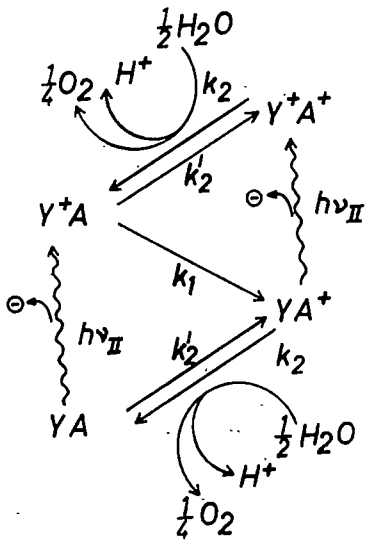


Fig. 3. Detailed scheme of Fig. 1 for the reactions between water and Chl-a_{II}. After long dark times 70 per cent of the total amount of Y and A is in the state YA, and 30 per cent in the state YA^+. The waved arrows indicate excitation of Chl-a_{II} by $h\nu_{II}$. Excited Chl-a_{II} transfers an electron from Y to DPIP (Fig. 1). Y^+ is reduced by A, and A^+ by water. $k_1 = 10^3$ sec^{-1}, $k_2 = 10$ sec^{-1}, $k'_2 = 4{\cdot}3$ sec^{-1}

authors. The results of Tamiya *et al.* were different from those of Emerson *et al.* Exciting by long flashes, they found: (*a*) that the half-time is $t_{d1/2} \approx 10^{-1}$ sec (by short flashes $t_{d1/2} \approx 2 \times 10^{-2}$ sec); (*b*) that the maximum oxygen-yield per flash ($t_d \rightarrow \infty$) is about twice the yield in short flashes; (*c*) that the maximum oxygen-yield is a function of temperature, whereas the maximum yield in short flashes is temperature independent.

As yet no satisfactory explanation for this discrepancy could be given. A calculation on the basis of the reaction scheme (Fig. 3), however, gives for the relative oxygen-yield $\alpha''(t_z)$ in periodic flashes of long duration (flash duration $t_f > 3/k_1$):

$$\alpha''(t_d) = k_2 t_f + (1 + c_1)(1 - e^{-k_2 t_d}),\ c_1 = 0{\cdot}7$$

$\alpha''_{max} = k_2 t_f + 1{\cdot}7$. This calculation agrees with the results of Tamiya and Chiba[21].

(*a*) The time dependence of α'' is a function of k_2 only; $\ln 2/k_2 = t_{d1/2} = 7 \times 10^{-2}$ sec. This half-time equals the experimental value of Tamiya *et al.*

(*b*) $\alpha''_{max} = 0{\cdot}10 + 1{\cdot}7 = 1{\cdot}8$, for $t_f = 10$ msec. This value is nearly twice the maximum yield which was found by Emerson *et al.* using short flashes.

(*c*) The maximum yield α''_{max} is a function of k_2. k_2 is dependent on temperature, therefore, α''_{max} is a function of temperature, too. In the case of short flashes, however, the maximum yield is $\alpha_{max} = 1$ and, therefore, independent of temperature.

The application of flash groups and the measurements on the separated reaction system (arrows, Fig. 2) opens a way to detect details of the reaction path of water splitting. The reaction scheme derived from these experiments gives a quantitative explanation for the manifold and apparently discrepant results obtained by flash groups and periodic excitation with short and long flashes.

As manganese has been shown to be an essential co-factor in water oxidation[22], it may be possible that the intermediate A is closely related to manganese. Finally, it may be noted that the mechanism for YA as depicted in Fig. 2 is supposed to be valid for the reaction of Chl-a_I·*Cyt-f*, also.

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MOLECULAR SIEVING BY CELL MEMBRANES OF *Bacillus megaterium*

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MOST bacterial cells are enclosed by two permeability barriers: a cell wall and an underlying cytoplasmic membrane. The wall in the Gram-positive bacteria seems to be an irregular meshwork of mucopeptide strands with openings (pores) permeable to water and certain solutes[1]. Solutes of high molecular weight are excluded, the threshold size varying among different bacteria[2–4]. Isolated cell walls of *Bacillus megaterium* KM seem to be heteroporous and they exclude dextrans or polyethylene glycols above molecular weight 57,000 (ref. 4). The prevailing concept of a triple-layered cytoplasmic membrane, with two protein sheets and an intermediate lipid layer, emphasizes the role of lipid solubility in permeation. A possible hexagonal arrangement in the ultrastructure[5,6] suggests that the membrane is isoporous and may contain water-filled channels. Oligosaccharides larger than disaccharides do not diffuse through the cytoplasmic membrane of representative Gram-positive bacteria[2,7].

The relationship between the cell structure of *B. megaterium* KM and permeating molecules has been investigated, using a series of commercially available ethylene glycols ranging from mono-ethyleneglycol to poly-ethyleneglycol, molecular weight 70,000 (Union Carbide Co. and Dow Chemical Co.). *B. megaterium* KM was grown in 10-litre batches of half-strength trypticase-soy-broth (Baltimore Biological Laboratory) at 30° C under constant aeration and agitation in a glass and stainless-steel fermentor. Late in the log phase of growth the bacteria were separated by centrifugation, washed twice in distilled water at 4° C, and suspended in water. The thick suspension or space technique[4,8] was used to determine the percentage of intracellular weight (R^w) occupied by the solute at 'diffusion equilibrium'. This technique is based on several assumptions: well mixed extra- and intra-cellular compartments; equal distribution of the solute in the entire available intracellular space; no changes, or correction for changes, in the cell volume; and determination at diffusion equilibrium of the final solute concentration in the extracellular compartment. Experiments were performed at 4° C under semi-anaerobic conditions (thick suspension) to preclude active transport or metabolism, although neither was demonstrated.

Polymer concentrations of the suspensions were determined by precision refractometry, using a correction for ultra-violet-absorbing material. Leakage of absorbing material was identical in polymer-containing and control suspensions. All the polymer was recovered by subsequent washings of the suspension after exposure. Cell viability was not affected by the polymers.

A linear relationship occurred between initial polymer concentration and amount of polymer taken up by the cell pack (Fig. 1), but because of possible association of polymers[9] and plasmolysis at higher solute concentrations, initial concentrations of 0·25–3·0 per cent (w/v) were used. Under these conditions, no change in cell volume was detected by determining ^3H_2O space during and after the experiments.

Extracellular space was $21{\cdot}3 \pm 4{\cdot}1$ per cent (w/w) standard deviation, when measured with non-permeating dextrans (Pharmacia,* Inc.) of molecular weight ($\bar{M}_w$) 158,000–2,000,000 and cells centrifuged at 21,000g for 90 min. Water space, measured with tritiated water, was $78{\cdot}0 \pm 1{\cdot}55$ per cent of cellular weight. This result corresponds with the total water content of 78·5 per cent in *B. megaterium*[4] and suggests that all intracellular water is available for diffusion.

A semilogarithmic plot of intracellular space occupied by polymers as a function of molecular weight showed a complex curve (Fig. 2). Three segments were distinguished: (I) polymers of molecular weight above 30,000 occupied no intracellular space; (II) polymers of molecular weight decreasing from 30,000 to 1,500 occupied an increasing intracellular space, to $R^w \sim 18$ per cent; (III) polymers of molecular weight from 2,000 to 62, and to 20 for ^3H_2O, occupied still more cell space, up to $R^w = 78$ per cent, as molecular weight decreased.

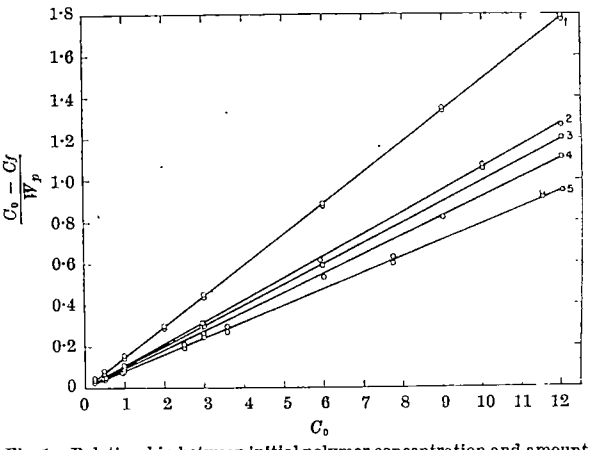


Fig. 1. Relationship between initial polymer concentration and amount of polymer taken up at diffusion equilibrium by *Bacillus megaterium* KM. C_0, initial concentration of polymer (per cent w/v); C_f, final concentration; W_p, weight of wet cell pack. Curve 1, ethyleneglycol; 2, methoxypolyethyleneglycol ($\bar{M}_n$, 550); 3, polyethyleneglycol ($\bar{M}_n$, 1,000); 4, polyethyleneglycol ($\bar{M}_n$ 6,750); 5, dextran ($\bar{M}_w$, 285,000)

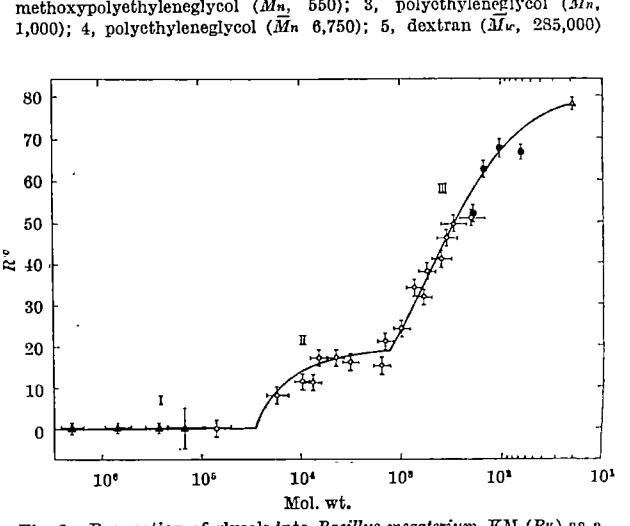


Fig. 2. Permeation of glycols into *Bacillus megaterium* KM (R^w) as a function of molecular weight ($\bar{M}_n$). △, ^3H_2O; ●, monodisperse ethyleneglycols; ○, polydisperse polyethyleneglycols; ▲, dextrans. Standard deviations are indicated for each point. Segments of the curve indicating cell exclusion, cell wall uptake, and cytoplasmic membrane penetration are indicated by I, II, and III, respectively

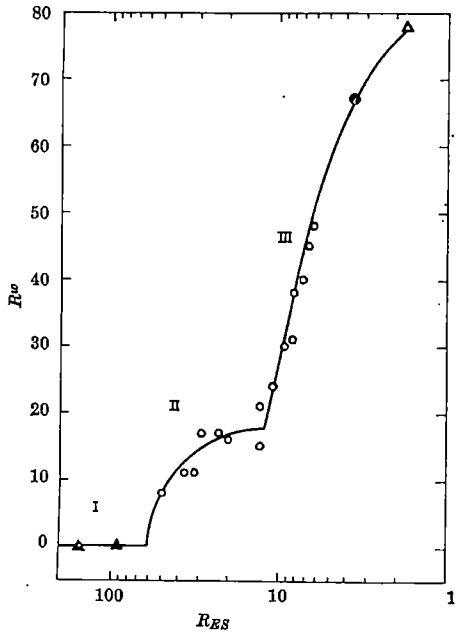


Fig. 3. Permeation of glycols into *Bacillus megaterium* KM (R^w) as a function of the Einstein–Stokes diffusional radius in Å (R_{ES}). The symbols are the same as in Fig. 2

Cell-wall weight is about 20 per cent of total cell weight, when estimated in the logarithmic growth phase[10]. Relative volumes, determined from electronmicrographs of thin sections of *B. megaterium* KM, indicated that cell-wall weight was 20–23 per cent of total cell weight (volume × 1·10 specific gravity = weight)[4]. Segment II of the curve in Fig. 2, approaching $R^w = 20$ per cent, may correspond to the cell wall region of *B. megaterium* while segment III may represent uptake through the cytoplasmic membrane into the cytoplasm.

Lipid solubility and molecular size are the two main criteria generally used to explain permeation phenomena with uncharged molecules[11]. Since lipids are not present in measurable amounts in cell walls of *B. megaterium* KM[12], they should not influence cell-wall uptake. Lipid solubility of glycols increases with increasing molecular weight[11], but solute space values decreased. Furthermore, polypropyleneglycols have a higher partition coefficient in oil–water systems than do polyethyleneglycols[11]; but both series, up to molecular weight of 425, gave the same solute space values. Thus, lipid solubility does not seem important in permeation of glycols into *B. megaterium*.

Diffusional size[13] of solute molecules, principally a function of molecular weight, is apparently important in dialysis and molecular sieving[13,14]. Molecular radii of the polyglycols were taken directly or by extrapolation from experimental data[15,16]. Fig. 3 shows a semi-logarithmic plot of diffusional size of glycols as a function of their uptake values in *B. megaterium*. The same three segments as in Fig. 2 were observed. Polymers with a diffusional radius above 60 Å were excluded from the cell wall, while smaller molecules penetrated it. Polymers of diffusional size above 10 Å radius could not penetrate the cytoplasmic membrane. The exclusion threshold for the cytoplasmic membrane was higher than a previous estimate[2], but for the cell wall was comparable to that obtained with isolated cell walls[4].

Molecular shape may be important in the penetration of macromolecules into bacterial cells. Polymers used in these experiments are 'high-pressure' polydisperse compounds, readily soluble in water[16–18]. A constant of 0·72–0·76 obtained for the Mark–Houwink equation indicated that none of the polyethyleneglycols was spherical in water[15,16]. Those of molecular weights less than 1,000, however, were found to be spherical in water, while higher polyethyleneglycols became more rod-shaped[17]. A comparison of glycols with differently shaped compounds might indicate the importance of molecular shape in penetration.

This work was supported by U.S. Public Health Service research grant *AI*–00619–11 from the National Institutes of Health.

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URINARY EXCRETION OF BETA-AMINO-ISOBUTYRIC ACID AND TAURINE IN MONGOLISM

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THE theoretical basis for the distortion of phenotypic ratios among human trisomics has been partially established[1–3]. Such distortions provide another avenue for the detection of linkage. The only locus reported as possibly located on chromosome 21 and supported by extensive data is that regulating the ABO blood group[3].

The urinary excretion of β-amino-isobutyric acid (BAIB) has been thought to be regulated in part by genetic factors, that is, high excretors are thought to be homozygous for an autosomal recessive gene[4–6]. Wright and Fink[7] and

* Recipients of Research Career Development Awards, U.S. National Institutes of Health.

Lundin and Gustavson[8] have reported high excretors to be more frequent among mongoloids than among either non-mongoloid mental retardates or normal individuals. Perry, Shaw and Walker[9] were unable to confirm Wright and Fink's observation. However, previous investigations have been based on one- or two-dimensional paper chromatographic analyses of urine, with their inherent limitations[10]. The importance of detecting linkage in man, the relatively high-frequency of the gene regulating high BAIB excretion[1-3], and the fact that the reported excess of high excretors among mongoloids exceeded theoretical expectation[11], prompted examination of urinary amino-acid excretion patterns of mongoloid and non-mongoloid subjects using the Technicon amino-acid analyser system, which permits higher resolution and greater reproducibility than paper chromatographic methods.

Urine was collected from 33 (19 male, 14 female) institutionalized mongoloids. Of the samples, 26 were accurate 24-h specimens and 7 were haphazard (not 24-h) specimens; 24-h urines from 20 normal subjects were examined as controls. All mongoloids were karyotyped and found to have the usual 21 trisomy abnormality. Urine was kept frozen until analysed. The analytic procedure was as previously described[12]. Among the 70–80 ninhydrin-positive peaks found in the usual pattern under these conditions, only two have been quantified thus far, namely, those produced by BAIB and by taurine.

BAIB excretion is expressed in terms of mg BAIB/day for urine samples with volumes greater than 600 ml. (39 subjects) and as mg BAIB/l. for specimens with volumes of 600 ml. or less (14 subjects). The latter was found to be a close approximation of mg BAIB/g creatinine, another mode of expression sometimes used with haphazard specimens.

BAIB is considered to be derived only from the breakdown of thymine and its urinary excretion rate appears normally to be independent of diet, age and sex, except that younger subjects (< 5 years of age) appear to excrete increased amounts. Haphazard urine samples collected months apart from two mongoloid subjects contained 88, 111 and 115 mg BAIB/l. in one and 43 and 63 mg BAIB/l. in the other, bearing out the relative constancy of excretion. In the only previous investigation using comparable methods, Armstrong *et al.*[14] reported a range for 3 oriental high excretors of 92·8–150 mg BAIB/day. The mean value (104·7) for the first-mentioned subject was the only value falling within this range among both mongoloids and controls. The small number of subjects (9) under 10 years of age precluded regression analysis of BAIB excretion on age. Sex differences were not detected ($t = 0{\cdot}63$, $P > 0{\cdot}50$ excluding 9 youngest subjects, 8 of whom were males).

The positive skewness in both mongoloid and normal distributions of BAIB excretion was normalized by conversion to their logarithms (Table 1). Variability in BAIB excretion among mongoloids was significantly higher than that among normals ($F = 3{\cdot}28$, $P < 0{\cdot}01$). Using the Fisher–Behrens test, the mean log of BAIB excretion was also significantly higher for mongoloids than for normal individuals ($d = 5{\cdot}49$, $P < 0{\cdot}01$). However, when the 9 mongoloids 10 years of age or younger are excluded from the calculation (the highest excretor is in the younger group), the significance disappears ($d = 0{\cdot}86$, $P > 0{\cdot}05$). Hence, our results corroborate those of Perry, Shaw and Walker[9], but with the suggestion of an excess of higher excretors among mongoloids which may become significant in the larger sample being collected.

Table 2 compares our results for mongoloids with those of Wright and Fink. The two classes with highest excretion levels were lumped because of small expected numbers. Our distribution of mongoloids included significantly fewer with high and more with low levels of BAIB excretion ($\chi^2 = 16{\cdot}8$, $P < 0{\cdot}001$). Whether the disparity arose from differences in age distributions or methodology cannot be rigorously determined because Wright and Fink did not indicate the ages of subjects. They did state that ages of mongoloid and normal subjects were comparable and that normal subjects were drawn from a pædiatric clinic, suggesting that all were under 15 years of age. Most of our mongoloids were adolescents and young adults (mean age = 15·1 years). However, if age were the important variable, one would expect our normals to differ from theirs, since our controls were all 18 years of age or older. A test revealed no difference between the distribution of BAIB levels in the two control groups ($\chi^2 = 1{\cdot}43$, $P > 0{\cdot}25$), though the small size of our sample does not permit a very powerful test of the hypothesis. Whatever the causes, our results do not confirm the results of Wright and Fink.

Table 1. URINARY EXCRETION OF β-AMINO-ISOBUTYRIC ACID AND TAURINE AMONG MONGOLOID AND NORMAL SUBJECTS

Log. (mg BAIB/day) Mongoloids		Log. (mg BAIB/day) Normals	Log. (mg taurine/day + 1) Mongoloids		Log. (mg taurine/day + 1) Normals
1·11	1·63*	1·11	0·50	1·53*	1·62
1·27	0·93*	1·25	0·99	1·63*	1·58
1·34	0·87	0·87	1·44	0·43	1·60
1·03	1·05*	0·72	1·13	0·41*	1·88
1·75	0·72	0·95	1·92	1·32	1·41
1·62	1·60	0·78	0·35	1·67	2·11
0·43	0·75	1·26	1·15	1·02	2·13
0·45	0·63	0·79	1·47	1·04	1·96
0·60	1·29	0·89	1·08	1·25	1·95
1·45*	2·02*†	1·19	1·29	0·26*†	1·66
1·65*	1·83*†	0·89	1·17	0·00*†	2·00
1·02	1·45*†	0·98	1·52	1·00*†	2·00
0·84*†	0·81*†	0·75	0·93*	0·00*†	1·53
1·54*†	0·99*†	1·04	0·90*	1·20*†	2·12
1·00*†	1·57*†	1·10	1·20	1·76*†	0·73
0·77	0·37	0·38	0·56*†	0·28*†	2·08
1·45		1·45	0·60*†		
		1·22			
		0·93			
		1·17			

* Mg/l. (see text for explanation).
† Subjects under 10 years of age.

Table 2. COMPARISON OF RESULTS FROM THIS WORK WITH THOSE OF WRIGHT AND FINK[7]

BAIB in mM/l.	Frequency Present work	Frequency Wright and Fink
0–0·14	18	21
0·15–0·49	13	29
0·50–1·70	2	25
1·80–9·00	0	12
Totals	33	87

Using one-dimensional chromatography of urine, Lundin and Gustavson[8] have reported an increased proportion of high excretors of BAIB among mongoloids. In duplicating their procedure, eluting the region corresponding to BAIB and column-chromatographing the eluate, we found the region of the paper containing the upper half of the BAIB spot to contain also tyrosine and urea, the respective approximate molar proportions being 8 : 1 : 27; the lower half contained BAIB and urea, in the proportions 1 : 56. Partially independent evidence that Lundin and Gustavson were measuring components other than BAIB is provided by the level of BAIB they designated as high excretion, namely, 10 per cent or more of the total optical density of the pattern being attributable to BAIB. Column chromatography of our highest BAIB excretor showed that the area of the BAIB peak was about 1/10 the combined areas of the peaks produced by glycine, alanine, lysine and urea alone. In the light of these findings, a comparison of our data with those of Lundin and Gustavson is obviously meaningless.

The fact that the column chromatographic method yields data on BAIB excretion which do not agree well with the results of previous paper chromatographic investigations suggests the need for re-examination of the level selected for discriminating high and low excretors before the hypothesis that the presumed recessive gene regulating BAIB excretion is on chromosome 21 can be tested. As will be shown elsewhere[11], making the assumptions that there is such a gene on chromosome 21, that it is absolutely linked to the centromere, that non-disjunction occurs in the first division, and that heterozygous mongoloids with a double dose of the recessive gene will

resemble disomic homozygotes (a dosage effect), there will be 12·7 per cent high excretors among mongoloids if the frequency of disomic high excretors is 5 per cent and 23·7 per cent if the frequency of disomic high excretors is 10 per cent. The expected excess of high excretors among mongoloids under these assumptions is considerably less than the 4–6-fold excesses previously reported[7,8]. Further, if these assumptions are not met, the excess will generally be smaller.

Taurine is considered to be derived from cysteine via cysteine sulphinic acid[15]. Its urinary excretion is highly variable and reflects primarily protein intake as evidenced by its lowered excretion under fasting conditions. Gartler, Dobzhansky and Berry[16] and others have found little evidence of heritability in the excretion rates of taurine, though the difference between mono- and di-zygotic twins was in the direction expected if genetic factors were involved. In the independent urine samples from the same individuals mentioned above, taurine excretion levels were 0·64, 1·00 and 0·87 mg/l. for the one mongoloid and 32·7 and 35·2 mg/l. for the other.

Table 1 provides the results obtained on taurine excretion from the present subjects. Because several values were under 1 mg, taurine is expressed in terms of the logarithm (taurine in mg/day or mg/l. + 1). Three subjects with exceedingly low excretion rates were excluded from the control group because they were on a taurine-deficient but nutritionally complete synthetic diet and one because he was fasting. No sex differences in taurine excretion were detected. The mean log for mongoloids was significantly lower than that for controls ($t = 5{\cdot}33$, $P < 0{\cdot}001$). As might be expected, taurine excretion was lower for younger subjects, but the difference between mongoloids and controls persisted when subjects less than 10 years of age were excluded ($t = 4{\cdot}90$, $P < 0{\cdot}001$), and when subjects less than 18 years of age were excluded ($t = 4{\cdot}77$, $P < 0{\cdot}001$). Though protein intake for these subjects was not rigorously established, the diet available to the mongoloids, based on an 1,800 calorie intake, includes 80 g protein, 80 g fat and 180 g carbohydrate. Subjects are served milk three times a day, meat twice a day and eggs once each day. Hence, it does not appear that the protein intake is low. The low heritability previously shown for taurine excretion and the large proportion of mongoloids with low excretion rates discourage the inference that a gene or genes regulating taurine metabolism is on chromosome 21. On the other hand, the absence of a theoretical genetic basis for calculating the expected proportion of low taurine excretors among mongoloids prevents testing such a hypothesis.

Other differences in amino-acid excretion patterns of some mongoloid subjects were observed and will be reported elsewhere[11], together with further details of the experiments reported here.

Addendum. Work under way at the time the foregoing paper was submitted has been completed[17] which establishes a theoretical genetic basis for the high frequency of low taurine excretors as well as for the high galactose-1-phosphate uridyl transferase activity[18], and high neutrophil alkaline phosphatase activity[19] among mongoloids. These striking differences are probably due to overdose effects, that is, to genes which when present in triple dose give rise to phenotypes different in quantity or kind from the effects of the same gene in double dose. With assumptions that crossing over and timing of non-disjunction are independent of parental genotype, it can be shown that the various trisomic genotypes occur in the proportions:

Genotype	Frequency
AAA	$(p^3 + 1/2\ p^2qK)\ (1-m) + mp^2$
AAA'	$(3p^2q + 1/2\ pqK(1-3p))\ (1-m) + mpq$
$AA'A'$	$(3pq^2 + 1/2\ pqK(1-3q))\ (1-m) + mpq$
$A'A'A'$	$(q^3 + 1/2\ pq^2K)\ (1-m) + mq^2$

where p = frequency of A, q = frequency of A', m = proportion of post-fertilization non-disjunction, and $K = c + 2t - 3ct$, where c = frequency of crossing over, and t = proportion of second division meiotic non-disjunction. The excess of high excretors of BAIB among mongoloids previously reported[7,8] is not compatible with any assumptions regarding dominance relationships in the proposed model and is also dubious for the reasons stated. In contrast, the low taurine-levels and reported enzyme differences[18,19] are compatible with overdose effects in the model described, though the few data presently available and the lack of knowledge of gene frequencies preclude rigorous tests.

We thank Dr. C. Nash Herndon for his advice. This work was supported in part by grant *AM*–08050 from the U.S. National Institutes of Health and by a grant from the United Medical Research Foundation of North Carolina.

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EFFECTS OF ELECTRIC CURRENTS ON BONE *IN VIVO*

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It now seems evident that bone functions as a transducer, converting mechanical energy to electrical energy. This behaviour has been ascribed to solid state or semiconductor characteristics in bone by some observers[1–3], and to classic piezoelectric phenomena, such as those observed when quartz crystals are stressed, by others[4–6]. Regardless of the mechanism by which these electrical

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potentials are produced, the question of how they function remains obscure. While it has been recognized for many centuries that bone structure adapts to functional demands[7], the immediate controlling factors have not been identified. In 1962 it was suggested that the stress-generated electrical phenomena were capable of directing the activity of bone cells and were responsible for the orientation and/or aggregation pattern of macromolecules in the extracellular space[1]. Subsequent work in these laboratories demonstrated that collagen fibrils were oriented by weak, direct, electric currents (1 μamp) as they were reconstituted from solution[2]. The pattern of fibril orientation which occurred near the cathode was field-dependent, and at right-angles to the field. The present investigation was initiated to determine the effects of similar low-level, direct electric currents on bone, *in vivo*.

Electric currents were developed in bone by implanting units consisting of 2 iridium–platinum electrodes, a battery and a resistor. Within the units a 1·4 V mercury cell battery was connected in series to a resistor with a value of $1{\cdot}5 \times 10^6$, $1{\cdot}5 \times 10^5$ or $1{\cdot}5 \times 10^4$ ohms, so that the approximate current yield would be 1 μamp (microampere), 10 μamp or 100 μamp respectively. All connexions between battery, resistor and platinum electrodes were made with silver-epoxy cement. The entire unit was finally 'potted' in epoxy and coated with RTV silicone prior to implantation (Fig. 1*a*). Two holes, 13 mm apart, were drilled in the lateral, middle portion of the femora of 18 adult dogs, through an otherwise intact periosteum. Battery packs were inserted so that the platinum electrodes projected through the cortex, and 3–4 mm into the medullary canal. Muscle, fascia and skin were closed over the packs. Non-active controls were implanted in the contralateral femora. Current was monitored *in situ* continuously for 30 min, with a Hewlet-Packard d.c. meter connected to a strip recorder.

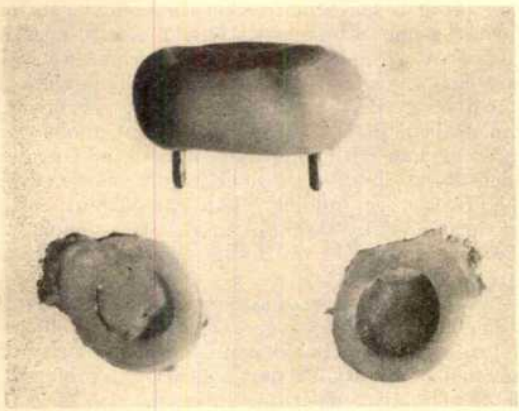


Fig. 1. (*a*) Silicone-encapsulated battery-resistor pack, with iridium–platinum electrodes which are 'plugged in' to holes drilled in femur so that tips of electrodes project 3–4 mm into medullary canal. (*b*) Cross-section of femur with 100-μamp pack in place for 14 days. Cathode left, anode right. Note the mass of intramedullary bone, nearly filling marrow cavity in specimen on left. The reaction about the anode, on the right, is approximately equal to that of the non-active control specimens

The results, depicted in Fig. 2, indicate that, most likely, as electrode polarization increases, resistance external to the pack increases and current falls. It seems reasonable to conclude that stability may be reached when the rate of gas production at the electrodes equals the rate of gas solution in the extracellular fluid. After 21 days in the animal, the 100-μamp packs were still delivering 3 μamp. When these animals were killed by the intravenous administration of an overdose of pentobarbital, the current fell rapidly to 2 μamp. This also suggests that current flow is dependent on the amount of gas being liberated and dissolved at the electrodes.

Cross and longitudinal sections of both femora were obtained following a perfusion of the hind quarters with 10 per cent neutral formalin, 14 and 21 days after operation. In the gross specimens, a mass of bone trabeculæ surrounded all platinum electrodes where they projected into the medullary canal. There was no difference between the amount of newly formed bone around cathode or anode of the active, 1-μamp packs and the inactive controls. Marked differences were observed, however, between controls and the specimens bearing the 10-μamp and 100-μamp packs. In both of these groups with active packs, massive production of new bone was noted around the cathode, while the reaction about the anode resembled that of the 2 inactive platinum electrodes in the controls (Fig. 1*b*). The result was the same, whether the cathode was proximal or distal to the anode. Frequently with the 100-μamp packs a dense, cathodal bone mass nearly filled the medullary canal. Bone production in the region of the negative electrodes had apparently reached a peak at about 2 weeks, since continuation of the stimulation for 21 days did not increase the trabecular masses. Although the differences were small, it appeared that animals with 100-μamp packs formed more bone at the cathode than did animals with 10 μamp packs.

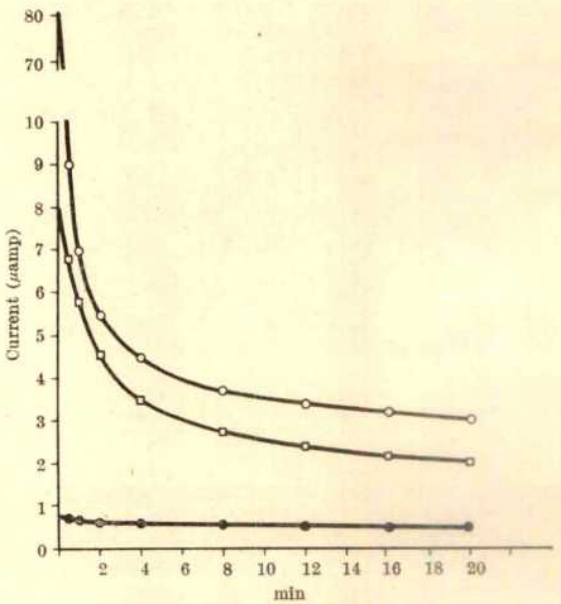


Fig. 2. Graph of current flowing in living bone after various intervals. Initial current values were 0·8, 8·0 and 80 μamp. ○, 100-μamp pack; □, 10-μamp pack; ●, 1-μamp pack

Microscopic examination of sections, prepared from specimens decalcified in formic acid, and embedded in paraffin, and stained with hæmatoxylin and eosin, paralleled the results listed here. There was bone formation about all active and control electrodes. While the reaction about control electrodes, cathodes and anodes was qualitatively similar, with minor exceptions, there was a greatly increased quantity of non-oriented, young, trabecular bone about the cathodes of all 10- and 100-μamp packs.

Osteogenic activity was judged by the number of osteoblasts on trabecular surfaces, the number of large lacunar spaces, and by the general cellularity. On the basis of these criteria, more bone was being formed about all control and active electrodes on the 14th than on the 21st post-operative day. At the cathodes of the 10- and 100-μamp packs, however, the picture at 14 days was one of florid osteogenesis. Many of these specimens contained microscopic fields which were extremely cellular

and in which the newly formed osseous matrix was poorly organized or non-existent (Fig. 3). In some areas, the tissue might have been mistaken for osteogenic sarcoma, had the cells appeared slightly more pleomorphic. Few of these histological characteristics were present in the cathode region of the 10 and 100 μamp packs at 21 days, and these were uniform fields of maturing fibre bone (Fig. 4). Osteoclasis was not observed at the anode. Multinucleated cells were frequently found on the trabeculæ and at some distance from them, but they were distributed uniformly throughout the areas of reaction. A sheet of brownish, homogeneous material, 30–40μ thick, possibly denatured protein, encased the active anode, but not the other electrodes. In the femoral cortex, between the cathode and anode of the 10 and 100 μamp packs, remodelling of osteones occurred and seemed to increase in magnitude between the 14th and 21st days. Bone resorption and deposition were evident along the course of the Haversian and Volkmann's canals in these specimens. Osteoblasts and osteoclasts did not appear to accumulate selectively in the region of the anode or cathode.

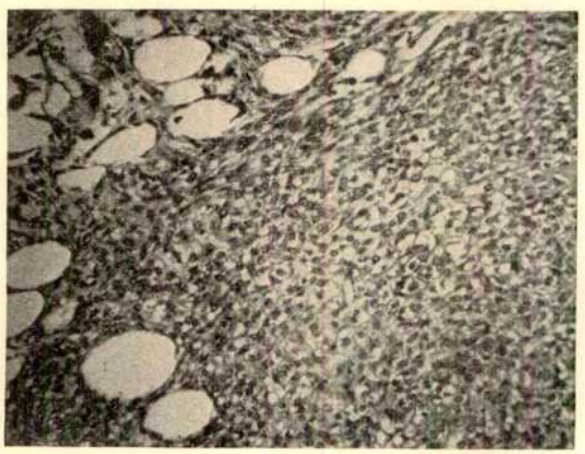


Fig. 3. Specimen 1(*b*), near cathode. Note marked cellularity, large vascular spaces and narrow 'seam' of osteoid at right of photomicrograph. (Hæmatoxylin and eosin, × 80)

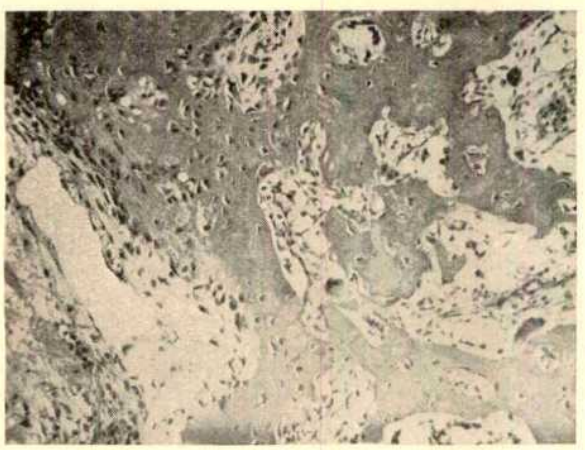


Fig. 4. Specimen 21 days after operation. Note fewer cells and more abundant extracellular matrix than are seen in Fig. 3. This picture was characteristic of the newly formed bone in most specimens with either active or non-active control packs. (Hæmatoxylin and eosin, × 80)

This work was begun without knowledge that investigators in Japan[9,10] had attempted similar experiments in 1956 and 1957, with results similar, in part, to those presented here. This present work, however, was undertaken as a test of the validity of a theoretical control system regulating bone structure and regeneration. The control system was proposed[2] as follows: mechanical deformation applied to the multiple apatite-collagen *pn* junctions in the bone matrix produces an electrical signal, which in turn stimulates differential cellular responses and causes the oriented deposition of newly formed collagen fibres to produce a structural change in the bone, enabling it better to resist the applied, deforming force. When the proposal was advanced in 1963, data were available to support each of its facets, with the exception of the relationship between electric currents and bone cell activity. Our results seem to indicate that such a relationship does, in fact, exist.

The massive osteogenesis occurring at the cathodes was probably not the result of electrophoretic action on collagen or mineral constituents. Had electrophoresis played a dominant part in the observations, a gradient effect might have been expected, with a greater or less amount of bone formed nearer the electrodes. Furthermore, on the basis of other studies (to be published), a preferred orientation of collagen fibres might also have been expected. Certainly, the mass of new bone was associated with an absolute increase in the number of young mesenchymal cells and osteoblasts. It is impossible to account for this degree of cellularity, unless more cells migrated to the region about the cathode, or the cells already present divided more frequently. Of the two possibilities, an increase in the mitotic rate seems more feasible, and the histological picture supports this. Furthermore, most cell membranes are negatively charged, and move towards the anode in electrophoretic chambers, under the influence of current densities vastly greater than the ones employed in this investigation[11,12]. In this connexion, it should be emphasized that while the current rating of packs before implantation ranged between 1 and 100 μamp, only 0·7 to slightly more than 3 μamp were actually delivered.

It was tempting to predict, on the basis of earlier studies[1,2], that bone formation on the concave surface of a deformed bone was related to its electro-negativity, while bone resorption on the convex surface was related to the relative positivity of that area. This work does not corroborate this hypothesis entirely. However, the experimental conditions, that is, direct continuous current, electrodes in line with long axis of bone and projecting into medullary canal, were deliberately chosen for simplicity. It is very unlikely that the current and field patterns produced by the implanted battery packs would ever be encountered in the living system in the natural course of events.

However, it seems that, under the influence of low level, direct electrical currents, bone formation mechanisms are affected in some way that depends on the polarity of the applied current, to produce new bone growth preferentially in regions of relative electro-negativity.

We thank P. R. Mallory and Company, Inc., Allen-Bradley Company, and Sigmund Cohn Manufacturing Company, Inc., for technical assistance and for materials used in this work, which was supported by grants from the Easter Seal Research Foundation and U.S. Public Health Service grant *AM* 07822–01.

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NEUTRALIZING ANTIBODY RESPONSES OF GUINEA-PIGS TO INACTIVATED RUBELLA VIRUS VACCINE

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A NUMBER of investigators have recently reported the isolation of rubella virus in tissue cultures inoculated with specimens from patients with clinically diagnosed rubella. Parkman, Buescher and Artenstein[1] observed that inoculation of primary African green monkey kidney cell monolayers with rubella virus rendered the cells resistant to super-infection with ECHO 11 virus. Sever, Schiff and Traub[2] used Coxsackie *A*-9 virus for challenge. Weller and Neva[3] noted specific cytopathogenic changes in cultures of primary human amnion cells infected with rubella virus. Utilizing these techniques, rubella virus isolations were afterwards reported by Veronelli, Maassab and Hennessy[4], Sigurdardottir, Givan, Rozee and Rhodes[5], Plotkin, Dudgeon and Ramsay[6], Lehmann and Ferris[7], Forbes, Bennett and Gray[8], Selzer[9] and Heggie and Robbins[10].

This article describes the frequency of isolation of rubella virus from naval recruits with clinically diagnosed disease and the neutralizing antibody responses of guinea-pigs following vaccination with non-viable rubella virus vaccine prepared from one of the rubella virus isolates.

Specimens were collected during April 1962 from naval recruits with clinically diagnosed rubella (lymphadenopathy, general malaise, nausea, headache and erythematous macular or maculopapular eruption persisting for 2–4 days) within 24 h after onset of rash. Control specimens were collected from some patients in the midst of the rubella outbreak with complaints other than rubella (that is, rubeola), and throat washings were obtained from a number of 'rubella-contacts' who failed to exhibit any clinical evidence of disease. The throat washings (in 0·5 per cent lactalbumin hydrolysate–Hanks's balanced salt mixture) were stored at −60° C in sealed glass ampules. Serum samples were collected at the same time and approximately 3 weeks later.

Rubella virus isolations were accomplished in culture tubes containing monolayers of African green monkey kidney cells. The monolayers were prepared, following treatment of kidneys with trypsin, with 2 per cent heat-inactivated 'Agamma' calf serum*–Medium 199 mixture containing SV_5 antiserum, and the monolayers, when confluent, were maintained with Medium 199. All media contained 1,000 units of penicillin and 1,000 μg of streptomycin per ml. No fewer than 10 cultures were inoculated with 0·1 ml. of throat washings and incubated on a roller apparatus at 36° C. After 7–9 days, a *p*H adjustment was made by the addition of small quantities of 2·4 per cent $NaHCO_3$. After 12–15 days, fluids were pooled and frozen at −60° C and 1·0 ml. of Medium 199 containing 100 TCD_{50} per 0·1 ml. of Coxsackie *A*-9 virus added to each tube. The cultures were incubated for an additional 7 days and resistance of the monolayers to super-infection with challenge virus was considered presumptive evidence for the presence of rubella virus. At least 3 sub-passages of pooled culture fluids were made before a specimen was considered not to contain rubella virus.

Rubella virus infectivity titrations were performed by the inoculation of 0·1 ml. of serial 0·5 $\log_{10}$ dilutions of rubella virus in Medium 199 to each of 4 culture tubes. After 12 days, the fluids were replaced with 1·0 ml. of Medium 199 containing 100 TCD_{50} per 0·1 ml. of Coxsackie *A*-9 virus. Infectivity titres (TCD_{50} per 0·1 ml.) were recorded 5 days later as the highest dilution of rubella virus rendering cells resistant to Coxsackie *A*-9 virus super-infection. Along with infectivity titrations, as with rubella virus isolations and sub-passages, uninoculated culture fluids were passed. Hæmadsorption tests with a variety of erythrocytes and animal and chicken embryonated egg inoculations were frequently made to exclude the presence of agents other than rubella virus.

Neutralizing antibody titres were determined by incubating 0·5 ml. quantities of serial ten-fold dilutions of rubella virus (diluted in 0·5 per cent 'Agamma' calf serum–Medium 199 mixture) with 0·5 ml. of serial two-fold dilutions of unheated serum for 90 min at room temperature. 0·2 ml. of mixture was then inoculated into each of 3 culture tubes and the monolayers incubated at 36° C for 11 days. After fluid removal, 1·0 ml. of Medium 199 containing 100 TCD_{50} per 0·1 ml. of Coxsackie *A*-9 virus was added to each tube. Five days later, neutralizing antibody titres were recorded as the highest (original) dilution of serum capable of suppressing the control rubella virus infectivity titre at least ten-fold ($10^{-4.1}$–$10^{-3.1}$ per 0·1 ml. in Table 1).

Table 1. Neutralizing Antibody Titres in Guinea-pigs to Rubella Virus Vaccine

Animal	Weeks and antibody titre (reciprocal) 0*†	1*	2*	3†	7	13*	16	20†
1	<4	<4	<4	128	64	16	32	32
2	<4	<4	64	512	512	64	256	128
3	<4	<4	<4	<4	<4	<4	<4	<4
4	<4	<4	32	64	32	4	64	256
5	<4	<4	64	64	32	16	128	32
6	<4	<4	<4	<4	<4	<4	16	16
7	<4			32	64	8	64	32
8	<4			256	64	16	128	128
9	<4			32	64	8	256	64
10	<4			128	512	32	32	32
11	<4			64	4	32	256	64
G.M.‡				1 : 45	1 : 34	1 : 11	1 : 60	1 : 42

* Intramuscular injection of 1·0 ml. vaccine.
† Serum (1 : 4) failed to neutralize Coxsackie *A*-9 virus.
‡ Geometric mean of neutralizing antibody titres.

The rubella virus vaccine was prepared as follows: 1-l. capacity bottles containing confluent African green monkey kidney cell monolayers maintained with 65 ml. of Medium 199 were inoculated with 1·5 ml. of undiluted rubella virus (isolate *GL*6, 3rd monkey kidney culture passage, $10^{2.3}$ TCD_{50} per 0·1 ml.) and incubated for 13 days at 36° C, during which time the *p*H was occasionally adjusted with dilute $NaHCO_3$. The cells and fluids were frozen in an alcohol–dry ice mixture, thawed once, pooled and centrifuged at 2,000 r.p.m. at 5° C for 30 min. The *p*H of the pool was adjusted to 7·2 and inactivation of virus infectivity ($10^{3.8}$ TCD_{50}/0·1 ml.) was directly performed with liquid ethylene oxide at a final concentration of 1 per cent for 1 h at 5° C. The ethylene oxide was completely volatilized during further incubation at 36° C for 14 h. The vaccine was stored at 5° C for the duration of the investigation. The vaccine probably did not contain any viable rubella virus or any other viruses since inoculation of monkey kidney cell cultures with the vaccine and subsequent challenge of some

* Obtained from Hyland Laboratories, Los Angeles, California.

cultures with 500–1,000 TCD_{50} per 0·1 ml. of Coxsackie *A*-9 virus failed to reveal the presence of such agents. Two additional sub-passages yielded similar results as did a single test performed in rabbit kidney cell monolayers according to the technique reported by McCarthy, Taylor-Robinson and Pillinger[11]. Suckling mice and chicken embryonated eggs were inoculated with the vaccine, also. Tests for detection of viable microbes other than viruses were similarly negative.

The results of virus isolation investigations indicate that the frequency of rubella virus isolation was greater from throat washings collected 1–12 rather than 13–24 h after rash onset. From 30 clinically diagnosed cases of rubella, 18 rubella virus isolates were obtained: 10/12 (83 per cent) from throat washings collected 1–12 h and 8/18 (44 per cent) from specimens collected 13–24 h following onset of rash. Rubella virus was recovered from 1 of 14 'rubella contacts' and rubeola virus from 2 individuals with illnesses clinically diagnosed as measles. Each rubella virus isolate was further characterized in that: (1) none was neutralized by homologous- or heterologous-acute phase serum samples but each was cross-neutralized by 3-week convalescent sera (antibody titres 1/32–1/512); (2) the infectivity of each isolate was neutralized by 4 pools of human γ-globulin† (antibody titres 1/128–>1/512); (3) each was neutralized by a standard reference rubella virus antiserum‡ (antibody titres 1/16–1/1,024); (4) all the convalescent but none of the acute phase serum samples neutralized a standard reference rubella virus preparation‡ (antibody titres 1/64–1/1,024). Various virus hyperimmune antisera failed to react with any of the isolates in neutralization and complement-fixation tests. Based on these results, the rubella virus isolates appeared to be antigenically similar to, or identical with, one another and the reference rubella virus preparation.

To determine whether the vaccine would induce the formation of neutralizing antibodies, 11 male guinea-pigs weighing 600–700 g were injected intramuscularly with 1·0 ml. (0·5 ml. injected into each of 2 sites) of vaccine and serum was collected at intervals noted in Table 1. Two additional animals as controls were inoculated with 1·0 ml. of normal tissue culture fluid treated with ethylene oxide. Neutralizing antibody titres were determined in a single neutralization test employing rubella virus isolate *GL*8 rather than *GL*6 used in the preparation of the vaccine. It can be seen from Table 1 that neither pre-vaccination sera nor serum specimens collected 1 week following a single injection of vaccine contained rubella virus antibodies. One week after a second injection of vaccine, neutralizing antibody titres were detected in 3 of 6 guinea-pig sera (antibody titres 1/32–1/64). Significantly higher titres (1/32–1/512) were found in serum samples collected 1 week after a third inoculation of vaccine (geometric mean 1/34) and these titres appeared to decline within 10 weeks (geometric mean 1/11). However, a single injection of vaccine at this 10-week interval resulted in an increase in the geometric mean of the neutralizing antibody titres to 1/60. Moreover, this additional inoculation resulted in the seroconversion of one guinea-pig (No. 6). Antibodies were not detected in undiluted serum samples collected from another (No. 3). None of the sera collected from immunized and control animals neutralized Coxsackie *A*-9 virus.

The results of these investigations indicate that rubella virus neutralizing antibodies appear in sera of guinea-pigs following immunization with rubella virus vaccine not containing detectable viable rubella virus. Since infection with rubella virus may result in serious congenital malformations, the results suggest that vaccination of susceptible human beings with non-viable rubella virus vaccine may induce the formation of antibodies protective against this virus infection.

I thank Captain L. F. Miller (U.S.N.), Lieut. J. Maisel (U.S.N.R.), Dr. R. E. Jewett (University of Kansas Medical School), Mr. A. E. Cadilek (CIBA), Mr. O. F. Andersen (CIBA) and Mr. N. F. McHugh (CIBA) for their assistance.

† Kindly supplied by Dr. J. Inman, Ortho Research Foundation, Raritan, New Jersey.

‡ Kindly supplied by Dr. G. Schiff, University of Cincinnati Medical School.

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RELATIONSHIP BETWEEN RADIATION DOSE AND GIANT CELLS IN THE SURVIVING CELL POPULATION OF MOUSE MARROW AS DETERMINED BY INTERFERENCE MICROSCOPY

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WE have reported previously the dry mass distribution of the normal bone marrow cell population of the mouse[1] and the subsequent changes during the first 24-h post-irradiation period[2], and suggested that the dry mass changes might help to determine the degree of radiation injury of the marrow and the chances of survival of the patient. This article is the result of an investigation of the quantitative relationship between radiation doses and the changes in cellular dry mass of the giant cells in the surviving marrow cell population 24 h following irradiation. The changes, as we observed, are approximately proportional to the radiation dose; thus the information may be of value in the estimation of radiation injury to the marrow caused by a source known or unknown as to its strength.

LAF_1 male mice of approximately 20–33 g body-wt. and about 8–9 weeks of age were supplied by Jackson Memorial Laboratory. Each animal was exposed to a single dose of total-body X-irradiation (200 kVp, 20 m.amp. high-voltage layer, 1·35 mm copper) in an individual plastic container. Some of these doses, namely, 1,000 and 1,500 r., were composed of two successive exposures with an interval between them of no more than 10 min. Twenty-four hours after the irradiation, the animal was

killed in a glass jar with an overdose of ether. Slide preparations for interference microscopy were made from the femoral marrow cells suspended in normal saline. The techniques of slide preparation and interference microscopy were described in our previous publications[1,3,4].

Two thousand cells from 40 non-irradiated control animals and 1,000 cells from 20 irradiated animals for each of the following X-radiation doses were measured for their total cellular dry mass with the aid of an *A-O* interference microscope and a half-shade eyepiece. The doses tested were: 100, 200, 300, 400, 500, 600, 700, 800, 900, 1,000 and 1,500 r. Of 50 cells from each animal, 25 from each femur were measured. The megakaryocytes, which were too variable in size and often difficult to measure because of the difference in dry mass concentrations between cytoplasm and nucleus, coupled with the irregularity in the shape of the nucleus, and cells smaller than the mature neutrophiles (about 8–10μ in average diameter when flattened in the slide preparation), which include the erythrocytes, small lymphocytes and small nucleated erythrocytes, were excluded from measurement. The erythrocytes, the small lymphocytes and the small nucleated cells in the erythrocyte series were found without discernible change in dry mass following lethal irradiation in our previous experiments. The mature granulocytes, which also showed little or no change in dry mass, however, were included in our measurements because we needed a base line for calculating the percentages of giant cells. Except for the excluded cell types mentioned above, all the cells found in each microscopic field of a 100× objective were measured. By and large there were only a few cells in each field, as the cells must be well separated for interference microscopic measurements. To find 25 measurable cells from a single slide, it must have an average of 5–10 fields, thus for 1,000 cells as many as 200–400 microscopic fields were needed. This provided satisfactory compensation for the lack of uniform distribution of different types of cells in the marrow.

We chose to study the post-irradiation period at the 24-h level because we found that the maximum dry mass increase in the marrow cells was reached at the end of that period[2].

As we have already stated, our main interest in this work is the quantitative relationship between radiation doses and the changes in the giant cells of the surviving marrow cell population. The death of the giant cells which may occur at higher dose-levels, and their rate of death are, however, not included in our study. This relationship is three-fold: (1) the distribution of giant cells, of different dry mass values in the surviving population; (2) the changes in mean dry mass per cell of the surviving giant cell population; (3) the changes in percentages of giant cells in the surviving cell population, as related to radiation doses. A giant cell is arbitrarily defined as any cell which has a total dry mass of more than 110 relative units, with the exception of megakaryocytes which were excluded from our measurements. The line of demarcation was drawn at 110 relative units due to the fact that, with the exception of megakaryocytes, only 0·1 per cent of the normal marrow cells reached the dry mass level between 110 and 130 relative units among 2,000 cells measured in our non-irradiated control animals, and none was found beyond this limit. The demarcation line could more appropriately set at 130 relative units had it not been for the fact that the percentage of cells between 110 and 130 relative units in dry mass increased rapidly as the radiation dose increased even at the non-lethal levels where no evidence of cell death was found. These cells must be considered as radiation-induced giant cells as well as the cells with more than 130 relative units of dry mass.

Distribution of giant cells of different dry mass values in the surviving cell population. As aforementioned, in the non-irradiated controls we found only 0·1 per cent of cells which had a dry mass value between 110 and 130 units and none over 130 units. In the irradiated animals, the giant cells not only increased in number rapidly but also gradually expanded its ranges to higher values on the dry mass scale as the radiation dose increased. The percentages of giant cells with lower dry mass values were generally higher than those of the cells with higher dry mass values. The changes in the distribution of the giant cells are obviously related to the dose. For easy comparison, the distribution changes were illustrated in the histograms (Fig. 1).

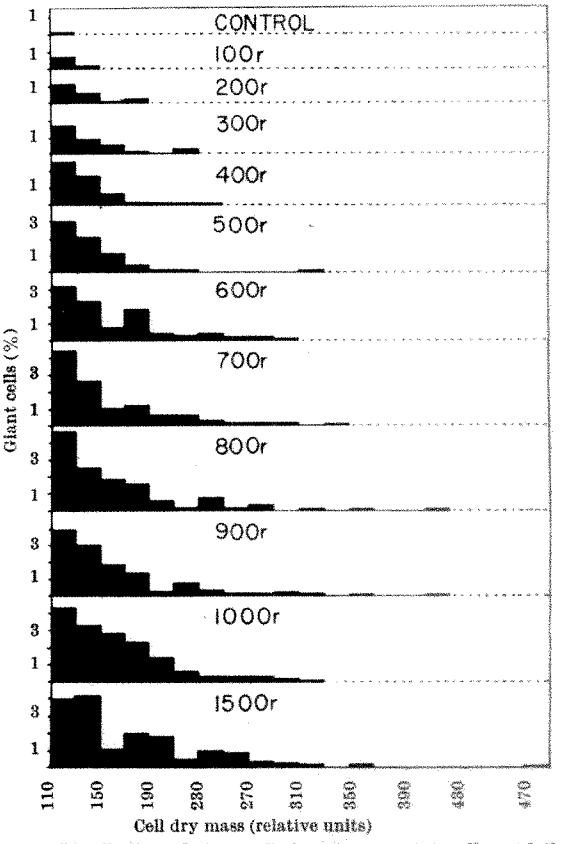


Fig. 1. Distribution of giant cells in mouse marrow cell population according to their dry mass values. Except for megakaryocytes, all the cells with more than 110 relative units in dry mass are considered as giant cells. The percentages of giant cells and their dry mass ranges increase with increasing radiation dose. The percentage of giant cells found in non-irradiated control animals is 0·1 per cent. Only the giant cells were presented in the histograms. The percentages of giant cells were calculated from 1,000 cells measured for each radiation dose tested and from 2,000 cells measured in the case of the non-irradiated control

Two factors contributed to the changes of giant cell distribution, namely, the increase in the number of giant cells and the increase in the dry mass of individual giant cells. The increase in number of the giant cells can be expressed as increase in percentage of the giant cells among the 1,000 cells measured from the surviving cell population for each dose, while the increase in dry mass of the individual giant cells can be expressed as an increase in mean dry mass per cell of the giant cell population.

Changes in mean dry mass per cell of the surviving giant cell population. From the fact that the giant cells showed a gradual spread towards the higher dry mass values in the increasing radiation doses as observed in the histograms (Fig. 1), one can almost predict that the mean dry mass of the giant cells will show a corresponding increase. When the mean dry mass of the giant cells for all the radiation doses as well as that of the non-irradiated control were calculated and plotted against the radiation doses in a curve, it became clear that the increase in mean dry mass per cell with the increasing radiation doses was approximately linear up to a limit—somewhere between 1,000 and 1,500 r. (Fig. 2). The linearity of the curve will probably improve with much larger cell samples

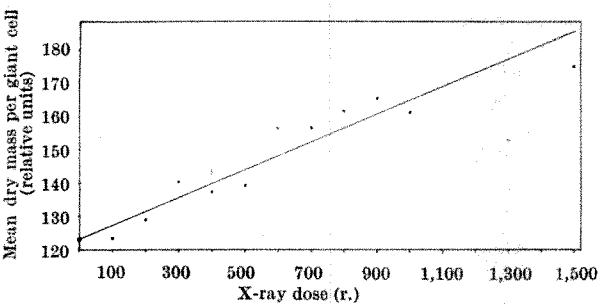


Fig. 2. Increase in mean dry mass per cell of the giant cell population of mouse bone marrow as plotted against increasing X-ray doses is approximately linear

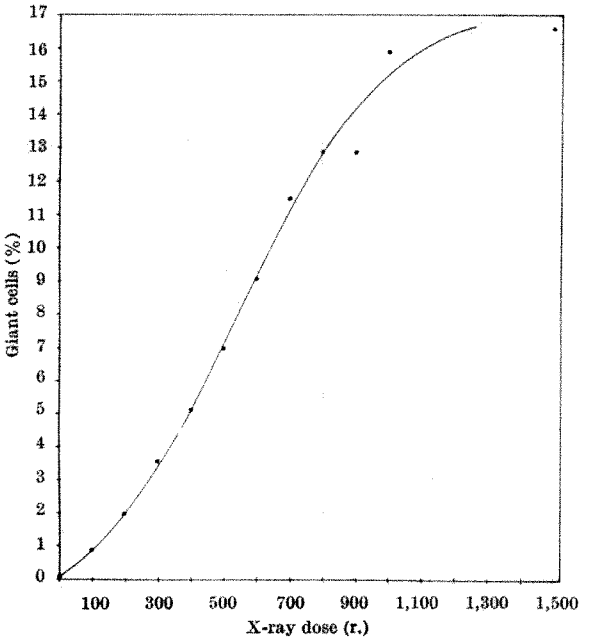


Fig. 3. Increase in the percentage of giant cells in the surviving marrow cell population of the mouse against the increasing X-ray doses results in a sigmoid curve. The middle portion of the curve between 400 r. and 800 r. is almost linear

measured. Thus the post-irradiational increase in mean dry mass per giant cell is also related to the dose.

Increase in percentages of giant cells in the surviving cell population. Another phenomenon related to the dose is the increase in percentages of giant cells in the surviving cell population which was demonstrated in both Puck's work[5] and ours. A gradual increase in the number of the giant cells as the radiation dose increased was clearly noticeable when the histograms of the giant cell distribution (Fig. 1) were compared. This gradual increase can be more clearly elucidated by plotting the percentages of giant cells of the non-irradiated control and of the irradiated animals against the radiation doses (Fig. 3). This results in a sigmoid curve. The middle portion of the curve from 400 to 800 r. is almost linear. The dose 400 r. is LD_2, very close to the non-lethal dose (LD_0), and the dose 800 r. coincides with LD_{100}. The increase of the giant cells is slow at the lower dose-levels but becomes faster at the 300-r. level; and a subsequent slowing down appeared at the last portion of the curve. This slowing down is probably due to the death of some giant cells at the higher dose-levels. The increase of the giant cells in the irradiated populations over that of the non-irradiated control is 52 times for 400 r., 129 times for 800 r. and 166 times for 1,500 r.

An increase in the size of cells following irradiation was observed in various organisms as well as mouse and human tumours in the late twenties through the early forties[6–9]. The appearance of radiation-induced giant cells in the animal bone marrow was first reported by Bloom in 1948 (ref. 10). The formation of the giant cells following irradiation was the result of mitotic inhibition without a corresponding inhibition in RNA and protein synthesis[11,12]. Puck found that, after X-irradiation, when the HeLa cells were plated, in tissue culture, the proportion of giant cells in the cells of the abortive colonies increased with the increase of the X-ray dose[5]. Cell death without any subsequent cell division following irradiation, however, occurred only at dose-levels above 600 r. Our results show that not only the percentage of giant cells in the surviving cell population increases with the increase of the radiation dose; the giant cell distribution pattern and the mean dry mass per giant cell are also related to the dose. The increase in percentages of giant cells seems to be the result of the fact that more cells are affected by a higher radiation dose in their mitotic process, while the increase in mean dry mass per giant cell can be explained by the increasing duration of mitotic inhibition of the higher radiation doses. It is a well-established fact that the duration of mitotic inhibition increases with increasing dose of irradiation[13]. The changes in the percentages of giant cells and the changes in the dry mass of the individual cells, together with the possibility of the death of some giant cells at higher dose-levels, contributed to the changes in the giant cell distribution.

Whether any radiation-induced death of the marrow cells in the sense of complete cessation of all the biological activities with subsequent disappearance from the population within 24 h following irradiation at dose-levels no greater than 600 r. may occur is open to question in the light of Puck's findings. If mammalian cells in tissue culture do not die without at least one or two cell divisions following direct exposure to radiation at the 600-r. level, one would tend to believe that marrow cells subjected to *in vivo* irradiation at levels of 600 r. will probably not die in statistically significant numbers. Thus cell death may have played little part in the changes we found in our results, at least not at the 600-r. level or below. At any rate, our concern is the relationship between the radiation doses and changes in the giant cells of the surviving cell population as an index to the degree of radiation-caused marrow injury. Whether or not any cell death might have occurred is of no significance.

These findings may have value in the estimation of the extent of radiation injury to the bone marrow and, accordingly, to the prognosis for a patient. Inasmuch as the response of individuals to a given dose of radiation differs greatly, knowledge of the dose a patient receives is helpful but far from being safe in permitting evaluation of the prognosis. A direct examination of the patient's response following the exposure is, therefore, of primary importance. This technique may offer possibilities as an aid in such investigations. The difficulty involved in the application of this technique for this type of study is that it is slow and tedious. With the information from our findings, we are at present exploring the possibilities of using much more rapid methods, such as a Coulter counter, for the measurement of corresponding changes in the size of the giant cells, and immersion refractometer for the measurement of the changes in cell dry mass concentrations at a populational level after irradiation of measured intensity.

This work was supported by U.S. Public Health Service grants *AM*0–7067 (01 and 02) and *AM*–04188 (03 and 04).

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CONTROL OF HÆMOGLOBIN A AND F SYNTHESIS: HÆMOGLOBIN FORMATION IN FŒTAL AND ADULT ERYTHROID CELLS

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THE work recorded here was designed to investigate the factors determining the rates of synthesis of fœtal and adult hæmoglobins. Examination of the mechanisms controlling the synthesis of hæmoglobin in human erythroid cells affords an opportunity for investigating certain aspects of cell differentiation. The hæmoglobin of human erythroid cells normally consists of a major type, hæmoglobin F ($\alpha_2\gamma_2$) in the fœtus, or hæmoglobin A ($\alpha_2\beta_2$) in the adult, and one or more minor components. Hæmoglobin A begins to replace hæmoglobin F as the major component during late fœtal development. In normal adults hæmoglobin F generally comprises less than 2 per cent of the total hæmoglobin[1]. In adults with certain types of thalassæmia, however, the proportion of hæmoglobin which is hæmoglobin F may exceed 40 per cent. Less striking elevations in hæmoglobin F concentration may occur in association with other types of hæmolytic anæmia. The α-globin chains in hæmoglobin F and adult hæmoglobin are probably identical in amino-acid composition[2].

Control mechanisms in protein synthesis may be exerted at various sites in the series of reactions involved in the translation of genetic information into the specific protein products. Previous investigations provided evidence that in thalassæmia there is a selective decrease in the capacity of polyribosomes to synthesize hæmoglobin A[3]. The rate of synthesis of hæmoglobin F in thalassæmic cells was found to be similar to that of cells from subjects with other hæmolytic anæmias not associated with impaired hæmoglobin A formation[4]. The synthesis of hæmoglobins A and F has now been further examined by determining the rate of formation of these proteins in fœtal reticulocytes and in cell populations from adults in which both hæmoglobins A and F are formed.

Ribosome content and amino-acid incorporation on ribosomes. It has been shown that ribosomes characterized by sedimentation coefficients greater than 100*S* (polyribosomes) are the site of protein synthesis in erythroid cells of adult subjects with thalassæmia and other types of hæmolytic anæmia[3]. The polyribosome content of reticulocytes from thalassæmic and non-thalassæmic persons is similar. In the present experiments, initial studies were designed to determine the content of ribosomes in reticulocytes of fœtal blood and to determine which ribosomes are active in protein synthesis in these cells. Fœtal blood was obtained from normal placentæ following full-term spontaneous delivery. The blood was collected in siliconized tubes containing 10 mg of dry heparin per 10 ml. of blood and immediately cooled in ice. The cells were recovered from the blood by centrifugation, re-suspended in 2 volumes of a modified Krebs-Ringer bicarbonate buffer[5] containing 6–10 μc. of ^{14}C-valine (20·3 μc./μmole) and ^{14}C-leucine (17·1 μc./μmole). Incubation was carried out with agitation under air at 37° for 80 min. Aliquots of the incubation suspension were removed at varying time-intervals from zero to 80 min for determination of radioactivity incorporated into the soluble protein[4]. In all studies the rate of incorporation of the ^{14}C-amino-acids into the soluble protein increased linearly during the entire period of incubation. Following the 80-min period of incubation, the cells were cooled in ice, separated by centrifugation and lysed, and ribosomes were recovered from the hæmolysate by the techniques described elsewhere[3]. The concentration of ribosomes was determined by absorbance at 260 mμ and the sedimentation characteristics analysed by sedimenting them through a 5–20 per cent linear sucrose density gradient[3]. The total amount of ribosomes and the proportion of the ribosomes with sedimentation coefficients in excess of 80*S* were estimated by analysis of the sucrose density gradient patterns.

In all cells, the incorporation of the radioactive amino-acids on the ribosomes was associated primarily with polyribosomes, ribosomes corresponding to sedimentation coefficients equal to or in excess of 110*S* (Fig. 1). Thus, in fœtal erythroid cells, as in rabbit reticulocytes[6–8] and human adult reticulocytes[3], protein synthesis proceeds primarily on polyribosomes.

The ribosomes recovered per unit of reticulocytes from the fœtal blood cells was 1·3 mg ± 0·7 mg/10^{10} reticulocytes. This value for ribosome content in reticulocytes is not significantly different from that found in cells of patients with thalassæmia major, sickle-cell anæmia, sickle-cell thalassæmia or acquired hæmolytic anæmia[3]. In fœtal erythroid cells, the proportion of the ribosomes which are polyribosomes was 37 ± 5 per cent, a value also in general agreement with that obtained for adult erythroid cells[3]. These findings suggest that differences between cord blood cells and adult blood cells with respect to the rates of synthesis of hæmoglobin A and hæmoglobin F do not reflect any gross differences in the content of polyribosomes.

Synthesis of hæmoglobins A and F in fœtal erythroid cells. Hæmoglobin A and hæmoglobin F were separated by chromatography of hæmolysates on 'Amberlite *IRC*-50' columns by the method of Allen, Schroeder and Balog[9]. In 20 chromatography runs, the average recovery of hæmoglobin was 97·6 ± 9·3 per cent, and of radioactivity, 90 ± 10·5 per cent. The homogeneity of the

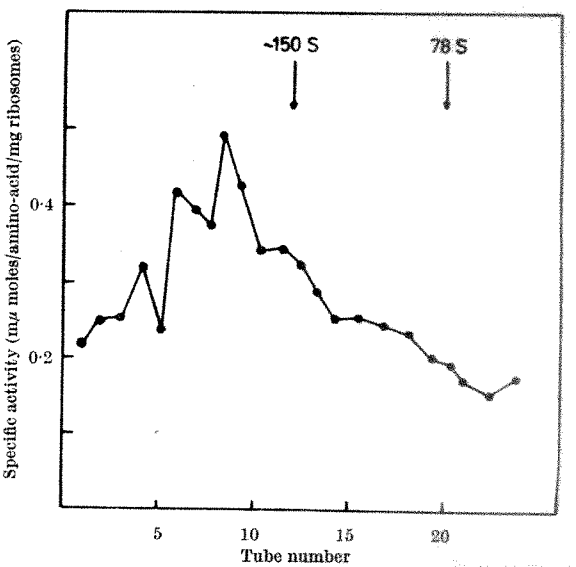


Fig. 1. The pattern of specific activity of ribosomes from fœtal blood cells labelled with ^{14}C-leucine and ^{14}C-valine. The ribosomes were layered on a 5–20 per cent linear sucrose density gradient. The sucrose was in a solution of $1·5 \times 10^{-3}$ M $MgCl_2$, 5×10^{-2} M KCl and 10^{-2} M *tris* HCl, *p*H 7·4. Centrifugation was in an *SW*-39 rotor at 39,000 r.p.m. for 40 min. The fractions of the sucrose gradient were collected and ribosome concentration and radioactivity determined as previously described[3]

fractions recovered from the column was evaluated by electrophoresis on starch gel. Fraction I contained, in addition to hæmoglobin F, non-hæmoglobin protein. The contaminating non-hæmoglobin protein was separated on DEAE cellulose[10] and never exceeded 5 per cent of the total counts incorporated into soluble protein. Fraction II included hæmoglobin A (or S) and A_2. The absence of hæmoglobin F in Fraction II was further documented by the following experiment. Hæmolysates were prepared from cord blood cells which had been labelled with ^{14}C-isoleucine. In human hæmoglobin, isoleucine is present only in the γ-peptide chain. The radioactivity in these hæmolysates was associated only with Fraction I[9] containing hæmoglobin F. There was no detectable ^{14}C-isoleucine in Fraction II containing hæmoglobin A.

The rate of incorporation of leucine and valine into total soluble protein by fœtal blood cells was similar to that observed for cells from patients with sickle-cell anæmia or other hæmolytic anæmias, and was markedly higher than that of cells from subjects with thalassæmia major (compare Table 1, col. 3, and Table 2, col. 4). In fœtal cells, hæmoglobin F accounted for, on the average, 90 per cent of the total hæmoglobin. However, in these cell populations hæmoglobin A was synthesized at a rate which averaged 28 per cent of that of hæmoglobin F. Thus, in each investigation of cord blood, the specific activity of hæmoglobin A was higher than that of hæmoglobin F. A ratio of specific activity of hæmoglobin A to specific activity of hæmoglobin F equal to one would be expected if the concentrations of the two hæmoglobins in cord blood was an accurate index of the capabilities of erythroid cells to form these proteins. The finding that the ratio of specific activities of hæmoglobin A–F is higher than one in cord blood is compatible with the concept that the capacity for hæmoglobin A synthesis increases during late fœtal development while the capacity for hæmoglobin F synthesis declines. At the time of birth, the major portion of the hæmoglobin in peripheral fœtal blood cells was formed at an earlier stage in fœtal development. This would account for the observation that, at birth, the ratio of the rates of synthesis of hæmoglobin A–F exceeds the ratio of the concentrations of hæmoglobins A–F.

Synthesis of hæmoglobins A and F in adult erythroid cells. Blood samples were obtained from patients with thalassæmia major, sickle-thalassæmia, sickle-cell anæmia and acquired hæmolytic anæmia. The rates of synthesis of hæmoglobin A and F by cells from these subjects were determined by methods indicated for the work on fœtal blood cells.

Peripheral erythroid cells from patients either homozygous or heterozygous for the thalassæmia gene, that is, patients with thalassæmia major or sickle-thalassæmia, synthesized protein at a markedly lower rate than cells from subjects with sickle-cell anæmia or acquired hæmolytic anæmia (Table 2). This decreased rate of protein synthesis in the presence of the thalassæmia gene was almost entirely due to a low rate of hæmoglobin A or S formation. The rate of hæmoglobin F synthesis in all adult groups was similar (Table 2).

It is clear from the results presented here that the capacity to form β-chains is not inversely related to the capacity to form γ-chains. Equally high rates of γ-chain or hæmoglobin F formation were observed in thalassæmia, with very low rates of β-chain or hæmoglobin A synthesis, as well as in other hæmolytic anæmias without impaired β-chain synthesis. Further, in fœtal cells, despite high rates of γ-chain formation, the rate of β-chain synthesis exceeded that of the cells from thalassæmic subjects. These data rule strongly against the concept[11] that β-globin synthesis, within a population of erythroid cells, is inversely related, in a specific fashion, to γ-globin synthesis.

If the relative rates of synthesis of hæmoglobins A and F remain constant during the maturation of erythroid cells, and if the survival of these two proteins in the peripheral blood are the same, the ratio of specific activities of hæmoglobin A–F would be equal to one. In studies with cells from subjects with sickle-cell anæmia or acquired hæmolytic anæmia, the average ratio of the specific activity of hæmoglobin A or S to the specific activity of hæmoglobin F was greater than one (Table 2). These results are in agreement with previous observations[4,12,13]. By contrast, in the patients with thalassæmia major or doubly heterozygous for thalassæmia and sickle hæmoglobin genes, the average ratio of the specific activity of hæmoglobin A or S to the specific activity of hæmoglobin F was lower than one (Table 2). Several mechanisms could account for the observed deviations from one in the ratio of specific activities of hæmoglobins A and F. Among these mechanisms are: (*a*) alterations in the relative rates of synthesis of messenger RNA (m-RNA) for different globins; (*b*) different rates of decay in the capacity of erythroid cells to form the different globins as these cells

Table 1. Hæmoglobin A and F Concentrations and Rates of Synthesis in Fœtal Erythroid Cells of Peripheral Blood

	(1) Hb concentration*		(2)	(3) Protein synthesis†		(4)	(5)	(6)
	A %	F %		Total mμmoles leucine + valine incorporated/mg ribosome		A	F	Specific activity of Hb A / Specific activity of Hb F
Average‡	10	90		467		101	354	3·4
S.E.	±8	±8		±190		±54	±140	

*Hæmoglobin A and F concentrations are expressed as the proportion of the total hæmoglobin as determined by column chromatography according to the method of Allen *et al.*[9].
†Average value for 5 studies. *S.E.*, standard error.
‡Total protein synthesis is expressed as the mμmoles of leucine plus valine incorporated into protein in 80 min/mg of ribosomes recovered. The rate of incorporation is referred to ribosome content since synthetic activity of the peripheral red blood cells is more directly related to ribosome content than either the proportion of cells which are reticulocytes or the total number of erythroid cells[17]. The amount of hæmoglobin F and A synthesized was determined by the counts incorporated into the proteins separated by column chromatography on 'Amberlite *IRC*-50'[9]. The non-hæmoglobin proteins were separated by DEAE chromatography[10]. Differences between the sum of columns 4 and 5 and column 3 represent non-hæmoglobin proteins.

Table 2. Hæmoglobin A and F Concentrations and Rates of Synthesis in Adult Erythroid Cells of Peripheral Blood

(1) Subject*	(2) Hb concentration‡		(3)	(4) Protein synthesis‡		(5)	(6)	(7) Specific activity of Hb A / Specific activity of Hb F
	A %	F %		Total mμmoles leucine + valine incorporated/mg ribosome		A	F	
AHA (6)†								
Average	92	8		383		356	23	1·4
S.E.	±3	±3		±230		±225	±10	
S–S (5)								
Average	85	15		365		316	43	1·3
S.E.	±4	±4		±183		±150	±29	
S–Thal (4)								
Average	88	12		143		109	28	0·5
S.E.	±4	±4		±80		±54	±24	
Thal (5)								
Average	55	45		39		19	21	0·7
S.E.	±15	±15		±15		±6	±7	

* Abbreviations: *AHA*, acquired hæmolytic anæmia; *S–S*, sickle-cell anæmia; *S–Thal*, sickle-thalassæmia; *Thal*, thalassæmia major; *S.E.*, standard error.
† No. in parenthesis indicates number of patients studied in each group.
‡ See footnotes for Table 1.

mature; (c) different survival times for hæmoglobin A and F in peripheral blood.

The finding that in fœtal cells the specific activity of A–F exceeds one is most probably explained, as has been previously suggested[11], by a conversion from synthesis of m-RNA for γ-chains to that of m-RNA for β-chains during late fœtal life. The increase in synthesis of hæmoglobin F in cells from subjects with certain hæmolytic anæmias may reflect a similar mechanism, namely, regulation at the level of DNA with an increase in the synthesis of m-RNA for γ-globin[11,14]. However, the present findings with cells from adult subjects can be explained by mechanisms regulating the amounts of hæmoglobins A and F formed which involve sites of control other than at the level of DNA.

In an earlier investigation[15] it was demonstrated that as erythroid cells mature, their capacity to synthesize hæmoglobin decreases, in association with a decay in polyribosome content. It is possible that the templates for different globins decay at different rates. It was previously suggested that the increased level of hæmoglobin F formation in hæmolytic anæmias might result from a decrease in the rate of decay in the capacity of erythroid cells to synthesize this protein as these cells mature under conditions of erythropoietic stress[4]. Such a change in the capacity to form hæmoglobin F could result from the fact that erythropoietic stress is associated with an altered pattern of maturation of erythroid cells, characterized by circulating reticulocytes being derived from early erythroid forms[16,17].

The observation that in reticulocytes from sickle-cell anæmia or acquired hæmolytic anæmia the ratio of the specific activities of hæmoglobin A–F exceeds one could result from a faster rate of decay in the capacity of erythroid cells to synthesize γ-globin (hæmoglobin F) than to synthesize β-globin (hæmoglobin A). A shorter survival of hæmoglobin A than F in the peripheral circulation could also result in a ratio of specific activities of hæmoglobin A–F of greater than one. Investigations of hæmoglobin turn-over in sickle-cell anæmia[18] and thalassæmia[19] demonstrated that hæmoglobin A or S survival is shorter than that of hæmoglobin F. However, a shorter survival of hæmoglobin A or S than F is not an adequate explanation of the findings in patients with the thalassæmia gene. In this work, the specific activity of hæmoglobin A was lower than the specific activity of hæmoglobin F. These findings can be explained by the hypothesis that the amount of a particular globin synthesized by erythroid cells reflects, in part, the relative rate of decay in the template for this protein as the cells mature. The presence of a thalassæmia gene may lead to a selective acceleration in the rate of decay in the template for β-globin.

The hypothesis that different rates of decay of globin templates is a factor controlling the rates of hæmoglobin A and F synthesis predicts that in normal subjects the capacity to form hæmoglobin F should decrease more rapidly than the capacity to form hæmoglobin A. According to this prediction it would be expected that in normal bone marrow the ratio of synthesis of hæmoglobin A–F would be less than the ratio of the concentration A–F in peripheral blood. In bone marrow cells of normal subjects, the ratio of the rate of synthesis of hæmoglobin A–F is less than the ratio of the concentration of A–F in peripheral blood[20,21].

The work recorded here provides evidence that in patients heterozygous or homozygous for the thalassæmia gene there is a marked selective decrease in hæmoglobin A formation. The defect in hæmoglobin A ($\alpha_2\beta_2$) synthesis in this group of patients appears to reflect a primary failure in β-globin synthesis. This is suggested by the finding that the rate of synthesis of hæmoglobin F ($\alpha_2\gamma_2$) is similar in cells from patients with a variety of hæmolytic anæmias with or without the thalassæmia gene. The capacity for hæmoglobin F synthesis in erythroid cells from these subjects is only a fraction of that of fœtal erythroid cells at birth. It is suggested that regulation at the level of the rate of decay in the templates for different globins may be important in the control of hæmoglobin A and F formation in erythroid cells of adult subjects. The conversion from hæmoglobin F to A synthesis in late fœtal life probably involves a different mechanism, such as control of the rates of synthesis of the different m-RNA's.

This work was supported in part by grants *GM–07368* and *CY–02332* from the U.S. Public Health Service and grant *GB–1817* from the U.S. National Science Foundation.

One of us (E. R. B.) was a trainee in hæmatology under the auspices of the U.S. Public Health Service (*TI–AM–5231*).

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DEMONSTRATION OF SYNGENEIC PREFERENCE *IN VITRO*

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As a rule, tumours of homozygous origin take more frequently and after a shorter latency period when small cell numbers (usually below 10^6) are transplanted to syngeneic recipients than to various semi-isologous F_1 hybrid hosts[1–3]. This phenomenon has been termed 'syngeneic preference'[4]. It can be demonstrated with sarcomas[3] and carcinomas[3] as well as with lymphomas[1,2]. Probably analogous findings have been made with hæmatopoietic cells, transplanted to lethally irradiated hosts[5,6].

In an attempt to examine the mechanism of syngeneic preference, iso-antigenic variant sublines were used, isolated from a mouse lymphoma of *A/Sn* × *A.SW* F_1 hybrid origin by selective passage in one of the parental strains[1]. The variants were selectively compatible with the parental strain of selection and had irreversibly lost the

H-2 isoantigens specifically derived from the opposite parental strain[7]. When compared with the *A* × *A.SW* F_1 tumour line, from which they had been selected, in *A* × *A.SW* F_1 recipients, the variants grew more slowly[1]. However, in their compatible parental strain they grew faster than in the hybrid, and behaved in the same way as did the unselected line in the hybrids. The differential behaviour of the variants in homozygous versus F_1 hybrid animals was maintained when the mice had been pre-irradiated with 450–500 r. in order to decrease their immunological reactivity[1] and it did not increase after pretreating the mice with sub-limit doses of the variant cells or normal cells of the strain of variant selection[8]. Since the *A* and *A.SW* strains are co-isogenic resistant lines differing mainly at the *H*-2 locus, the decreased growth capacity of the variant cells in the F_1 hybrids could be tentatively attributed to their loss of *H*-2 antigens resulting in a difference between graft and host on their inoculation into F_1 hybrids.

Among conceivable mechanisms of syngeneic preference, one possibility is that exposure of the cells to foreign histocompatibility antigens may have a cytopathogenic or growth inhibitory effect[1]. This article describes experiments designed to test this possibility. Mouse tumour cells cultured *in vitro* were exposed to homogenates prepared from a foreign mouse strain or to antigenic extracts, prepared according to the technique of Haughton[9], similar to that described earlier by Davies[10].

Two methylcholanthrene - induced sarcomas were used: *MC57S* of *C57BL* origin, and *MACD* of *A/Sn* × *A.CA* F_1 hybrid origin. Both tumour lines had been maintained by serial transplantation in syngeneic hosts for 20–30 and 45–52 passages, respectively. Tissue cultures were prepared by methods described previously[11], which involved addition of penicillin (100 I.U./ml.) and streptomycin (100 μg/ml.) to the medium. Trypsinized cell suspensions were made from transplanted tumours to start primary cultures. Secondary cultures were made from these 2–4 days afterwards, when roller tubes were inoculated with $1{\cdot}5 \times 10^6$ cells in 1 ml. of medium, consisting of 10 per cent (v/v) heat-inactivated calf serum in Earle's salt solution, to which 0·5 per cent (w/v) lactalbumin hydrolysate had been added. When the cells had formed layers, 48 h after explantation, the medium was replaced by 1 ml. of a mixture of 2 per cent (v/v) heat-inactivated horse serum in Parker 199 solution, to which homogenates or antigenic extracts were added. The tubes were placed in a drum, rotated at a speed of 4 r.p.m. As a rule, the number of trypan-blue unstained cells in each tube was determined two days later for 2–6 tubes of each series, after the cells had been detached from the glass by versene treatment. The tubes were given code numbers, unknown to the person performing the counting.

The homogenates were prepared under sterile conditions from the spleens of *C57BL*, *A/Sn* or *A/Sn* × *C57BL* F_1 hybrid mice weighing about 20 g. Three spleens of each type were homogenized into 3 ml. of Earle's salt solution. In order to kill possibly surviving cells, the homogenates were frozen in dry ice and thawed four times, after which they were kept at +4° C and used within 2 weeks. The homogenates were added in 0·1-ml. volumes to the medium of the cultures, either undiluted or after 1 : 2, 1 : 3 or 1 : 10 dilution (Fig. 1). The antigenic extracts were prepared according to the technique of Haughton[9]. They contained 33, 25–50 and 30 antigenic units per mg, as defined by Haughton[9]. Extracts were made from the strain *A/Sn* carcinoma *TA*3, from *C57BL* spleen cells and from lymphoma *EL*4 of *C57BL* origin. Lyophilized antigenic extracts were sterilized by ultraviolet irradiation, and suspensions of 75-mg lyophilized antigenic extract were made in 8 ml. of Earle's salt solution, and were added in 0·1-ml. volumes to the cultures, either undiluted or after 1 : 2 or 1 : 10 dilution.

The results are presented in Figs. 1 and 2. Fig. 1 shows that cultures exposed to allogeneic homogenates contained regularly fewer trypan-blue unstained cells than control groups, exposed to syngeneic homogenates. Since all preparations were tested on *MACD* and *MC75S* cells as well, a non-specific toxic effect could not explain the results obtained. The two tumours examined were sensitive to about the same degree. In a few experiments (not included in Fig. 1) *MACD* and *MC57S* cultures were exposed to homogenates prepared from *A* × *C57BL* F_1 hybrids in a similar way as from *A* or *C57BL*. Cultures treated with these homogenates (allogeneic to both tumours) contained regularly fewer cells than cultures treated with syngeneic homogenates.

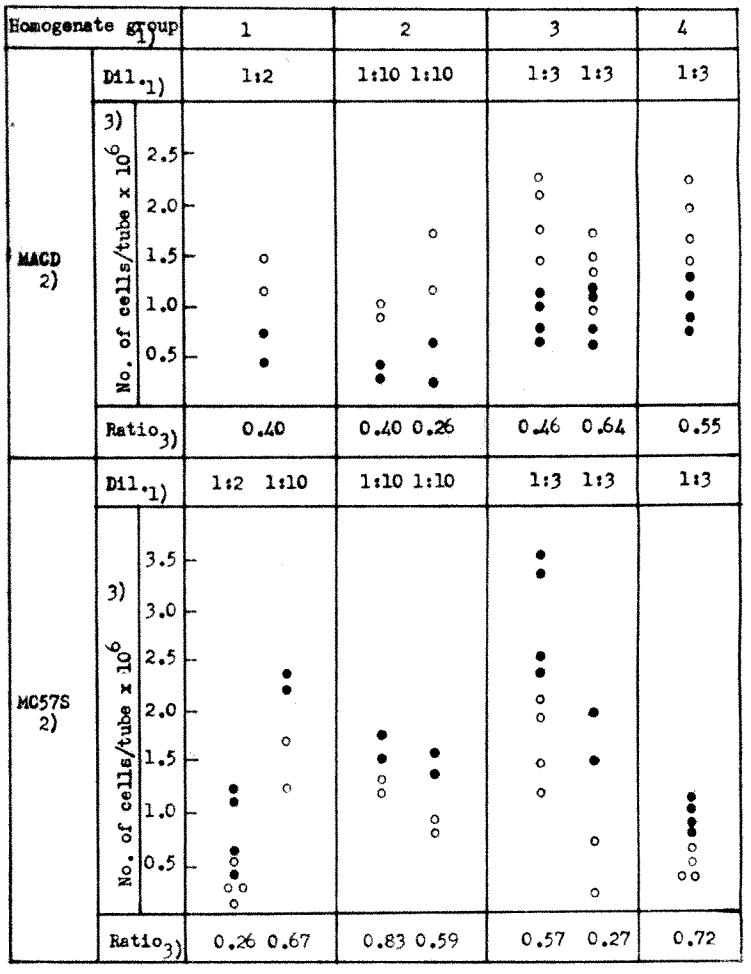


Fig. 1. Effect of allogeneic and syngeneic homogenates on cultured tumour cells. (1) Four different groups of homogenate were used, each group consisting of one sample of *A/Sn* and one sample of *C57BL* homogenate. The spleens of 3 *A/Sn* and 3 *C57BL* mice were homogenized into 3 ml. Earle's salt solution, after which the homogenates were diluted 1 : 2, 1 : 3 or 1 : 10. (2) *MACD* and *MC57S* are both methylcholanthrene-induced fibrosarcomas, of *A* × *A.CA* F_1 and *C57BL* origin, respectively. (3) The number of cells was determined two days after addition of the homogenates to the cultures, that is, 4 days after explantation. The cells were detached from the glass surfaces by versene. The number of trypan-blue unstained cells in millions per tube is shown for each tube counted. ○, Tube treated with *A/Sn* homogenate, ●, Tube treated with *C57BL* homogenate. The ratios $\frac{\text{cell No. with allogeneic homogenate}}{\text{cell No. with syngeneic homogenate}}$ were calculated

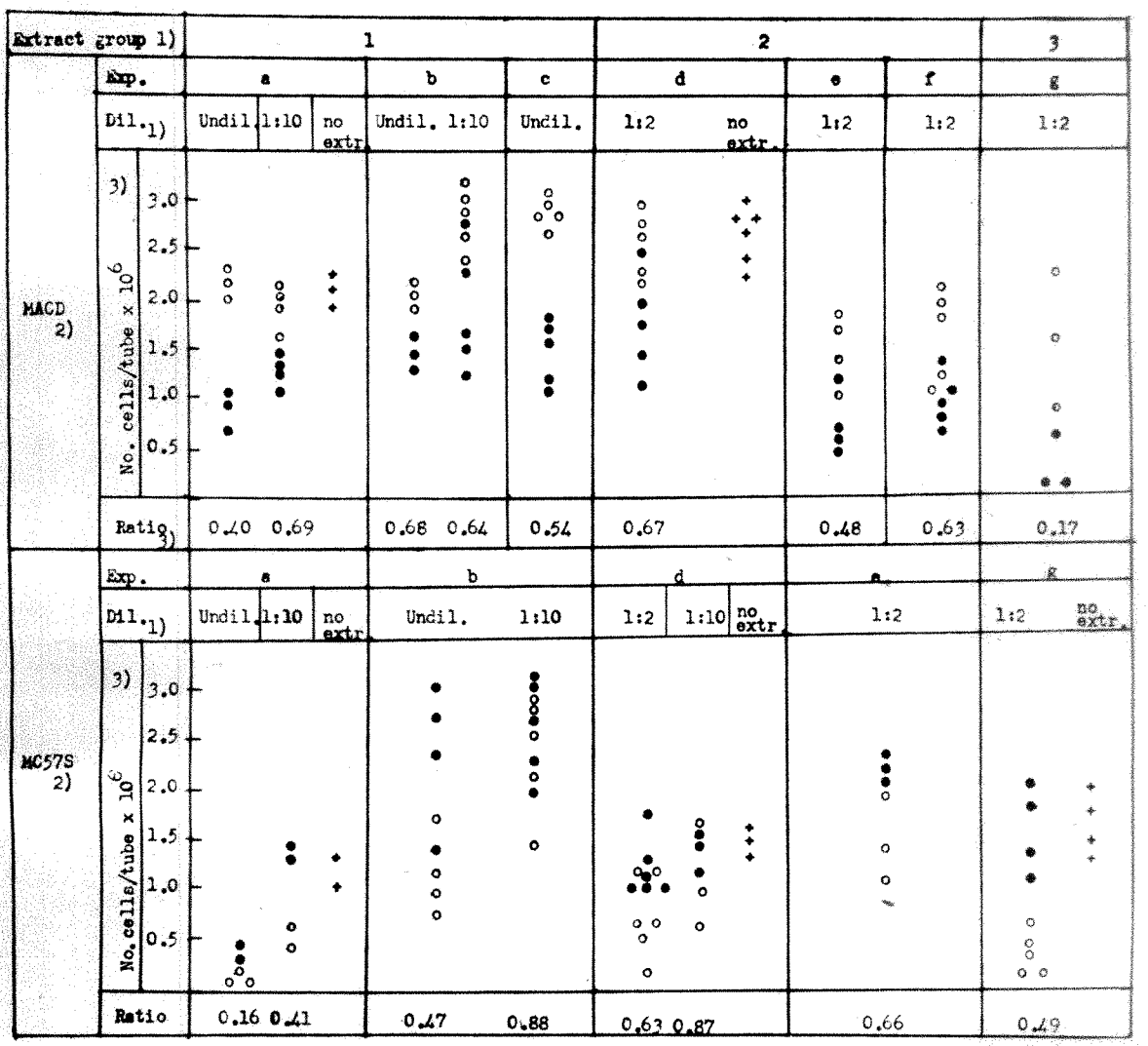


Fig. 2. Effect of antigenic extracts containing foreign *H*-2 isoantigens on cultured tumour cells. (1) Three different groups of antigenic extract were used, all containing one extract of *A/Sn* origin (from tumour *TA*3) and one extract of *C57BL* origin. In groups 1 and 3 the *C57BL* extract was prepared from spleen cells (preparations made on two different occasions), and in group 2 from ascites lymphoma *EL*4 of *C57BL* origin. The *TA*3 extract contained 33 antigenic units/mg, the *C57BL* spleen extracts 25–50 units/mg and the *EL*4 extract 30 units/mg, as defined by Haughton[9]. Cultures received 0·94 mg undiluted extract/tube. (2) See footnote 2 to Fig. 1. (3) See footnote 3 to Fig. 1. ○, Tube treated with *A/Sn* antigenic extract. ●, Tube treated with *C57BL* extract; +, no antigenic extract added

Confirmatory results were obtained with the antigenic extracts (Fig. 2): cultures exposed to allogeneic extracts contained fewer cells than cultures exposed to syngeneic extracts. There was no difference in the number of living cells between groups which received syngeneic extracts and those which did not receive any extract except in one experiment with *MC57S*, where cultures receiving undiluted *C57BL* extract contained fewer cells than untreated controls or cultures receiving *C57BL* extract diluted 1 : 10.

The findings presented in Figs. 1 and 2 concern cell numbers two days after addition of homogenate or antigenic extract. This interval was chosen for the majority of experiments, since cell layers often became detached from the glass surfaces at later intervals in untreated controls as well. A clear-cut difference was, however, already found between the cell numbers in experimental and control groups 36 h after the addition of the antigenic material and was maintained for 6–8 days, whereafter the majority of cells became detached from the glass surfaces.

Although it seems most likely that the inhibitory effect found with allogeneic homogenates and antigenic extracts is due to their content of foreign isoantigens, it remains to be definitely proved that the antigenicity, *per se*, is involved. Experiments performed with extracts and tumours from co-isogenic-resistant mouse strains, known to differ only at locus *H*-2, may help to elucidate this point. Furthermore, it may be worth attempting to neutralize the inhibitory effect of allogeneic antigenic extracts by specific isoantibodies.

It follows from the findings described that syngeneic preference, detected by transplantation of tumour cells into mice, is probably due to the exposure of the grafted cells to the foreign isoantigens of the host and not, for example, to recessive histocompatibility factors.

It may be postulated that syngeneic preference has a homeostatic function helping to maintain the genetic equipment of the somatic cells constant by eliminating cells which lack certain isoantigens (as a result of somatic mutation, for example). The present findings make it possible to approach this question by studying the interaction between cells and foreign isoantigens under well-defined conditions and to determine the effects of various amounts and various types of antigen added ('strong' and 'weak' histocompatibility antigens, tumour specific antigens). It will also be possible to investigate whether differences in sensitivity to the foreign isoantigens exist between dividing and non-dividing cells and whether the cellular sensitivity can be modified by various means.

This investigation also suggests that it may be possible to select *in vitro* for cells that have gained certain antigens, for example, by fusion[12], from populations where the majority of cells lack the same antigen. Such selection has

been successfully performed *in vivo* in model experiments, involving artificial mixtures of tumour cells of different genotypes[13]. Selection *in vitro* is an advantage since cells which do not grow progressively *in vivo* can also be studied.

This work was supported by grants from the Swedish Cancer Society, the Swedish Medical Research Council, the Wallenberg Foundation, and by grant C–3700 to Prof. G. Klein from the National Cancer Institute, U.S. Public Health Service. One of us (G. H.) is grateful to the International Union against Cancer for an Eleanor Roosevelt Fellowship. We thank Prof. G. Klein for his support and Misses Barbro Jansson, Maj Britt Sondell and Elsbeth Zimmermann for assistance.

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FAILURE TO RECONSTITUTE NEONATALLY THYMECTOMIZED MICE BY 'SUCCESSFUL' RAT THYMUS TRANSPLANTATION

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THE immunological deficit and growth failure of neonatally thymectomized mice may be prevented by several procedures performed within the first three weeks of life: grafting of syngeneic or allogeneic thymus[1,2]; injection of adult syngeneic spleen or lymph node cells[2–4]; injection of new-born or adult allogeneic or syngeneic thymus cells[5,6], and intraperitoneal implantation of 'Millipore' chambers containing thymic tissue[7,8]. This last group of investigations confirmed the existence of a subcellular, humoral, and presumably hormonal, factor in the mouse thymus which permits development of the peripheral lymphoid tissues and immunological competence. Such a factor had been suggested by the findings of Miller[1] and Dalmasso *et al.*[2] that the lymphoid cells of the spleens of neonatally thymectomized mice grafted with allogeneic thymus were largely of host origin, as determined by the *T6* marker[1] and histocompatibility characteristics[2]. Significant restoration of lymphoid tissue structure and immunological function in neonatally thymectomized rats by thymus tissue contained in 'Millipore' membranes has also been reported recently[9].

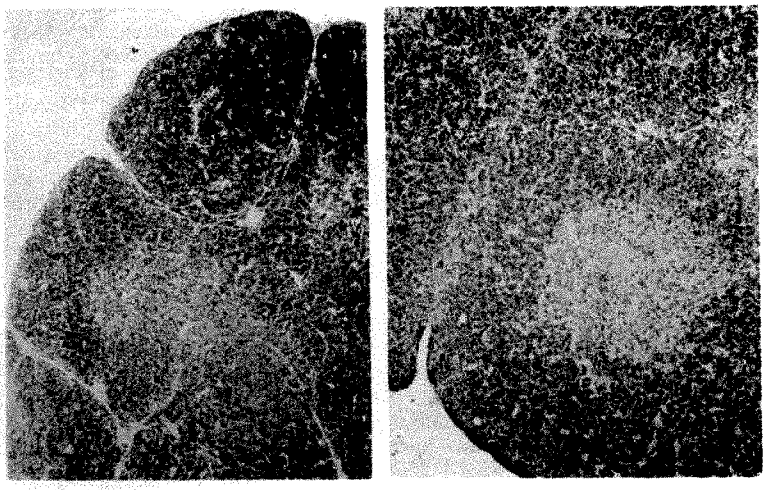
The work to be reported here was designed to ascertain whether xenogeneic thymus grafts can be successfully accomplished and prevent the growth failure and immunological deficiency of neonatally thymectomized mice. These experiments stemmed directly from the preliminary observations of Dalmasso *et al.*[2] that rat thymus grafts may take in neonatally thymectomized mice but do not seem to reconstitute the capacity of the spleen cells of the animals to exercise a graft versus host reaction.

Mice of the *C3H* strain of both sexes were thymectomized within 24 h after birth by the method routinely performed in this laboratory[10]. One group was not treated further; three others received thymus grafts, either one lobe of thymus from new-born albino rats of the Holtzman line, an entire new-born syngeneic thymus, or an entire new-born hemi-allogeneic $(A \times C3H)F_1$ thymus. All grafts were cut part way through in two or three places and placed into the subcutaneous tissue of the back. Eight rat-thymus-grafted mice were killed 5, 10, 15 and 20 days after grafting for microscopic evaluation of the graft. Lymphocyte counts were done at 4–5 weeks of age for animals in all the groups. At 5–6 weeks of age, several rat-thymus-grafted and syngeneic-thymus-grafted mice were killed, the subcutaneous tissues of the back examined both grossly and microscopically for presence of thymus graft, and the capacity of their spleen cells to exercise graft versus host reactions in F_1 hybrid recipients was compared with that of intact *C3H* mice and ungrafted neonatally thymectomized animals. Two additional rat-thymus-grafted mice were splenectomized at 40 days of age, the thymus grafts removed, and the animals followed until death. The assay of graft versus host reactivity of Simonsen was used[11]: it involves administration of 30 million spleen cells to 8-day-old mice hybrid to the host histocompatibility characteristic. Splenomegaly is assessed 8 days later by determination of the relative spleen weight (mg/100 g body-wt.) and finally calculation of the spleen index, the mean relative spleen weight of the experimental group divided by the mean relative spleen weight of hybrids of the same litters receiving syngeneic cells (negative controls). An index of 1·30 or more is, in these laboratories, considered indicative of significant graft versus host activity. The remaining animals were weighed every third day. All mice dying of wasting disease, killed for the Simonsen assays, or surviving at 120 days were



Fig. 1. Portion of a rat-thymus graft removed from a neonatally thymectomized *C3H* mouse five days after placement. There is some depletion of the cellular elements (compare with Fig. 2), although not so much as has been observed in mouse thymus grafts in some studies

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A *B*

Fig. 2. Comparison of a rat thymus graft, removed from a neonatally thymectomized *C3H* mouse 24 days after placement (*A*), with a normal rat thymus of the same age (*B*). The two show comparable cellularity, and the essential structure of the normal thymus seems to be present in the graft

autopsied. The retrosternal areas of all neonatally thymectomized animals were examined for thymic remnants under the dissecting microscope, and the soft tissues of the mediastinum sectioned for microscopic study. Mice found to be incompletely thymectomized are not included in this report. In all thymus-grafted animals the graft found in the subcutaneous tissue of the back was examined grossly and microscopically.

These experiments confirm the earlier preliminary finding that neonatally thymectomized mice regularly accept rat thymus grafts, just as they sometimes accept rat skin[3]. The successful grafts included all eight of the animals killed 5–20 days after grafting, four of the five splenectomized or killed at 5–6 weeks for graft versus host assays, and eight of the 12 dying of wasting disease. At 5 and 10 days, the grafts showed some necrosis and some loss of lymphoid elements or both (Fig. 1), similar to that described by others[12,13] in sequential investigations of thymus grafts. After 10 days and up to 39 days, the vascularized rat thymus grafts had epithelium, Hassall's corpuscles, approximately normal numbers of lymphoid elements, and even organization into cortex and medulla (Fig. 2). Morphologically they were very similar in appearance to the successful syngeneic and hemi-allogeneic thymus grafts examined. Chromosome analyses of the grafts were not carried out, and the relative proportion of rat and mouse lymphocytes in the repopulated grafts was not determined.

Despite the apparent success of the rat thymus grafts, early mortality was as characteristic of neonatally thymectomized mice receiving thymus grafts from new-born rats as it was of untreated neonatally thymectomized mice. These results contrast with the high percentage of survival at 120 days of the neonatally thymectomized mice grafted with new-born syngeneic or hemi-allogeneic thymus (Table 1). Fig. 3 shows the growth curve, age at death, and graft status of each of the rat-thymus-grafted animals. Despite the presence of the well-vascularized, normal-appearing rat thymus graft in most of these animals, they all developed weight loss, diarrhœa, hunched appearance, and ruffled fur, the typical post-thymectomy wasting syndrome, and succumbed between 29 and 66 days of life. For comparison, mean body-weights of neonatally thymectomized mice are given in the figure, as are comparable data for neonatally thymectomized mice receiving hemi-allogeneic thymus grafts. Fig. 4 shows that the transplantation of new-born rat thymus did not restore the numbers of circulating lymphocytes of neonatally thymectomized mice toward normal. By contrast, studies in our laboratories[14] and elsewhere[3] have shown restoration of circulating lymphocyte levels as a regular consequence of early transplants of syngeneic or allogeneic thymus.

Recorded in Table 2 are investigations indicating that ungrafted or rat-thymus-grafted neonatally thymectomized mice do not develop immunological competence, as assessed by the graft versus host reaction of Simonsen. By contrast, intact mice or syngeneic thymus-grafted neonatally thymectomized animals had spleen cells with

Table 1. Effect of Thymus Grafts from New-born Mice and Rats on the Longevity of Neonatally Thymectomized *C3H* Mice

Thymus graft donor	Survival rate at 120 days
None	0/24*
New-born *C3H* mice	7/8
New-born $(A \times C3H)F_1$ hybrids	5/6
New-born rat	0/12*

* All animals in these groups had died before 85 days of age.

Table 2. Graft-versus-host Assay of Spleen Cells from Neonatally Thymectomized Mice receiving Syngeneic and Xenogeneic Thymus Grafts

Recipient	Thymus graft donor	Test litters	Spleen indices*
Intact *C3H*	None	$(C3H \times DBA/2)F_1$	1·85, 2·40, 3·40, 3·80, 4·20
Neonatally thymectomized *C3H*	New-born *C3H*	$(C3H \times DBA/2)F_1$	1·14, 1·51, 2·28, 2·86, 3·20
Neonatally thymectomized *C3H*	New-born rat	$(C3H \times DBA/2)F_1$	0·85, 0·89, 1·03, 1·14, 1·28
Neonatally thymectomized *C3H*	None	$(C3H \times DBA/2)F_1$	0·82, 0·98, 0·99, 1·03, 1·03, 1·09, 1·15, 1·17, 1·32

* The spleen index is calculated by dividing the relative spleen weight (mg/100 g/body wt.) of the test animals by the mean relative spleen weight of the negative controls.

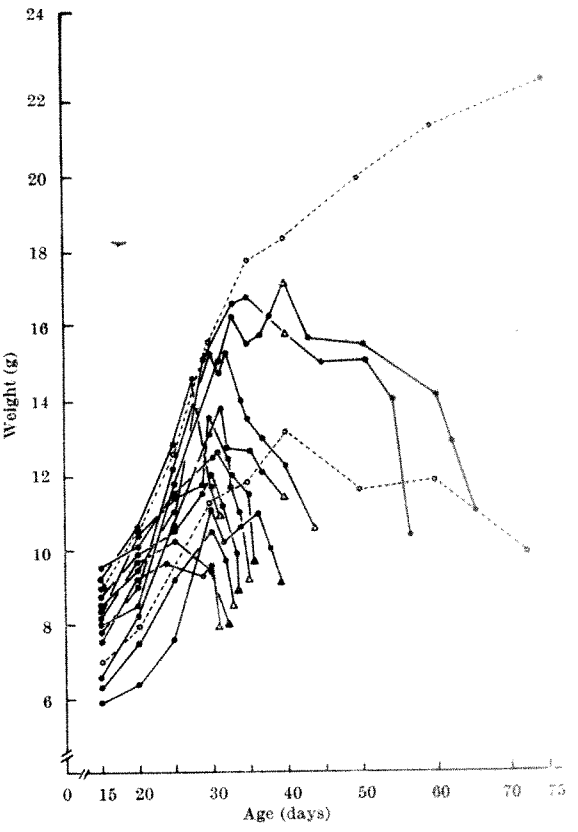


Fig. 3. Weight curves of neonatally thymectomized *C3H* mice bearing rat thymus grafts (solid lines): an open triangle indicates a vascularized thymus graft at autopsy or biopsy, a solid triangle a rejected graft. The lower broken line represents the mean weights of neonatally thymectomized mice not receiving grafts; the upper broken line represents the mean weights of neonatally thymectomized mice receiving hemi-allogeneic $(A \times C3H)F_1$ grafts. None of the rat-thymus-grafted mice escaped the wasting syndrome

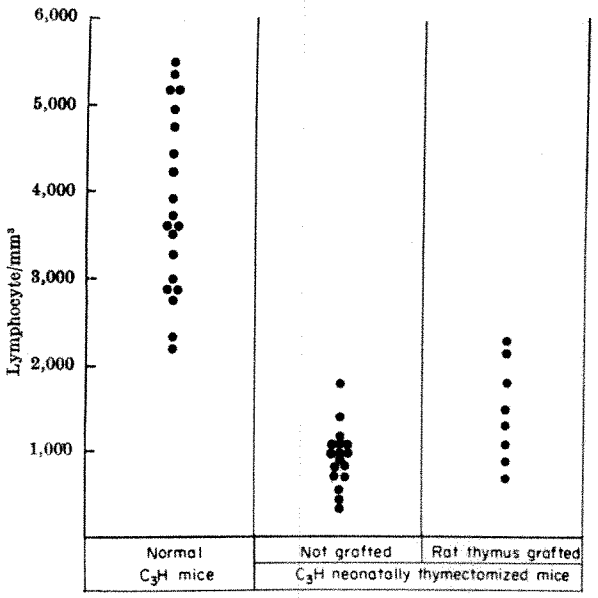


Fig. 4. Lymphocyte counts, at 4–5 weeks of age, of rat-thymus-grafted neonatally thymectomized *C3H* mice compared with those of normal animals and untreated neonatally thymectomized mice of the same strain

vigorous capacity to produce splenomegaly after intraperitoneal injection into appropriate 8-day-old hybrid mice.

The lack of effect of the 'successful' rat thymus grafts on the wasting disease, lymphoid tissue depletion, and immunological deficit of neonatally thymectomized mice suggests that the thymic hormone(s) may be proteins and may operate through mechanisms demanding species specificity. There is, however, a precedent for a thymic effect on lymphopoiesis across species barriers in the finding that extracts of human thymus produce a lymphocytosis in baby mice[15].

It is possible, of course, that quantitative factors are involved in our results, and that the effect of the rat thymus graft in these animals was below the sensitivity of the assay technique used. In the present experiments the rat thymus grafts were at least as large and, from a morphological point of view, as successful as the mouse thymus grafts, but the possibility exists that a larger number of rat thymus grafts, or even rat thymus grafts in different locations, might provide evidence of reconstitution. It may also be that immunological processes insufficient to result in morphological rejection of the graft could inhibit production of a hormonal factor by the rat thymus stroma in the mouse.

Whatever the explanation, our observations indicate clearly that rat thymus grafts can regularly be apparently successful in neonatally thymectomized mice and yet fail to give evidence of significant physiological or immunological effects on neonatally thymectomized mice.

We thank Mr. K. Sjodin for performing the thymectomies in the neonatal mice.

This work was aided by grants from the U.S. Public Health Service (*HE*–02085, *AI*–00798, *C*–3511), the National Foundation, the American Cancer Society and the American Heart Association.

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GENERALIZED HOVERING-FLIGHT CORRELATION FOR INSECTS

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It has been proposed[1–4] that the three major forces on a fluttering wing-body system are gravitation, air acceleration and aerodynamic lift such that the resultant produces body oscillation superposed on continuous directional motion. Under hovering conditions digitally computed flight trajectories have shown[3,4] that, for four insects and a bird, the aerodynamic lift arising from wing flutter represents on average 25 per cent of the force needed to cancel gravity, the remaining 75 per cent being generated by air acceleration resulting from the non-uniform wing motion (wave-form); while the aerodynamic lift contribution is not small, it is by no means enough to balance gravity, an observation that explains why most of the earlier theories based on aerodynamic lift considerations alone were inadequate to explain the phenomenon of insect and bird flight. Furthermore, the average experimental accuracy of most insect flight data[5–7] of value in the context of the present analysis is of the order of ± 25 per cent, as estimated by Weis-Fogh and Jensen[8], the aerodynamic lift contribution being, therefore, of the same magnitude as the experimental error, so that for purposes of simplicity it would seem feasible, within this error, to approximate the body velocity $u(t)$ by only two terms, one due to gravity, $-gt$, and the other due to non-uniform air acceleration as follows:

$$u(t) \approx -gt - (\gamma\varphi/M)\int_0^t P_{\tau\tau}(k, \omega\tau + \varepsilon)\cos[\varphi P(k, \omega\tau)]\mathrm{d}\tau \quad (1)$$

where

$$\gamma = 8\rho(1 + \varkappa)\varphi S m_2 \quad (2)$$

$$m_2 = (1/S)\int_0^L r^2 W(r)\mathrm{d}r \quad (3)$$

$$S = \int_0^L W(r)\mathrm{d}r \quad (4)$$

The periodic wave-form $P(k, \omega\tau)$ of overall angular frequency, ω, can be simulated by two half sinusoids of different frequencies either joined at their bases (S-wave-form) or at their peaks (Z-wave-form), the ratio of the highest to the lowest frequency being the duration factor k—because of inertial and viscous effects the air acceleration force may lag behind the wing motion by a phase angle ε as shown in equation (1). In this connexion digital computations have shown[4] that the integral air acceleration term of equation (1) is the sum of two parts, one of which increases linearly with time and can be represented by $(\mu\gamma\varphi/M)(\omega_U^2 - \omega_D^2)t$, the remaining part being a smooth oscillation simulated by $-(\gamma\varphi\omega^2/M)\int_0^t \cos(\omega\tau + \varepsilon)$

$\cos(\varphi \cos \omega\tau)d\tau$ so that equation (1) can be replaced by the following functional approximation:

$$u(t) \approx [-g + (\mu\gamma\varphi/M)(\omega_U^2 - \omega_D^2)]t - (\gamma\varphi\omega^2/M)\int_0^t \cos(\omega\tau + \varepsilon)\cos(\varphi\cos\omega\tau)d\tau \quad (5)$$

where μ is an empirical constant, the value of which must be adjusted to fit the particular air acceleration term under consideration, it being possible to obtain fits within 5 per cent using just the one constant.

According to relation (5) the effect of gravity can be nullified by setting the coefficient of t equal to zero, while motion away from Earth can be obtained by making the same coefficient positive, so that using expression (2) for the acceleration factor γ and remembering that $\omega_U = (k + 1)\omega/2$ and $\omega_D = (k + 1)\omega/2k$, these being the upstroke and downstroke frequencies respectively with $k = \omega_U/\omega_D$, then the criterion for levitation or antigravity motion is:

$$-Mg + 8\rho(1 + k)\mu\omega^2\varphi^2 KSm_2 \geqslant 0 \quad (6)$$

where $K(k) = (k - 1)(k + 1)^3/4k^2$; $k > 1$

In criterion (6) $\varkappa$ is the coefficient of virtual mass for the air displaced by the wings when acting as paddles, the digitally computed flight trajectories for a bird, locust, moth, fly and bee having shown[3] that $1 + \varkappa = 2{\cdot}3 \pm 0{\cdot}7$ with a Z-wave-form at $\varepsilon = -3\pi/4$—similar calculations[4] under the same hovering conditions have revealed that the dimensionless constant μ must have values of 0·125, 0·056, 0·099, 0·047 and 0·096 respectively to approximate the acceleration term for the same bird, locust, moth, fly and bee, the overall average being $\mu = 0{\cdot}085 \pm 0{\cdot}026$, no trend with either animal size or wing frequency being detectable for either $\varkappa$ or μ. The average deviations of $\pm 0{\cdot}7$ and $\pm 0{\cdot}026$ respectively for the dimensionless coefficients $1 + \varkappa$ and μ correspond to variations of ± 30 per cent from the mean, this average deviation being the same magnitude as the average experimental error (± 25 per cent) in measuring the wing frequency ω and the angular half amplitude φ—that the use of mean values for $\varkappa$ and μ is quite reasonable is justified by the final correlation in Fig. 1 of the extensive insect data of Table 1, the theoretical line based on criterion (6) roughly bisecting the experimental points, thereby lending statistical significance to the use of fixed values of $\varkappa$ and μ. Before inequality (6) can be applied to the quantitative behaviour of insects and birds, it is first necessary to generalize, if possible, the position of the second wing moment m_2, which takes into consideration possible variations in wing geometry and size—since previous workers did not foresee any need for taking into account wing shape, except qualitatively, such geometrical data are not available in the literature.

However, I have made geometric measurements on the wings of a variety of insects including bees, wasps, flies, flying beetles, moths and butterflies and have found the second moment defined by relation (3) to be remarkably consistent in that it can be located with respect to the overall length of the wing L by the simple relationship $m_2 = (0{\cdot}36 \pm 0{\cdot}03)L^2$, the deviation being the average. While not used in the present analysis, it is also interesting to note that the first wing moment, $m_1 = (1/S)\int_0^L rW(r)dr$, which locates the centre of mass or centroid of the wing, can be similarly generalized by the relation $m_1 = (0{\cdot}54 \pm 0{\cdot}03)L$. The consistency with which m_1 and m_2 may be simply predicted for a wide range of insects indicates that the first and second wing moments are either inherently insensitive parameters or that a process of evolution has standardized them at aerodynamically optimum values, regardless of wing size and shape. The procedure for

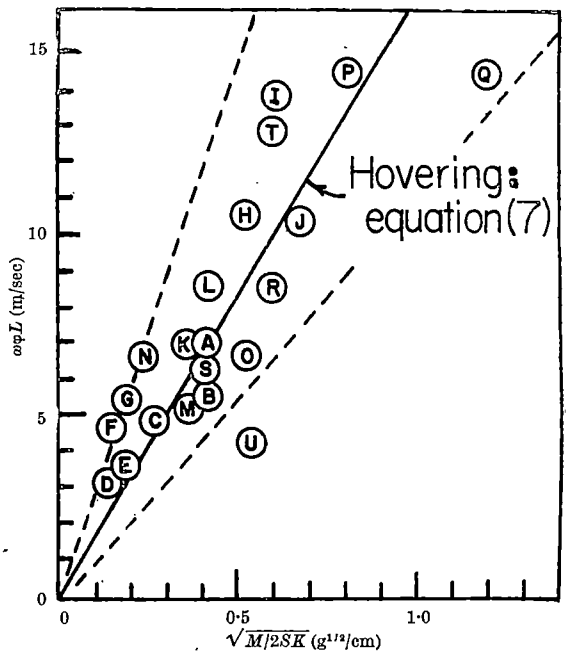


Fig. 1. Correlation of the insect results from Table 1 by hovering (equation 7). The broken lines define the experimental error and the letters denote particular insects in Table 1

Table 1. Dimensions and Performance Data of Insects after Magnan[5], Osborne[6] and Sotavalta[7]

Letters for Fig. 1	Insect	Mass M (g)	Wing surface S (cm^2)	Length of wing L (cm)	Wing loading $M/2S$ (g/cm^2)	Stroke half-angle φ (radians)	Observed frequency f (c/s) Magnan	Sotavalta	Duration factor (k)
	Lepidoptera								
A	*Macroglossa stellatorum* L.	0·345	2·0	2·0	0·086	0·70	85	73	1·3
B	*Plusia gamma* L.	0·144	2·2	1·8	0·033	1·05	48	48	1·1
	Neuroptera								
C	*Brachytron pratense* Mull.	0·557	6·0	3·65	0·046	0·65	33	—	1·4
D	*Calopteryx splendens* Harr.	0·120	4·2	3·0	0·014	1·00	16	—	1·6
E	*Pyrosoma minium* Harr.	0·038	1·78	2·5	0·011	0·87	27	—	1·2
F	*Panorpa communis* L.	0·030	0·88	1·45	0·017	1·3	28	50	1·6
G	*Aeschna mixtra* Latr.	0·530	6·9	3·95	0·038	0·61	38	30 to 40	1·7
	Coleoptera								
H	*Melontha vulgaris* Fabr.	0·961	2·01	2·8	0·24	1·13	46	62	1·5
I	*Cetonia aurata*	0·537	1·30	2·0	0·20	(1·2)*	86	100	1·3
J	*Lucanus cervus*	2·60	4·00	3·6	0·33	1·40	33	—	(1·4)†
K	*Telephorus fuscus*	0·109	0·58	1·25	0·094	1·26	72	—	1·4
	Diptera								
L	*Tabinus bovinus*	0·276	0·92	1·55	0·15	0·79	96	130	1·5
M	*Sarcophaga carnaria* L.	0·45	0·18	0·70	0·12	0·66	160	200	1·5
N	*Musca domestica*	0·012	0·10	0·55	0·056	0·79	190	200	1·7
O	*Volucella pellucens* Meig	0·073	0·38	1·2	0·096	0·66	120	150	1·2
	Hymenoptera								
P	*Xylocope violacea*	0·614	0·86	1·8	0·36	(1·0)*	130	—	1·3
Q	*Bombus terrestris* Fabr.	0·388	0·71	1·6	0·27	(1·0)*	130	158	1·1
R	*Vespa germanica*	0·187	0·49	1·4	0·19	0·79	110	139	1·3
S	*Vespa crabo* L.	0·567	1·30	2·25	0·22	0·44	100	105	1·8
T	*Apis mellifica* L	0·078	0·21	0·85	0·19	(1·0)*	250	240	1·3
U	*Amonophila sabulosa* V. del	0·045	0·21	0·90	0·11	0·61	120	140	1·2

* Angular amplitude adopted by Osborne (ref. 6). † Duration factor indicated by computer calculations.

determining the wing geometry was to trace carefully the outline of the wing on transparent graph paper so that, after drawing a straight line from the root of the wing to the tip, corresponding values of the chord length $W(r)$ as a function of distance r from the root could be taken and graphically integrated to obtain m_1 and m_2; in effect such a procedure transforms the asymmetric wings found in Nature into their axially symmetric equivalents of the same area S as defined by expression (4). Thus, using the generalizations that $\varkappa = 1{\cdot}3$, $\mu = 0{\cdot}085$ and $m_2 = 0{\cdot}36L^2$, inequality (6) is readily transformed into the following hovering-flight criterion:

$$\omega\varphi \geqslant \omega_c\varphi_c = \frac{1{\cdot}33}{L}\sqrt{\frac{Mg}{K\rho S}} \qquad (7)$$

According to criterion (7), if the product of wing frequency and angular half amplitude exceeds a certain critical value $\omega_c\varphi_c$ then the fluttering wing-body system will rise against gravity, while $\omega\varphi = \omega_c\varphi_c$ corresponds to hovering and $\omega\varphi < \omega_c\varphi_c$ to fall.

Criterion (7) predicts that, as the wing loading (mass per unit area, $M/2S$) increases, the frequency ω must in general increase, since there are limitations on increases in the stroke half-angle φ. Table 1 summarizes the insect data of Magnan[5] as supplemented by Osborne[6] and Sotavalta[7], and it is interesting to note that the insect orders can be roughly arranged in a sequence of ascending wing loadings with corresponding ascending wing frequencies as follows: Lepidoptera (moths and butterflies) $\lesssim$ Neuroptera (lacewings and snake flies) $\lesssim$ Diptera (flies and mosquitoes) $\lesssim$ Hymenoptera (bees and wasps)—the Coleoptera (flying beetles) appear to be an exception presumably because of the high stroke angles and because the wing loadings of Table 1 and Fig. 1 do not include the area of the stationary wing covers or elytra which could contribute somewhat to the lift by being immersed in the velocity field created by the fluttering membranous wings, the area of the latter only being used as the basis for the present calculations. Although not shown in Table 1 because the duration factors were not available, most of the Lepidoptera reported by Osborne[6] exhibited wing frequencies of 10–12 c/s with correspondingly low wing loadings of 0·007–0·013 g/cm^2.

Another interesting observation from critical relation (7) is that, as the air density ρ decreases with increasing altitude, the insect or bird should increase either its flapping frequency or stroke angle to attain the same gravitational stability. The results of Chadwick[9–11] and Williams[9] for insects show that, although ω and φ in fact increase as ρ decreases, a rigorous analysis of density effects is obscured by small compensations in numerous other parameters. Furthermore, since the single wing area S will be a function of the wing length L, then criterion (7) predicts that the wing frequency is proportional to $L^{-3/2}$ if the width parameter for the wings is held constant and proportional to L^{-1} if the total wing surface is invariant. Previous models for insect flight behaviour based on considerations of wind forces or wing mass indicate that the beat frequency may vary as $L^{-1/2}$ (refs. 12 and 13), $L^{-2/3}$ (ref. 7) and L^{-1} (refs. 14 and 15). The inverse relationship between wing frequency and wing length is supported by the careful experimentation of Weis-Fogh[16] on the swarming desert locust—he found that ω was proportional to $L^{-2/3}$, it being pointed out by Weis-Fogh and Jensen[8] that the somewhat crude methods of estimating the length parameter do not permit a distinction to be made between powers of L in the range $-0{\cdot}5$ to $-1{\cdot}0$. In the experiments of Weis-Fogh and Jensen, L was taken as the geometric average of the fore-wing length and the length of the hind femur.

In order to test the validity of criterion (7) for the insect data of Magnan[5], Osborne[6] and Sotavalta[7] in Table 1, Fig. 1 has been constructed using the wing-tip velocity $\omega\varphi L$ as the ordinate and the group $\sqrt{M/2SK}$ as the abscissa. The 'hovering line' that divides the field into rising and falling regions corresponds to the critical hovering relation $\omega\varphi L = \omega_c\varphi_c L = \sqrt{1{\cdot}8Mg/K\rho S}$ with $\rho = 1{\cdot}25 \times 10^{-3}g/$ cm^3 and $g = 981$ cm/sec^2. Clearly the hovering line passes symmetrically through the data, the scatter on either side being probably due to experimental variations of ω, φ and k, since, according to Weis-Fogh and Jensen[8], the experimental error in ω and φ is on average about ± 25 per cent for the data of Magnan[5]—the possible effects of such experimental errors are shown by the broken lines in Fig. 1 which provide an error band for the hovering line. The more recent frequency data of Sotavalta deviate from the earlier measurements of Magnan by on average 22 per cent, the largest deviation being 80 per cent, so that whenever two frequencies are available in Table 1 the mean has been used in constructing Fig. 1. Furthermore, the duration factor k is only reported to the nearest $\pm 0{\cdot}1$, so that when all possible causes of deviations are taken into consideration it would seem that the correlation predicts the values and trends reasonably well, particularly for a natural phenomenon of this kind. There is also some reason to believe that the k factors of Magnan[5] might have an experimental error in excess of $\pm 0{\cdot}1$ since attempts to simulate the vertical flight of the stag beetle (*Lucanus cervus*) on the digital computer indicate that k should be at least 1·4 and not 1·0 as reported by Osborne[6]; the value of 1·4 would also agree better with the values of 1·3–1·5 reported for the other flying beetles in Table 1. That the deviations and scatter of the data in Fig. 1 are probably due mostly to random experimental errors is also indicated by the distribution of the 21 experimental points around the hovering line, 13 insects rising and 8 falling.

Another more concise, but perhaps less pictorial, method of specifying the gravitational stability of insects would be in terms of the dimensionless number N generated from criterion (7) such that $N = \omega^2\varphi^2L^2K\rho S/Mg \geqslant 1{\cdot}8 \approx 2$; for $N > 2$ the fluttering wing-body system will climb, hovering corresponding to $N = 2$, and fall to $N < 2$. Such critical dimensionless numbers are common in fluid mechanics usage—for example, the critical Reynolds number of 2,000 corresponds to the ratio of the fluid inertia to viscous friction that must be exceeded before laminar flow in pipes may become turbulent. In the present instance the dimensionless number N denotes the ratio of the kinetic energy per unit volume dissipated by non-uniform air motion ($\frac{1}{2}\rho\omega^2\varphi^2L^2K$) to the gravitational force per unit wing area ($Mg/2S$).

In conclusion—while criterion (7) correlates, within experimental error, the values and trends observed for a wide range of insect flight behaviour, the results reveal a need for more exact experimentation on both insects and birds along the lines of the classic work of Weis-Fogh[8,16] and Jensen[8,17] on the desert locust, particularly with a theory in view, so that all the pertinent flight parameters are determined simultaneously and precisely.

I thank R. L. Houghton for supplying the insect specimens needed to determine the generalized wing geometries.

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LETTERS TO THE EDITOR

PHYSICS

Solar Radiation on Inclined Surfaces

CALCULATION of the probable intensity of solar radiation on a surface which is inclined to the horizontal and at any attitude or azimuth relative to the Sun's meridian is frequently necessary in solar engineering research. The usual practice has been to perform such a calculation by separating the direct component of insolation and the diffuse component of sky radiation and to combine these according to the geometry of the system[1]. The assumption is made that the sky radiation is uniformly distributed from all directions, but this is known not to be the case for bright Sun or cloudless conditions, when the diffuse radiation is more intense in the vicinity of the Sun[2]. Hence the normal procedure as already described here is not valid except for surfaces which are horizontal.

The work recorded here is based on the results of four years' continuous recording of the solar radiation received by surfaces at various attitudes and has the object of unifying the method of presentation of the data for bright Sun conditions*. The total radiation received by a surface at any attitude may then be calculated directly in terms of the total radiation received by a horizontal surface at the same instant. Briefly, the system of unification is based in the first instance on the resolution of the direct radiation from the Sun into components that are respectively perpendicular and parallel to the inclined surface, only the former being effective. Hence equation (1) is derived for these simplified conditions, and this is afterwards modified to allow for the diffuse radiation component.

$$\Phi I = \left(\frac{I_i}{I_h} - \cos\theta_H\right)\frac{\tan a}{\sin\theta_H} = \cos A_r \qquad (1)$$

where I = intensity of direct radiation; D = diffuse radiation; E = total radiation = $I + D$. The suffix i added to the foregoing symbols refers to intensities on an inclined surface, the suffix h to a horizontal surface. θ_H = angle of inclination of the surface to the horizontal; a = solar altitude above horizon; A_r = relative azimuth of surface, that is, the angle between the facing direction of the surface and the Sun's meridian.

The relationship shown by equation (1) when plotted graphically gives the 'ideal radiation characteristic line', which is universally valid for all conditions, except zenith Sun, if the diffuse component of radiation is ignored. As shown in Fig. 1, ΦI plotted against cos A_r gives a straight line of slope unity provided a is greater than θ_H. If a is less than θ_H, the relationship becomes a horizontal line at the relative azimuth angle at which the Sun's rays graze the surface; this is at $A_r = 90°$ or cos $A_r = 0$ for $\theta_H = 90°$ (vertical surfaces) or in general when cos $A_r = -\tan a/\tan\theta_H$.

If the terms for total radiation, namely, direct plus diffuse components, are substituted for the direct radiation in equation (1), then the data show that the conditions may be represented by equation (2) here, that is, there is still a straight-line relationship between ΦE and cos A_r, but the characteristic lines do not pass through the point 0,0 and have a slope less than unity.

$$\Phi E = \left(\frac{E_i}{E_h} - \cos\theta_H\right)\frac{\tan a}{\sin\theta_H} = C + M\cos A_r \qquad (2)$$

* The expression bright Sun conditions implies that the vicinity of the Sun is free from cloud, that the total cloud does not exceed 3/10, and that there is an absence of haze. Perfectly cloudless conditions are so rare that there would be a paucity of data and the periods of application would be limited.

The constant C is the value of ΦE when cos $A_r = 0$ and M is the slope of the characteristic lines; the deviation of these from the ideal is determined by the quality

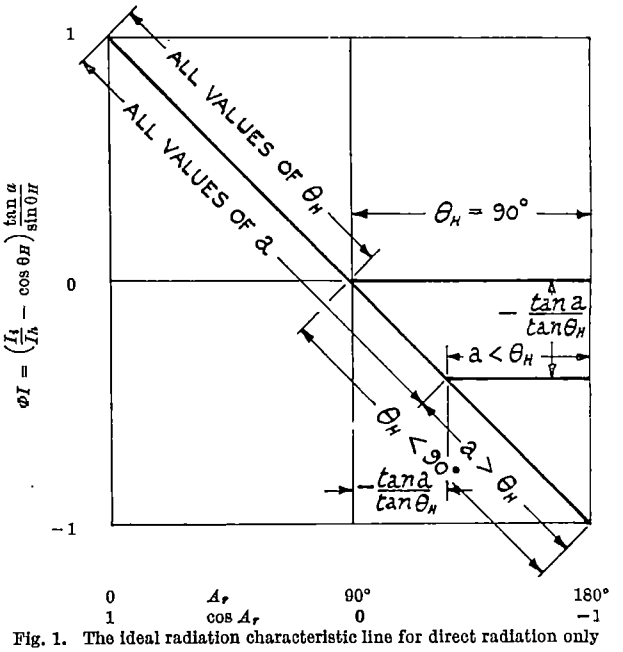


Fig. 1. The ideal radiation characteristic line for direct radiation only

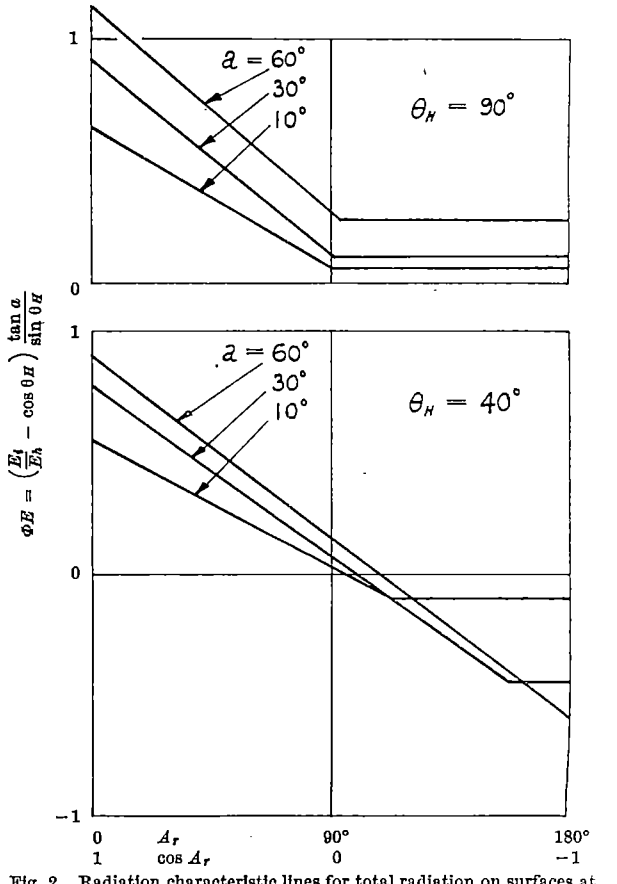


Fig. 2. Radiation characteristic lines for total radiation on surfaces at 90° and 40° to the horizontal and for various solar altitudes

of the radiation, that is, the relative magnitudes of the direct and diffuse components or of the ratio D_h/E_h. If it is assumed that for a given locality and season of the year the quality of the radiation is predominantly a function of the solar altitude α, then this latter factor is a convenient parameter on which to base a system of characteristic lines for a given value of θ_H. Typical characteristic lines are shown for $\theta_H = 90°$ and $\theta_H = 40°$ in Fig. 2. An analysis of the theory shows that if the diffuse component of radiation were uniformly distributed the slope M of the characteristic lines would be $(1 - D_h/E_h)$, but as the distribution is not uniform $(1 - D_h/E_h)$ is slightly smaller than M. The total radiation on a steeply inclined surface also includes some radiation from the heated surface of the ground, but this ceases to be appreciable when the angle of inclination θ_H is below 60°.

It is intended to publish charts or tabulated values of the constants C and M for various solar altitudes throughout the yearly cycle in the London area, which will enable the values of E_t/E_h to be calculated for any surface. The primary object of this communication, however, is to promote interest in the research in other countries and to stimulate the determination of similar data which will extend knowledge of the availability of solar radiation.

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Electron Temperature in a Laser-heated Plasma

When a high-power laser beam is focused on to a material target, a small inertially confined plasma is generated near the target surface. Theoretical work by Basov and Krokhin[1,2], verified by Dawson[3], suggests that very hot plasmas ($\sim 10^7$ °K) can ultimately be produced in this way. A spectroscopic investigation of plasmas generated by a small Q-switched ruby laser has already been reported[4]. Further experiments have now been carried out using a larger Q-switched neodymium glass laser, and an estimate of the electron temperature attained in a carbon plasma is reported in this communication. Other results will be described in detail elsewhere.

The peak power density at the focus of the neodymium glass laser beam was about 10^{11} W cm^{-2}. The output wave-length was 10,600 Å. Each laser pulse lasted about 1 μsec and consisted of three spikes. The energy in a pulse was about 1 joule. The focused beam broke down atmospheric air, so the target was placed in a vacuum. The plasma formed by the laser beam was imaged on the entrance slit of a Hilger medium spectrograph. When a graphite target was used, a single laser pulse was sufficient to produce a spectrogram on which lines of the CI, II, III, IV and V spectra were identified.

Kaufman and Williams[5,6] have pointed out that under certain conditions the specific intensities of lines from atoms of the HeI isoelectronic sequence give a useful indication of the plasma electron temperature even when thermodynamic equilibrium is absent and the electron and ion densities are only very approximately known. A number of assumptions must be made whose validity will be discussed in a later paper. In particular, a Maxwellian distribution of electron velocities is assumed. The expression given by these authors for the specific intensity, I, divided by the product of the number densities of electrons, n_e, and of quadruply ionized carbon atoms in the ground state, n_t, has been evaluated for the observed CV transition $2s^3S_1$–$2p^3P_2$ at 2270·91 Å and is plotted as a function of the electron temperature, T_e, in Fig. 1.

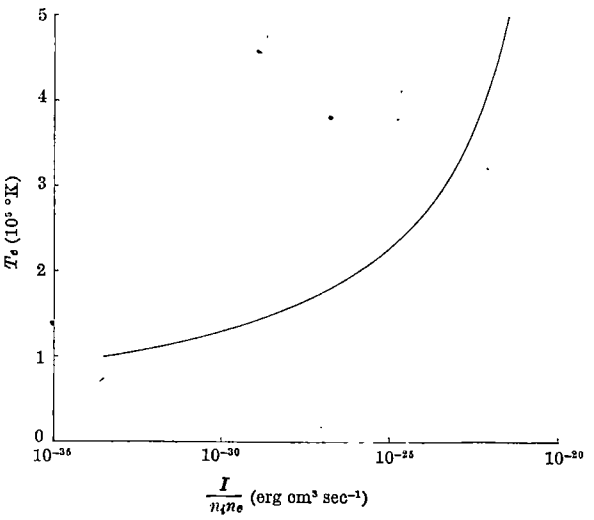


Fig. 1

Taking into account geometrical considerations, the observed blackening of the spectrographic plate indicates that the specific intensity of this CV line was at least 3×10^{11} erg cm^{-3} sec^{-1}. An extreme upper limit of 10^{21} cm^{-3} for n_t is obtained from the volume and density of the graphite removed from the target by the laser pulse, and the volume of luminous plasma emitting CV light. The corresponding maximum electron density is 6×10^{21} cm^{-3}, because the carbon plasma was very pure. Thus $I/(n_t n_e) \geq 5 \times 10^{-32}$ erg cm^3 sec^{-1}. From Fig. 1, the lower limit for the peak electron temperature is therefore $1{\cdot}1 \times 10^5$ °K. As may be seen from Fig. 1, at this temperature large errors in density or intensity estimates produce only small errors in the derived temperature. Nevertheless, as the true densities may well be orders of magnitude lower than the values used in the calculation the true electron temperature is probably somewhat higher.

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Saturation Characteristics of a Spherical Ionization Chamber and a Determination of Boag's Constant for Air

Thomson and Rutherford's[1] initial investigations of the saturation characteristics of a parallel plate ionization chamber were followed by theoretical treatments by Thomson[2], Mie[3], Seeliger[4] and others. More recently, Boag and Wilson[5] have shown by dimensional analysis that the collection efficiency, f (that is, the ratio of the measured current, i, to the ideal saturation current, i_s), is a function of $d^2q^{\frac{1}{2}}V^{-1}$ where d is the distance between the plates, q is the rate of ionization and V is the applied voltage. Through the introduction of certain assumptions, they derived the following equation for the generalized saturation characteristic:

$$i_s = i\left[1 + \frac{i}{i_s} \cdot \frac{1}{6} \cdot \frac{\alpha/e}{k_1 k_2} \cdot \frac{d^4 q}{V^2}\right] \qquad (1)$$

or $$f = 1 - \frac{1}{6} f^2 \xi^2$$

where $$\xi = m\left(\frac{d^2 q^{\frac{1}{2}}}{V}\right) = \sqrt{\frac{\alpha}{e k_1 k_2}}\left(\frac{d^2 q^{\frac{1}{2}}}{V}\right)$$

k_1 and k_2 are the mobilities of the positive and negative ions respectively (that is, it is assumed that the current carried by electrons is negligible); α is the recombination coefficient; e is the charge per ion; m is Boag's constant.

Greening[6] has surveyed experimental data and concluded that, while equation (1) was valid very near saturation, Mie's theory[3], as simplified for the case of air, is true at least down to $f = 0{\cdot}7\left(i \cdot e \frac{i}{i_s} > 0{\cdot}7\right)$ and gives an equation for the generalized saturation characteristic:

$$i_s = i\left[1 + \frac{1}{6}\frac{\alpha/e}{\left(\frac{k_1 + k_2}{6}\right)\left(k_1 k_2\right)^{\frac{1}{2}}} \cdot \frac{d^4 q}{V^2}\right] \qquad (2)$$

or $$f = 1 - \frac{1}{6} f \xi^2$$

Although in equation (2) m is defined as:

$$m^2 = \frac{\alpha/e}{\left(\frac{k_1 + k_2}{2}\right)\left(k_1 k_2\right)^{\frac{1}{2}}}$$

it differs numerically from the definition given with equation (1) by only 0·5 per cent. Greening also obtained from his survey a value for m of 36·7 ± 2·2, which was higher than an earlier suggestion[5]. Boag[7] has shown theoretically that both equations (1) and (2) can be extended to a spherical ionization chamber provided the electrode separation $(a-b)$ is modified by a geometric factor, K_{SPH}:

$$(a-b)\, K_{SPH} = (a-b)\sqrt{\frac{1}{3}\left(\frac{a}{b} + 1 + \frac{b}{a}\right)}$$

where a and b are the radii of the outer and inner electrodes, respectively. Notwithstanding this, he points out that Mie's theory (equation (2)) may not necessarily be valid down to $f = 0{\cdot}7$ for spherical geometry.

A spherical ionization chamber has been constructed of copper with the radii of the inner and outer electrodes 3·13 cm and 5·08 cm, respectively, and irradiated in the γ-ray fields from a 2,000-c. cobalt-60 unit and a 1,000-c. cæsium-137 unit.

The wall thickness of the outer electrode, 2·64 mm of copper, was sufficient to establish electron equilibrium. The polarizing voltage was supplied by dry batteries and the current measured on a vibrating reed electrometer. A voltage calibration of the electrometer was obtained by means of a standard cell together with a potential divider. The input resistors were calibrated by a capacity leak method.

The experimental saturation characteristics obtained at two different exposure rates from the two radiation sources are shown in Fig. 1. Measurements made at other exposure rates lay on the same curve. The value of Boag's constant resulting from Greening's survey, 36·7, was used to calculate the generalized saturation characteristic from Boag's theory (Fig. 1, *B*) and Mie's theory (Fig. 1, *M*). The agreement at different exposure rates and different γ-ray energies confirms Boag's theoretical deduction that the generalized saturation curve derived for a parallel-plate chamber can be applied to a spherical ionization chamber, provided an appropriate geometric constant is introduced.

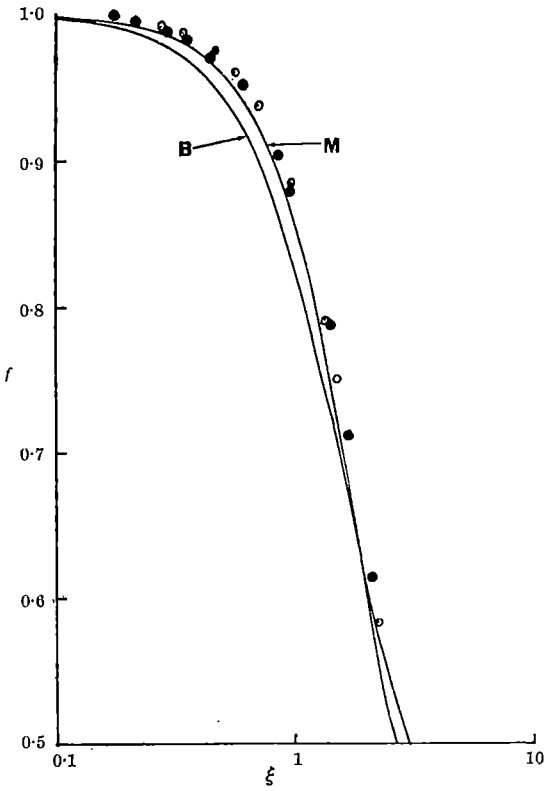


Fig. 1. The generalized saturation characteristics of a spherical ionization chamber. *M*, Mie's theory; *B*, Boag's theory; ○, Experiment, cobalt-60 γ-rays, q, 1·28 E.S.U./cm³ sec; ●, Experiment, cæsium-137 γ-rays, q, 0·51 E.S.U./cm³ sec

The experimental results tend to coincide more closely with Mie's theory. In fact, when the value of Boag's constant, which is derived later, is used to calculate the theories, the experimental points are superimposed on the curve representing Mie's theory. However, a better way of examining the validity of the two equations is to plot f against f^2/V^2, which according to Boag's theory should be a straight line, and also against f/V^2, which according to Mie's theory should be a straight line. This is done for one set of measurements in Fig. 2, where it will be observed that the plot of f against f^2/V^2 exhibits a distinct curvature, while the plot of f against f/V^2 lies on a straight line. The same results were obtained with the other sets of measurements. This concurs with Greening's finding that Boag's theory is valid only very near saturation, but Mie's theory is valid over a wider range. In addition, it is noted in Fig. 2, and in other measurements not presented here, that the straight line for the plot of f against f/V^2 is maintained down to at least $f = 0{\cdot}7$. This suggests that

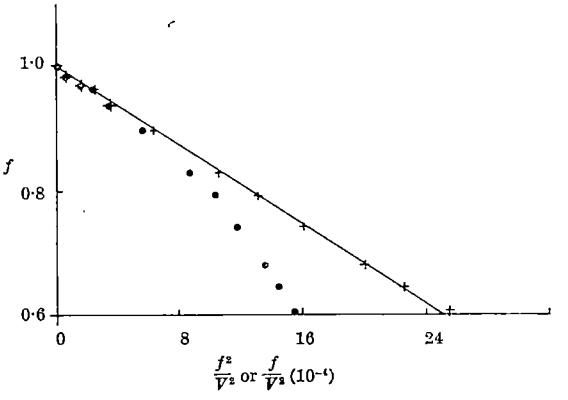


Fig. 2. Examination of validity of Mie's theory and Boag's theory. +, Mie's theory; ●, Boag's theory

Table 1

Radiation source	q (E.S.U./cm³ sec)	m
^{60}Co	1·28	34·6
^{60}Co	0·76	33·8
^{137}Cs	0·51	33·7
^{137}Cs	0·047	34·8

the generalized saturation curve derived from Mie's theory is valid for currents greater than 0·7 of the saturation current for spherical ionization chambers, as well as for parallel-plate ionization chambers. As the slope of the straight line in Fig. 2 is $-\frac{m^2d^4q}{6}$ (see equation (2)) Boag's constant can be determined from these data. This has been done for the two sets of measurements represented in Fig. 1 and also for the other two sets of measurements. The rate of ionization, q, and the calculated value of Boag's constant are listed in Table 1. The mean value obtained for Boag's constant was 34·2.

We thank Mr. D. E. A. Jones for allowing us to use the radiation facilities at Mount Vernon Hospital, Northwood, and Prof. J. F. Fowler and Mr. J. Yarwood for their advice. Dr. J. W. Boag kindly let us see the text of his chapter on "Ionization Chambers" prior to its publication in *Radiation Dosimetry*.

This work was supported by the Department of Scientific and Industrial Research.

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Forward Scattering of Light from a Laboratory Plasma

The scattering of electromagnetic radiation by a plasma in thermal equilibrium has been investigated by several authors[1–3] following the remarkable result of Bowles[4], who found that the spectrum of the radiation back-scattered from the ionosphere has a breadth comparable to the Doppler shift at the thermal velocity even though the electrons do the scattering. Rosenbluth and Rostoker[5] have extended those calculations to certain non-equilibrium cases. These calculations all show that the form and breadth of the spectrum of the scattered radiation are determined principally by the parameter defined as follows:

$$\alpha = \lambda/(4\pi\ \lambda_D \sin\theta/2)$$

where λ is the wave-length of the incident radiation, λ_D the Debye length of the plasma and θ the scattering angle measured with respect to the forward direction. If $\alpha \ll 1$ the electrons scatter as independent particles and the resulting spectrum is gaussian with a half-breadth given by the Doppler shift corresponding to the electron thermal velocity. If $\alpha > 1$ the spectrum is dominated by collective effects and consists of a central peak broadened at the Doppler shift corresponding to the ion thermal velocity which, however, is gaussian in shape only for large frequency shifts and has two weak maxima at the half-breadth positions and two electron satellites at $\pm\omega_p$, the electron plasma frequency. These electron satellites each contain only $1/\alpha^2$ of the total scattered energy.

Since λ_D for a typical θ-pinch plasma is of the same order as the wave-length of the ruby emission, if α is to be greater than unity the scattering angle must be less than $(2\pi)^{-1}$. This communication reports the first results obtained on a laboratory plasma for scattering at angles sufficiently small for collective effects to be dominant as contrasted with other work[6–10] at large angles.

Fig. 1 shows the experimental arrangement. Light from a Q-switched ruby laser is sent down the axis of a θ-pinch discharge and caught in a light trap beyond. The laser emits a single pulse of 20-nsec duration and a power of either 1·5 or 15 MW depending on the temperature of the ruby.

The light scattered at angles round 3° is collected from the entire azimuth and length of the plasma by the combination of a lens, axicon, and focal plane stop. The light then passes through an interference filter and a Fabry–Perot etalon. The etalon is followed by a lens-axicon combination which for a given wave-length shrinks one of the circular interference fringes to a point so that it can pass through a focal-plane stop to a photomultiplier (RCA 7265). The spectrum is scanned by displacing the axicon. (This method of scanning is described in detail in an article to be published in *Applied Optics*.) A second photomultiplier monitors the total signal to correct for variations in the laser output. The fraction of light scattered at all wave-lengths was $\sim 10^{-12}$.

The θ-pinch consisted of a coil 7 cm diam. × 16 cm long. The condenser bank was 4 μF charged to 36 kV. The period of the discharge was 4·8 μsec and all measurements were made 3·7 μsec after initiation. Hydrogen was used throughout at a pressure of 200μ.

The electron spectrum is taken with the etalon set for a free spectral range of 30 Å and the laser operated at a power of 15 MW. Fig. 2 shows a spectrum taken over two orders. The dispersion is, of course, quadratic and the wing toward the short wave-lengths is an instrumental effect. It is noted that the photoelectric signal is always enhanced in the presence of plasma within the instrumental breadth of the etalon. Outside the signal falls to the level of the noise except at certain points to the short-wave-length side of the main peak where a large enhancement is noted in the presence of plasma. The arrows show the corresponding point on the long-wave-length side where a small enhancement is noted. It would seem that the short wave-length electron satellite is much stronger than the long wave-length one.

The ion spectrum is taken with the etalon adjusted for a free spectral range of 0·8 Å. The laser is refrigerated to emit a narrow line. The resulting power is 1·5 MW. Fig. 3 shows the results. It is seen that the maximum of the ion peak is shifted toward shorter wave-lengths. The results are very sporadic in the neighbourhood of the maximum emission wave-length of the laser, as the latter was found to shift frequency from shot to shot. These variations are

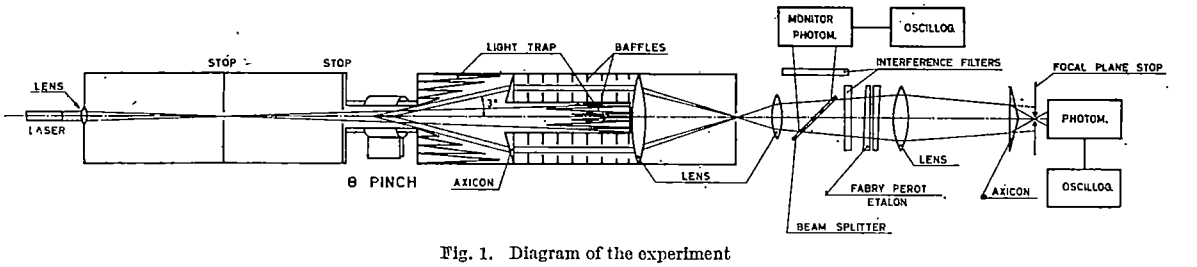


Fig. 1. Diagram of the experiment

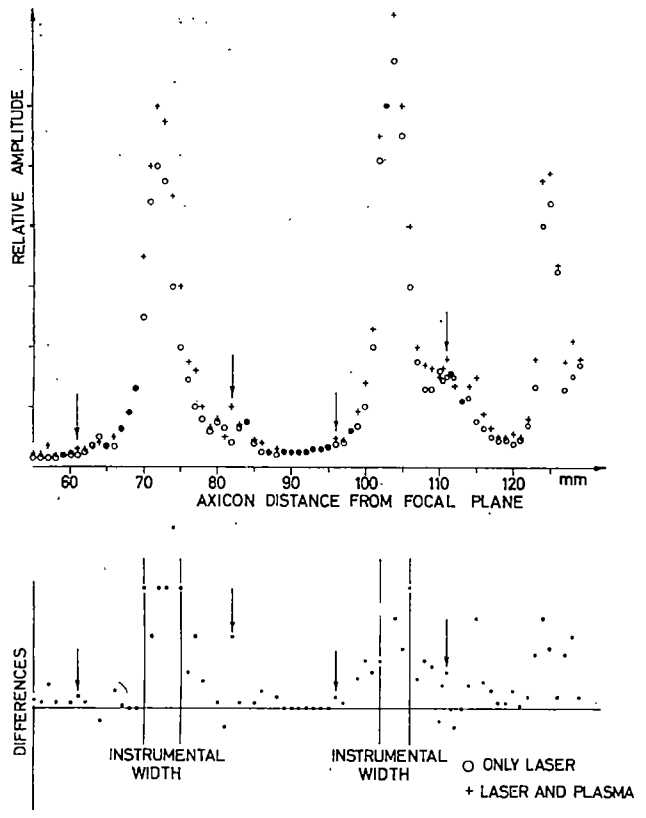


Fig. 2. Spectrum of scattered light at low-resolution etalon free spectral range 30 Å

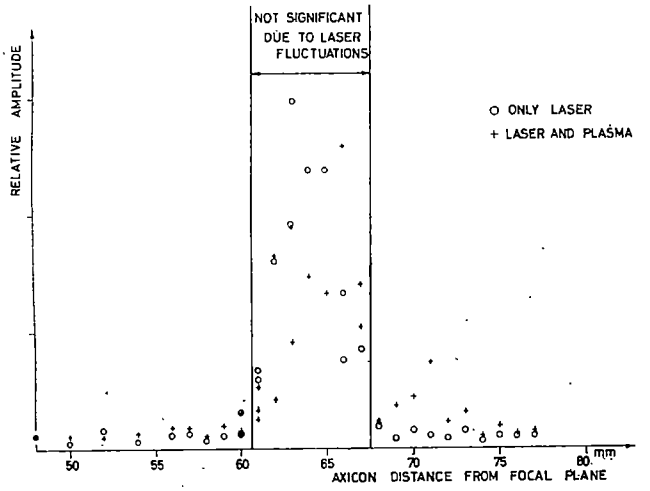


Fig. 3. Spectrum of scattered light at high-resolution etalon-free spectral range 0·8 Å

not sufficient to give a false indication of the displaced ion peak, however, because with the axicon set to accept such a large frequency shift the large-amplitude signal occurred only in the presence of plasma.

If one neglects any thermal correction to the electron satellites the resulting electron density from the frequency shift is $3{\cdot}2 \times 10^{15}/cm^3$. This agrees with the value of $2{\cdot}4 \times 10^{15}/cm^3$ deduced from the total scattered intensity. Setting the displacement of the ion peak equal to the ion thermal Doppler shift gives an ion temperature of 70 eV. If we assume the electron temperature is equal to the ion temperature the resulting value of α for our scattering angle of 3° is ~ 2. Except for the asymmetries toward shorter wave-lengths these data are consistent with the theory and the expected behaviour of a small θ-pinch. The asymmetry in the ion peak can be attributed to an electron drift velocity relative to the ions of the order of the thermal velocity according to the previously cited work of Rosenbluth and Rostoker. Such a drift could also account for the enhancement of the short wave-length electron satellite.

We thank Prof. Brunelli, director of the Laboratorio Gas Ionizzati, and other staff members of the laboratory for their advice. We also thank R. Marchetti and M. Baldascini for carrying out the measurements. One of the authors (J. K.) was a Euratom Research Fellow during 1961–63.

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Attenuating Blast Waves produced by an Instantaneous Release of Thermal Energy

It has been observed that, when an explosive charge of TNT is intimately surrounded by a light crushable porous solid of large surface area and high heat capacity, the subsequent blast wave is rapidly attenuated. The mechanism of attenuation is hazarded as mainly heat transfer. The crushable nature of the surrounding porous solid (no elasticity–zero velocity of sound) would prevent immediate transmission of the blast wave energy and allow time for multiple reflexions, hence heat transfer and therefore attenuation, as gas flow is established through the porous solid.

Although a large number of materials would be suitable as the attenuating medium, initial experimentation has been confined to exfoliated mica, that is, vermiculite. The advantages of this material are its almost insignificant cost and its chemical inertness.

Experience has indicated that the use of vermiculite is a very convenient laboratory tool. A charge of TNT weighing 1/3 lb. can be detonated at the centre of a simple wooden box (4 ft. × 4 ft. × 4 ft.), filled with vermiculite, with complete safety. In fact very little audible signal is received outside the box.

Extrapolation emphasizes the possibility of obscuring the detonation of some 1,000 tons of TNT in a pile of vermiculite of the order of 100 ft. in height. The attenuating mechanism becomes more effective the higher the temperature of the explosive source.

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GEOLOGY

Gibbsite in Altered Granitic Rock in North Wales

My attention was directed by the Nature Conservancy's chief warden for Snowdonia, Mr. Evan Roberts, to an unusual form of rock weathering on the ridge of Y Llymllwyd, which projects into Nant Ffrancon between Cwm Cywion and Cwm Coch. These are two well-developed late-glacial corries. The rock in question is exposed over an area of about 100 sq. m. in a col on the crest of the ridge at approximately 750 m (2,450 ft.), *O.D.*, grid reference *SH* 631609.

Sections show a thin greyish-white surface horizon overlying white loose material, sandy in initial texture but easily crushed and earthy. This becomes more compact and massive at about 25–30 cm, in the form of soft altered rock to a known depth of 1·5 m. This soft rock retains a recognizable original fabric.

Williams[1] showed that granophyric intrusions probably of Llandeilo age, behaving generally as laccoliths, occurred in the area. These intrusions include the Bwlch-y-Cywion granite, which outcrops over an area of about 2·4 km × 0·4–0·8 km. This intrusion includes four lithological variants identified by Williams of which the most extensive, a microporphyritic granite, occupies the ridge of Y Llymllwyd. This rock, greyish-white or pinkish in colour, consists of a ground-mass of interlocking quartz and alkali felspars, mainly orthoclase, containing small tabular felspar phenocrysts and flakes and patches of biotite and chlorite. The felspars are partly decomposed to sericite and paragonite, but there is no reference to areas of deep weathering or decomposition. The complete chemical analysis of this rock[1] includes figures for SiO_2 and Al_2O_3 of 74·23 and 13·39 per cent respectively.

Examination of the clay fraction of the 0–25 cm horizon showed it to be composed of gibbsite (Al_2O_3 $3H_2O$) as the only crystalline component. X-ray powder photographs (Philips 114·6-mm cameras) give a strong pattern almost identical with that given by Rooksby[2] or on the American Society for Testing Materials (A.S.T.M.) data card 7–324. Differential thermal analysis (Netzsch equipment) gives a curve of the general type published for gibbsite[3] although peak positions are not identical to those of published data (which are themselves variable). The differential thermal analysis curve indicated that no substantial amount of any other clay mineral was present with the gibbsite except possibly allophane, evidenced by a small endotherm and exotherm at 160° C and 910° C respectively. The probability of some amorphous mineral of allophane type being present is shown by a semi-quantitative X-ray spectrographic analysis which gave aluminium as dominant, with silicon present to the order of 10–15 per cent SiO_2. Calcium, potassium, manganese and iron are present only as trace amounts. 44–75μ size-fractions from the same 0–25 cm horizon contain the order of 50 per cent SiO_2.

An occurrence of gibbsite as the dominant product of alteration or weathering of a granitic rock is believed to be unique in Britain. Gibbsite has, in Britain, been previously recorded in Tertiary bauxitic weathering products in the Antrim inter-basaltic horizon[4], in the Ayrshire bauxitic clays derived by contemporaneous weathering of Carboniferous lavas[5], and, associated with allophane, in segregations in chalk[6] at Clifton Hill, Brighton. The implications of this record from North Wales are of geological interest if it results from hydrothermal alteration and of wider interest if, as is presently believed, the gibbsite is a relic product of sub-aerial weathering. Work, in collaboration with Dr. D. A. Jenkins, University College of North Wales, Bangor, is continuing on the field relationships, petrography and chemistry of this clay, its parent rock and the 'weathered' zone with which it is associated.

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Grand Banks Earthquake of 1929 and the 'Instantaneous' Cable Failures

The Grand Banks earthquake and the associated disturbances have received attention from all students of marine geology, particularly since the publication of the classical interpretation of the submarine cable failures by Heezen and Ewing[1]. In their recent publication, "Grand Banks Slump", Heezen and Drake[2] have attributed the virtually instantaneous cable failures around the epicentre of the earthquake to an initial slump, triggered by the shock. In their original interpretation, Heezen and Ewing attributed the later, systematic cable failures for a period of 13 h 17 min to the action of the turbidity current dislodged by the initial shock. The cause behind the almost instantaneous failure of six cables lying 150–1,800 fathoms on the continental slope, however, remained unexplained at that time. The original authors attributed these, rather vaguely, to "initial ground motion, or almost instantaneous slumps and slides".

The 'epicentral area' of this earthquake was about 30 miles in radius[2], and the instantaneous damage within this area was obviously due to the initial shock, ground movements and slumping. The fact which remained unexplained was that the southernmost cable, which broke instantaneously, lay but 10 miles away from the cable which broke 59 min later. Apparently, therefore, the almost instantaneous breaks were caused by a phenomenon somewhat different from and faster than the turbidity current which damaged the other cables at progressively increasing times from the 59th minute of the shock.

Heezen and Drake have suggested an initial slumping to explain the 'instantaneous' cable breaks. Gravitational slumping in the area is indicated by a reflexion seismic profile which shows irregular and curved sub-bottom horizon dips.

The idea of initial slumping supported by Drake's reflexion profile seems to be quite a plausible solution for part of the problem. From Fig. 1 of Heezen and Drake, however, it appears that the farthest cable which broke 'instantaneously' lay at a distance of about 60 miles from the epicentre. Considering the extensive area which, according to Heezen and Drake, was 'instantaneously' affected by a gravity slumping, I have been wondering if the almost instantaneous cable breaks outside the epicentral area could be attributed to some phenomenon faster than the postulated gravity slumping.

Surface wave disturbances in experimental seismograms are quite commonly experienced by the explosion seismologists. Travelling along the free solid-surface with about nine-tenths the velocity of the shear wave, these waves (particularly Rayleigh or the so-called 'ground roll') progressively hit the geophones at successively greater distances from the explosion. A similar phenomenon of elastic wave propagation might explain at least a part of the cable failures outside the 'epicentral area'.

Specific data on the shear wave velocity in underwater sediments are rather meagre. The available information from experimental studies on sediment cores by Sutton, Berckhemer and Nafe[3] roughly suggests a compressional wave velocity of 1·5–1·8 km/sec (or a mean velocity of about 1·65 km/sec) near the water-sediment interface. The corresponding shear wave velocity derived from velocity–porosity relations[4] is about 0·6 km/sec. Travelling along the free surface of the sediment the corresponding Rayleigh wave would traverse a distance of 60 miles in about 2·98 min. The distance of 30 miles from the edge of the 'epicentral area' to the farthest cable which broke almost instantaneously will be covered in only about 1·49 min—a rather small time-interval compared with the next cable break 59 min after the shock. Effects of the compressional and shear waves within this zone would, of course, be still faster (0·49 and 1·34 min, respectively). In view of the dispersive nature of the Rayleigh wave these velocity computations might be modified to some extent.

It is interesting to note in this connexion the earlier observation by Officer (in Nafe and Drake[4]) that the topmost part of the sediment near the water-sediment interface might have slightly lower velocity than the velocity of water for frequencies of about 100 c/s.

As regards the feasibility of large-scale damage in unconsolidated sediments by elastic wave propagation, the instrumental observations by Gutenberg (in Swain[5]) on the so-called "jelley theory" which indicates that displacements in unconsolidated medium may be two to ten times greater than displacements from similar shocks in solid rocks, are worth noting.

These observations are recorded not as alternative suggestions to the idea of initial gravity slumping in the area which has been confirmed by Drake's reflexion profile. This is only an attempt to indicate the possible specific mechanism which might be responsible for: (1) the initial gravity slumping within and around the 'epicentral area'; (2) the almost instantaneous cable breaks in an extensive area outside the epicentral zone where gravity slumping at the instant of the earthquake seems to be a rather remote possibility.

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CHEMISTRY

Isotope Effects in the Infra-red Spectra of some Carbon-14-labelled Acids, an Ester and Sodium Salt

ISOTOPE shifts in the infra-red have been reported for a number of compounds containing deuterium, nitrogen-15 and oxygen-18, for example, the work of Pinchas *et al.*[1,2], but little has been published on carbon-13- or carbon-14-labelled compounds. George[3] has examined the spectrum of 1-^{13}C-propene-1, and Karabatsos[4,5] some ^{13}C-di-iso-propyl ketones and 2,2-dimethyl propionic acid; as regards carbon-14 compounds, only the spectra of carbon dioxide and barium carbonate appear to have been reported hitherto.

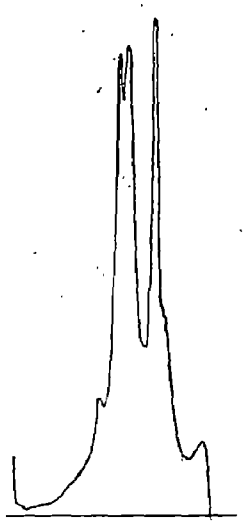


Fig. 1. Palmitic acid-1-^{14}C
μ: 5·68, 5·83, 5·90, 6·11, 6·20
cm^{-1}: 1,760, 1,715, 1,695, 1,637, 1,613 (*sh*)

This communication reports the spectra of some carbon-14 acids, an ester and sodium salt labelled at high specific activity in the 1-position.

The spectra were obtained on a Grubb-Parsons *G.S.*3 double-beam spectrophotometer from carbon tetrachloride solutions of the compounds, with the exception of sodium formate which was incorporated into a potassium bromide disk. The activities and radiochemical purities determined either by thin-layer chromatography, paper chromatography, or dilution analysis as appropriate were as shown.

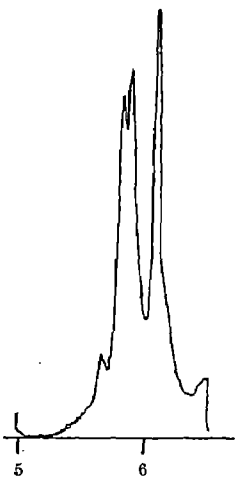


Fig. 2. Stearic acid-1-^{14}C
μ: 5·68, 5·83, 5·90, 6·11, 6·20
cm^{-1}: 1,760, 1,715, 1,695, 1,637, 1,613

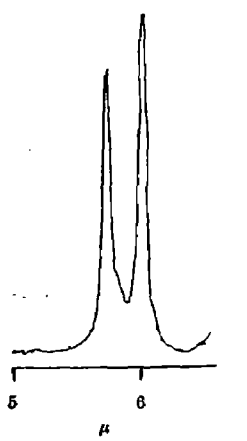


Fig. 3. Methyl stearate-1-^{14}C
C=O(^{13}C), 5·74μ, 1,742 cm^{-1}; C=O(^{14}C), 6·02μ, 1,661 cm^{-1}

It will be seen that the spectrum of the methyl stearate shows an additional peak at 1,661 cm^{-1} corresponding to ^{14}C=O stretching, and at a frequency close to that calculated. Similarly in the spectrum of sodium formate each of the peaks from 1,667 cm^{-1} to 741 cm^{-1} is also split. It is possible to detect ^{14}C–H stretching as a weak shoulder at approximately 3,260 cm^{-1}. However, it is worth noting that the free acids exhibit instead of the expected effects a complex band system, the origin of which is as yet unexplained.

Table 1

Name	Activity (mc./m.mole)	Radiochemical purity*
Palmitic acid-1-^{14}C	30·8	*P.C.*, *R.P.P.C.*, *T.L.C.*
Stearic acid-1-^{14}C	35	*P.C.*, *R.P.P.C.*, *T.L.C.*
Methyl stearate-1-^{14}C	35	*P.C.*, *R.P.P.C.*, *T.L.C.*
Sodium formate-^{14}C	13·4	*D.A.*
Palmitic acid (inactive)	—	—

**P.C.*, paper chromatography; *R.P.P.C.*, reversed phase chromatography; *T.L.C.*, thin-layer chromatography; *D.A.*, dilution analysis (for sodium formate reaction with *O*-phenylenediamine).

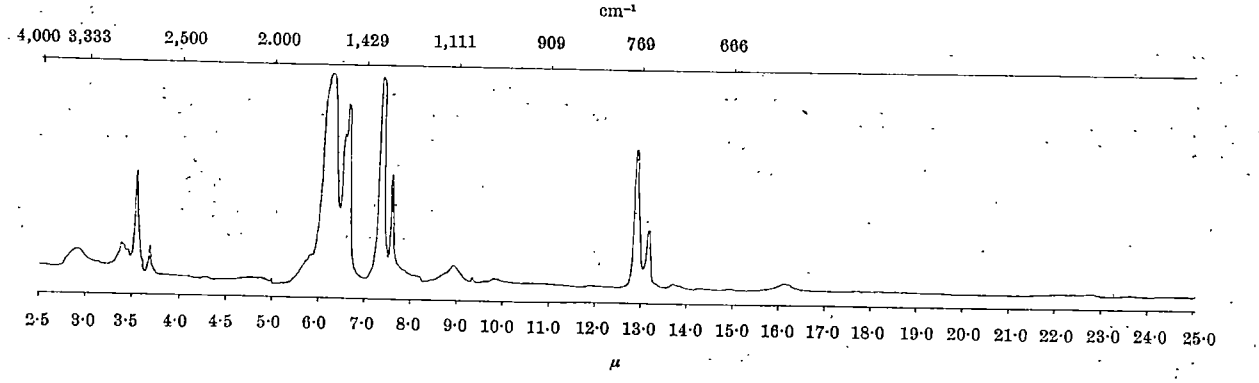


Fig. 4. Sodium formate-^{14}C

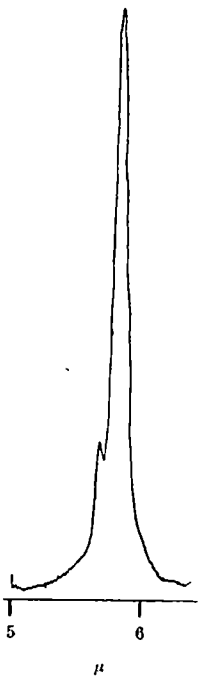


Fig. 5. Palmitic acid (inactive)
5·68μ, 1,760 cm⁻¹; 5·82μ, 1,718 cm⁻¹

We would like to direct attention to possible errors due to isotope shifts which might arise in purity determination of carbon-14-labelled compounds by infra-red spectrometry. Unsaturated fatty acids having multiple unconjugated double bands may be cited as an example, in this case absorption due to $^{14}C=O$ could easily be confused with the strong band arising from small amounts of conjugated impurities. The spectrum of a molecule generally labelled at high specific activity might well be so altered as to be unrecognizable as that of the same compound unlabelled. It is suggested that in such instances the evidence of infra-red spectrometry alone should not be accepted as a criterion of purity and recourse should be made to methods of assay independent of isotope effects.

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A Sensitive Chemical Actinometer for Ultra-violet Radiation

The most widely used chemical actinometer for ultra-violet radiation is based on the photochemical decomposition of oxalic acid in solutions of oxalic acid and uranyl oxalate, and subsequent titration with hot potassium permanganate[1]. This actinometer requires relatively large volumes of solution, and since the ultimate sensitivity depends on the titration difference before and after irradiation, ultra-violet sources of low dose-rate require relatively long exposure times. Actinometers in which the products of photolysis are directly determined are capable of greater sensitivity, depending on the sensitivity of the method used for analysis. Direct spectrophotometry, with high absorption of the photolysis products, introduces problems of inner filters and non-linearity of response. Parker[2] has devised a chemical actinometer with high sensitivity which depends on the decomposition of potassium ferrioxalate, spectrophotometric determination of the ferrous ion formed by the addition of phenanthroline and buffer solution, and subsequent measurement of absorption at 5100 Å. However, even dim electric light produces rapid decomposition of the potassium ferrioxalate, and all manipulations must be performed in a dark room.

Fluorometry is inherently about 20,000 times more sensitive than colorimetry for measuring concentrations of substances, although fluorescence is not so universal a process as absorption, because of competing quenching processes which tend to decrease the yield of fluorescence.

During the course of our investigation of the benzoic acid dosimeter for ionizing radiation of Armstrong and Grant[3], we found that ultra-violet irradiation in the region below 300 mμ of aqueous solutions of benzoic acid caused the formation of fluorescent products[4]. A large fluorescence reading is obtained which is linear with dose, even for prolonged irradiation doses. No significant change is observed in the ultra-violet absorption spectrum of the benzoic acid as a result of irradiation (except for a small increase for very long irradiation times), thus assuring that a constant fraction of actinic light is absorbed during the photochemical reaction, as is seen in Fig. 1. The fluorescence production is extremely sensitive; doses of the order of 1×10^{-7} N quanta/cm^2 of 254 mμ radiation are easily detected. We estimate the minimum detectable amount of energy at this wavelength, with a 1×10^{-3} M benzoic acid solution at pH 12 irradiated in air, to be of the order of 2×10^{-8} N quanta/cm^2. This is much lower than can be measured with the uranyl oxalate actinometer, though it is not more sensitive than the Parker actinometer. The principal advantage of this actinometer, besides its sensitivity and stability in room illumination, is the fact that only small volumes (2–3 ml.) are required, and that measurements can be made directly with no further manipulations, if the irradi-

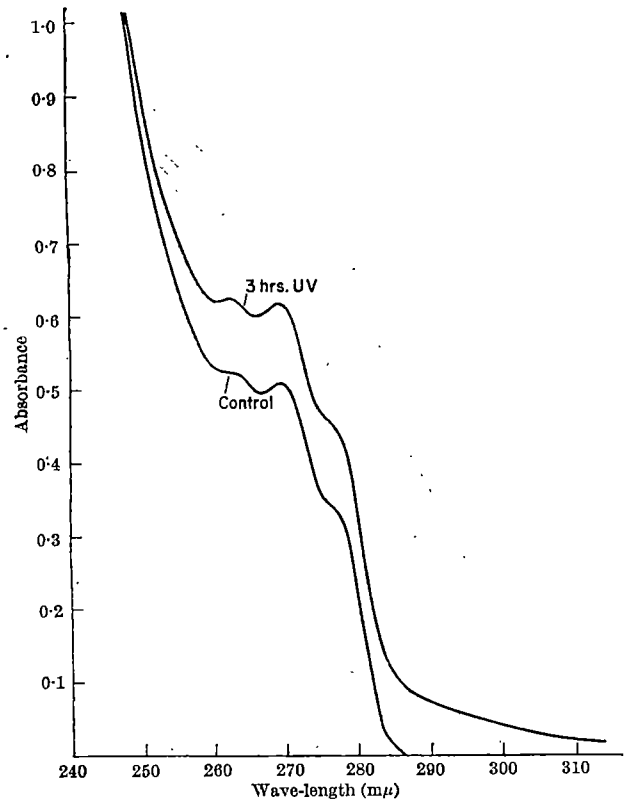


Fig. 1. Ultra-violet absorption spectra of actinometer solution before and after irradiation with germicidal lamp ($1{\cdot}4 \times 10^{-7}$ $Nh\nu$/min/cm^2). Solution is 1×10^{-3} M benzoic acid brought to *p*H 12 with NaOH. This dose increases the fluorescence intensity from nine arbitrary fluorometer units to 1,010 units

ation is carried out in a fluorometer cuvette. Fluorescence was determined with a standard fluorometer (Photovolt Corporation, New York, Model 520 *M*), using a Hanovia S-4 mercury lamp, fitted with an *RCA* 931-*A* photomultiplier tube. A primary filter (Corning 754 in conjunction with Wratten 34*A*) isolated the band with a maximum at 320 mμ for excitation, while a Baird-Atomic *A*-11 dielectric interference filter was used to isolate the 295 mμ band for shorter wave-length excitation. A secondary filter (Balzer 434-11-*B*-40) passed the fluorescent light with peak transmission at 405 mμ. The fluorometer was standardized before use by setting the fluorescence response to zero fluorometer units with distilled water, and to 1,000 fluorometer units with a solution of 6×10^{-6} molar *ortho*-hydroxy benzoic acid (salicylic acid) made alkaline to *p*H 12 with NaOH. Monohydroxy and dihydroxy benzoic acids have maximum fluorescence in alkaline solution where they are maximally ionized.

The fluorescent response of a 1×10^{-3} M benzoic acid solution was measured at different *p*H values against ultra-violet dose using a Mineralight 2537*SL* lamp, emitting primarily 254 mμ radiation at a dose-rate of $1{\cdot}4 \times 10^{-7}$ $Nh\nu$/min/cm^2, at a distance of 7·0 cm. Maximum fluorescence response to radiation was obtained at *p*H 12, similar to the response with ionizing radiation. These irradiations were carried out in air, with no bubbling or agitation. A slightly enhanced fluorescence response was noted if the irradiations were carried out under oxygen bubbling conditions, and the fluorescence response was slightly decreased under nitrogen bubbling conditions. The fluorescence is a linear function of dose for as long as 3 h duration. Curve *B*, in Fig. 2, shows the linear response of fluorescence intensity for doses of only several min duration. At 254 mμ, the fraction of light absorbed in a 1 cm cell containing 1×10^{-3} M benzoic acid at *p*H 12 is 75 per cent.

In an investigation designed to increase further the sensitivity of this actinometer. it was found that the addition of various compounds which are well-known radiation protective agents, namely, cysteine, cysteamine, glucose, and chloro-mercuri-benzoic acid, enhanced the fluorescence response by a factor of between 2 and 11 depending on the additive. For example, the addition of cysteamine (1×10^{-3} mole/litre) increased the fluorescence response by a factor of about 10, as shown in curve *A* of Fig. 2. The mechanism of this enhancement of sensitization is unknown at present; it cannot be due to increased absorption of light, since cysteamine at this concentration has an absorbance at 254 mμ of only 0·12, nor can it be due to fluorescent species formed by the photolysis of cysteamine, since control solutions of cysteamine at *p*H 12, similarly irradiated, developed no fluorescence.

From the data of Table 1 we can see that the ratio of arbitrary fluorometer units, F_{295}/F_{320}, found for peroxide-treated benzoic acid is very similar to that of ultra-violet irradiated benzoic acid.

Table 1. Comparison of Fluorescence Intensity at *p*H 12 with 320 mμ and 295 mμ Excitation

	Solution	Arbitrary fluorometer units F_{320}	F_{295}	F_{295}/F_{320}
a	Benzoic acid (1×10^{-3} M)	9	45	4·7
b	*ortho* Hydroxy-benzoic acid (6×10^{-6} M)	1,000	5,900	5·9
c	*meta* ,, ,, ,, ,,	2,120	6,850	3·2
d	*para* ,, ,, ,, ,,	20	75	3·7
e	Equal mixture of *b*, *c*, and *d*	1,040	4,275	4·1
f	H_2O_2 (2×10^{-3} M)-treated benzoic acid	1,020	4,350	4·3
g	NaOCl (2×10^{-3} M)-treated benzoic acid	0	0	—
h	Ultra-violet irradiated benzoic acid	1,000	4,500	4·5
i	4,000 rads from cobalt-60 gamma-rays	1,101	4,140	3·7

The activation process is due to the absorption of ultra-violet quanta, and subsequent photolysis of the type:

$$RH + h\nu \longrightarrow R\cdot + H\cdot$$

These radicals react readily with oxygen to form organic peroxides which may afterwards decompose with the formation of hydroxy-benzoic acid, similar to the action of hydrogen peroxide. However, the powerful oxidizing agent sodium hypochlorite does not cause any fluorescence in benzoic acid. We have calculated the quantum yield of hydroxy-benzoic acid production at *p*H 12 to be 0·081, assuming the fluorescence is due to an equi-molar ratio of hydroxy-benzoic acids. From the ultra-violet absorption spectra of benzoic acid at different *p*H values, the fraction of light absorbed and the quantum efficiency of isomer production at each *p*H can be calculated. The larger quantum yield at *p*H 12 may reflect the alkali-catalysed cleavage of peroxides, rather than differences in light absorption or ionization.

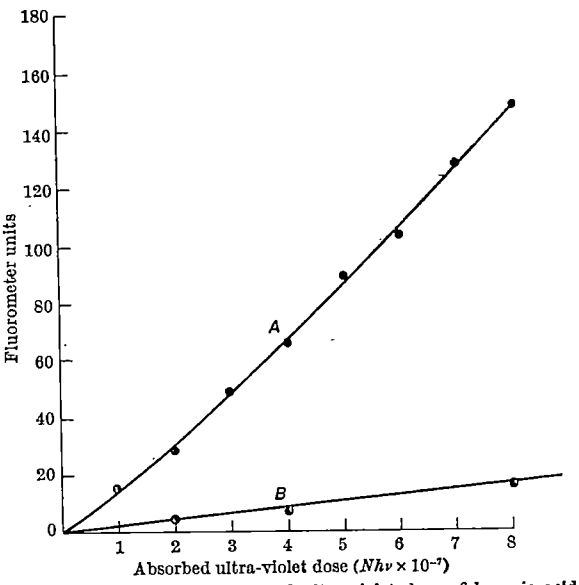


Fig. 2. Fluorescence intensity and ultra-violet dose of benzoic acid actinometer. Curve *A*: 1×10^{-3} M benzoic acid at *p*H 12 plus 1×10^{-3} M cysteamine. Curve *B*: 1×10^{-3} M benzoic acid at *p*H 12. Excitation is with 320 mμ band

Because this system does not absorb light at wavelengths above 300 mμ, excitation with the 320 mμ band in the fluorometer has no actinic effect on the benzoic acid. Only the products of photolysis, presumably the hydroxy isomers of benzoic acid, absorb at this wave-length and emit fluorescent radiation. Our system is more convenient and rapid in use than other conventional chemical actinometers.

This work was supported by the National Cancer Institute, contract *C* 3811.

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Intermolecular Potential Energy Functions for Simple Molecules

THE development of an intermolecular potential energy function for simple molecules based solely on an investigation of properties arising from two-body interactions[1] may prove more valuable than the alternative approach which considers also the properties of solids[2]. The latter approach involves the assumption of the pair-wise additivity of potential energy, the validity of which must be regarded as unproven. Unfortunately, the second virial coefficient, which is the most convenient two-body property to analyse, is not capable of defining a unique potential energy function[3]. However, recent low-temperature data provide a valuable test of the adequacy of potential energy functions and illustrate clearly the failure of many widely-used potentials[4]. Although some evidence points to a more complex form of the potential function, it seems appropriate to examine first simple two-term functions of the $(n–6)$ type, which may be written:

$$U(r) = \varepsilon\left[\left(\frac{6}{n-6}\right)\left(\frac{r_m}{r}\right)^n - \left(\frac{n}{n-6}\right)\left(\frac{r_m}{r}\right)^6\right]$$

when r_m is the separation of the molecular centres at the potential minimum. The collision diameter σ is related to r_m by:

$$\sigma = r_m\left(\frac{6}{n}\right)^{\frac{1}{n-6}}$$

An analysis of the second virial coefficients of a number of simple gases shows that, as expected, many potentials of this type are capable of interpreting the experimental data for relatively high temperatures. However, analysis of the low-temperature results leads to the definite conclusion that, of the $(n–6)$ potentials, only those with n in the range $16 \leqslant n \leqslant 20$ are acceptable. (It is of interest to note that the shape of the (16–6) potential is similar to that of the Kihara function as applied to the inert gases[5]). This is illustrated for the very-low-temperature ranges in Fig. 1. Further evidence in favour of such values of n may be obtained from investigations of the transport properties of gases. It has been shown that an $(n–6)$ potential with $n > 12$ is superior to the traditional (12–6) potential when applied to such properties[6], and a quantitative investigation is in progress. Of the potentials we have considered, the (18–6) potential has previously been used to interpret the properties of globular molecules and found to be as good as the (28–7) potential which was expressly developed for this purpose[7].

On the basis of all the foregoing considerations we conclude that the (18–6) potential will provide a much more useful basis for the description of second virial coefficients and the transport properties of gases than any of the simple potential functions[11] in general use.

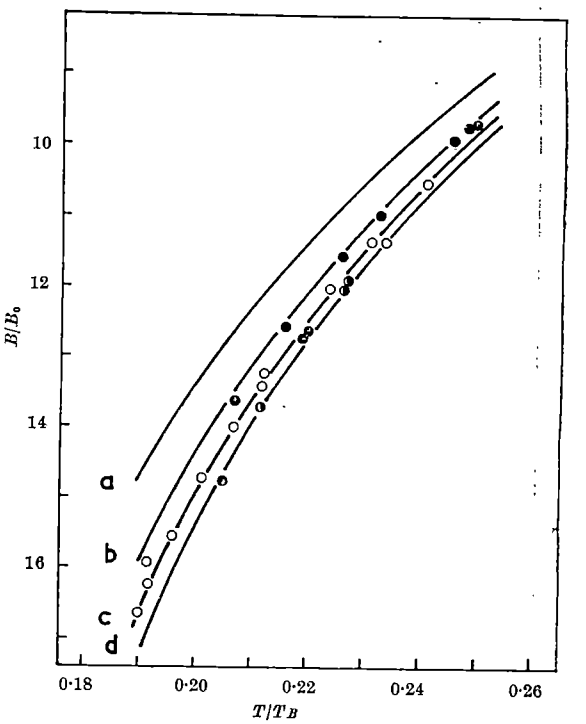


Fig. 1. The second virial coefficients at low reduced temperatures. The reduction procedure used was based on the Boyle temperature of the gases, $T_B.B_0$ is defined as the second virial coefficient at $T = 0{\cdot}7\ T_B$. Results are available for three classical gases in this region: ●, argon, refs. 4, 8, 9; ○, krypton, refs. 4, 10; ◑, oxygen, ref. 8. The solid curves *a*, *b*, *c* and *d* represent the theoretical values for $(n–6)$ potentials when n has the values 12, 16, 18, 20, respectively

We have calculated values of the reduced third virial coefficient over a range of reduced temperatures on the basis of the (18–6) potential by direct numerical integration, making the assumption of pair-wise additivity of the potential energy. The results are given in Table 1

Table 1. REDUCED THIRD VIRIAL COEFFICIENT FOR THE (18–6) POTENTIAL

T^*	C^*	$\Delta C^*/a^*$
0·7	−0·640	—
0·9	+0·513	—
1·0	+0·572	8·71
1·2	+0·535	5·73
1·5	+0·448	3·69
2·0	+0·373	2·27
3·0	+0·339	1·28
5·0	+0·338	0·69

$T^* = kT/\varepsilon$, $C^* = C/b_0^2$ where $b_0 = \frac{2\pi}{3}N\sigma^3$. $a^* = a/\sigma^3$ where a is the polarizability. $\Delta C^*/a^*$ is correction factor for non-additivity.

and are accurate to better than 0·5 per cent. A comparison with the rather uncertain experimental data shows the agreement to be reasonable, but in no way superior to that given by the (12–6) potential. However, if correction is made for non-additivity of the potential energy[12], the agreement is considerably improved.

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Negative Ion Formation in the Atomic Oxygen – Acetylene Reaction

PREVIOUSLY[1,2] we have reported on the production of positive chemi-ions in low-pressure atomic oxygen–acetylene reactions. Now we have also observed large concentrations (estimated as 10^{11}–10^{12} c.c.$^{-1}$) of negative ions in this system and have concluded that they are formed predominantly by electron attachment processes. This medium thus offers a much-needed new opportunity for the examination of electron attachment and charge transfer phenomena and the reactions of negative ions with positive ions and neutral species. Both the 'natural' ions of the reaction environment and those ions formed in the presence of additives can be investigated.

The same mass spectrometer atomic-diffusion flame apparatus used for positive-ion studies was used in this investigation after electronic modifications for negative-ion operation[3]. An atomic oxygen stream, produced in a side tube by subjecting a 96 per cent helium–4 per cent oxygen mixture to a 2,450-Mc/s, 125-W microwave discharge, diffused into the central portion of a 12-in.-diam. 'Pyrex' cross through which C_2H_2 flowed. The resulting reaction zone was oxygen-atom rich in the early stages of the reaction (near the O-atom inlet) and hydrocarbon rich farther downstream. The reaction pressures were 1–10 torr and the temperature varied from a maximum of 700° K near the centre of the reaction zone to near room temperature far out in the 'Pyrex' cross.

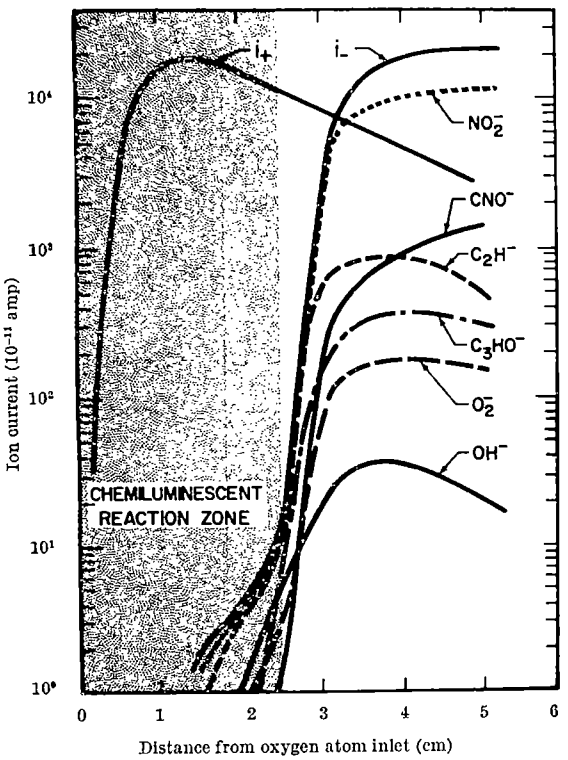


Fig. 1. Ion profiles. The magnitude of individual currents is not directly comparable to that of total current. i_+ and i_- indicate total positive and negative ion currents, respectively. C_2H_2 flow, 17·0 c.c. (S.T.P.) sec^{-1}; helium flow, 14·5 c.c. (S.T.P.) sec^{-1}; oxygen flow, 0·6 c.c. (S.T.P.) sec^{-1}

The negative ions observed include masses 16, 17, 24, 25, 26, 32, 42, 46, 49, 53 and 59. The hydrogen content of these ions has been determined in experiments where C_2H_2 was replaced with C_2D_2. Masses 17, 25, 53 and 59 all contain one hydrogen atom apiece; mass 49 may be a mixture of two species, having one and five hydrogen atoms, respectively. The other ions contain no hydrogen. Commensurate with these results, the ions observed have been tentatively designated as the following: O^-, OH^-, C_2^-, C_2H^-, CN^-, O_2^-, CNO^-, NO_2^-, C_4H^- (and possibly $CH_5O_2^-$), C_3HO^- and $HCNO_2^-$. The presence of nitrogen-containing ions in large relative concentrations is indicative of the well-known high electron affinities of their parent compounds, which are present as impurities only. Addition of halocarbons to the acetylene stream results in atomic halogen ions becoming dominant; addition of SO_2 yields large quantities of SO_2^- (or S_2^-) and some S^-.

Some individual ion current profiles are shown in Fig. 1, together with total positive and negative ion current profiles; the magnitude of individual currents is not directly comparable to that of total currents. The maxima in these total ion current profiles are approximately equal, but their relative magnitude in the luminous zone indicates that the negative charge carriers there are mainly electrons. Negative ions become important charge carriers only in the gases surrounding the reaction zone, indicating that they are formed predominantly by attachment processes. Concentrations of O^-, C_2^- and CN^- were too small, relative to OH^- and C_2H^-, respectively, to allow us to take meaningful profiles. Masses 49 and 59 are present in maximum concentrations comparable to O_2^- and C_3HO^-, respectively. Large relative concentrations of heavier ions up to at least mass 130 have also been observed. Little effort has been given thus far to identification of these ions because of poor mass spectrometer resolving power at higher mass numbers. These heavier ion species, however, maximize further from the O-atom inlet than do most of the lighter ion species. They are, therefore, thought to be the products of ion-clustering processes.

It is interesting to compare the results obtained here to those obtained in low (1–10 torr)[3] and atmospheric pressure[4] C_2H_2-O_2 combustion flames. The low-pressure combustion shows relatively small negative-ion to positive-ion ratios and a preponderance of lighter ions. The atmospheric pressure flames behave much like the present system, which shows that both electron attachment and ion clustering are favoured by lower temperatures and higher pressures. The same observation with regard to clustering can be made for the positive ions in these three systems[2,5].

Fite and Rutherford[6], working with afterglows in atmospheric gases, reported the presence of significant quantities of CO_3^- and (NO_2^-·H_2O), due to carbon and water impurities. There are no detectable quantities of these ions in the present system, notwithstanding the presence of carbon, NO_2^-, and at least some H_2O; water has been found to be a minor reaction product[7], and its presence here is also substantiated by the observation of an H_3O^+ peak[1,2].

A new mass spectrometer is being built to examine the discussed processes in further detail in electrical discharge flow systems of the Wood-Bonhoeffer type[1,2].

This work was sponsored by Project *SQUID* which is supported by the Office of Naval Research, Department of the Navy, under contract *Nonr* 3623(00), *NR*-098-038.

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Preferential Adsorption of Long-chain Normal Paraffins on MoS_2, WS_2 and Graphite from *n*-Heptane

I REPORTED recently that cast iron[1] and graphite[2] adsorb preferentially long-chain normal paraffins from hydrocarbon solvents such as *n*-heptane or benzene. It was thought that the adsorption is relevant to effective lubrication of sliding surfaces of cast iron and graphite by mineral oils. Adsorbed films of hydrocarbons are most likely to reduce the extent to which the rubbing surfaces come into contact, under a given pressure, which would result in less transfer of solid material across the interface and, therefore, a reduced rate of wear.

It was reported in the literature that films of MoS_2 and WS_2 deposited on steel surfaces reduce their wear rates when lubricated by mineral oils[3]. These two sulphides were examined, therefore, in the flow micro-calorimeter to discover whether they also have the property of preferentially adsorbing long-chain paraffins from low-boiling solvents. The flow calorimeter used has already been described[2]. The solvent in which the adsorption was examined was *n*-heptane purified by percolation through silica gel and alumina. *n*-Heptane percolated through a bed of MoS_2 or WS_2 in the calorimeter cell and μg quantities of dotriacontane were injected into *n*-heptane. Contact between dotriacontane and graphite was accompanied by a heat effect registered by a potentiometric recorder.

MoS_2 and WS_2 used in the experiments were commercially available materials. Examination by X-ray diffraction showed that these disulphides have a high degree of crystallinity, the diffraction patterns corresponding to those of MoS_2 and WS_2. Some weak unidentified lines were present in the WS_2 pattern. The Brumauer–Emmett–Teller surface areas of the disulphides were 8·3 and 2·2 m^2/g respectively. The amount of the disulphides placed in the calorimeter was 0·15 g. Results of the preferential adsorption experiments are illustrated in Figs. 1 and 2.

It can be seen that as the amount of *n*-C_{32} that comes into contact with the disulphides increases from 2 to 40 μg the heat effects increase. This increase is approximately linear for WS_2. For MoS_2 the heat effects per unit weight of solute decrease with the amount injected. Thus, although for 2-μg quantities of *n*-C_{32} the heat effects are very similar for both MoS_2 and WS_2, the heat effects produced when 40 μg of *n*-C_{32} come into contact with the disulphides are higher for WS_2 than they are for MoS_2. These results are obtained in spite of the fact that the surface area of MoS_2 is four times that of WS_2. Thus in the calorimeter cell the total Brumauer–Emmett–Teller area available for the adsorption of *n*-C_{32} is 1·25 m^2 for MoS_2 and 0·32 m^2 for WS_2. (If all this area was available for adsorption and the adsorbed molecules lay flat on the surface, a monolayer would be formed by 75 μg of dotriacontane on WS_2.)

In separate experiments it was established that up to 40 μg of dotriacontane was adsorbed almost completely on 0·15 g of both disulphides when injected into *n*-heptane percolating through the solids. This made it possible to estimate heats of adsorption of dotriacontane on the disulphides from *n*-heptane. The results are given in Table 1. It can be seen that the heats of adsorption on MoS_2 are somewhat lower than those on WS_2. The difference is, however, small in comparison with that between the heat adsorption on graphite and WS_2 or MoS_2, the latter heats being about one-third of the heat of adsorption on graphite. This indicates that dotriacontane adheres to graphite surfaces more strongly than it does to the surfaces of the disulphides.

Table 1. Heat of Adsorption of n-Dotriacontane from *n*-Heptane on Some Solid Lubricants

Solute adsorbed (μg)	Heat of adsorption (kcal/mol)		
	Graphite	MoS_2	WS_2
2	50	12·3	12·3
10	36	10·0	12·0
20	34	10·1	12·4
40	—	7·9	12·3

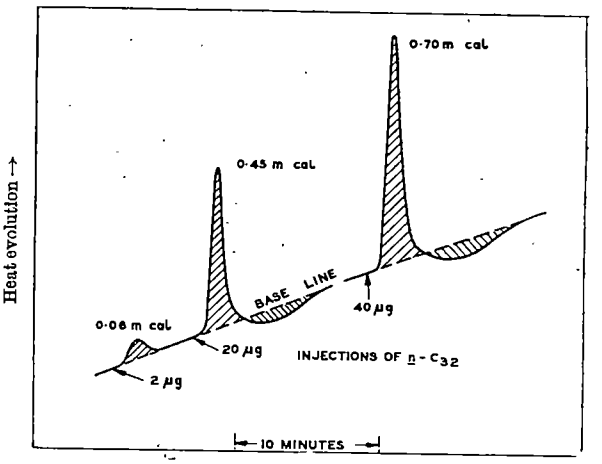


Fig. 1. Adsorption of *n*-C_{32} on MoS_2

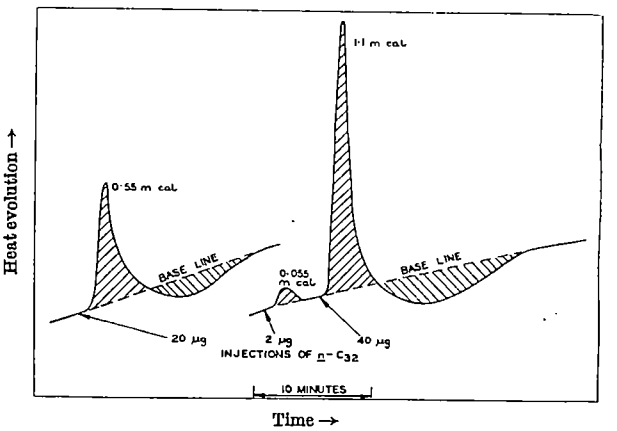


Fig. 2. Adsorption of *n*-C_{32} on WS_2

In view of the observed specific adsorption properties of graphite, MoS_2 and WS_2 and, also, the fact that these solids are reported to increase the load-carrying capacity of metal surfaces on which they are deposited, it is possible that the relatively strong tendency of long-chain hydrocarbons to adhere to the surfaces of these layered solids is an essential factor in increasing their load-carrying capacity.

It was shown by various investigations[4] that adsorption of long-chain compounds on metal surfaces in sliding contact decreases their wear and friction. This leads me to postulate that the value of graphite, MoS_2 and WS_2 in their performance as solid lubricants depends in part on their property of adsorbing tenaciously compounds with long normal hydrocarbon chains, which are usually present in mineral oils. The adsorbed hydrocarbons may be expected to reduce the frequency of solid-to-solid contact, in much the same way as the adsorption of stearic acid reduces the contacts of steel surfaces on which it is adsorbed.

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3-Methylxanthosine

In view of the present-day interest in methylated purines as minor components of RNA and DNA[1], and their possible role in mutagenesis, carcinogenesis[2] and protein synthesis[3], the preparation of *N*-methylated purine nucleosides and nucleotides has attracted increased attention[4–6]. One of the aims of these investigations is to find the reactive sites of the purine bases toward alkylating agents.

With regard to methylation of xanthosine, Bredereck, Haas and Martini[7] recorded the synthesis of 1,3-dimethylxanthosine. Jones and Robins[5] prepared the 7-methyl derivative, indicating the reactivity of the N_7 position. We wish to report evidence for the formation of small amounts of 3-methylxanthosine, among other products, by the reaction of xanthosine with diazomethane. The structure of this compound was demonstrated by hydrolysis to 3-methylxanthine and D-ribose, which were identified by ultra-violet spectra and paper chromatography.

A chilled solution of diazomethane (5·5 mmole in all) in *N*,*N*-dimethylacetamide was added gradually, with stirring in the cold, to a suspension of 500 mg xanthosine in 1 ml. of the same solvent. The resulting clear solution was added to 50 ml. of cold chloroform. A precipitate formed, which was washed with cold chloroform and dried *in vacuo* (234 mg crude product).

Chromatography of an aqueous solution of the precipitate (25 mg) on a 'Dowex-1-acetate' (×8,200 mesh) column (2·5 × 66 cm) was carried out by developing and eluting with water. Collection of fractions (4·5 ml., 0·5 ml./min) began immediately after charging the column. The profile of the recorded absorbance at 260 mμ exhibited three peaks. The third peak, comprising fractions 113–150 (maximum at fraction 124), contained the presumed 3-methylxanthosine. Fraction 130 showed the absorption characteristics indicated in Fig. 1. The spectrum is similar to that reported by Pfleiderer and Nübel[8] for 3,9-dimethylxanthine, in analogy to the similarity of the spectra of xanthosine and 9-methylxanthine[8].

Hydrolysis of adjacent fraction 129, by acidifying to *p*H 1 with hydrochloric acid and heating for 2 h at 100°,

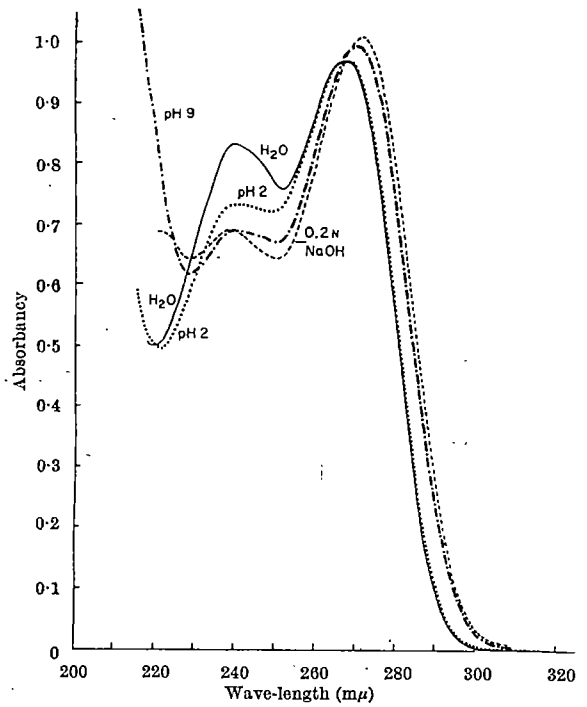


Fig. 1. Ultra-violet spectrum of 3-methylxanthosine (Fraction 130), maxima and minima (mμ): In H_2O, λ_{max} 239, 267, λ_{min} 220, 252; *p*H 2, λ_{max} 240 (*sh*), 267, λ_{min} 222, 251; *p*H 9, λ_{max} 240, 271 λ_{min} 228, 251; 0·2 N NaOH, λ_{max} 238, 272, λ_{min} 228, 250

Table 1. Comparison of Ultra-violet Spectra of Hydrolysed Fraction 129 with that of Authentic 3-Methylxanthine

	Hydrolysate (mμ) Max.	Min.	Authentic sample (mμ) Max.	Min.
*p*H 1	269	243	270	242
*p*H 9	271	245	272	245
0·2 N NaOH	273	235 (inflex)	273	235 (inflex)
		248		248

revealed an ultra-violet spectrum corresponding to that of authentic 3-methylxanthine (Table 1). The identity of the hydrolysate with 3-methylxanthine was further confirmed by R_F values in paper chromatograms. The summit of the third peak, which comprised a pool of fractions 120–128 containing the purest material, was concentrated to dryness *in vacuo* at room temperature. Half the dry material was hydrolysed in 0·1 N hydrochloric acid by heating for 2 h at 100°. The hydrolysate was concentrated *in vacuo* to a small volume, applied to Whatman No. 1 paper, and chromatographed in the following solvent systems: (I) ascending: *n*-butanol/acetic acid/water = 12 : 3 : 5 (v/v)[9]; (II) descending: *n*-butanol/*N*,*N*-dimethylacetamide/water = 2 : 1 : 1 (v/v); (III) descending: *n*-butanol/5 N acetic acid = 2 : 1 (v/v)[8] (Table 2).

The identity of the chromatographic spots obtained from the hydrolysate with 3-methylxanthine was again confirmed by the ultra-violet spectrum of the spot extract. Only one sugar spot was obtained from the hydrolysate, which was located with silver nitrate dipping reagent[9], and found to be identical with D-ribose (Table 2).

Table 2. Comparison of R_F Values of 3-Methylxanthosine Hydrolysate, 3-Methylxanthine, Xanthosine and D-Ribose in Three Solvents

R_F values	Solvents I	II	III
Authentic 3-methylxanthine	0·42	0·57	0·43
3-Methylxanthine in hydrolysate	0·43	0·55	0·44
Xanthosine	0·22	0·38	
1,3-Dimethylxanthine			0·67
Authentic D-ribose	0·33		0·28
D-Ribose in hydrolysate	0·33		0·29

Concentration of the eluate pool of fractions 120–128 was associated with considerable hydrolysis, reflected in a complete change of the ultra-violet spectrum and the appearance of spots on paper chromatograms indicating the presence of 3-methylxanthine and D-ribose.

We thank Miss Gertrude B. Elion, of Burroughs Wellcome Research Laboratories, Tuckahoe, New York, for supplying us with samples of methylated xanthines. This work was supported by U.S. Public Health Service grant *A*–162.

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BIOCHEMISTRY

Effect of Amitosis on the Distribution of Human Lactic Acid Dehydrogenase Isozymes

The lactic acid dehydrogenase (LDH) of mammalian tissues exists as five isozymes[1] which have different charges, structures and biochemical properties[2]. The pattern of isozyme distribution in embryonic and adult

Table 1. Distribution of LDH Isozymes in Various Human Tissues at Different Stages of Development

Tissue	Age	Isozyme distribution* I	II	III	IV	V
Ideal binomial distribution		6·25	25	37·5	25	6·25
Skeletal muscle	Embryo	8	28	36	21	8
	Adult	3	4	7·5	9·5	76
Heart muscle	Embryo	7	27	39	19	10
	Adult	60	30	5	3	2
Œsophagus muscle	Embryo	8	22	30	25	14
	Adult	5	18	40	24	13

* Values are the percentage isozyme activity related to the total LDH activity in the tissue homogenate (ref. 5). At pH 8·6 isozyme I shows the fastest migration to the anode and isozyme V the slowest.

tissues indicates that the fertilized ovum also contains five isozymes[5] which are identical to those of all other tissues[3,4]. In embryonic tissue isozyme III is the most abundant, but with cell differentiation the relative amount of isozyme III decreases (Table 1). There exists a parallel between the shift in isozyme distribution and the increase in cell nuclei population of human heart muscle. This increase (in the last phase through amitosis) comes to an end in the left heart muscle about six months after birth but is not finished even after one year in the right heart muscle[6,8]. It was, therefore, thought that the final distribution of isozymes would be achieved earlier in the muscle of the left heart ventricle than in the muscle of the right.

The heart muscle from the right and the left ventricles of human embryos and babies was obtained approximately 10–20 h *post mortem*. The methods for the extraction of LDH, the electrophoretic separation of the isozymes and the measurement of their activities were as previously described[5]. The results (Table 2) show a constant isozyme distribution during the mitotic phase, but during the amitotic phase the isozymes in the left heart muscle approach their final distribution sooner than those of the right. Human skeletal muscle has a similar shift in isozyme distribution during the post-mitotic phase (Table 1).

Table 2. Distribution of LDH Isozymes in Heart Muscle of Human Fœtuses and Babies*

	Length (cm)	Ventricle	Isozyme distribution I	II	III	IV	V	B/A†
Mitosis	15	Right	3	19	43	28	7	0·95
		Left	5	23	36	30	5	0·93
	23	Right	5	21	43	26	5	0·95
		Left	7	25	38	23	7	1·02
	33	Right	10	28	39	21	2	1·26
		Left	10	28	44	15	3	1·3
Post-mitosis	46	Right	12	28	42	15	3	1·36
		Left	12	40	39	8	1	1·74
	47·5	Right	16	56	23	4	1	2·4
		Left	38	40	17	4	1	3·4
	50	Right	26	39	27	7	1	2·4
		Left	37	43	16	3	1	3·54
	51	Right	20	39	29	10	2	2·0
		Left	26	42	24	6	2	2·44
	53	Right	45	37	11	4	3	3·8
		Left	54	34	9	2	1	5·5
	57	Right	52	35	9	2·6	1·4	5·0
		Left	54	35	7	3	1	5·5
	87	Right	52	28	10	6	4	4·0
		Left	60	32	5·5	1·6	0·9	6·8
	60 years	Right	64	30	5	1	0	8·3
		Left	62	33	3·1	1·3	0·6	7·8

* Values are the percentage isozymes related to the total LDH activity and are the mean of 3 determinations. Lengths are from the crown of head to the heel in cm.

† Values are the result given by dividing the sum of sub-units *B* by the sum of sub-units *A*.

Each isozyme of the LDH is a tetramer. There are two types of monomers, *A* and *B*, the combinations of which result in the five isozymes (4*A*; 3*A*, 1*B*; 2*A*, 2*B*; 1*A*, 3*B*; 4*B*)[10,11]. Therefore, there must be at least two gene loci. A binomial distribution of isozymes would be expected if the monomers *A* and *B* were synthesized to the same extent. This was found to be the case for mitotic tissues (Table 1). During the post-mitotic phase more of the extreme isozymes (I and II or IV and V) are produced[9]. This could be explained by an increased synthesis of *A* or *B*. The cells are polyploid since the chromosomes multiply before the amitosis[7]. If more *A* or *B* gene loci were produced then the shift in isozyme distribution would be explicable.

Amitosis occurs only in highly differentiated, specialized cells. This should lead to an increase in efficiency[6,7]. In tissues which function *in vivo* under relatively aerobic conditions there is a preponderance of isozymes I and II and a preponderance of IV and V in relatively anaerobic tissues. For example, in skeletal muscle isozymes IV and V catalyse the production of large amounts of lactic acid under high pyruvate concentrations whereby isozymes I and II are inhibited through large amounts of lactic acid[3]. In heart muscle the synthesis of lactic acid is optimal under low pyruvate concentrations since isozymes I and II have a higher affinity for pyruvate than IV and V. It thus appears that the metabolism controls enzyme synthesis to ensure optimal utilization of available metabolites. This control could be achieved by changes in the polyploid state of the chromosomes during the post-mitotic phase[12].

This work was carried out in the Medizinische Poliklinik der Universität Mainz.

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A Cryostat Method for the Extraction of Soluble Tissue Components

Established techniques used to release the intracellular components of tissues either into solution or suspension include homogenization[1,2], mincing[3], crushing[4], and ultrasonic vibration[5]. The degree of difficulty in producing tissue homogenates varies for different tissues. In the case of cellular tissues containing little connective tissue almost complete cell disruption can be obtained with any of these techniques. Hogeboom *et al.*[6] have shown that in unfiltered rat liver homogenates they were able to find only 7×10^6 intact cells/g liver as compared with 268×10^6 free nuclei. However, as the proportion of connective tissue increases, so does the work necessary to produce the same degree of cell disruption, and loss of labile cell contents generally follows as a result of prolonged exposure of the biological material to adverse physical conditions.

Perhaps the most widely used technique involves the use of a Potter–Elvehjem homogenizer[1]. There are many disadvantages to this technique, and the main ones are as follows: (*a*) Homogenization is carried out by a shearing force applied to the material for some time. The length of time necessary for complete homogenization is dependent on the type of tissue and this can lead to incomplete and non-reproducible degrees of cell membrane disruption. (*b*) Despite external cooling of the tube, the shearing force must result in localized heating which may in turn produce denaturation of the tissue components. (*c*) In order to allow the full shearing forces to develop it is necessary to reduce the viscosity of the suspension being homogenized by the addition of buffer, the volume of buffer required depending on the physical nature of the tissue. The concentration of the extract is therefore determined to some extent by the nature of the tissue.

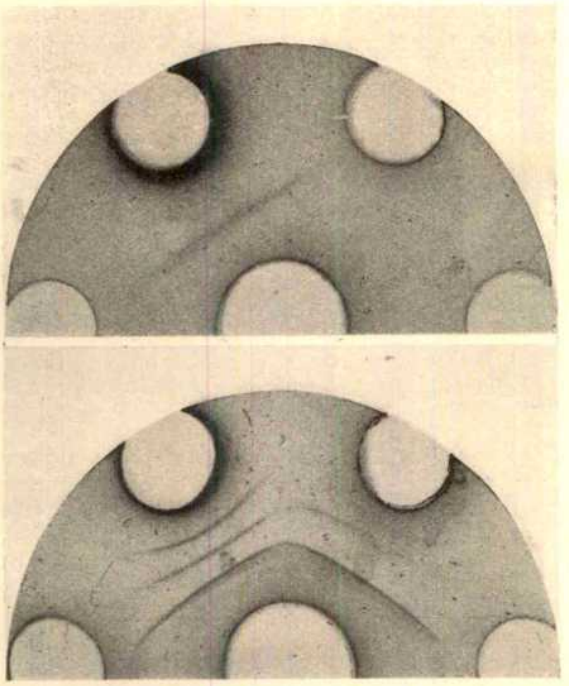


Fig. 1. Agar-gel diffusion plates of antitissue antiserum versus tissue extracts showing (*A*) an additional component, and (*B*) an increased concentration of components in the cryostat extracts. Central wells, antitissue antiserum; upper left hand well, cryostat extract; upper right hand well, Potter extract

In order to compare extracts from different tissues it is often necessary to concentrate them first—a process which can again lead to varying degrees of protein denaturation. Because of the viscosity effect one is restricted to the use of aqueous eluants and the use of organic or organic-aqueous solvents for the elution of specific cell components is precluded.

Some of these disadvantages are obviously common to the other techniques.

A very elegant steel block homogenizer, operating at temperatures below 0° C and using the freezing point depression occurring at high pressures, has been described by Ludvigsen[7]. Unfortunately the apparatus appears to be costly and its operation fairly complicated.

As a result of our preliminary attempts to use the established methods to isolate the soluble components from tissues, including human tumours, it became evident that the yields of cell components varied considerably according to the amount of connective tissue present. This made valid comparisons of the antigenic components from different specimens extremely difficult. In order to overcome this difficulty, a slicing technique, involving the use of a refrigerated microtome, has been developed which produces a degree of cell disruption at least equivalent to that of the homogenates produced by more conventional methods. The degree of cell disruption obtained was reproducible for a wide variety of tissues.

A block of the tissue, approximately 1 c.c., was frozen on to the chuck of a Cambridge rocking microtome using solid carbon dioxide. 5-μ sections of the tissue were then cut, either in a deep-freeze cabinet at −20° C or in a Bright's cryostat and collected into weighed pots. If required, sections were taken for histological or histochemical examination. After re-weighing, the pots containing the sections were stored at −20° C until required. The sectioned tissue was eluted with normal saline buffered with phosphate, *p*H 7·6 (3 ml./g sliced tissue). The slurry of tissue and buffer was either agitated gently for 3 h on a rotary mixer or left to stand overnight at 2° C. The suspension was then centrifuged at 15,000*g* for 15 min to pack down the cell debris and the clear supernatant pipetted off. The supernatants were stored at −20° C until required.

A comparison was made of the proteins present in the extracts from equal samples of the same tissues obtained by use of the cryostat, the Craigie Mincer[3], and the Potter homogenizer respectively. The extracts were adjusted to equal volumes and examined by starch-gel electrophoresis, agar-gel immunoelectrophoresis and Ouchterlony gel-diffusion technique.

Very little difference was found between the extracts prepared by the Potter homogenizer and the tissue mincer; but the cryostat extracts showed additional protein components or an increased quantity of proteins (Fig. 1). Similar extraction efficiency has been obtained with a variety of tissues, in particular human tumours.

In order to compare the degree of cell disruption produced by the Potter and cryostat techniques, smears were prepared from the tissue residues after homogenization. These smears, after fixing in Schaudinn's fluid and staining with hæmalum and eosin, were examined microscopically for the presence or absence of both free nuclei and intact cells.

Intact cells and clumps of cells were invariably present in all the Potter residues examined, while the cryostat residues only occasionally contained a few nuclei or intact cells (Fig. 2).

The advantages of this technique are summarized as follows: (*a*) Maximum cell membrane disruption was achieved. (*b*) Temperatures below 0° C were used for the homogenization. (*c*) No eluant was added until after homogenization and no restriction was made as to choice of eluant. (*d*) The homogenate could be stored. (*e*) The method required only readily available apparatus. This technique has been used with success in this laboratory for the past two years. The use of a tissue slicer for

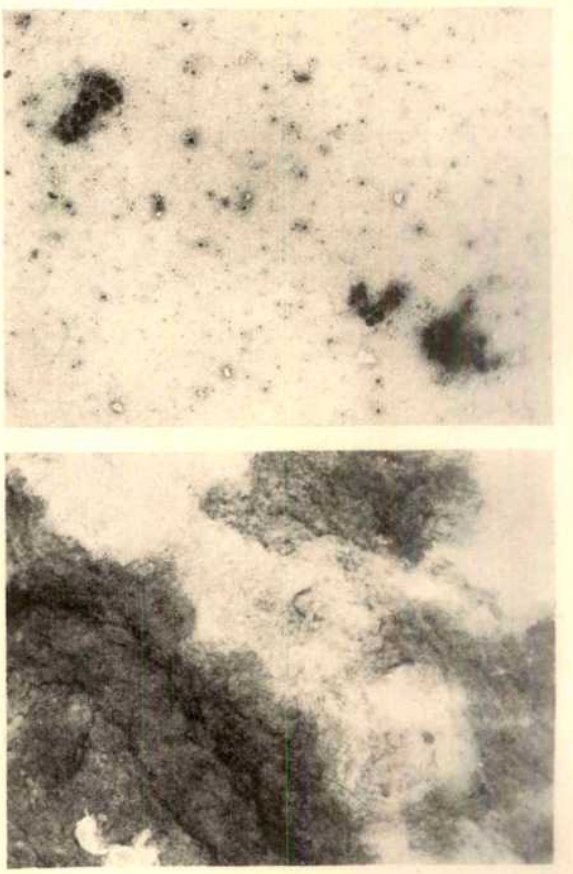


Fig. 2. Photomicrographs of tissue residue smears. (× *c.* 100.) *A*, After Potter homogenization, showing intact cells; *B*, after cryostat method, showing mainly acellular debris

tissue homogenization has been reported previously[4,8], but in this case the cells were not disrupted by sectioning but by freeze drying the slices and then bursting the cells by immersion in buffer.

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Detection of Enzymes in Agar Electropherograms

To demonstrate the proteolytic enzymes in an electropherogram, Uriel *et al.*[1,2] dipped an electropherogram into a substrate solution of an appropriate *p*H and, after an incubation period of some hours, the electropherogram was stained with amido black. Light spots indicated the presence of proteolytic enzymes. In their immunological work, Raunio and Gabriel[3] used a second slide covered with substrate in agar to find the precipitation lines that had proteolytic activity. Here, too, the disappearance of substrate through enzymatic activity is demonstrated by staining the protein. With these publications in mind, we developed a simple and rapid method for localizing enzymatic activities in agar electropherograms.

We carried out the electrophoresis of a protein extract on a microscope slide. The substrate solution in 1 per cent agar was poured on to a second slide. After electrophoresis the slide with the agar substrate gel was placed, with the gel layer down, on to the electropherogram. After an incubation time of 5–20 min, at 37° C, the two slides were separated and the remaining substrate was precipitated by an appropriate fixing agent. Transparent spots indicated where enzymatic activity had occurred.

The advantage of precipitating rather than staining the substrate lies in the possibility of constantly observing the process. A short fixation only precipitates the uppermost layer of the substrate gel and thus reveals even very slight activity. On the other hand, the narrowing of the transparent spots after a long fixation time allows a more exact localization of the stronger enzymatic activities.

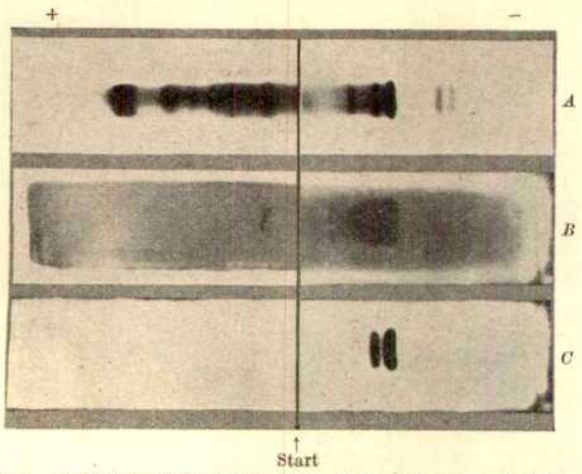


Fig. 1. *A*, The stained electropherogram of rat pancreas extract electrophoresis on microscope slides, covered with 2 ml. 1 per cent agarose in veronal buffer *p*H 8·6 ($\mu = 0{\cdot}033$), 35 min, 18 m.amp (approx. 20 V/cm); *B*, the substrate slide, after 5 min fixation in ethanol-acetic acid; *C*, the substrate slide, after 30 min fixation

This method can be used for the demonstration of several enzymes. It has been applied to the localization of amylase in electropherograms of a rat pancreas extract. The extract was obtained by grinding rat pancreas with sand under 0·14 M NaCl in a mortar. The extract was concentrated and subjected to electrophoresis on agarose[4] in a veronal buffer, *p*H 8·6.

For the substrate slide, 1 ml. 1 per cent hydrolysed starch in 1 per cent agar in the same buffer was used and after 15 min incubation at 37° C the starch was precipitated by immersing the slide in 70 per cent ethanol containing 5 per cent AcOH.

The presence of amylase activity is betrayed by the transparent spots on a milky white background, as seen in Fig. 1.

Three components with amylase activity could be distinguished in the rat pancreas extract. One of them occurred in such a low concentration that its effect was only visible after a short fixation time. The transparent spot disappeared after a prolonged fixation time.

This method, which can easily be adapted to the detection of other enzymes because of its flexibility, has helped us to locate proteolytic enzymes and nucleases. In the latter case, we used 0·5 N HCl as the precipitant.

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A New Mould D-Amino-acid Oxidase

D-amino-acid oxidase has been found in mammalian tissues[1], bacteria[2,3] and moulds[4–7], and the enzyme has been used for many purposes, such as the determination of D-amino-acids, the optical resolution of DL-amino-acids and the preparation of α-keto acids.

On D-amino-acid oxidase from moulds, the constitutive existence of its activity has been reported in *Neurospora crassa* by Horowitz[4], in *Neurospora sitophila* by Burton[5] and in *Penicillium chrysogenum* by Emerson *et al.*[6]. Besides these reports, adaptive formation of D-glutamic acid oxidase activity, which also exhibits some activity towards D-aspartic acid in *Aspergillus ustus*, was reported by Mizushima *et al.*[7].

We also investigated extensively the D-amino-acid oxidase activity in moulds and found that an enzyme activity, which is highly active towards a wide variety of D-amino-acids, was formed in *Aspergillus flavus* and *parasiticus*.

The screening experiment of the moulds which could utilize D-amino-acids was carried out first. To the basal medium containing 2 per cent glucose, 0·2 per cent K_2HPO_4 and 0·1 per cent $MgSO_4 \cdot 7H_2O$, was added D-methionine, D-phenylalanine, D-tryptophan or D-valine as a sole source of nitrogen at a final concentration of 0·5 per cent. 43 typical strains of moulds were cultured with shaking at 30° for 3–6 days in test-tubes containing the foregoing screening media. Most of the tested moulds grew by utilizing the D-amino-acids.

Oxidase activity towards several D-amino-acids of the 8 strains, which showed better growth among those tested, was measured by a Warburg manometer. As a result, *Aspergillus flavus* and *parasiticus* were found to show the highest activities.

Thus with these two moulds, cultural conditions for the formation of D-amino-acid oxidase were investigated. Cultures of the moulds were prepared in 500-ml. shaking flasks containing 100 ml. of the respective media prepared by supplementing 0·2 per cent L-, D-, or DL-

amino-acids to Czapek medium, *S*–9 medium (2 per cent glucose, 2 per cent corn steep liquor, *p*H 5·0), and *S*–10 medium (2 per cent glucose, 2·5 per cent 'Pharmamedia' (Traders' Protein Division, Traders' Oil Mill Co., Fort Worth, Texas), *p*H 5·0). After 3–4 days culture at 30° on a reciprocal shaker, the mycelia were collected, homogenized, extracted with water, and the supernatant solution was used as cell-free extracts for the assay of oxidase activity.

Table 1. Effect of D-Amino-acids and DL-Amino-acids on the Production of D-Amino-acid Oxidases

Media		D-Amino-acid oxidase activity*	
Basal media	Amino-acids added	*A. flavus*	*A. parasiticus*
Czapek medium	No addition	< 1	< 1
	DL-Ala	43	76
	D-Asp	< 1	0
	Gly	4	< 1
	D-Lys	5	18
	D-Met	28	48
	L-Met	0	0
	DL-Met	12	37
	D-Phe	65	96
	L-Phe	2	—
	D-Thr	11	60
	L-Thr	2	0
	D-Try	—	111
	D-Val	38	94
	L-Val	< 1	—
S–9 medium	No addition	46	44
	DL-Ala	362	697
	Gly	174	94
	D-Lys	157	94
	DL-Met	—	65
	DL-Orn	—	102
	D-Thr	422	265
	L-Thr	132	100
	DL-Thr	346	—
S–10 medium	No addition	0	0
	DL-Ala	—	423
	DL-Met	—	275
	D-Thr	130	254

Enzyme activity was measured at 37° C on a Warburg manometer. Each flask contained 1·0 ml. of the cell-free extract and 1·0 ml. of H_3BO_3–KCl–NaOH buffer (*p*H 9·0) in the reaction chamber, 0·5 ml. of 0·04M D-valine in the side arm and 0·5 ml. of 20 per cent KOH in the centre well. Oxygen consumption was measured for a period of 5–10 or 5–15 min.
* Uptake of oxygen (*μ*l./h/ml. of culture medium).

Table 2. Substrate Specificity of the D-Amino-acid Oxidases*

Substrates \ Moulds	*A. flavus*†	*A. parasiticus*‡
D-Val	44·7	25·3
D-Met	44·2	25·3
D-Phe	39·2	24·4
D-Leu	32·8	21·8
D-Ala	28·3	18·6
D-Glu NH_2	28·1	17·5
D-Tyr	25·6	15·4
D-Try	22·7	11·6
ε-N-Bz-D-Lys	22·2	14·4
D-Ileu	14·7	12·9
D-His	11·8	5·3
D-Lys	11·1	4·0
D-Orn	9·5	5·0
DL-Arg	9·1	4·8
DL-Pro	8·6	5·4
D-Ser	7·8	5·2
D-Thr	4·6	3·0
D-Glu	4·4	0·9
D-Asp NH_2	4·0	2·5
D-Asp	0·8	0
Gly	0·4	0·3
L-Amino-acids	0	0

Conditions of enzyme assay were the same as described in Table 1.
*Uptake of oxygen *μ*l./h/mg of dry mycelia.
†*A. flavus* grown on *S*–9 medium containing DL-alanine.
‡*A. parasiticus* grown on Czapek medium containing D-valine.

As shown in Table 1, higher D-amino-acid oxidase activity was observed in the media containing D- or DL-amino-acids, although the activity was scarcely detected in the case of no addition or the addition of L-amino-acids. In *S*–9 medium, however, some activity was formed without the addition of D-amino-acids. This may be due to the presence of partially racemized amino-acids and/or glycine in corn steep liquor. The results suggest that the D-amino-acid oxidases of *Aspergillus flavus* and *parasiticus* are a kind of adaptive enzyme.

Using the cell-free extracts of *Aspergillus flavus* and of *Aspergillus parasiticus*, oxidative activities towards a number of D- and L-amino-acids were measured manometrically to compare their specificities.

As shown in Table 2, both preparations showed similar specificity. The D-isomers of neutral and basic amino-acids are readily oxidized, while acidic amino-acids and glycine are oxidized slowly or not at all. None of the L-isomers of all tested amino-acids was susceptible.

Judging from its higher activity, inducible nature and substrate specificity, the present enzyme activity appears to be quite different from any other previously known D-amino-acid oxidases from moulds[4–7] (summarized in Table 3).

Several enzymatic properties were examined with the enzyme from *Aspergillus flavus*. Although optimum *p*H varies to some extent according to the amino-acids and buffer solutions, it is situated at 8·0–9·5 in most cases. Reaction velocity attains its maximum at 47° C, and beyond this point the velocity decreases rapidly. The activity is inhibited by heavy metal ions, Ag^+ and Cu^{++}, monoiodoacetic acid, potassium cyanide and 2,4-dinitrophenol. The Michaelis constant is $2{\cdot}1 \times 10^{-3}$ M for D-valine, $2{\cdot}2 \times 10^{-3}$ M for D-phenylalanine, $4{\cdot}8 \times 10^{-3}$ M for D-lysine and $1{\cdot}1 \times 10^{-3}$ M for D-glutamine.

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A Selective Reagent for the Study of Base Sequence in Nucleic Acids

Some time ago, we proposed an electron microscopic procedure for the determination of the sequence of bases in nucleic acids[1]. This method requires that electron-dense, base-specific groups be attached to the nucleic acid molecule. On examination in the electron microscope, the visible electron-dense groups indicate the positions of those bases to which they are specifically attached.

It was shown that 8-amino-1,3,6-naphthalenetrisulphonic acid can be attached to DNA by a diazocoupling

Table 3. D-Amino-acid Oxidases of Various Moulds

Moulds	Enzyme formation	Substrate specificity	D-Amino-acid oxidase activity*		
			Substrate	Per ml. of culture medium	Per 100 mg of fresh mycelia
Neurospora crassa (ref. 4)	Constitutive	Non-specific	DL-Met	0·8–1·3	10·7
Penicillium chrysogenum (ref. 6)	Constitutive	Non-specific	DL-Leu	—	20·8
Aspergillus ustus (ref. 7)	Adaptive	D-Glu, D-Asp specific	D-Glu	13·2	—
Aspergillus flavus†	Adaptive	Non-specific	D-Val	422	840
Aspergillus parasiticus†	Adaptive	Non-specific	D-Val	697	1,070

* Uptake of oxygen (*μ*l./h). † Present communication.

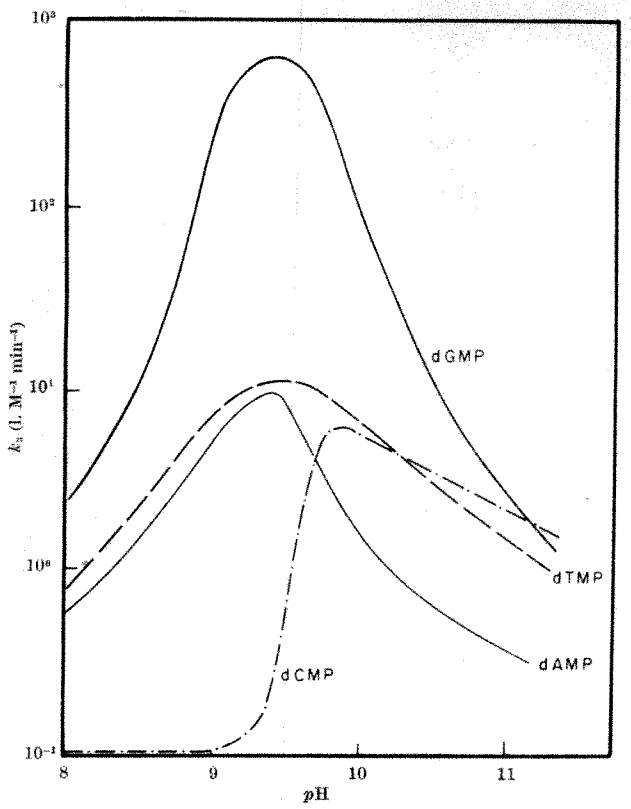


Fig. 1. Rate constants of reaction of diazotized 2-amino-1,4-benzenedisulphonic acid with the deoxyribonucleotides between *p*H 8 and 11

reaction and that, after staining with uranyl salts, it is detectable in the electron microscope. Here we report our results on the chemical specificity of a similar though somewhat smaller marker: diazotized 2-amino-1,4-benzenedisulphonic acid. The reactions of this reagent with the deoxynucleotides were investigated in aqueous solution at *p*H values from 7 to 11. The rate of coupling was inferred from the rate of disappearance of diazonium salt in the presence of the nucleotide. The concentration of unused diazonium salt was determined colorimetrically on addition of excess β-naphthol. Assuming second-order kinetics for the reactions, rate constants were calculated for all the nucleotides. These are given in Fig. 1.

It is clear that at *p*H 9 the reaction is highly *G* specific.

A fuller report of this work will be published elsewhere. This work was supported by National Institutes of Health grant *RG* 08968. We thank Dr. F. H. White for his assistance.

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Beta-Galactosidase of *Helix pomatia*

The digestive juice of *Helix pomatia* contains a remarkable number of enzymes; many of them are carbohydrases, and they include an enzyme that hydrolyses lactose or β-methyl-galactoside[1,2]. This communication describes the properties and activities of the enzyme that hydrolyses *o*-nitrophenyl-β-D-galactopyranoside (ONPG).

Digestive juice was stored at 2° C with no added antiseptic and was diluted before use. Liberation of *o*-nitrophenol from ONPG, in 0·2 M Na_2HPO_4–0·1 M citric acid buffer, was measured as described by Conchie *et al.*[3]. The effect of *p*H on the rate of hydrolysis was determined over the *p*H range 2·2–8·0 (Fig. 1). The *p*H optimum was 5·3, a higher value than that for other galactosidases (4·5 for plant glycosidases[4]; 3·6 for mammalian glycosidases[3]). The optimum *p*H was the same for each of two substrate concentrations (2·5 mM and 6·0 mM).

The effect of various substrate concentrations on the reaction velocity was investigated at *p*H 5·3 (Fig. 2). The limiting velocity was never attained, and there was no indication of inhibition by excess of substrate. Fig. 3 shows the variation in activity with enzyme dilution (the amount of substrate split in unit time increased linearly with the amount of enzyme, up to 30 per cent hydrolysis of the substrate), and Fig. 4 shows the variation in activity with time of action. The rate was unchanged for an incubation period of up to 2 h, after which there was some

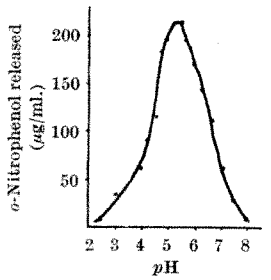


Fig. 1. Effect of *p*H on the rate of hydrolysis of *o*-nitrophenyl-β-D-galactoside (6·0 mM) in phosphate-citrate buffer; juice dilution 1/7,000, 1 h; 37° C

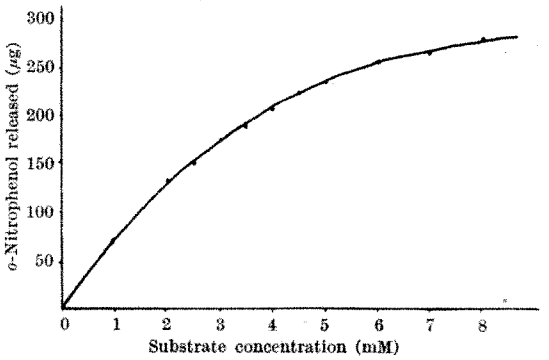


Fig. 2. Effect of varying the substrate concentration on the rate of hydrolysis of ONPG in phosphate-citrate buffer *p*H 5·3; juice dilution 1/6,000, 1 h; 37° C

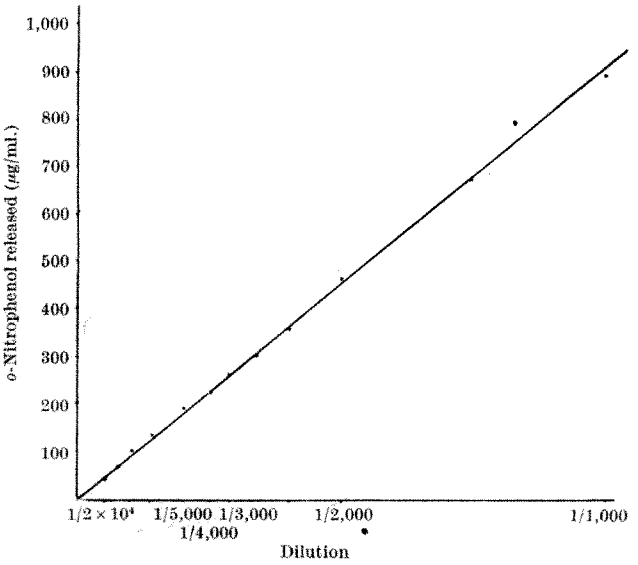


Fig. 3. Variation of β-galactosidase activity with amount of enzyme; 2·5 mM ONPG, *p*H 5·3, 1 h; 37° C

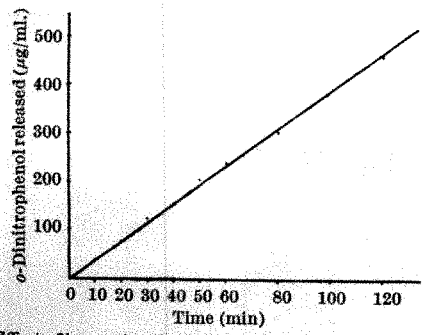


Fig. 4. Effect of increasing the period of incubation on the hydrolysis of 6 mM ONPG, *p*H 5·3, 37° C

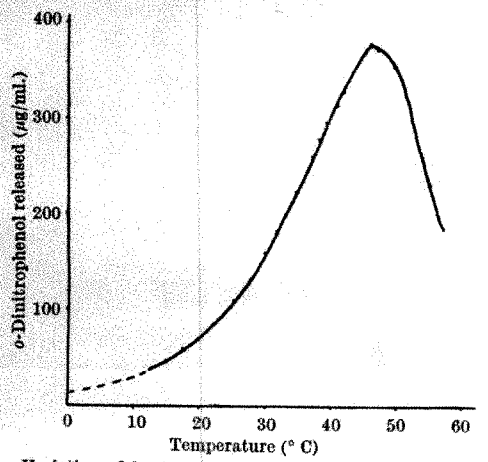


Fig. 5. Variation of β-galactosidase activity with temperature; 6 mM ONPG, *p*H 5·3, 10 min; 37° C, *p*H 5·3; juice dilution 1/1,000

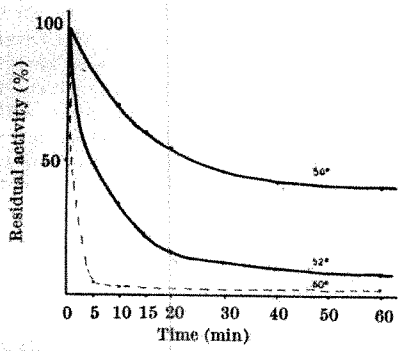


Fig. 6. Residual activity of β-galactosidase after varying period of pre-incubation at 50° C, 52° C and 60° C; *p*H 5·3

inactivation. With a 1/500 juice dilution and 6·0 mM substrate concentration, 90 per cent of ONPG was hydrolysed in 7 h, at *p*H 5·3 and 37° C.

Fig. 5 illustrates the variation in activity with temperature. Inhibition occurred at 50° C. Partial heat inactivation is plotted in Fig. 6, the enzyme being pre-incubated for various times in the absence of substrate at 50° C, 52° C and 60° C, at *p*H 5·3.

The rat epididymis is among the richest known sources of β-galactosidase[3]. It is difficult to compare results expressed as μg *o*-nitrophenol released per g moist tissue (47,000) and per ml. digestive juice (1,500,000), but the enormously high activity of β-D-galactosidase in the digestive juice of *Helix pomatia* makes this a most convenient source of the enzyme.

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Tryptophan Modification in Polypeptide Chains

It is known that tryptophan in proteins is completely destroyed during acid hydrolysis or performic oxidation of disulphide bridges[1]. Our purpose is to remove some difficulties that occur during sequential investigations of tryptophan-containing polypeptides and proteins.

In previous work[2] it was shown that ozone treatment in formic acid at room temperature converted tryptophan, methionine, cystine and tyrosine, respectively, to N'-formyl-kynurenine, methionine sulphone, cysteic acid and aspartic acid. In the same conditions all the other amino-acids were stable. In the present work we have investigated the selectivity of ozone oxidation towards the aforementioned amino-acids. When ozone is allowed to bubble slowly through an equimolecular solution of tryptophan, methionine, cystine and tyrosine in formic acid at 8° C, the observed order of reactivity is:

tryptophan > methionine > cystine > tyrosine

In an attempt to minimize the extent of the oxidation of tyrosine we have examined the utilization of some aromatic compounds. We have found that in the presence of resorcinol the oxidation of cystine and of tyrosine begins with a delay proportional to the quantity of resorcinol added (Fig. 1). In a typical experiment we have interrupted the ozone oxidation of a 10^{-2} M solution of tryptophan, methionine, resorcinol, cystine and tyrosine after complete disappearance of tryptophan followed spectrophotometrically at 283 mμ. The reaction mixture was analysed according to Spackman, Stein and Moore[3] after removal of formic acid and deformylation of N'-formyl-kynurenine[2]. The results are shown in Table 1.

Table 1. Products of Selective Ozonization of a Mixture of Oxidizable Amino-acids in Formic Acid at 8° C with Resorcinol added

Compound	Recovery after ozonization (%)	Products from ozonization	Yield (%)
Tryptophan	0	Kynurenine*	96
Methionine	0	Methionine sulphoxide	97
Cystine	93	Cysteic acid	5
Tyrosine	100	—	—

* Kynurenine emerges as a separate peak on the effluent curve before lysine.

It is clear that the resorcinol allows a quantitative conversion of tryptophan and methionine to kynurenine and methionine sulphoxide while tyrosine and cystine are quantitatively recovered.

In another experiment, after ozone oxidation until tryptophan had completely disappeared, we have added performic acid at 0° C as described by Hirs[4] and analysed the mixture again according to Spackman, Stein and Moore[3]. In Table 2 are shown results concerning recovery of kynurenine and tyrosine after performic oxidation, with conversion of cystine to cysteic acid and methionine sulphoxide to methionine sulphone.

Table 2. Products from Selective Ozonization of Oxidizable Amino-acids in Formic Acid at 8° C with Resorcinol added and further Performic Oxidation at 0° C

Compound	Recovery after oxidation (%)	Products from oxidation	Yield (%)
Tryptophan	0	Kynurenine	94
Methionine	0	Methionine sulphone	95
Cystine	0	Cysteic acid	92
Tyrosine	97	—	—

In peptides of tryptophan the conversion

$$\text{tryptophan} \xrightarrow{O_3} \text{kynurenine}$$

takes place without break-down of peptide bonds[5]. We suggest that such a conversion in a polypeptide chain could be used: (1) for the analytical determination of tryptophan in proteins by acid hydrolysis (since kynurenine is stable in such conditions); (2) for the performic oxidation of —S—S— bridges in polypeptide chains containing tryptophan; and (3) for the synthetic preparation of unknown kynurenine peptides from corresponding peptides of tryptophan.

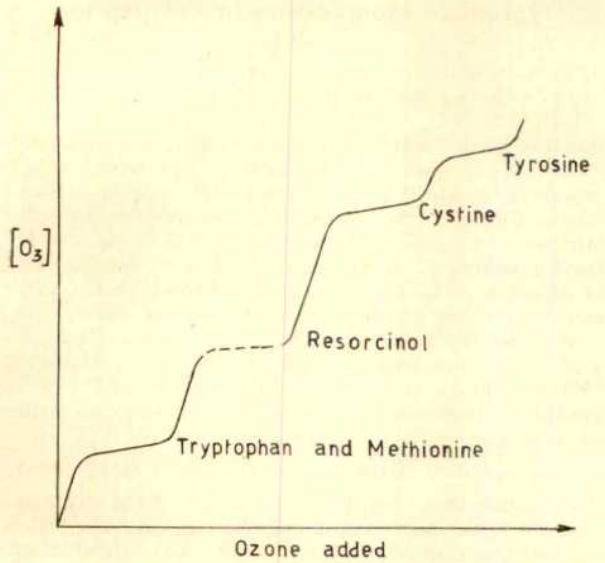


Fig. 1. Hypothetical increase of ozone concentration as a function of oxidizable amino-acids and resorcinol

The selective oxidation of the aforementioned amino-acids could be conveniently used to investigate the biological activity of modified polypeptides and proteins.

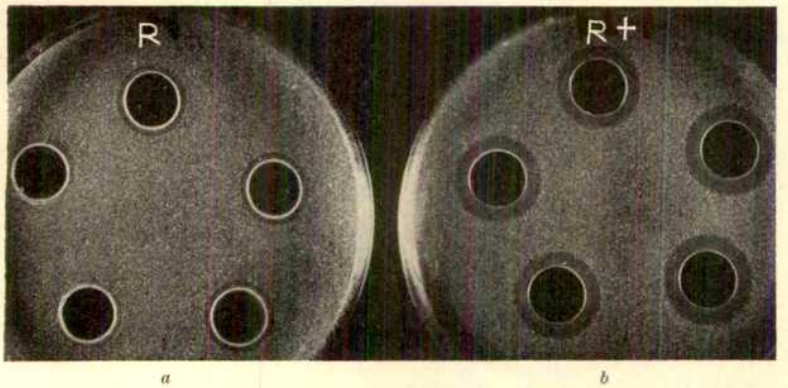
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An Inhibitory Substance present in Milk

During investigations into the efficiency of ultra-high-temperature sterilizing plants, Franklin *et al.*[1] observed that the test organism, a strain of *Bacillus stearothermophilus*, was inhibited by the heated milk. Inhibition became apparent when comparisons were made between the recoveries obtained from direct plate counts and from dilution counts in which the ultra-high temperature sterilized milk was used as diluent; a similar but weaker effect on *B. subtilis* spores had been previously noted by Franklin *et al.*[2].



a *b*

Fig. 1. (*a*) Zones of inhibition from *R*, raw milk, and in a clockwise direction, milk heated to 80° for 20 min, to 80° for 60 min, to 90° for 20 min and to 90° for 60 min. (*b*) Zones of inhibition obtained with factors prepared from milk. *R*+, obtained from raw milk, and in a clockwise direction, from milk heated to 80° for 20 min, to 80° for 60 min, to 90° for 20 min and to 90° for 60 min

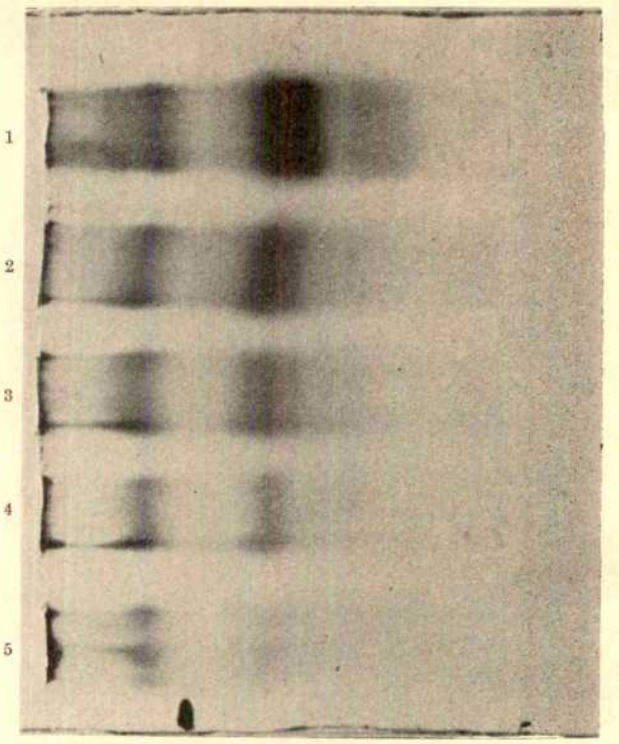


Fig. 2. Electrophoresis patterns of inhibitory material prepared from milk samples as described in text. (1) From raw milk; (2) from milk heated to 80° for 20 min; (3) from milk heated to 80° for 60 min; (4) from milk heated to 90° for 20 min; (5) from milk heated to 90° for 60 min. The anode is on the right

In a preliminary communication Jayne-Williams[3] reported that the inhibitory factor(s) occurred also in raw, pasteurized and dried milk and were active against 23 out of 60 strains of thermophilic spore-formers tested. The material was shown to be associated with the 30 per cent $(NH_4)_2SO_4$ precipitate obtained from milk serum at *p*H 6·5, to be stable to heat and low *p*H and to maintain activity in the presence of sulphydryl compounds. The stability of the factor(s) to heat is demonstrated in Fig. 1(*a*). The extent of inhibition against *B. stearothermophilus* was the same for material obtained from raw skim milk and from milk heated at 80° for 20 and 60 min and at 90° for the same times. Autoclaving at 121° for 15 min, however, destroyed the activity.

It has been shown recently that the factor(s) can be obtained in good yield by the following technique. Skim milk is acidified with concentrated H_2SO_4 to 0·2 N; the precipitate formed is removed by centrifuging and ethyl alcohol is added to the supernatant to a final concentration of 20 per cent (v/v). The precipitate which forms on standing overnight at 4° is removed by centrifuging, re-dissolved in water and the *p*H adjusted to 6·5–7·0 with N NaOH. The activity of the material obtained in this way from raw milk and the same milk heated as outlined above is shown in Fig. 1(*b*). The material was concentrated five-fold compared with the original milk.

The factor(s) is not dialysable, separates with the milk protein on filtration on 'Sephadex *G*–25' and loses activity rapidly on treatment with trypsin or chymotrypsin. Activity is also lost in the presence of $FeSO_4$ (*c.* 0·02 M) and to a lesser extent in the presence of $MgSO_4$.

Analysis of the amino-acids present after acid hydrolysis of material derived from skim milk heated at 90° for 60 min

revealed no exceptional amounts of either acidic or basic amino-acids.

Electrophoretic patterns of the material are shown in Fig. 2. Separation was performed in 7·5 per cent polyacrylamide gels with 0·005 M *tris*-citrate buffer (*p*H 7·0) for 5·5 h at 300 V. The protein was afterwards stained with amido black. The effect of heating is to reduce the quantity of faster-moving major components but to leave a slower-moving component unaltered. There is some indication that activity is associated with this slower-moving fraction but, as only part of the initial activity was recovered from this zone, it may be that other components, as yet not determined, contribute either singly or synergistically to the total activity.

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PHYSIOLOGY

Derivation of the Partial Differential Equations utilized in a Model describing the Na Concentration Profile in the Renal Medulla

THE purpose of this communication is to derive the partial differential equations and to clarify the mathematical details described in connexion with a model used to obtain the sodium concentration profile in the renal medulla[1]. An elementary operating unit of the model is shown in Fig. 1. A generalized segment of a vascular or tubular channel is shown in the top half of Fig. 2. Consider the case where fluid moves in a direction from left to right, corresponding to motion in the descending limbs of the vas rectum or the Henle loop. The net change of sodium concentration ΔC(g/cm³) in the differential volume element of length ΔX shown is given by equation (1).

$$\Delta C_m A_m \Delta X = \Delta t U_m A_m [C_m|_x - C_m|_{x+\Delta x}] + \delta_m \Delta X \Delta t (J - C_m) \quad (1)$$

A_m is the cross-sectional area of the tubule in cm², J is the interstitial concentration of sodium, and δ_m is a membrane constant of dimension cm²/sec. U_m is the magnitude of the rate of flow of fluid in the channel in cm/sec. Δt is a differential element of time (sec). The subscript m may be used to refer to a particular channel. In the original work, C was the concentration in the descending limb of the vas rectum, C' that in the ascending limb of the same element, C_h the concentration in the descending limb of the Henle loop, and C'_h the concentration in the ascending limb of the loop of Henle. It is assumed that δ is the membrane constant throughout the vas rectum, δ_h the constant in the thin segment of the Henle loop and δ'_h the appropriate constant in the thick segment of the Henle loop. Passing to the limit as Δt and ΔX both approach zero, the defining partial differential equation (2) is obtained.

$$\frac{1}{U_m}\frac{\partial C_m}{\partial t} = -\frac{\partial C_m}{\partial X} + \frac{\delta_m}{U_m A_m}(J - C_m) \quad (2)$$

For fluid moving in the ascending limbs, equation (1) becomes:

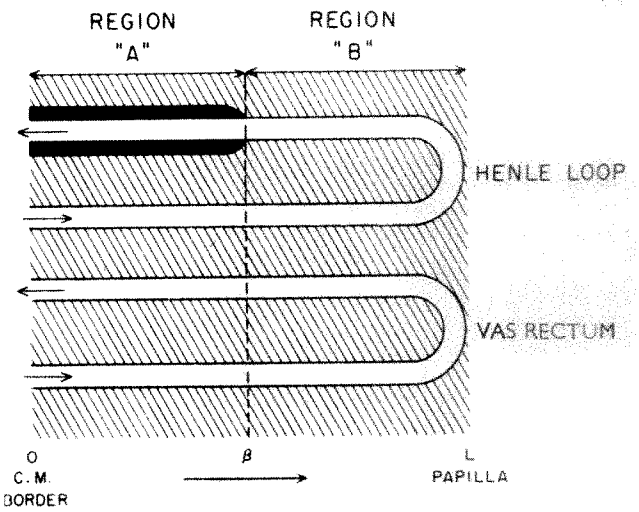


Fig. 1. Elementary operating unit

$$\Delta C_m A_m \Delta X = \Delta t U_m A_m [C_m|_{x+\Delta x} - C_m|_x] + \delta_m \Delta X \Delta t (J - C_m) \quad (3)$$

producing partial differential equation (4) in its limit:

$$\frac{1}{U_m}\frac{\partial C_m}{\partial t} = \frac{\partial C_m}{\partial X} + \frac{\delta_m}{U_m A_m}(J - C_m) \quad (4)$$

The thick segment of the ascending limb of the Henle loop, which is the only portion in which active transport of sodium is assumed to occur, produces the difference equation (5), and partial differential equation (6).

$$\Delta C'_h A_h \Delta X = \Delta t U_h A_h [C'_h|_{x+\Delta x} - C'_h|_x] - \delta'_h \Delta X \Delta t C'_h \quad (5)$$

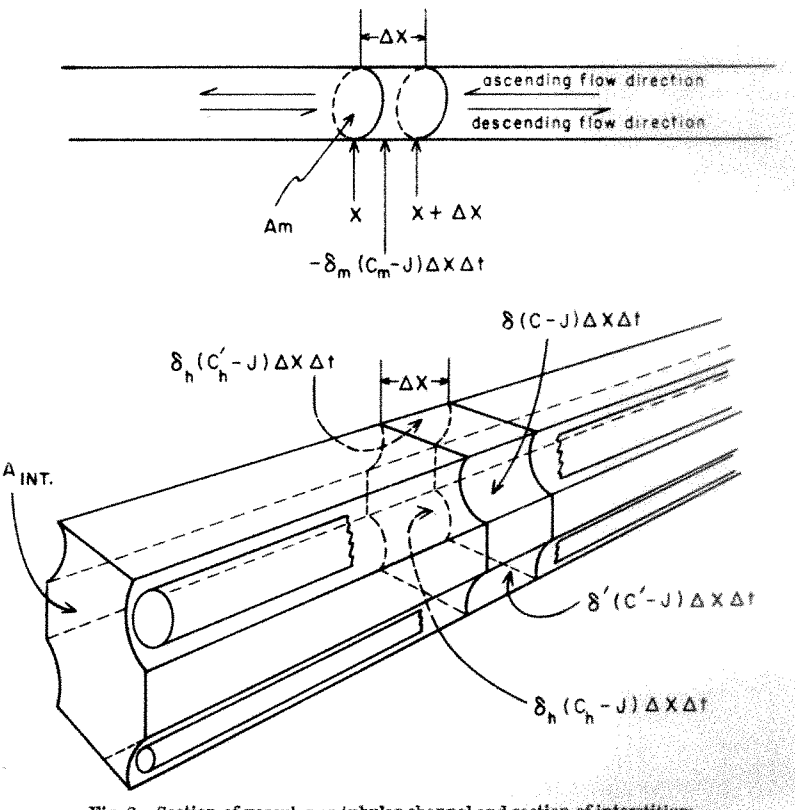


Fig. 2. Section of vascular or tubular channel and section of interstitium

$$\frac{1}{U_h}\frac{\partial C'_h}{\partial t} = \frac{\partial C'_h}{\partial X} - \frac{\delta'_h}{U_h A_h} C'_h \qquad (6)$$

The equation for the interstitium may be derived as follows: Consider a differential element of length ΔX as shown in the lower half of Fig. 2. In this case there is no fluid motion across the faces parallel to the channel directions, but only diffusion across the channel boundaries, into the interstitial region as shown in the figure. The net change of sodium concentration J in the element, neglecting longitudinal diffusion, is:

$$\Delta J A_{\mathrm{INT}} \Delta X = \Delta X \Delta t[\delta(C-J) + \delta(C'-J) + \delta_h(C_h-J) + \delta_h(C'_h-J)] \qquad (7)$$

for the region in which the ascending limb of the Henle loop is thin, and (8) where it is thick-walled.

$$\Delta J A_{\mathrm{INT}} \Delta X = \Delta X \Delta t[\delta(C-J) + \delta(C'-J) + \delta_h(C_h-J) + \delta'_h(C'_h)] \qquad (8)$$

The corresponding partial differential equations become:

$$\frac{\partial J}{\partial t} = \frac{1}{A_{\mathrm{INT}}}[\delta(C-J) + \delta(C'-J) + \delta_h(C_h-J) + \delta_h(C'_h-J)] \qquad (9)$$

$$\frac{\partial J}{\partial t} = \frac{1}{A_{\mathrm{INT}}}[\delta(C-J) + \delta(C'-J) + \delta_h(C_h-J) + \delta'_h(C'_h)] \qquad (10)$$

These equations, along with the boundary conditions that the solutions must match where physically realizable, that is, at turning points of tubes, etc., and that the concentrations of sodium at the input to the Henle loop and vas rectum are equal, can readily be evaluated to obtain the time-dependent solution of sodium concentration in the medulla, and are at present being solved by us.

The time-independent solution, reported on previously[1], is obtained from the foregoing partial differential equations by passing to the limit as time approaches infinity for a given set of input boundary conditions. This effectively makes all time differentiation terms zero, producing the time-independent equations utilized previously, with the following additional manipulation. Equation (9) is solved for J (in the time-independent case). The equation is differentiated with respect to X as shown:

$$\frac{\partial J}{\partial X} = \frac{1}{2(\delta+\delta_h)}\left[\delta\frac{\partial C}{\partial X} + \delta\frac{\partial C'}{\partial X} + \delta_h\frac{\partial C_h}{\partial X} + \delta_h\frac{\partial C'_h}{\partial X}\right] \qquad (11)$$

The values for the partial derivatives are obtained from the time-independent equations (2) and (4) utilizing appropriate values for the subscript m to denote which channel (vas rectum or Henle loop, ascending or descending) is utilized, giving equation (12).

$$\frac{\partial J}{\partial X} = \frac{1}{2(\delta+\delta_h)}\left[\frac{\delta^2}{AU}(J-C) - \frac{\delta^2}{AU}(J-C') + \frac{\delta^2_h}{A_h U_h}(J-C_h) - \frac{\delta^2_h}{A_h U_h}(J-C'_h)\right] \qquad (12)$$

In addition, the condition of equilibrium may be written as equation (13):

$$UC_0 + U_h C_{h0} = UC'_0 + U_{hh}C'_0 \qquad (13)$$

The subscript zero refers to concentrations at the cortico-medullary boundary. C_0 and C_{h0} were set equal.

A similar result is obtained from equation (10) for the thick segment. These equations were the actual ones utilized in the initial work. The 'catch-all' notation (which included the direction of the flow in the channels) utilized in the initial article may have introduced difficulty and it is hoped that the preceding derivation will help to clarify the results previously obtained.

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Hepatic Pyrogen and Cortisone-antipyresis in Canine Endotoxin Fever

The pathogenesis of endotoxin fever has been much elucidated since the observation of the appearance in the serum of a fever-producing substance (endogenous pyrogen) after endotoxin[1]. The transient profound leucopenia which follows the intravenous injection of endotoxin has been cited in support of the idea that leucocytes are a source of endogenous tissue pyrogen. These observations on rabbits were further extended by Petersdorf and Bennett[2,3], who made their observations on dogs. However, these authors pointed out that under certain conditions the leucopenic response to endotoxin could not be correlated with the appearance of endogenous serum pyrogen and that the dissociation of the febrile response and levels of endogenous serum pyrogen was observed. This was noticed especially when endotoxin was given on successive days. Development of endogenous pyrogen markedly decreased on the second day, despite the full febrile and the leucopenic response.

Here, attention should be directed to the species differences in the febrile and the leucocytic response to endotoxin in dogs and rabbits[4]. In dogs, contrary to rabbits, the leucocytic response to endotoxin remains almost unaltered in the endotoxin tolerant state induced by daily administration of increasing doses of endotoxin, though the febrile response becomes markedly reduced. In the case of dogs there is no intimate relation between the initial leucopenic response and the fever. Moreover, after adrenalectomy the febrile response becomes markedly intensified and its latency is shortened when maintained with DCA, and the animals become extremely susceptible to endotoxin and succumb easily to collapse. Thus in dogs, contrary to rabbits, the febrile response seems to have a close correlation with the endotoxin intoxication and might be regarded as one of the signs of the endotoxin intoxication. The extreme susceptibility to endotoxin after adrenalectomy in dogs is attributed to the intensification of the metabolic response of liver to endotoxin after taking up endotoxin in its reticuloendothelial cells[5,6]. The liver glycogen becomes depleted almost completely within 1 h after endotoxin, and the liberation of an endogenous 'shock-inducing factor' from the liver follows, which can be proved also by passive transfer technique. Thus supposition will be made that the endogenous pyrogen in dogs might be liberated also from the liver as the result of the hepatic metabolic responses to endotoxin. This was tested as follows.

One hour after inoculation of commercial typhoid-paratyphoid vaccine (0·4 ml./kg, intravenously), blood was drawn for passive transfer test and the liver was removed under 'Nembutal' anæsthesia. The liver was cut into slices about 1 mm thick and soaked in physiological saline solution of about equal weight containing antibiotics (penicillin *G* 4,000 u., streptomycin 0·05 g in 100 ml.) and kept overnight at 4° C. The supernatant, after removing cellular debris by centrifugation, was given intravenously to the extent of 10 ml./kg to dogs which had been made tolerant to endotoxin beforehand. The recipients were also tested by cross-match blood test in order to avoid the homologous

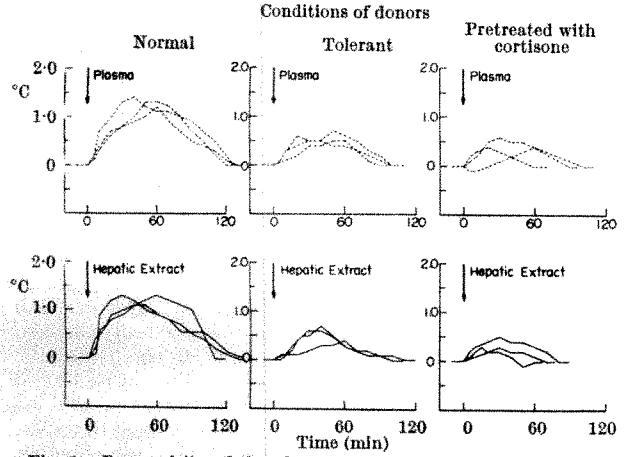


Fig. 1. Pyrogenicity of the plasma and the hepatic extract of dogs inoculated with endotoxin. Tested on normal recipient dogs, the condition of the inoculated donors varied as noted

blood syndrome[7]. As shown in Fig. 1, after infusion of the hepatic extract the rectal temperature immediately began to rise and after reaching a peak within 60 min declined slowly to the initial level.

Leucocyte counts made 15, 30, 60 and 120 min after the infusion showed no significant changes. This clearly indicated that the febrile response induced was not due to the endotoxin itself or some other macromolecular substances liberated in the extract. Saline extract of liver slices of non-inoculated dogs did not show any thermogenicity. Thus it is certain that the liver forms an easily extractable pyrogenic factor after taking up endotoxin in its reticuloendothelial cells.

This pyrogenic factor was found to be heat-unstable and lost its thermogenicity when heated at 56° C for 30 min. As shown in the same figure the passive transfer of the plasma (10 ml./kg) made the day before on the same recipients induced quite similar febrile responses. Thus the transferable endogenous pyrogen after endotoxin in dogs seemed to be due to the liberation of the hepatic pyrogen *in vivo*.

When the febrile response to vaccine was markedly reduced after making dogs tolerant to endotoxin by administering increasing doses of endotoxin (10–200 μg/kg *Salmonella typhosa*, Difco) in one week, the extractable pyrogenic factor in the liver and the plasma pyrogen after vaccine were both markedly reduced as shown in Fig. 1. The tolerant dogs, however, could show full febrile response on infusion of the effective hepatic extract. Thus the tolerance in fever response might result from the absence of the development of the hepatic pyrogen. The latter seemed to be due to the lessening of the hepatic metabolic response in tolerance[6,8].

Petersdorf and Bennett[3] have reported a puzzling result concerning the antipyretic effect of glucocorticoids. Three dogs given 50 mg of prednisone intravenously 1 h before, at the time of, and 1 h after the administration of 3 ml. of commercial typhoid vaccine developed transferable serum pyrogen, although the donor fever responses were suppressed. This was never the case when glucocorticoids of moderate doses were given more chronically. When cortisone (5 mg/kg, intramuscularly) was given for 3 days and 10 mg of prednisone was given intravenously 2 h prior to the administration of vaccine, no vomiting occurred in spite of the first-time inoculation, and the febrile response was markedly suppressed. As shown in Fig. 1, both the plasma and the saline extract of liver had only slight pyrogenicity in the recipient dogs. The antipyretic effect of glucocorticoids seemed to be due to the suppression of the hepatic metabolic responses including the liberation of the pyrogenic factor. This coincided well with the observations on adrenalectomized dogs previously mentioned.

In the absence of glucocorticoids the hepatic metabolic responses to endotoxin become intensified and not only the hepatic pyrogenic factor but also the 'shock-inducing factor' are liberated from the liver. The liberation of both factors together with the depletion of hepatic glycogen can be prevented by pretreatment with glucocorticoids[5,6]. Glucocorticoids, however, could not inhibit the fever induced by the effective hepatic pyrogenic extract, just as they fail to prevent the effect of the 'shock-inducing factor'[5,6]. It is certain that the antipyretic effect of cortisone on endotoxin fever was not due to the depression of the responsiveness of the central nervous system to the pyrogenic stimulus.

In passing, it may be mentioned that similar hepatic saline extract of rabbits did not show such a distinct and regular thermogenicity. However, the rabbit's extract, contrary to that of the dog, showed marked leucocytosis-inducing effect[9]. There exist also marked species differences in the hepatic response to endotoxin.

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Uptake of Potassium at Low Temperatures in Kidney Cortex Slices of Hibernating Mammals

In hibernating hamsters and ground squirrels cationic concentration gradients between tissue cells and plasma are undiminished[1,2]. This fact probably accounts for the ability of the excitable tissues of these species to retain high resting potentials[3], to conduct action potentials[3–5] and to regulate fibre water content[6] at the exceptionally low body temperatures of hibernation. In most mammalian tissues at normal, high body temperature the maintenance of cationic gradients across cell membranes is an active process requiring the expenditure of metabolic energy by mechanisms which transport cations against electrochemical gradients in the face of passive leaks. The question arises whether gradients continue to exist during hibernation because these mechanisms still function or because membranes are less permeable to ions at low temperature.

Kidney cortex when prepared in thin slices is a convenient mammalian tissue for examining transport *in vitro*, because it can rapidly re-accumulate K and extrude Na in measurable net quantities[7,8] and has a high rate of oxidative metabolism, a large fraction of which is associated with cationic pumping[9]. The experiments reported here were designed to show whether kidney cortex taken from two species of mammals which hibernate was capable of establishing as well as maintaining a high tissue K and low tissue Na concentration *in vitro* at low temperature and whether such ability depends on concurrent metabolism.

The methods used have been described in detail elsewhere[9]. Hamsters (*Mesocricetus auratus*) or ground squirrels (*Citellus tridecemlineatus*) were killed by a blow on the head and the kidneys were removed through a mid-line incision. Slices of kidney cortex were made free-hand with a razor blade and were placed temporarily in a Petri dish containing medium at room temperature. When the slicing was completed, the slices were transferred

Table 1. Tissue Concentration of Potassium and Sodium in Slices of Kidney Cortex from Hamsters and Ground Squirrels after Incubation for 1 h

Species and conditions	(No. of cases)	Potassium (μ-equiv./g dry wt.) Initial	38°	5°	Sodium Initial	38°	5°
Hamster	(7–11)						
Normal medium		193 ± 8	259 ± 5	291 ± 7	431 ± 25	253 ± 9	303 ± 14
DNP + IAA		193 ± 8	120 ± 4	187 ± 9	431 ± 25	796 ± 44	499 ± 24
Sodium-free medium		211 ± 8	112 ± 5	225 ± 7	102 ± 10	31 ± 3	51 ± 5
Ground squirrel							
Awake	(3)						
Normal medium		223 ± 6	281 ± 7	284 ± 8	441 ± 36	288 ± 32	340 ± 23
DNP + IAA		223 ± 6	101 ± 5	224 ± 8	441 ± 36	825 ± 4	472 ± 13
Ouabain (125 μM)		223 ± 6	124 ± 11	217 ± 5	441 ± 36	421 ± 31	449 ± 32
Sodium-free medium		223 ± 6	167 ± 8	251	441 ± 36	46 ± 5	66
Hibernating	(4)						
Normal		289 ± 6	307 ± 10	294 ± 10	462 ± 54	274 ± 14	388 ± 41
DNP + IAA		289 ± 6	143 ± 6	286 ± 10	462 ± 54	836 ± 23	466 ± 15
Ouabain (125 μM)		289 ± 6	156 ± 14	249 ± 9	462 ± 54	442 ± 20	441 ± 48
Sodium-free medium		289 ± 6	188 ± 14	286 ± 14	462 ± 54	38 ± 14	46 ± 14

The index of variation after each mean value is the standard error. Means without *S.E.* are based on only two values. The normal medium contained 143 mM sodium ion. In the normal medium containing metabolic inhibitors the final concentration of dinitrophenol (DNP) was 0·02 mM and the final concentration of iodoacetate (IAA) was 0·1 mM. In the sodium-free medium NaCl was replaced with choline chloride. This medium was only completely 'sodium-free' at the outset of incubation because the small amount of sodium in the slice was eluted into it during the experiment.

to Warburg flasks containing 4 ml. of medium. After 1 h of incubation at 38° or 5° C, slices were removed from the flasks, blotted on hardened filter paper, placed in tared tissue beakers and were dried at 105° C overnight to obtain dry weight. Sodium and potassium contents were determined by internal standard flame photometry on samples of tissue which had been digested in concentrated nitric acid.

The incubation medium contained NaCl (143 mM) as its principal constituent and also $CaCl_2$ (3 mM), $MgSO_4$ (1·5 mM), potassium-phosphate buffer (3 mM, providing 5·5 mM K ion) and glucose (10 mM). Flasks incubated at 38° C were gassed with 100 per cent oxygen. For experiments in which a sodium-free medium was desired, choline chloride was substituted for NaCl.

The brief soaking of kidney cortex slices in such media prior to incubation results in a considerable reduction in concentration of potassium in the tissues from *in vivo* values[7–9]. In the experiments recorded here the objective was to measure net uptake or loss of potassium by comparing the final tissue concentration of potassium after incubation with this initial, depressed value. In slices of hamster kidney cortex, this 'initial' concentration of potassium after soaking in the normal, sodium-rich medium was 193 μequiv./g dry wt. (Table 1), compared with an *in vivo* value of 339 (ref. 2). Conversely, pre-soaking in a sodium-rich medium also led to a great increase in concentration (431 of sodium in the tissues compared to an *in vivo* value of 242 in hamster kidney), and the extent of net extrusion of sodium during incubation could be determined by reference to this initial high value. Slices of ground squirrel kidney cortex lost potassium and gained sodium in a similar way when soaked in sodium-rich medium. Hamster slices soaked in choline medium lost both potassium (to 211) and sodium (to 102). Slices of cortex from kidneys of ground squirrels, however, were prepared only in the ordinary sodium-rich medium and, hence, were not soaked in choline medium before incubation in this medium.

The principal finding of this investigation, shown in Table 1, is that in the kidney cortex slices of both hamsters and awake ground squirrels there was an uptake of potassium at 5° C equal to or greater than that occurring at 38° C. Incubation of slices with a combination of two metabolic inhibitors, dinitrophenol (0·02 mM) and iodoacetate (0·1 mM), blocked this re-accumulation of potassium at both temperatures. At 38° C these inhibitors caused a decrease in concentration of potassium in the tissues to about 100, but at 5° C the concentration of potassium in the tissues was unchanged from the initial value. These results are in marked contrast to those obtained in kidney cortex slices of such non-hibernating species as rabbits and guinea-pigs[7,8], in which incubation at a low temperature is as effective a block to uptake of potassium as inhibition of oxidative metabolism.

Previous investigations have shown that in slices of kidney cortex from rabbits uptake of potassium may also be prevented directly by replacing all sodium in the medium with choline, thus obviating transport of sodium, or by incubating the slices with the cardiac glycoside, ouabain, which is a specific inhibitor of cation transport[9]. Neither of these methods interferes directly with oxidative metabolism.

Incubation of kidney cortex slices of hamsters and awake ground squirrels in sodium-free medium blocked uptake at both 38° and 5° C. As with the metabolic inhibitors, incubation in sodium-free medium caused a fall in concentration of potassium in the tissues at 38° C, but no change occurred at 5° C. In kidney slices of awake ground squirrels ouabain (125 μM) had the same effect on concentration of potassium in the tissues as metabolic inhibition: it blocked net uptake at both temperatures, caused a loss of potassium at 38° but not at 5° C.

In two experiments ouabain in a concentration of 125 μM, which is sufficient to produce maximum inhibition of uptake of potassium in rabbit kidney cortex, had no effect whatever on concentration of potassium in the tissues of hamster kidney slices at either temperature. This apparent resistance is probably a specific example of the hamster's well-known general insensitivity to ouabain[10].

In slices of kidney cortex from ground squirrels which were hibernating at an ambient temperature of 5° C with a body temperature less than 7° C when killed, the 'initial' concentration of potassium in the tissues after the usual slicing procedure was 66 μequiv./g dry wt. higher than in 'initial' slices of kidney cortex from awake ground squirrels. Consequently, no net uptake occurred during subsequent incubation. At 5° C metabolic inhibitors and incubation in sodium-free medium caused no decline from this high initial value and ouabain caused only a slight decrease. At 38° C each of these agents depressed the concentration of potassium considerably, but even so the potassium values after 1 h of incubation with metabolic inhibitors were higher than in the comparable slices from kidneys of awake ground squirrels. The greater concentration of potassium after preliminary soaking reflects, presumably, the higher *in vivo* concentration of potassium of this tissue during hibernation[2], but whether the higher values of potassium after incubation with metabolic poison can be so explained is not certain. Conceivably, kidney cortex may acquire during hibernation a higher fraction of intrinsic or 'bound' potassium[11] which cannot be further diminished by leaching or poisons.

Because the extracellular space was not determined in these experiments, little emphasis has been placed on the tissue concentrations of sodium in these experiments. The values for concentration of sodium in the tissues given in Table 1, however, show that in general the movements of this ion are reciprocal to those of potassium. Thus, there is an extrusion of sodium at both 38° and 5° C which is reversed at 38° and only blocked at 5° C by dinitrophenol plus iodoacetate. In sodium-free, choline chloride medium the sodium content of the tissue is virtually abolished.

Unlike metabolic inhibitors, ouabain in these experiments did not cause a further increase in concentration of sodium in the tissues above the initial value produced by leaching. As in rabbit kidney slices[9], however, the concentration of sodium in slices after incubation with ouabain at 38° C was greater than that of slices incubated without ouabain by an amount (130 μequiv./g dry wt.) which is equal to the difference in concentration of potassium in the tissues (124) in these two conditions.

This work was supported by a grant (*GM*11494) from the General Medical Institute, National Institutes of Health. I thank Mrs. Christine Bullock for her assistance.

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Thyroid Function of Friesian Cows during the Œstrous Cycle and in Conditions of Ovarian Abnormality

The physiological symptoms of heat in cows do not appear unless there is an optimal level of the thyroid hormone[1]. There is evidence that thyroid activity varies during the œstrous cycle, with maximal activity during œstrus[2–4]. Thyroidectomized cows failed to show physical signs of heat[1,5]. A low level of protein-bound iodine in the blood of infertile cows has also been demonstrated[6]. The levels of thyroid and thyrotrophic hormones in the blood of Friesian cows during œstrous cycle as compared to those affected with ovarian inactivity and cystic ovaries have been investigated as follows.

Fifty ml. of blood was obtained from the jugular veins of 58 Friesian cows, 3–7 years old. Ten were in œstrus, 10 in dioestrus, 23 affected with ovarian inactivity and 15 with cystic ovaries. The procedure for the determination of thyroid hormone was that adopted by D'Angello and Gordon[7], namely, 'the stasis tadpole method'. Tadpoles were divided into five groups of 20 each; one group was injected with saline; the other groups received five injections of 0·01 ml. of serum obtained from the four groups of cows on alternate days. Twenty-four hours after the last injection, the tadpoles were killed with chloroform and stored in 80 per cent alcohol. Their hind limbs were then measured and their length used as a criterion of thyroid hormone activity. Thyrotrophic hormone extracted from the sera of cows[8] was assayed by the use of one-day-old male chicks[9]. The chicks were injected daily with a subcutaneous injection of 0·5-ml. serum for 4 days. They were then killed by ether 24 h after the final injection. The thyrotrophic hormone level was indicated by its effect on the weight of the thyroid and expressed in terms of chick units (a chick unit represents 50 per cent increase above control).

Table 1. Levels of the thyroid and thyrotrophic hormones in the blood of Friesian cows during the œstrous cycle in comparison with those affected with ovarian inactivity and cystic ovaries

	Saline control	Œstrus	Dioestrus	Inactive ovaries	Cystic ovaries
Length of tadpole hind limbs (mm)	0·99 ± 0·33	2·42 ± 0·33	1·77 ± 0·19	1·83 ± 0·11	3·16 ± 0·17
Thyrotrophic hormone (chick unit)	—	0·34	1·59	0·26	0·00

± Standard error.

It appears from Table 1 that the level of thyroid hormone was significantly high during œstrous as compared to that of dioestrous cows. The level of thyroid hormone in cows with cystic ovaries was even higher than that observed at œstrus, but in cows affected with inactive ovaries it was similar to that of the dioestrous cow.

The thyrotrophic hormone level was highest during dioestrus and low at œstrus and in cows with inactive ovaries, while it was totally absent in cows with ovarian cysts.

In the case of œstrous cows and those with ovarian cysts the increase in the level of thyroid hormone was accompanied by a decrease in thyrotrophic hormone. On the other hand, during dioestrus decreased thyroid hormone level was accompanied by a sharp rise in thyrotrophic hormone level. In the case of animals with inactive ovaries, thyroid and thyrotrophic hormone levels were low, suggesting that such a condition accompanies pituitary hypofunction.

The increased thyroid activity at œstrus and in animals with ovarian cysts is most probably due to an increase of œstrogen in the blood[10].

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Serum Calcium and Phosphorus in Rabbits during Fracture Healing, with Reference to Parathyroid Activity

The level of serum androgens, and thyroid activity, are both increased in the second and third weeks after bone fracture during cellular regeneration of the callus[1,2]. The early period of catabolism was found to be accompanied by increased adrenal function[3].

The present work was planned to investigate the levels of serum calcium and inorganic phosphorus during fracture healing in rabbits. The hormone content of the parathyroid glands of these animals was also determined.

Thirty-five Boscat male rabbits of an average weight of 1·5 kg were used. They were divided into 7 groups of 5 animals each. One group was left intact and served as a control. The other groups were subjected to experimental fracture of the left tibia by a closed method under ether anæsthesia[4]. The operations were so arranged that rabbits were obtained 1, 2, 4, 8, 15 or 21 days after fracture. The animals were killed and blood samples were obtained. Double determinations of serum Ca (ref. 5) and inorganic phosphorus[6] were made. Analysis of variance was carried out to evaluate the differences between the serum Ca and P levels in the experimental rabbit groups.

The thyroid and parathyroid glands of the rabbits of each group were kept in acetone and preserved in the deep freeze. Parathyroid hormone was extracted[7] and a saline suspension of the extract was prepared so that the hormone from each animal was contained in 1·0 ml. saline. It was assayed by the method depending on its antagonism to magnesium narcosis[8]. Groups of 10 mice were injected subcutaneously with 3 doses of 0·1 ml. of the parathyroid suspension at intervals of 3 h; 90 min after the final injection the animals were given 20 mg of magnesium sulphate contained in 0·1 ml. saline. After half an hour the dead, narcotized and unnarcotized animals were counted. The percentage of animals unnarcotized is

proportional to the parathyroid hormone content of the extract. A standard log–dose response curve was established under the same conditions, using parathyroid extract ('Paroidin'; Parke, Davis).

Table 1 shows that the serum Ca level fell significantly 2 days after fracture and rose significantly during the fourth and eighth days. It then returned to a level approximating to normal. Serum inorganic phosphorus was significantly increased 24 h after fracture, and then returned to normal level, with a temporary depression below the normal level about 15 days after fracture.

As shown in Table 2, it appears that the parathyroid hormone was only detectable in normal rabbits and 8 days after fracture.

Table 1. Levels of Calcium and Inorganic Phosphorus in Rabbit Serum during Fracture Healing

Days after fracture	Calcium (mg/100 ml.)	Phosphorus (mg/100 ml.)
Control (normal)	13·41 ± 0·45	6·81 ± 0·44
1 day	14·86 ± 0·23	8·42 ± 0·39
2 days	10·80 ± 0·97	6·44 ± 0·36
4 days	17·63 ± 1·95	7·32 ± 0·28
8 days	16·32 ± 0·34	6·76 ± 0·20
15 days	14·50 ± 0·61	5·35 ± 0·08
21 days	15·06 ± 0·49	6·84 ± 0·36
F value	7·09*	3·14†
d.f.	60, 6	61, 6

± Standard error.
* Significant differences between groups at 1 per cent level of probability.
† Significant differences between groups at 5 per cent level of probability.

Table 2. Hormone Content of Parathyroids of Rabbits during Fracture Healing (Antagonism to Magnesium Narcosis)

Days after fracture	No. of mice 20 g body-weight Dead	Narcotized	Unnarcotized	
Saline	5	4	1	
Controls (normal rabbits)	3	2	5	(56·53 U.S.P. units/rabbit)
1 day	5	3	2	
2 days	4	4	2	
4 days	2	6	2	
8 days	4	–	6	(92·13 U.S.P. units/rabbit)
15 days	5	3	2	
21 days	4	4	2	

Table 3. Slope, Standard Error and Fiducial Limits of the Standard Log–Dose Response Curve of Parathyroid Hormone

b =	0·47 ± 0·06
Fiducial limits at 5 per cent	0·11
Fiducial limits at 1 per cent	0·15
X (average log.-dose)	1·16
Y (average response)	0·47

The early rise in P level in the blood corresponds to the necrotic disintegration of the cells at the site of fracture. This is followed by a drop in serum Ca level which favours the resorption of bone spicules resulting from the trauma. By 4–8 days after fracture there is a pronounced rise in serum Ca level, bringing about a state of supersaturation of the body fluids, favourable for the deposition of bone salts at the sites of healing. At this time great waves of new callus tissue are sweeping towards the break from the osteogenic layer of the periosteum[9]. Serum Ca then returns to its normal level and new bony trabeculæ replace the cartilage until the trabeculæ from each fragment meet and join.

The rise in the level of P in the blood of rabbits 24 h after fracture, and the pronounced drop in serum Ca on the next day, might be expected to stimulate the secretion of parathyroid hormone[10]. This is possibly why the parathyroids of such animals had no detectable amounts of the hormone. At this time, stress is accompanied by increased adrenal function, and ACTH markedly lowers the Ca level in the blood of rabbits[11]. By the fourth day adrenal function has returned to normal and parathyroid hormone predominates, resulting in a sharp rise in Ca level in the blood. It brings about hypersaturation by its general effect on the skeleton[4]. This, by a feed-back mechanism, evidently acts on the parathyroid glands by the eighth day after fracture, interfering with the release of parathyroid hormone. The hormone content of the parathyroids of such rabbits was high. Hypercalcæmia is also believed to release a hypocalcæmic factor (calcitonin) from the parathyroids, and this, by its antagonism to parathyroid hormone, brings blood Ca back to the normal level[12]. Such a condition probably prevails during the period 15–21 days after fracture.

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Volume Change accompanying Passive Stretch of Frog Muscle

The existence of a volume change accompanying passive stretch of frog muscle was first noted by Ernst and Tigyi[1]. They concluded that passive stretch of a pre-shortened frog muscle was accompanied by a volume decrease, but they did not record the magnitude of the decrease.

A method of measuring volume changes in frog muscle, previously described by Abbott and Baskin[2], has been modified. This modification involves a measurement of the pressure change, which is caused by the muscle contracting in a closed vessel. In fact, since the volume changes are small the results by a volume transducer method are exactly the same as those using a pressure transducer method. The muscle is mounted in a chamber (Fig. 1) and the chamber is sealed. The top of the muscle is attached to a wheel which is mounted concentrically through the chamber on to a smaller shaft to which is attached a piece of chain. The chain goes over the pulley

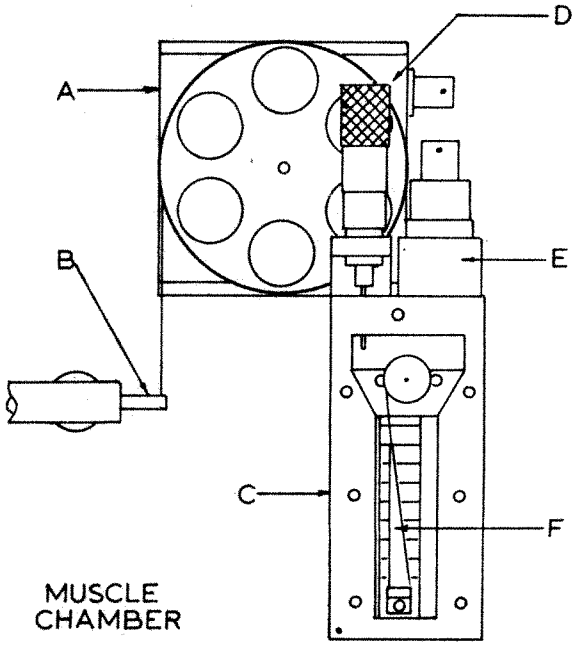


Fig. 1. Diagram of muscle chamber. *A*, muscle length reference transducer; *B*, Levin–Wyman ergometer; *C*, muscle chamber; *D*, micrometer volume adjustment; *E*, pressure transducer; *F*, sartorius muscle

and is attached to a Levin–Wyman ergometer. By this method the length of the muscle may be varied by the Levin–Wyman ergometer without the process of stretching the muscle in itself introducing any pressure change into the chamber. A calibration is made by use of a micrometer-controlled rod which is pushed into the chamber to a precisely determined amount. The pressure change accompanying this is recorded through a special amplifier circuit and displayed on an oscilloscope. The oscilloscope tracings are photographed.

Using the new chamber, the volume changes accompanying passive stretch of pre-shortened frog muscle have been measured. A muscle is placed in the chamber and attached at reference length to the wheel. It is then given three shocks to bring it down to a length determined by a setting on the Levin–Wyman ergometer. The chamber is next sealed and filled with Ringer's solution. The system is allowed to equilibrate for 20 min. A release is then allowed and the Levin–Wyman ergometer stretches the muscle to a specified length. The pressure changes accompanying this are noted and are reported in terms of volume change. The studies of Ernst and Tigyi have been confirmed. A volume decrease results when a pre-shortened muscle is passively stretched. Table 1 lists six experiments. In each the muscle has been stretched by a different amount. An average value for the volume change per millimetre of

Table 1. VOLUME CHANGE ACCOMPANYING PASSIVE STRETCH

Exp.	Passive stretch (mm)	ml./g. mm
1	4·5	$9·5 \times 10^{-6}$
	4·5	10·9
	4·5	10·5
	4·5	15·7
2	6·0	$12·6 \times 10^{-6}$
	6·0	6·2
3	7·0	$4·0 \times 10^{-6}$
	7·0	5·4
	7·0	6·4
	7·0	3·0
	7·0	5·9
4	2·5	$9·0 \times 10^{-6}$
	2·5	7·5
	2·5	8·0
5	8·5	$9·5 \times 10^{-6}$
	8·5	4·0
6	5·0	$14·0 \times 10^{-6}$
	5·0	6·0

stretch is approximately 1×10^{-5} ml./g of muscle. The velocity of stretch, controlled by the Levin–Wyman ergometer, does not affect the magnitude of the volume change. Within the accuracy possible in this measurement it appears that the magnitude of the volume decrease is a function of the amount of stretch only, and not of the position of the muscle at the start of stretch. That is, the volume decrease varies linearly over the stretching distance used in this investigation.

This observation is of special interest in view of the recent demonstration of volume changes accompanying contraction and relaxation in isolated myofibrils by Baskin[3]. It is postulated that the volume changes observed in the isolated frog sartorius muscle have the same origin as the volume changes which are observed in isolated rabbit muscle myofibrils under the influence of ATP. The origin of these changes has been ascribed to molecular rearrangement, that is, folding or unfolding occurring in the actomyosin. These results indicate the existence of large configurational changes taking place in actomyosin during both shortening and stretch of pre-shortened muscle. These changes are not accompanied by large amounts of tension. Since these volume changes have been shown to occur in isolated glycerol extracted myofibrils under the influence of ATP, it appears impossible to ascribe their origin to biochemical changes occurring in creatine phosphate or other muscle constituents. It seems likely that these changes occur in the actomyosin molecules, either alone or in combination with ATP. They might have their origin in the exposure of previously infolded groups or in the ionization of previously unionized groups.

This work was supported in part by U.S. Public Health Service grant *GM*–09898 and by a grant from the Life Insurance Medical Research Fund.

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PHARMACOLOGY

Effect of Methylamphetamine on the Psychomotor Depression produced by Adrenaline in Mice

INTRAPERITONEAL injections of catecholamines produce a marked depression of the psychomotor activity of mice, accompanied by vegetative phenomena, such as tachycardia, exophthalmos and pilo-erection. These vegetative phenomena are, however, independent of the psychomotor depression, for they can be suppressed by phentolamine ('Regitin *R*'), an adrenergic blocking agent, without modifying the depressed behaviour. Furthermore, isopropylarterenol produced, in higher doses, a similar psychomotor depression, without vegetative symptoms[1].

Catecholamines, especially noradrenaline, are generally considered to be central neuro-humoral agents with ergotropic functions[2]. Notwithstanding this, their administration to animals or human beings may also produce, in certain circumstances, signs of a central nervous depression (analgesia, stupor, etc.[2]). The mechanisms of these phenomena are unknown. Some workers consider them to be a consequence of central inhibitions due directly to an excess of catecholamines; other authors suppose the existence of feed-back mechanisms, triggered by stimulation of baro- or chemo-ceptors. It is possible that the depression of psychomotor activity of mice after an intraperitoneal injection of catecholamines, described by us[1], pertains also to these phenomena. In this case, a feed-back mechanism seems more likely than a direct inhibition by an excessive local concentration, for it is not very probable that catecholamines can reach the central nervous system in important amounts after an intraperitoneal administration. The supposed feed-back mechanism does not seem to depend on vegetative phenomena, such as vasoconstriction (baroceptors), etc., because the administration of an adrenergic blocking agent did not suppress it[1]. It is a well-known fact that amphetamine and similar drugs produce a central sympathetic stimulation, characterized also by an elevated psychomotor activity. The adrenergic action of amphetamine-like substances is mainly of indirect character, that is, produced through liberation of endogenous catecholamines. Nevertheless, it seems that the amphetamine action has also a direct adrenergic component (substitution of catecholamines in their receptive sites[2]).

The work recorded here refers to the effects of a combined treatment of mice, with adrenaline and methylamphetamine, on their psychomotor activity. The experiments were performed in order to see if it is still possible to raise the psychomotor activity by methylamphetamine above the normal after an intraperitoneal injection of adrenaline and if a subsequent injection of this substance by the same route abolishes the psychomotor excitation caused by a previous administration of methylamphetamine.

The technique of our experiments was essentially the same as in our earlier investigations[1]. The mice received two intraperitoneal injections (adrenaline–methylamphetamine, adrenaline–distilled water, methylamphetamine–

distilled water, distilled water–distilled water), in both orders of sequence with an interval of 15 min. The mice were placed in an actophotometer 15 min after the final injection, measuring their locomotion during 10 min. Every group consisted of seven animals.

Table 1

First injection:	Distilled water	Second injection: Methylamphetamine 1·0	Methylamphetamine 2·0
Distilled water	67 ± 7	100 ± 3	118 ± 5
Adrenaline 0·25	38 ± 4	72 ± 7	—
Adrenaline 0·50	23 ± 2	31 ± 4	31 ± 3
Adrenaline 1·00	17 ± 5	30 ± 3	29 ± 4
Adrenaline 2·00	7 ± 2	13 ± 2	18 + 3

Table 1 shows the results obtained when the mice were injected first with adrenaline and 15 min later with methylamphetamine, using always the intraperitoneal route. The doses are expressed in mg/kg, the values correspond to the actophotometer reading ± the standard deviation of the mean.

It can be seen that ascending doses of adrenaline depress progressively the psychomotor activity in these conditions. Methylamphetamine produces a hyperactivity only when injected into mice not treated previously with adrenaline. After an injection of adrenaline, methylamphetamine only lessens the degree of psychomotor depression, without abolishing it completely, except for the slight depression caused by 0·25 mg/kg of adrenaline, that did not appear in methylamphetamine-treated mice. If the sequence of injections is reversed, the results are somewhat different (Table 2).

Table 2

First injection:	Adrenaline 0·50	Second injection: Adrenaline 1·0	Adrenaline 2·0
Methylamphetamine 1·0	33 ± 5	44 ± 13	45 ± 17
Methylamphetamine 2·0	36 ± 3	41 ± 3	49 ± 8

The results in Table 2 show that methylamphetamine reduces the psychomotor depression caused by adrenaline more efficaciously if injected first. For example, 2·0 mg/kg of adrenaline alone depresses the psychomotor activity to a value of 7 ± 2; this value is raised after a following injection of 2·0 mg/kg of methylamphetamine to 18 ± 3 (Table 1), but it will be nearly normalized by a preceding injection of this substance (value of 49 ± 8). It is also interesting to note that in the last case the locomotion remains somewhat depressed but the animals show signs of excitation of other kinds (incessant rotating movements, cleansing and other small movements).

We believe that our results support the supposition that intraperitoneal injection of adrenaline depresses the psychomotor activity by some feed-back mechanism involving central adrenergic ergotropic functions, blocking herewith also the indirect stimulating effect of methylamphetamine, but leaving unblocked its direct stimulating action, so that a reduction of the psychomotor-depressing effect of adrenaline can be achieved.

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Distribution of Cobalt-60 in the Rat as influenced by Chelating Agents

The recent development of quantitative co-ordination chemistry is paralleled by remarkable advances in the therapeutic use of chelating agents, for example, in the removal of toxic or radioactive metals from the body.

In the particular case of radiocobalt, however, our knowledge about the efficacy of chelators is rather scanty: ethylenediamine-tetraacetic acid (EDTA)[1–5], diamino-*cyclo*hexanetetraacetic acid[6], diethylenetriaminepentaacetic acid (DTPA)[2], cysteine (Cy)[4,5] and cysteamine (CyA)[4,5] have been tested and found to be effective, DTPA being slightly superior to the other compounds.

In order to find chelators with still higher efficacy, one must take into account the fact that Co(II) is a transition metal. With regard to co-ordination properties, the transition metal ions are known to behave quite individually and to react not only with oxygen as ligand atom, but also with nitrogen and sulphur. Therefore, screening tests with a greater number of corresponding chelators were necessary.

Albino rats of the Heiligenberg strain (6–10 weeks old, 170–210 g) were intravenously injected with approximately 5 μc. of carrier-free $^{60}CoCl_2$. To achieve maximum effectiveness, the different compounds were administered immediately after the injection of ^{60}Co. The dosage was 200 μmoles per animal, with the exception of the relatively toxic 2-mercapto-ethyliminodiacetic acid (MEIDA) (25 μmoles). The animals were killed 48 h later, and the radioactivity of the organs assayed by means of a KI(Tl)-scintillation crystal.

In good agreement with earlier investigations, ^{60}Co was eliminated rather quickly from the body, and the total retention after 48 h amounts to 8–11 per cent. The highest concentrations are observed in liver and kidneys. As can be seen from Table 1, our data confirm the findings of Foreman[2], that is, the superiority of DTPA over EDTA. This accords with the ratios of the relevant stability constants $K^{Co}_{CoL}/K^{Ca}_{CaL}$: $10^{8\cdot4}$ for DTPA[7] and $10^{5\cdot5}$ for EDTA[8]. DTPA does not occupy, however, the same unique position as it does with other metal ions[9], and its efficacy is equalled by triethylenetetraminehexaacetic acid (TTHA), 2 : 2′-*bis*[di(carboxymethyl)-amino]diethyl ether (BADE), 1 : 2-*bis*[di(carboxymethyl)-aminoethoxy]ethane (BAE), MEIDA, Cy and CyA and clearly surpassed by the sulphur-containing 2 : 2′-*bis*-[di(carboxymethyl)amino]diethyl sulphide (BADS), 1 : 2-*bis*[di(carboxymethyl)aminoethylthio]ethane (BATE) and D-β,β′-dimethylcysteine (DMCy). Although the participation of the sulphur atoms in chelating Co(II) is beyond doubt, it is not yet clear (since the K^{Co}_{CoL}-values are not available) whether the high efficacy of the sulphur

Table 1. ^{60}Co-CONTENT (MEANS OF 5–6 RATS AND STANDARD ERRORS) IN DIFFERENT ORGANS 48 H AFTER INJECTION

The chelating agents (with the exception of DMCy, Cy and CyA, given as Ca-Na-chelates) were administered intraperitoneally, immediately following the injection of the radionuclide

Chelate	Percentage recovery of ^{60}Co dose in: Kidneys	Liver	Muscle	Bones
Control	0·836 ± 0·014	4·857 ± 0·148	2·137 ± 0·034	0·585 ± 0·017
EDTA	0·959 ± 0·049	2·605 ± 0·110	1·453 ± 0·083	0·370 ± 0·014
DTPA	1·197 ± 0·018	1·830 ± 0·046	0·875 ± 0·059	0·106 ± 0·004
TTHA	0·965 ± 0·047	1·150 ± 0·027	1·225 ± 0·031	0·154 ± 0·004
BADE	0·436 ± 0·019	2·108 ± 0·053	0·748 ± 0·035	0·202 ± 0·005
BAE	0·581 ± 0·014	3·684 ± 0·124	1·616 ± 0·075	0·377 ± 0·008
BADS	0·204 ± 0·005	0·408 ± 0·005	0·647 ± 0·026	0·075 ± 0·005
BATE	0·155 ± 0·004	0·763 ± 0·021	0·613 ± 0·041	0·077 ± 0·002
Cy	0·385 ± 0·005	1·774 ± 0·038	1·112 ± 0·038	0·303 ± 0·011
CyA	0·734 ± 0·014	1·701 ± 0·057	1·017 ± 0·033	0·313 ± 0·006
DMCy	0·200 ± 0·025	0·858 ± 0·113	0·730 ± 0·052	0·260 ± 0·034
DMCy*	0·151 ± 0·005	0·576 ± 0·026	0·592 ± 0·016	0·138 ± 0·008
MEIDA†	0·268 ± 0·022	2·228 ± 0·219	1·070 ± 0·096	0·441 ± 0·042

* The dose of 20 μmoles DMCy was given orally.
† The MEIDA contains ~10 per cent of cystaminetetraacetic acid as impurity.

compounds is due only to their low K^{Ca}_{CaL}-values or also to high K^{Co}_{CoL}-values.

The chelators show different patterns of effectiveness, that is, the differences in effectiveness are more pronounced in relation to the kidney than in other organs: EDTA, DTPA, TTHA, CyA are not able to reduce the retention of ^{60}Co by the kidney, whereas the other compounds were found to be markedly effective. The reasons for this discrepancy are not yet fully understood. Tentatively, different metabolic behaviour of particular chelates as well as the formation of ternary complexes in the kidneys (which are known to possess a high concentration of SH-groups[10]) can be assumed.

From the practical point of view the therapeutic use of BADS and BATE in cases of internal contamination with ^{60}Co is obvious. However, the highest significance must be attributed to D-dimethylcysteine (commonly known as penicillamine). Its high efficacy compares favourably with BADS and BATE. Our experimental data show that it can be administered orally without loss of effectiveness. Finally, its low toxicity must be stressed. More detailed studies on the effectiveness of these and other chelators, as influenced by several factors and, in particular, the time of their administration, are now under way and will be published elsewhere.

The generous supply of the polyamino-acids by J. R. Geigy AG., Basle, and of D-dimethylcysteine by Heyl and Co., Berlin, is gratefully acknowledged.

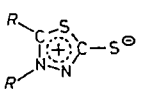
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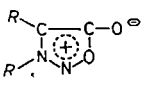
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A New Class of Antimicrobial Agents

The mesoionic 1,3,4-thiadiazoles, I, were first reported by Busch[1], who assigned a bicyclic structure to the compounds.



(I)



(II)

Schoenberg[2] later re-examined the structure of Busch and offered a resonance hybrid structure, now accepted as being typical of a large group of heterocycles described as being mesoionic[3]. We became interested in the mesoionic 1,3,4-thiadiazoles as a natural extension of our interest in the pharmacology and pharmaceutical chemistry of the sydnones II[4–7]. It occurred to us that since the two ring systems are isoelectronic (that is, being mesoionic they both possess an aromatic, positively charged, 5-membered ring, and a balancing negative charge on an exocyclic atom) the possibility might exist that the two ring systems could also be bioisosteric. That is, the ring itself may behave as a pharmacologically active functional group and that this already expressde similarity between the ring systems may impart similar pharmacological activity. A more conservative speculation would be that perhaps the mesoionic 1,3,4-thiadiazole ring system would have some prominent pharmacological activity.

Accordingly, a number of these compounds were synthesized in our laboratory. The synthesis of the *C*-ethyl, *N*-phenyl derivative will be used as a typical procedure. Phenylhydrazine (32 g, 0·3 mole) was dissolved in 350 ml. of absolute ethanol and carbon disulphide (24 g, 0·3 mole) was added with stirring. A precipitate rapidly formed. This was not isolated but was treated directly with an ethanolic solution of potassium hydroxide (17 g in 120 ml., 0·3 moles). The precipitate dissolved and, after stirring, a precipitate of potassium phenyldithiocarbazinate formed in 85 per cent yield, m.p. 144°–46°. This acid salt (22 g, 0·1 mole) was suspended in 500 ml. of toluene and the slurry warmed to 60°. To this was added propionyl chloride (9·3 g, 0·1 mole) at a slow rate, maintaining the temperature at 60°. After the addition, the reaction mixture was cooled and the 4-phenyl-5-ethyl mesoionic 1,3,4-thiadiazole precipitate removed and recrystallized from ethanol to give a 5 per cent yield, m.p. 191°–192° (found: N, 53·84; S, 28·96). The nomenclature recommended by Katritzky for mesoionic compounds[8] becomes anhydro-4-phenyl-5-ethyl-2-thio-1-thia-3,4-diazolinium thiol.

In the course of a broad screening of these compounds for potential medicinal activity we assayed them for antibacterial activity by the standard disk method against *Staphylococcus aureus*, *Diplococcus pneumoniae* and *Escherichia coli*. Disks saturated with these compounds were incubated with brain heart infusion agar plates of the organisms and observed at 24- and 48-h intervals for zones of inhibition of growth. Penicillin was used as an antibacterial reference. Four of the 4-phenyl derivatives, namely, the 5-methyl, 5-ethyl, 5-isopropyl and the 5-propyl, were found to produce significant zones of growth inhibition of *S. aureus* and *D. pneumoniae*. The extent of inhibition was the same as that produced by the penicillin reference. The effect on the *E. coli* was minimal.

Preliminary pharmacological tests indicated a low order of acute intraperitoneal toxicity in mice. The acute median lethal dose was found to be greater than 500 mg/kg for the four compounds cited here.

These investigations indicate that these compounds might be safe antibacterial agents against at least some Gram-positive organisms.

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Reversal of Hormonal Effects as a Result of Chronic Morphinization

Morphine accelerates the rate at which the rat diaphragm takes up glucose from synthetic media, to an extent comparable with the effect of insulin. With tissue from a chronically morphinized animal this acute effect of the drug is reversed, and the rate of glucose-uptake is retarded[1]. These effects of morphine are antagonized by hydrocortisone[2]. Hydrocortisone alone, in the concentration used for the experiments ($7{\cdot}7 \times 10^{-4}$ M) with morphine, has no significant effect on the rate of glucose-uptake by either normal or chronically morphinized tissue. We have confirmed this by additional experiments, and have concluded that the previously observed effects of the hormone in the presence of added morphine may be attributed entirely to a suppression by the hormone of the effects of the drug.

We now find, however, that hydrocortisone in lower concentrations has a marked and apparently direct effect on the glucose-transport mechanism. The effect on tissue from the chronically morphinized animal is the reverse of that on normal tissue. Following the experimental procedure previously described[2], we find that hydrocortisone, in concentrations of $3{\cdot}85 \times 10^{-4}$ M and below, strongly retards the uptake of glucose by diaphragm from normal rats, but accelerates its uptake by diaphragm from rats which had received daily injections of morphine sulphate (30 mg/kg body wt.) for 4–6 weeks. As previously emphasized[2], there is no significant difference between the normal and chronically morphinized rat diaphragm in the rates of glucose-uptake in the absence of added hormone or drug. Results are presented in Table 1.

Differences between normal and chronically morphinized rats in tissue response to adrenaline have also been observed in a study of the effects of hydrocortisone and adrenaline[3] analogous to that with hydrocortisone and morphine. There is a possible analogy here between the reversal of hormonal effects by morphine and the reversal of the effect of vitamin B_1 on yeast fermentation as a result of growth in the presence of cocaine[4,5], for, in both cases, reversal of the effect of the active substance is induced by chronic exposure to a drug which to some extent it resembles in chemical structure. The morphine molecule embodies both a steroid-like and an adrenaline-like structure and it is to be expected that the drug would interfere with the normal action of both types of hormone.

While we cannot as yet offer a full explanation for our observations, it would seem that, in the hormonal control of metabolic processes, a hormone or other active substance such as a hormone-simulating drug may either activate or inhibit a process according to its concentration relative to that of other active substances, and depending on the state of the tissue on which it acts, this latter factor being related to the established hormonal balance of the organism. There is ample evidence that morphine disrupts the normal hormonal balance so far as adrenal hormones are concerned[6,7].

Table 1. *In vitro* Effects of Hydrocortisone on Glucose-uptake by Diaphragm of Normal and of Chronically Morphinized Rats

	Glucose-uptake, mg/100 g wet tissue/h (Mean ± S.E.)			
	Control	+Hydrocortisone ($7{\cdot}7 \times 10^{-4}$ M)	Difference	*t*-test
Normal rats (17)	205 ± 31	171 ± 34	−34 ± 81	N.S.
Chronically morphinized rats (12)	139 ± 28	134 ± 46	−5 ± 44	N.S.
		($3{\cdot}85 \times 10^{-4}$ M)		
Normal rats (6)	193 ± 41	52 ± 27	−141 ± 27	($P < 0{\cdot}01$)
Chronically morphinized rats (7)	228 ± 29	325 ± 35	+97 ± 35	($P < 0{\cdot}05$)
		($1{\cdot}92 \times 10^{-4}$ M)		
Normal rats (8)	253 ± 27	163 ± 26	−90 ± 30	($P < 0{\cdot}02$)
Chronically morphinized rats (8)	196 ± 25	276 ± 20	+80 ± 13	($P < 0{\cdot}001$)

Our observations lead us to suggest that morphine, directly as well as indirectly, induces a change in the tissues which are targets for hormonal action, somewhat in the nature of an adaptation to the changed hormonal balance, with the additional complication that morphine itself participates in that balance by virtue of its hormone-simulating properties. We suspect that, in this way, morphine induces a biological dependence in tissues which are sensitive to the adrenal hormones and that this is the basis of morphine addiction.

We gratefully acknowledge grants from the China Medical Board of New York, Inc., and from the University of Hong Kong.

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HÆMATOLOGY

Inhibition of ADP-induced Platelet Aggregation by Substituted Amino-acids

Aggregation of platelets in the presence of adenosine diphosphate (ADP) appears to be important in hæmostasis and in the genesis of platelet thrombosis. There is evidence that this reaction is common to platelet clumping induced by a variety of stimuli, including collagen, adrenaline, long-chain fatty acids, and perhaps weak solutions of thrombin. Although this phenomenon is under investigation in many laboratories, the mechanism by which ADP causes platelet aggregation is largely unknown. Certain inhibitors of platelet clumping have been described (for example, inhibition of thrombin-induced platelet aggregation by heparin[1] or of ADP by adenosine[2]), and investigation of the inhibitory reaction has afforded some insight into the underlying process.

We have examined platelet clumping induced by ADP, using the turbidimetric technique of Born[3]. This reaction is strongly inhibited by certain substituted α-amino-acids, such as arginine methyl ester. Arginyl esters in which the α-amino group is also blocked, such as BAME, BAEE and TAME, are equally effective. The degree of inhibition varies with the concentration of the inhibitor. The inhibition becomes manifest immediately on addition of the substituted amino-acid and is not enhanced by incubation before addition of ADP, unlike inhibition produced by adenosine. Indeed, if the addition of the inhibitor is delayed until the maximum effect of ADP has been achieved, the platelet aggregates promptly disperse. Fig. 1 illustrates this phenomenon with benzoyl-arginine methyl ester (BAME); similar data have been obtained with other inhibitory agents in this family.

If the reciprocal of the velocity of platelet aggregation in platelet-rich citrate plasma is plotted against the reciprocal of the concentration of added ADP by the technique of Lineweaver and Burk[4], a straight line is obtained (Fig. 2, control) which intersects at the ordinate with a similar curve derived in the same manner by adding ADP to platelet-rich plasma in the presence of arginine methyl ester. Analogous curves may be derived with other concentrations of inhibitor and with other members of the family of inhibitors, and the lines characteristically con-

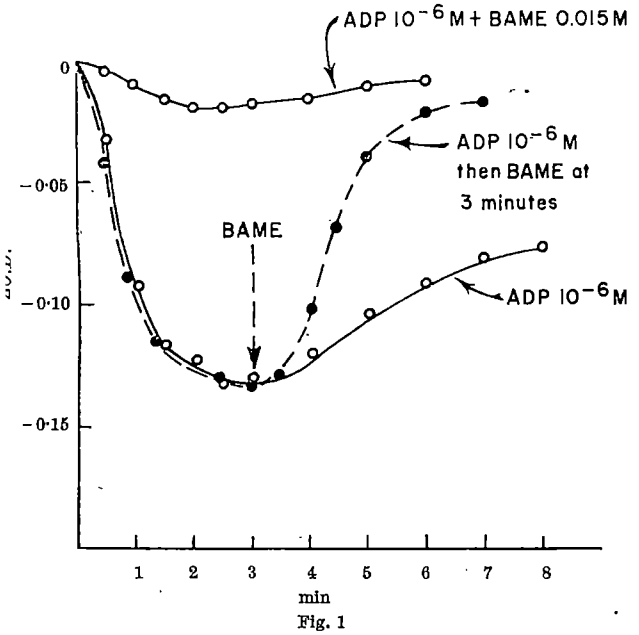


Fig. 1

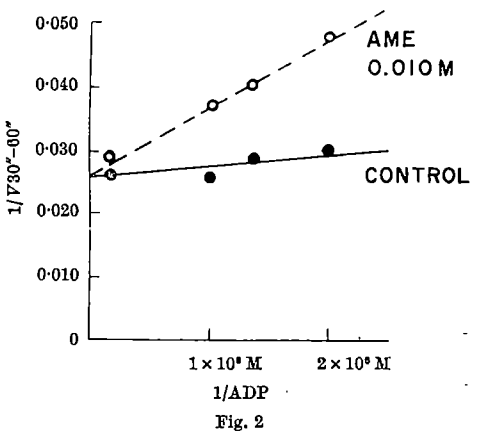


Fig. 2

verge on a single point on the ordinate. As conventionally interpreted, this type of plot indicates competitive inhibition. The components of the reaction blocked by AME are not known, but these results suggest that ADP is one of the reactants.

The potency of various amino-acids and their relatives in blocking ADP-induced platelet clumping is shown in Table 1. All substances were tested in equimolar concentration. Derivatives of arginine inhibit effectively whether the carboxyl group is substituted by an ester or an amide and whether the α-amino group is free or blocked. Arginine does not inhibit ADP-induced aggregation, however, nor does guanidine, which resembles the non-carboxyl end of the arginine molecule.

Table 1. INHIBITORS OF ADP-INDUCED PLATELET AGGREGATION
(tested in 0·015 M)

Upper 1/3		
	Arginine methyl ester (AME)	Arginyl amide (AA)
	Benzoyl arginine methyl ester (BAME)	Tosyl arginyl amide (TAA)
	Benzoyl arginine ethyl ester (BAEE)	Cysteine methyl ester
	Tosyl arginine methyl ester (TAME)	Histidine methyl ester
Middle 1/3		
	Tyrosine methyl ester	Lysine methyl ester
	Phenylalanine methyl ester	Aspartic acid β-methyl ester
	Guanidino-butyric acid methyl ester	Cysteine
Lower 1/3		
	Leucine methyl ester	Isoleucine methyl ester
	Glycine ethyl ester	Serine methyl ester
	Proline methyl ester	Reduced glutathione
	Epsilon amino caproic acid methyl ester	2-Mercaptoethanol
No inhibition		
	Arginine	Methionine
	Alanine	Alanine methyl ester
	Glutamine	Glutamic acid ethyl ester
	Epsilon amino caproic acid	

Guanidino-butyric acid methyl ester, which resembles AME but lacks the α-amino group, is moderately effective as an inhibitor. Epsilon amino caproic acid methyl ester, which bears the same relation to lysine methyl ester, is only slightly less potent, but non-esterified epsilon amino caproic acid has no effect. It would appear that the presence of a substituted carboxyl group is necessary for these compounds to function as inhibitors of ADP-induced aggregation. Of the free amino-acids tested, only cysteine exhibited any inhibitory capacity, and this disappeared if its free sulphydryl group was blocked, as in methionine. Reduced glutathione has a modest ability to inhibit the effect of ADP, as does 2-mercapto-ethanol. Simultaneous addition of cysteine and AME produces greater inhibition of ADP's effect than does either alone, suggesting diverse sites of action. Methionine does not enhance AME's potency as an inhibitor.

These results are consistent with the hypothesis that an enzymatic reaction is involved in aggregation of platelets by ADP, and that this reaction is competitively inhibited by these synthetic substrates. It would appear that sulphydryl groups are involved. The case for an enzymatic reaction is supported by the high degree of specificity of the aggregation phenomenon, induced by ADP and by a few of its closest relatives but not by a large group of related nucleotides tested by Gaarder[5]. Since guanidine does not block ADP-induced platelet clumping, while BAME is a potent inhibitor, the inhibitory effect does not seem to be a non-specific one based on ionic strength. The substituted carboxyl group appears to be required, perhaps by blocking an active enzyme site.

As many workers have shown, platelet aggregation induced by thrombin can be blocked by BAME and its α-amino substituted analogues[6,7]. However, if an enzyme is, in fact, involved in platelet aggregation produced by ADP, the enzyme is probably not thrombin. Heparin inhibits platelet clumping produced by thrombin and blocks the thrombin-fibrinogen reaction but does not inhibit ADP's effect on platelets. Arginine methyl ester, on the other hand, blocks platelet aggregation produced by either ADP or thrombin. Unlike its α-amino-substituted relatives, such as BAME, however, AME is a weak inhibitor of the thrombin-fibrinogen reaction. This observation provides additional support for the thesis[8,9] that platelet clumping produced by dilute thrombin is mediated through ADP.

Although BAME and the other synthetic substrates studied are known to be hydrolysed by certain proteolytic enzymes and thus to compete with the natural protein substrate (as, for example, with trypsin or plasmin), the aggregation of platelets induced by ADP is not inhibited by such naturally-occurring proteolytic enzyme inhibitors as ovomucoid, lima bean trypsin inhibitor, and 'Trasylol'. In addition, we were unable to demonstrate rapid hydrolysis of BAME[10] in platelet-rich plasma in the presence of ADP, although inhibition of the effect of ADP on platelets was almost immediate. Therefore, the inhibitory reaction appears to be of a nature different from that involving proteolytic enzymes.

Platelet adhesiveness *in vitro*[11] is impaired by mixing native blood with BAME and other substances capable of inhibiting ADP's effect. Platelet adhesiveness is deficient in von Willebrand's disease, and there is much evidence that this abnormality is the result of a missing plasma factor[12,13]. It occurred to us that this plasma factor might be the enzyme postulated here. However, the ability of a given concentration of AME to inhibit platelet aggregation in von Willebrand's disease proved to be the same as in normal subjects. Since the sensitivity of ADP-induced aggregation to AME is not enhanced in this disease, it appears that the enzyme we postulate is not the deficient 'von Willebrand's factor'.

Arginine does not inhibit ADP's effect and, if one adds both arginine and arginine methyl ester to platelet-rich plasma, the inhibition of ADP is less than with AME alone. The explanation for this observation is not clear, but may reside in the differing affinities of arginine and AME for the postulated enzyme.

The proof that platelet aggregation induced by ADP is an enzymatic reaction must await isolation of the pertinent enzyme and identification of its natural substrate. Meanwhile, the demonstration that this reaction can be blocked by a family of compounds typified by arginine methyl ester provides stimulus for further experiments.

Certain of the compounds tested were furnished through the kindness of Dr. Sol Sherry. This work was supported by grants from the U.S. Public Health Service and the American Heart Association. We thank Dr. G. Mechanic for his advice.

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Abnormal Hæmoglobin in Sheep

In man there exists a large number of abnormal genetically determined hæmoglobins. To our knowledge no abnormal hæmoglobins are yet reported found in domestic animals. When investigating the hæmoglobin phenotypes of experimental sheep with the technique of starch-gel electrophoresis an animal having atypical hæmoglobin was found.

This abnormal type is shown in Fig. 1, which is a photograph of a gel stained with amido-black and where samples representing the two normal sheep hæmoglobin phenotypes AB and AA have been run for comparison. We have in this communication used the nomenclature of Evans *et al.*[1]. For better demonstration of the two bands in the type AB this sample was less concentrated than the other three shown in Fig. 1. The abnormal hæmoglobin type is shown on the right of Fig. 1. It was first found in a sample from a 7-months-old lamb and appeared as a band having a rate of migration on starch gel which was markedly slower than that of the B band. This special band was of approximately the same strength as the A band and has preliminarily been named N (for Norway). The lamb with this band had been heavily invaded by intestinal parasites during the last three months, primarily *Haemonchus contortus*. Accordingly the animal was anæmic, having a hæmoglobin value of only 7·1 g/100 ml. The lamb had lost 5·2 kg in weight in the last three months and had a considerably lower live weight than the average of the lambs in the control group. The parents of the lamb were of hæmoglobin phenotypes AA and AB, respectively. Testing of the lamb was repeated after a few days on a new blood sample and with the same result. However, when investigating another blood sample four weeks later, which is also shown in Fig. 1, the N band was very faint. Unfortunately the death of the lamb prevented any further investigations.

A year later another group of experimental lambs was hæmoglobin typed. One 7-months-old animal was found which also had the N band. This is shown in Fig. 2, which is a photograph of a gel stained with benzidine and where samples representing the three normal sheep hæmoglobin phenotypes were run for comparison. This animal was also anæmic, due to parasite invasion (mainly *Haemonchus contortus*). The lamb has been tested every week for 2 months. Its hæmoglobin value averaged 4·9 g/100 ml. This lamb has not gained any weight during the last four months, whereas the control group showed an average increase of 6·9 kg. The strength of the N band varied from approximately 2 per cent to 15 per cent of total hæmoglobin.

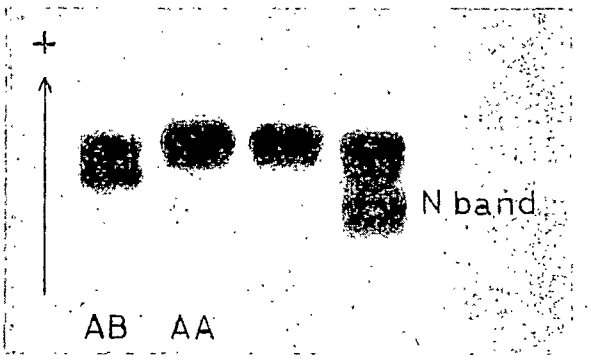


Fig. 1. Photograph of a stained gel showing two examples of the abnormal hæmoglobin type in sheep

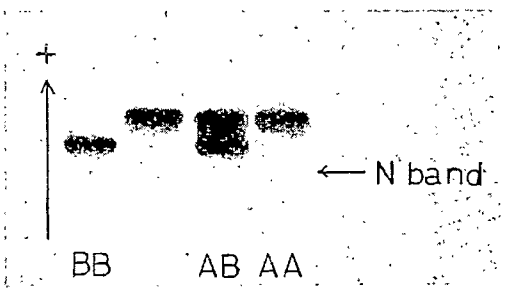


Fig. 2. Comparison between a third example of the abnormal type and the three normal sheep hæmoglobin phenotypes

Recently we have also found the abnormal hæmoglobin type in adult sheep used in special feeding experiments. They did not, however, have any abnormally high number of intestinal parasites.

A definite explanation for the occurrence of this abnormal hæmoglobin type cannot be given at this stage. It is perhaps not genetically determined in the same sense as the many abnormal human hæmoglobins. A condition showing deviations in relative amounts of the two hæmoglobin components has been found in strongly anæmic sheep of phenotype AB by Huisman *et al.*[2] and Efremov[3]. Of special interest, in our opinion, would be the possibility of producing the abnormal hæmoglobin type N in sheep experimentally.

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Oxygen Equilibria of Red Cell Hæmolysates of Adult Individuals, Heterozygous for Different Rare Abnormal Hæmoglobins

In the past few years, oxygen equilibria of various normal and abnormal adult human hæmoglobin types have been studied. No differences were observed between normal Hb-A ($\alpha_2\beta_2$) and the β-chain abnormalities Hb-S (ref. 1), Hb-C (ref. 2) and Hb-E (ref. 3). Investigations of the isolated Hb-A_2' ($\alpha_2\delta_2$) and its abnormal variant Hb-A_2 revealed a considerable increase in oxygen affinity when compared with normal Hb-A (ref. 4). Hb-Lepore, which is composed of normal α-chains and 'β' chains being related to the β-chain of Hb-A as well as to the δ-chain of Hb-A_2 (ref. 5), showed a high affinity for molecular oxygen similar to that of Hb-A_2 (ref. 1). The affinities of the α-chain lacking abnormal hæmoglobin types Hb-H (β_4) and Hb-Bart's (γ_4) for molecular oxygen were found to be extremely high, while no Bohr effect or hæm–hæm interactions were detectable[6,7].

This investigation was an additional step in our search for possible functional alterations of different abnormal human hæmoglobin types. The experimental situation made it necessary to limit our investigations to the determination of the oxygen affinity of red blood cell hæmolysates obtained from adult individuals, known to be heterozygous for various α- or β-chain abnormalities. The methods used for the oxygen equilibria determination and for the quantitation of abnormal components either by CM- or DEAE-cellulose chromatography have been described before[1,8,9]. Four β-chain abnormalities and three α-chain abnormalities were investigated. The electrophoretically fast-moving β-chain abnormalities, Hb-$J_{Georgia}$ (ref. 10) and Hb-Jenkins (ref. 11), each found in two Negro families, were present in the blood of heterozygous carriers in quantities exceeding 50 per cent (Table 1). The quantities of Hb-D_{Punjab} (ref. 12), discovered in several members of two families of Caucasian extraction, and of another Hb-D with unknown structural alteration of the β-chain, discovered in a Negro family (fam. M), were approximately one-third of the total hæmoglobin (Table 1). The three major α-chain abnormal hæmoglobins, Hb-Dα, found in two members of a Negro family, the electrophoretically fast-moving Hb-I (ref. 13) and Hb-Russ (ref. 14), both discovered in members of families of Caucasian extraction, were present in different quantities; the percentage of Hb-Dα amounted to approximately one-third, that of Hb-I to approximately one-fifth and that of Hb-Russ to approximately one-tenth of the total hæmoglobin present in the red cell hæmolysates of heterozygous carriers (Table 1).

Table 1. Oxygen Equilibria of Various Hæmolysates at 37° C and *p*H 7·0

Type of hæmolysates	Abnormal chain	% Abn. Hb.	% A_2	Log P_{50}*	Δ Log P_{50}	n
Normal			2·3–2·7	1·280		3·2
Hb-$J_{Georgia}$	β	55·5	2·3	1·280	0	3·2
Hb-Jenkins	β	51·7	2·3	1·275	−0·005	3·15
Hb-D (Fam. M)	β	37·8	2·4	1·270	−0·010	3·2
Hb-D_{Punjab}	β					
Fam. Jon.		35·7	2·4	1·245	−0·035	3·2
Fam. E		34·2	2·3	1·250	−0·030	3·2
Hb-D (Fam. J)	α	32·9	1·4†	1·270	−0·010	3·2
Hb-I (Fam. S)	α	19·4	1·6†	1·280	0	3·2
Hb-Russ	α	11·7	2·1†	1·265	−0·015	3·0

* The value given for normal hæmolysates is the mean of 13 determinations with a standard deviation of the mean of 0·010. All other values are mean values of duplicate determinations with the exception of those given for Hb-D_{Punjab} hæmolysates, which are the mean of four separate analyses.
† The percentages of the corresponding abnormal (in α-chain) Hb-A_2 components were 0·8 for Hb-Dα, 0·7 for Hb-I and 0·4 for Hb-Russ.

The results of the oxygen equilibrium investigations are also summarized in Table 1; the oxygen affinities are presented as the logarithms of the oxygen pressures required to half-saturate the different hæmolysates, while the slopes of the oxygen dissociation curves are described by the values found for the constant n in Hill's equation[15]. All different hæmolysates, except one, have oxygen affinities identical to that of a hæmolysate of normal adult red blood cells. The data obtained in several experiments with hæmolysates of red blood cells of an Hb_{Punjab} trait carrier indicate that the presence of this abnormal hæmoglobin increases the oxygen affinity, the difference being slightly greater than twice the standard deviation of the mean. A similar small difference was observed when blood samples of Hb-D_{Punjab} trait carriers were analysed. The values for the log P_{50} values at 37° C and *p*H 7·4 were 1·300 for the D trait carrier and 1·325 for the normal control. These results, which are in accordance with the observations made with isolated Hb-D_{Punjab} (ref. 1), suggest that the structural abnormality in this hæmoglobin type, namely, the replacement of a glutamic acid residue by glutamine in position 121 of the α-chain, does interfere with the affinity for molecular oxygen. It might be argued that the results from the experiments with the other six hæmoglobin solutions do not definitely prove that the structural changes in these abnormal hæmoglobin types do not interfere with their oxygen equilibria, since mixtures with varying percentages of normal hæmoglobin types were investigated. It seems, however, that such differences, if present, are too small to change the physiological characteristics of hæmolysates prepared from these red blood cells.

We thank Mr. R. Lee for his help in locating the carriers of the abnormal hæmoglobin types investigated. This work was supported by U.S. Public Health Services grant *H*–6982.

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Ellipsometer Recordings of Changes in Optical Thickness of Adsorbed Films associated with Surface Activation of Blood Clotting

Recent publications[1–4] indicate that when blood comes into contact with glass, Factor XII is adsorbed and in turn adsorbs Factor XI. Much of the resulting 'activation product' can be recovered from the adsorbing surface[1,3], but other indirect evidence shows that measurable amounts are released[4–7].

To observe adsorption directly at a wettable solid interface before and during surface activation, a recording ellipsometer was used in our work. Details of technique were described elsewhere[8,9]. As reflecting adsorbents, the following were used: tantalum-sputtered glass object slides, anodized to form an optically sensitive thickness of oxide, and with an approximately 25 Å thicker oxide step at one end of the slide for calibration of the recorder span at the beginning of each experiment; and slices of silicon crystal which could be rendered wettable by exposure to hot nitric acid. Normal intact (non-activated)

Table 1. Activation of Barium Stearate-treated Normal Intact Plasma

0·1 ml. normal intact plasma + 0·1 ml. of:	Clotting time (min)
Normal intact plasma recalcified immediately *	8½, 9½
Intact BaSt-treated plasma recalcified immediately *	9½, 10½
Activated BaSt-treated plasma recalcified immediately *	6½, 7
Intact BaSt-treated plasma incubated separately †	11½
Intact BaSt-treated plasma incubated together †	8½, 9
Activated BaSt-treated plasma incubated separately †	6½, 6½
Activated BaSt-treated plasma incubated together †	4, 4½

* All tests at 37° C in 12 mm × 75 mm polystyrene tubes; 0·2 ml. plasma mixture + 0·2 ml. 0·025 M $CaCl_2$, tilted at 15-sec intervals.
† Normal intact plasma sample and additive incubated for 10 min at 37° C in styrene tubes separately, or together as indicated; then mixture recalcified.
BaSt, barium stearate.

plasma was obtained by centrifuging fresh ACD blood for 30 min at 4° C and 1,000*g*, and then the collected plasma at 4° C and 40,000*g* for 60 min, using 'Nalgene' tubes. The plasma was then aspirated from under the lipid film that had formed, and incubated in 'Styrene' test-tubes for 1 h. Intact, Factor XI-deficient plasma was prepared from this 'whole' intact plasma by adsorption with 50 mg neutral barium stearate (Witco Chem. Co., New York) per ml.[8] for 20 min, followed by centrifugation. Activation was achieved by adding 1 part of quartz powder (No. 520, 3–5μ, Engelhart, Newark, New Jersey) or 'Speedex' (Great Lakes Carbon Corp., Los Angeles, Calif.) to 3 parts of plasma by volume, gently mixing for 10 min[3] at about 25° C, and centrifuging at 20,000*g* for 15 min. The recalcified clotting time of normal plasma in 'Styrene' tubes was shortened about five-fold by this treatment, and the ability of similarly treated barium stearate adsorbed plasma to correct the clotting time of whole intact plasma was improved (Table 1).

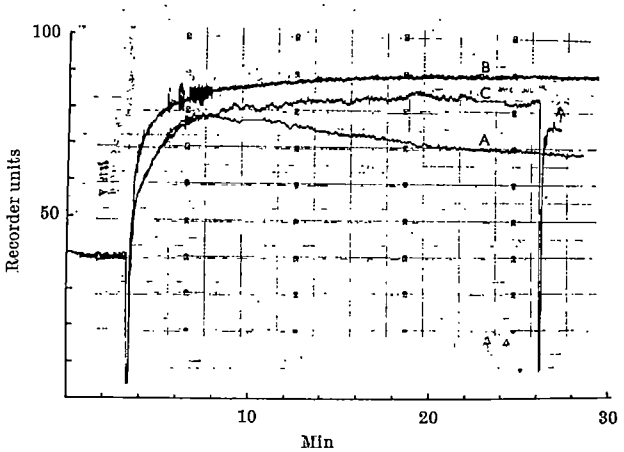


Fig. 1. Adsorption and spontaneous desorption of normal intact plasma (*A*), and adsorption of activated (*B*) and barium stearate-treated plasma (*C*). Superimposed transparent photocopies. 24 Å barium stearate film is equivalent to 80 recorder units

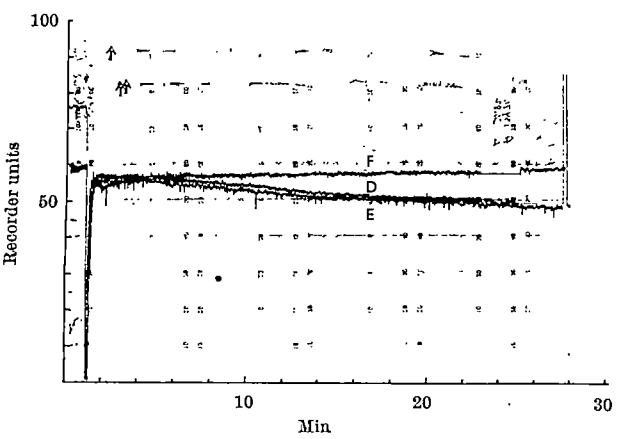


Fig. 2. Removal of barium stearate-treated plasma by normal intact (*D*) and Factor XII-deficient 'activated' plasma (*E*); no removal by normal activated plasma (*F*). Superimposed transparent photocopies

The recordings shown here (Figs. 1 and 2) were obtained with 5461 Å light polarized at 45° to the plane of incidence, and incident at 75°; for recording, the analyser was set about 6° from compensating position. Adsorption was initiated as follows: the tantalum/tantalum oxide slide was lowered into a strain-free glass cuvette containing 60 ml. of 1 part 'VBIS'[8] to 4 parts of normal saline, stirred continuously at 300 r.p.m.; when the recording indicated equilibrium was obtained, 1 ml. of plasma was added. At proper intervals, the instrument was switched from recorder to photometer and minimum settings of quarter wave plate and analyser were determined; these were then both returned to the original recording settings.

Since recordings obtained with the analyser set above its minimum position were more or less the mirror images of those obtained with the analyser set below its minimum position, none of the results reported here was due to significant changes in any optical properties other than those attributable to optical thickness of the reflecting film; moreover, the direction of recorded curves always corresponded to the changes in phase and amplitudes of reflected light components measured with quarter wave plate and analyser. The following results were obtained (Table 2 and Figs. 1 and 2):

(*a*) Adsorption of whole, intact plasma yielded at first a more or less hyperbolic curve, indicating steadily decreasing rate of adsorption; after about 4 min, it reversed to indicate slow partial desorption (Fig. 1*A*).

(*b*) Adsorption of quartz-treated or 'Speedex'-treated whole plasma, or of barium stearate-treated intact or quartz-treated plasma, is completed in about 10 min, and is not followed by desorption (Fig. 1 *B* and *C*).

(*c*) Whole intact plasma, and quartz-treated Factor XII-deficient plasma (obtained through the courtesy of Dr. L. Weisfuse, Garden City, New York), were able to remove constituents from adsorbed films of barium stearate-treated normal intact plasma (Fig. 2 *D* and *E*); quartz-treated normal plasma was not (Fig. 2*F*).

(*d*) No desorption could be induced either by normal intact plasma when it was brought into contact with a film left by activated (quartz-treated) barium stearate-treated plasma, or when barium stearate-treated serum instead of plasma was used as the substrate.

The following tentative conclusions are drawn from the corresponding findings: (1) when plasma which contains intact Factors XI and XII is adsorbed under our conditions, part of the adsorbate—possibly activation product—is soon released; (2) no adsorbate is removed spontaneously by plasma which does not contain intact Factors XI and XII both; (3) adsorbate formed from barium stearate-treated plasma can only be removed by plasma containing intact Factor XI; and (4) only if the adsorbate contains intact Factor XII.

These conclusions fit the presumption that Factor XII is adsorbed, and is removed by Factor XI only if both were initially intact.

The expression of quantitative data (Table 2) in Ångströms is based on the customary presumption that the optical properties of barium stearate layers are identical to those of protein[8,9]. This is not the case[10], and

Table 2. Change in Compensating Analyser Position, and its Barium Stearate Equivalent in Ångströms, after Adsorption and Desorption of Intact Plasma

Slide	Sample	Degree change (Å)	±	*S.D.*	Equiv. (Å) of compressed barium stearate	*N* *
TaW	Intact normal plasma	1·90	±	0·25	57	13
TaW	Spontaneous desorption of intact normal plasma	0·44	±	0·27	13	13
TaW	Intact barium stearate-treated plasma	1·91	±	0·18	57	11
SiW	Intact normal plasma	1·13	±	0·07	34	4
SiW	Spontaneous desorption of intact normal plasma	0·16	±	0·04	5	4

* No. of experiments.

efforts are planned to distinguish between changes of actual thickness and of refractive index of adsorbates[10].

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HISTOLOGY

Compensatory Growth of Villi in Postmature Human Placentæ

HISTOLOGICAL changes have been investigated in the postmature human placenta. This is an obstetric abnormality known to be associated frequently with antenatal asphyxia and stillbirth. Fœtal loss occurs as a result of hypoxia, attributed to functional placental insufficiency[1–4]. It is generally stated that in postmaturity the placenta displays signs of ageing and degeneration: widespread vascular obliteration, thrombosis and infarction with excessive fibrinoid and calcified deposits. The appearance of 'young' placental villi in postmaturity, reported by certain investigators[5–7], does not seem to have received adequate attention.

A total of 253 placentæ were studied. These included placentæ from cases of postmaturity of various grades (pregnancy extending to 46 weeks or longer), as well as 25 placentæ delivered at 38–40 weeks (normal labour) used as control.

Specimens for histological investigation were obtained from central, medial and marginal placental zones and embedded in paraffin after fixation in 10 per cent formol. Sections were stained with hæmatoxylin–eosin or with van Gieson's stain. Histological characteristics were related to the clinical data concerning the course of pregnancy and the outcome of labour for mother and child.

Contrary to the present-day view that signs of ageing, and widespread degeneration, tend to prevail in the placenta with prolongation of pregnancy, our observations pertaining to different degrees of postmaturity have demonstrated the occurrence of areas of complementary compensatory villous growth (Fig. 1) against a background of mature or 'ageing' placental structure. These areas were generally located in peripheral portions of the placenta, being more scarce in the central part. The newly-formed groups of terminal villi displayed unequal grades of maturity in different placentæ, suggesting that they probably originated at different stages of pregnancy. In a number of these placentæ, the new villi showed 'harmonic' development of stroma and fœtal capillaries, the latter tending mostly to underlie the plasmodiotrophoblast of the villi, and forming the so-called 'syncytial-capillary' membranes. Compensatory villous growth contributed to the enlargement of the placental surface available for exchange.

At certain sites, however, terminal villi proved to be set more closely. Their surfaces then tended to approximate while the intervillous spaces became more and more narrow. In some of these cases approximation of villi resulted in their conglutination (Fig. 2). Disappearance of intervillous spaces was brought about in this way, the circulation of maternal blood was interrupted and the villous surfaces were excluded from gas exchange and other metabolic processes. Fibrinoid deposition was occasionally seen at these sites.

In some of the placentæ, development of embryonic connective tissue and capillaries was also subject to variation in these foci of compensatory growth. In a number of cases capillary development was poor, the fœtal capillaries being extremely narrow. The distribution of these foci was often restricted to the central part of the villous, but they were occasionally found throughout the stroma. In either case, however, most capillaries were separated from the trophoblast by thick collagen fibres (Fig. 3). In other placentæ, there was excessive development of capillaries in the terminal villi within the foci of compensatory growth. These villi were completely occupied by a multitude of wide capillaries, pressed together and only separated by narrow streaks of connective tissue (Fig. 4).

Irrespective of the prolongation of the period of gestation, some of the placentæ presented the usual appearance of mature placental structure, occasionally

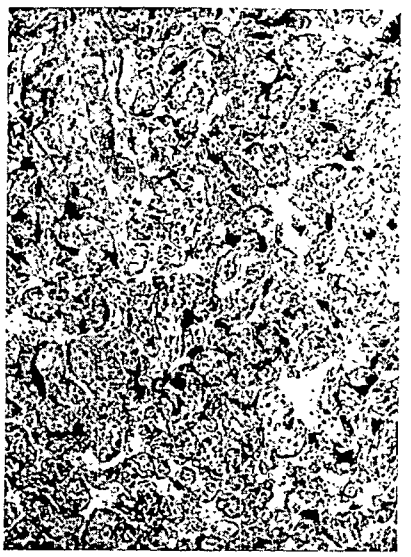


Fig. 1. Focus of compensatory villous growth in human placenta. Gestation, 300 days. (Hæmatoxylin–eosin, × *c.* 55)



Fig. 2. Conglutination of villi in foci of compensatory growth. Gestation, 300 days. (Hæmatoxylin–eosin, × *c.* 500)



Fig. 3. Young villi with deficient development of capillaries. Gestation, 388 days. (Hæmatoxylin–eosin, × *c.* 500)



Fig. 4. Young villi with excessive development of capillaries. Gestation, 319 days. (Hæmatoxylin–eosin, × *c.* 500)

showing signs of ageing, such as extensive accumulations of fibrinoid matter, white infarcts, obliteration of vascular trunks, etc.

All the available material could be classed under three large groups: (1) mature placentæ; (2) placentæ displaying compensatory growth of villi, (*a*) with normal fœtal capillaries or (*b*) with defective fœtal capillary development. Conglutination of villi may occur in (*a*) or (*b*); (3) 'old' placenta.

The distribution of these morphological groups among afterbirths from pregnancies of varying duration are given in Table 1. It is evident that with the prolongation of pregnancy beyond term, the percentage of mature placentæ decreases, while that of placentæ with compensatory growth of villi increases.

When the results of microscopic examination were related to the clinical course of pregnancy and its outcome for the child, a certain pattern could be revealed. In most of the women delivered, within 43–46 weeks, of living babies showing no signs of asphyxia, the placentæ displayed foci of compensatory villous growth without conglutination of the villi and with efficient capillary development. The abnormal development of capillaries in the foci of compensatory growth, associated with conglutination of villi, aggravated conditions detrimental to fœtal development by reducing the functional placental

Table 1

Group	Gestation (weeks)	No. cases	'Mature' No.	'Mature' %	Placenta Displaying compensatory growth of villi No.	Placenta Displaying compensatory growth of villi %	'Old' No.	'Old' %
I	38–40	25	20	80·0	5	20·0	—	—
II	40–42	35	14	40·0	21	40·0	—	—
III	42–44	106	31	29·2	72	68·0	3	2·8
IV	44–46 and over	87	23	26·4	61	70·1	3	3·4
	Total	253	88	—	159	—	6	—

surface area. This type of placental structure was revealed in three-quarters of the cases of stillbirth[8,9].

Thus, investigation of placentæ in postmaturity has shown that the outstanding morphological characteristic of the condition is the occurrence of foci of compensatory growth of terminal villi, rather than the processes of ageing and degeneration, which naturally tend to increase also with postmaturity. Compensatory growth of villi evidently sets in with any deficiency of oxygen and other vitally important substances for fœtal development. It can probably be regarded as a compensatory response of the placenta. In certain cases, compensatory growth of villi fails to bring about favourable results, due to abnormal development of the fœtal capillaries and the impairment of maternal circulation in the intervillous spaces, owing to conglutination of the villi. These changes affecting the placenta, particularly when associated with other unfavourable factors (uterine inertia, etc.), are among the major causes of oxygen lack and perinatal fœtal deaths.

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IMMUNOLOGY

Role of Debris of Tumour Ascitic Fluid in Transplantation Immunity

Examination of the chemical nature of histocompatibility antigens is not easy, because of the rapid inactivation of cell-free extracts and the difficulty in obtaining these antigens in a soluble form.

Attempts have recently been made to discover another source of these antigens, more favourable than cells. It has been found that cell-free tumour ascitic fluid possesses some antigenic activity. Hašková and Hilgert[1] detected transplantation antigens in cell-free ascitic fluid of sarcoma I by means of the second-set reaction. Davies[2] isolated from the ascites form of tumour *BP* 8 an iso-antigenic lipoprotein complex absorbing hæmagglutinins from specific antisera. Manson *et al.*[3] extracted soluble transplantation antigens from leukæmic cells grown *in vitro*.

The experiments recorded here were planned to find out whether the antigenic activity of cell-free ascitic fluid was

Table 1. ANTIGENIC PROPERTIES OF PLASMA AND 'DEBRIS' FROM TUMOURS *Sa I*, *EL*-4 AND *BP*-8 DETECTED BY 'SECOND-SET' REACTION

Preparation	Strain used	*Sa I* Volume	*Sa I* Results†	*EL*-4 Volume	*EL*-4 Results†	*BP*-8 Volume	*BP*-8 Results†
Plasma	*CBA*	3 × 1 ml.	± − − − − − − −				
		2 × 5 ml.	− − −				
	C57BL/6J	7·5 ml.	− − −				
	ASW	6 ml.	± − − − − −	3 ml.	− − −		
				5 ml.	+ − − −		
	C57BL/Ks					3 ml.	− − − −
						5 ml.	± − − − −
"Debris"	*CBA*	2 ml.*	− − − − − −				
		5 ml.*	− − − −				
	C57BL/6J	5 ml.*	± − −				
		8 ml.*	± − −				
	ASW	4 ml.*	± ± ± ± − − −	5 ml.*	+ + + + −		
		15 ml.*	± ± ± ±				
	C57BL/Ks					3 ml.*	± + + +
						5 ml.*	± + + + +

* Volume of ascitic fluid from which the debris were prepared. † Results of histological evaluation of grafts removed 6 days after transplantation +, complete destruction of all epidermal cells; ±, partly damaged layer of epidermal cells; −, proliferation of epidermal cells.

bound to soluble components or to particles of cellular origin (debris). Three ascitic tumours were used: *Sa I* (*A* strain specific), *BP*-8 (*C3H* strain specific) (kindly provided by Dr. Davies), and *EL*-4 (leukosis-*C57BL*, *H*-2^b-strain specific).

Cell-free ascitic fluid was prepared from ascites collected by four times repeated centrifugation for 10 min at 10,000*g* (maximum). After each centrifugation the supernatant was carefully transferred with a syringe. Absence of tumour cells in the last sediment was controlled by the intraperitoneal injection into the indigenous strain of mice of the tumour. That sediment never gave rise to growth of a tumour.

The debris and the plasma were separated from the cell-free ascitic fluid by centrifugation for 60 min at 105,000*g* (average).

Both fractions were injected intraperitoneally for the examination of the sensitizing activity by means of the 'second-set' reaction[4]. The dosage of debris was expressed by the original volume of ascitic fluid, from which it was extracted. Large volumes of plasma were injected; but they failed to induce any transplantation immunity. The debris exhibited a different sensitizing activity depending on the nature of the tumour from which they were extracted. The debris from the tumours *EL*-4 and *BP*-8 were highly active, but the debris from the tumour *Sa I* were only weakly active (Table 1).

The results observed *in vitro* were consistent with these findings. The debris of tumour *Sa I* inhibited hæmagglutination[5] to a lesser extent than similar preparations of tumours *EL*-4 and *BP*-8. These results were not surprising since cell-free extracts or non-repopulating cells (cell suspension irradiated by 15,000 r. prior to an accumulation) prepared from sarcoma I had a lower activity than similar preparations obtained from tumour *EL*-4 or *BP*-8 or spleen cells.

In the strain combination *A-CBA*, a fully sensitizing extract required a starting material of some 10×10^8 cells of sarcoma I, but only 2×10^8 spleen cells.

The extract of sarcoma I is a weak antigen for two main reasons: if *Sa I* cells are lacking some antigenic factors, or if they possess a smaller amount of antigenic factors per cell.

It is known that the cells of *Sa I* are deficient in some antigenic factors. O'Gorman and Mikulska[6] detected very weak or no antigenic factors *E* and *K* by means of specific antiserum. Kandutsch and Stimpfling[7], however, found *K* antigen to be present, but their preparations contained no antigen *C* and *E*. Davies[8] noted that the cells of tumour *Sa I* had no specificity *E* and *K*.

It may be concluded from the foregoing findings that:

(1) The debris seemed to be responsible for antigenic activity of cell-free ascitic tumour fluid. It was suggested that no transplantation antigens existed in its soluble form in that fluid. But debris was not an easy or economical material to handle for the preparation of some antigenic extract. The amount of debris in the ascitic fluid was mostly variable.

Since the cellular origin of the debris has been proved by electronmicroscopy, it would seem more logical to start from tumour cells rather than from debris for the extraction of iso-antigens.

(2) The extracts from sarcoma I were weakly antigenic and, since the sub-lines of this tumour have proved to possess different antigenic properties (detected *in vitro*)[9], they are not so suitable as the extracts from tumours *EL*-4 and *BP*-8 or spleen or epidermal cells for the investigation of transplantation antigens.

We thank Dr. Ivan Hršel, of the Institute for Experimental Biology and Genetics, Prague, for the electronmicroscopy investigations of ultrastructure of debris.

This work was supported by the Centre Anti-Cancéreux de Liège.

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Species Specificity of the Immunogenic Complexes of the Tapeworm Hexacanth Embryo

IN a previous article[1] evidence was reported supporting the hypothesis that at least two immune responses occur against larval tæniid tapeworms in the intermediate host; and that these responses can be elicited by a single parenteral injection of homologous activated embryos in previously unexposed animals. The initial response appears to occur before the penetration of the hexacanth embryo through the intestinal barrier, and the later response occurs at the site of election against the growing metacestode.

The question was raised as to whether the protection inducing immunogenic complexes of each species of hexacanth embryo are species specific, or some parts of the complex are shared to a greater or lesser extent by

Table 1. Average No. of Cystioerci established and surviving from an oral challenge of up to 2,500 Eggs in animals immunized with 1,000–50,000 Hexacanth Embryos

Antigen	Stage of embryo	Challenge infection with: *T. hydatigena* in sheep			*T. ovis* in sheep			*T. pisiformis* in rabbits		
		No. of animals	Average No. of: Viable cysts per animal	Average No. of: Dead and viable cysts per animal	No. of animals	Average No. of: Viable cysts per animal	Average No. of: Dead and viable cysts per animal	No. of animals	Average No. of: Viable cysts per animal	Average No. of: Dead and viable cysts per animal
T. hydatigena	Active	10	0·0	0·1	10	5·9	59·9	14	5·4	50·0
	Passive	10	0·2	86·7	10	2·8	52·6	14	17·1	56·1
	Dead	10	77·1	193·8	—	—	—	—	—	—
T. ovis	Active	10	31·5	181·2	10	0·1	16·7	14	7·7	79·7
	Passive	10	85·7	182·1	10	0·3	51·1	14	20·4	172·2
	Dead	10	149·0	230·0	—	—	—	—	—	—
T. pisiformis	Active	10	72·4	232·6	10	14·3	128·6	12	0·3	10·0
	Passive	10	141·4	234·4	10	11·3	90·3	12	0·3	2·7
	Dead	10	111·0	214·7	—	—	—	—	—	—
	Controls	10	144·8	302·2	10	12·7	115·1	32	25·7	112·8

species within the same genus as well as with some of those of different genera.

To assess the species specificity of the immunogenic complexes of cestodes within the same genus, three trials involving 170 worm-free (*Strongyloides* spp. excepted) sheep and 112 rabbits have been carried out, using the hexacanth embryo of *Taenia hydatigena*, *T. ovis* and *T. pisiformis* as the test antigen complexes.

The activated hexacanth embryo antigen complex for each species was prepared by immersing eggs expressed from gravid proglottids in Silverman's[2] hatching solutions. The viable but resting embryo antigen complex (passive) consisted of untreated eggs. The killed embryo complex was prepared by freezing viable eggs at −70° C for 24 h. After treatment, each antigen complex was suspended in lamb's serum (for *T. hydatigena* and *T. ovis*) or rabbit's serum (for *T. pisiformis*) and injected intramuscularly. The animals were approximately three months of age at vaccination. The time interval between vaccination and challenge with aliquot doses of up to 2,500 fresh eggs was about eight weeks. The interval between challenge and autopsy was also about eight weeks.

Table 1 gives the number of animals used to test the protective efficiency of each antigen complex and shows the number of embryos which became established (that is, the minimum number of challenge organisms which penetrated the intestinal barrier and reached the site of election), as well as the number which survived to reach maturity at the site of election in the immunized animals and in those comprising the control groups.

Table 2 shows the percentage of protective efficiency of each of the three species of embryo in different phases of metabolism in inducing an immune response to the establishment and subsequent survival of a homologous and a heterologous challenge.

The specificity of the response to the homologous oral challenge in immunized animals compared with that in the controls in each trial, together with the remoteness of the site of injection from the sites of entry and election of the challenge, give a reasonable basis for assuming an immunological origin for most of the resistance phenomena induced against the challenge of the immunized animals.

These data also show (Table 2) that the activated form of *T. ovis* induced a recognizable response at the 5 per cent level of significance against the invasion of *T. hydatigena*, and the activated embryos of *T. hydatigena* induced a similar response against the invasion of *T. ovis* through the intestinal barrier. The activated embryos of *T. ovis*, however, induced a higher overall protection against *T. hydatigena* than those of *T. hydatigena* elicited against *T. ovis*, because the activated embryos of *T. hydatigena* failed to induce any protection against the long-term survival of *T. ovis*, whereas those of *T. ovis* induced a protective response at the 1 per cent level of significance against the long-term survival of *T. hydatigena*.

Table 2. Percentage Protective Efficiency of the Antigen-complexes of the Hexacanth Embryo

Antigen / Parasite	Stage of embryo	Challenge infection with: *T. hydatigena* in sheep, Immunity at: Intestinal level	*T. hydatigena* in sheep, Immunity at: Site of election	*T. ovis* in sheep, Immunity at: Intestinal level	*T. ovis* in sheep, Immunity at: Site of election	*T. pisiformis* in rabbits, Immunity at: Intestinal level	*T. pisiformis* in rabbits, Immunity at: Site of election
T. hydatigena	Active	99·97*	100·0*	48·0†	10·7	28·06†	48·60†
	Passive	71·31*	99·52*	54·3†	51·8†	19·28	0·0
	Dead	35·87	16·97	—	—	—	—
T. ovis	Active	40·0†	63·2*	85·5*	94·6*	45·14†	25·15†
	Passive	39·74	1·78	55·6*	94·7*	0·0	8·75
	Dead	23·89	0·0	—	—	—	—
T. pisiformis	Active	23·02	35·04	0·0	0·0	92·47*	88·81*
	Passive	22·57	0·0	21·6	0·0	97·97*	58·52†
	Dead	28·93	0·0	—	—	—	—

* 1 per cent level of significance.
† 5 per cent level of significance.
The percentage immunity in each phase is calculated from the following:

$$\text{Immunity at the intestinal level is } \left[1-\frac{Nt+Vt}{Nc+Vc}\right]\times 100$$

$$\text{Immunity at the site of election is } \left[\frac{\frac{Nt}{Nt+Vt}-\frac{Nc}{Nc+Vc}}{1-\frac{Nc}{Nc+Vc}}\right]\times 100 \text{ or } \left[\frac{NtVc-NcVt}{Vc(Nt+Vt)}\right]\times 100$$

Where Nt is the number of necrotic lesions per animal within each test group. Nc, the number of necrotic lesions per animal within each control group. Vt, the number of viable cysticerci per animal within each test group. Vc, the number of viable cysticerci per animal within each control group.

The resting form of the embryos of *T. hydatigena* induced a recognizable response at the 5 per cent level of significance against the invasion and subsequent survival of *T. ovis*. The embryos of *T. ovis* injected in the same form appeared to induce some immunity at the intestinal barrier against *T. hydatigena*, but this response did not reach the 5 per cent level of significance.

The activated embryos of both *T. hydatigena* and *T. ovis* induced a recognizable response at the 5 per cent level of significance against the establishment and long-term survival of *T. pisiformis* in rabbits, but those of *T. pisiformis* did not appear to induce an equivalent protective response against either *T. ovis* or *T. hydatigena* in sheep.

Since some protection against a homologous challenge can be induced at the intestinal level as well as at the site of election by the prior parenteral injection of activated as well as resting but not by killed embryos, it would appear likely that metabolic, possibly enzymatic, rather than purely somatic antigen complexes may be involved. However, this cannot be determined on the evidence presented, since it is possible that protection may be given by the somatic tissue which is destroyed by freezing, or by new somatic tissue developed in the resting and particularly in the activated form of the embryos after injection.

The results obtained in these trials indicate that the whole antigenic complex is species specific, but that parts of the protection-inducing complexes are common to the embryonic as well as to the cystic forms of *T. ovis* and

T. hydatigena. The evidence, however, for a similarly shared immunogen between the embryos and cysticerci of the two sheep metacestodes and those of the rabbit metacestode *T. pisiformis* is less certain.

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ANATOMY

Effect of Light Deprivation on the Postnatal Development of the Optic Nerve

Gyllensten and Malmfors[1] examined *inter alia* the cross-sectional area and myelinated fibre distribution of the optic nerve in mice reared in the light or in the dark. In mice reared in complete darkness for 20 and 30 days, delay in myelination of the optic nerve fibres caused a reduction in the number of myelinated fibres of some 12 per cent. The number of larger fibres was affected to a greater extent than the number of smaller ones. At 20 days the cross-sectional area of the optic nerve was reduced by 3·4 per cent and at 30 days it was increased by 0·4 per cent; this was said to be insignificant. This latter finding prompts me to report some preliminary observations and a new experimental technique for light deprivation.

The classic experiments in this field are those of Held[2] (cited by Sattler[3]). "Bei neugeborenen Kaninchen, Katzen und Hunden öffnete er einseitig die Lidspalte und fand bei Untersuchung in polarisiertem Licht, 'einen deutlichen, wenn auch nicht sehr beträchtlichen Unterschied in der Markreife', während ein vollständig im Dunkeln gehaltener Hund, dessen eine Lidspalte vorzeitig geöffnet worden war, keinen Unterschied in der Markentwicklung zwischen beiden Sehnerven erkennen liess." In Held's experiments, since the closed eyelids of the new-born are translucent, the difference in stimulus between the two retinæ was one of degree only. It was thought that occlusion of all light from one eye from the time of birth would give a more clear-cut effect and would permit quantitative assessment of the effect of function on fibre size. Even in these experiments, however, the difference may be one of degree only, as the question of spontaneous activity[4] must be considered.

The occluding shield was made from tantalum foil (0·002–0·003 in.). Circular disks of 5 mm diameter were cut from the sheet and made ovoidal with a specially-shaped punch. Newborn mice were operated on within a few hours of birth. From a midline incision over the sagittal suture the skin was undercut over one eye and a shield was inserted. The shape of the shield kept it in position over the convexity of the eyeball and the incision was closed by holding the wound edges together with toothed forceps for a while. The mice were then returned to a foster mother of a hybrid strain who had littered a day or two earlier. The use of recently-littered or highly-strung pure-strain mice resulted in cannibalism. In experiments conducted so far the unoccluded eye opened on the 12th to 14th day but the lids of the occluded eye usually remained fused and the shield remained in position. The eyelids of control animals (operation but no shield) opened at the normal time. The animals were weaned 10 days later and anæsthetized at 75 days with intraperitoneal 'Nembutal'. The animals with occluded eyes were normal in weight and the eye beneath the shield was normal in size and structure. The calvarium was removed and the brain divided in the mid-coronal plane so that the optic nerves and chiasma were left *in situ* when the rest of the brain was removed. For light microscopy the nerves were prepared as follows: the nerve of the operated side was cut longer than the other for identification; the nerves and chiasma were then removed and processed *en bloc* so that the nerves received identical treatment; they were sandwiched between pieces of liver, the operated side being identified by a human hair placed alongside it, and placed in a cell; the cell was frozen in a CO_2–acetone mixture and transverse sections were cut at 7μ in a cryostat[6]. The sections were fixed and stained on a slide with potassium permanganate[5] and mounted in Farrant's medium.

The fresh-frozen section technique is particularly suitable for detecting a gross effect since it 'fixes' tissue fluid and preserves the relations of living tissues so that the shrinkage and distortion which accompany most routine methods of fixation and embedding[7] are absent. In the control animals the cross-sectional areas of the nerves of the two sides were similar. In the animals with occluded eyes the nerves of the operated side had a cross-sectional area some 10 per cent less than those of the unoperated side.

The question of which tissue component or components of the optic nerve was responsible for this gross effect could not be settled with certainty. Difficulties in quantification with this method may be related to the large number of small fibres and to the intimate membrane contacts which exist in this situation[8]. The visual impression gained was that both axon diameter and sheath thickness failed to reach normal values. Substantiation of this is being sought by electron microscopy; phase contrast microscopy is unsuitable for precise dimensional estimations of this kind[6]. Gyllensten and Malmfors[1] commented only on overall fibre diameter. Since their experiments affect the time of onset of myelination, which is an incremental process, it is likely that sheath thickness has been affected. Has axon diameter also been affected by light deprivation? Is the retardation of myelination secondary to retardation of axonal growth? One would expect, *a priori*, a primary effect on the axon, but the secondary effects of this cannot be predicted. While axons do not myelinate below a certain minimum diameter (which varies with site and species) the diameter of newly myelinated axons varies quite widely[9-11] and it may be that myelination is related more closely to neuronal type and maturity, as indicated by the pattern of Nissl substance[12], and to neuronal activity, which does not necessarily depend on connexions. Whatever the factors, it would be interesting to know whether the rate of incremental myelination (increase in sheath thickness) as well as its onset are affected by light deprivation. This is yet another situation in which analysis is incomplete without determination of both axon diameter and sheath thickness[13].

The pilot study was undertaken in the Department of Anatomy, Guy's Hospital Medical School. I thank Prof. R. Warwick for facilities and Mr. Graham Stevens for assistance.

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RADIOBIOLOGY

Aminothioamides as Protectors against the Effect of Gamma-rays in *Allium cepa*

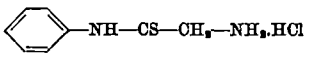
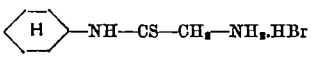
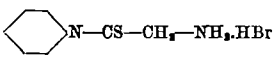
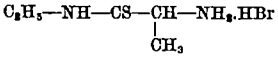
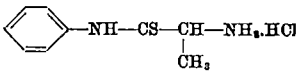
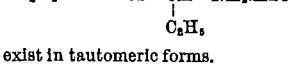
Several aminothioamides of the general type R_1—NH—CS—CH(NH_2)—R_2 have been synthesized[1]. As is generally known with thioamides, these aminothioamides can exist in solution in two tautomeric forms:

$$R_1\text{—NH—CS—CH—}R_2 \rightleftharpoons R_1\text{—N=C—CH—}R_2$$
$$\qquad\qquad\quad |\qquad\qquad\qquad\quad |\quad\ |$$
$$\qquad\qquad NH_2\qquad\qquad\quad SH\ NH_2$$

Notably in their thiol form they exhibit a free sulphydryl group as well as a free amino-group separated by two carbon atoms. Such arrangement was shown to favour protection against ionizing radiation[2–4].

The protective potentialities of a number of amino-thioamides (Table 1) were tested against the action of gamma-rays from a cobalt-60 source. *Allium cepa* seeds, variety 'White May', were germinated and seedlings with primary roots reaching 3–5 mm were selected and pre-treated for 1 h with a non-toxic concentration of the chemical under investigation. Then together with the respective solution the seedlings were irradiated.

Table 1. Aminothioamides Tested

	Chemical name	Formula
(1)	Amino-thioacetamide hydrobromide	H_2N—CS—CH_2—NH_2.HBr
(2)	*N*-Ethyl-amino-thioacetamide hydrobromide	C_2H_5—NH—CS—CH_2—NH_2.HBr
(3)	Amino-thioacetanilide hydrochloride	
(4)	*N-cyclo*hexyl-aminothio-acetamide hydrobromide	
(5)*	Amino-thioacetpiperidide hydrobromide	
(6)	*N*-Ethyl-D,L-α-aminothiopro-pionamide hydrobromide	
(7)	D,L-α-aminothiopropion-anilide hydrochloride	
(8)	*N*-Ethyl-D,L-α-aminothiobutyr-amide hydrobromide	

* This compound, in solution, does not exist in tautomeric forms.

The protective activity was assessed from: (*a*) the rate of growth of *Allium* roots which received a dose of 350 r. delivered as 5 r./sec; (*b*) from reduction in chromosome aberration at the same dose-level; (*c*) from the survival of roots against a lethal dose. In this communication, results obtained from the rate of growth are given (Fig. 1). Seedlings which were pre-treated with the chemical and then irradiated were thoroughly washed, transferred to Petri dishes containing moistened filter papers and then kept at 26° ± 1° C in a thermostat into which daylight was allowed through a glass door. The rate of growth of the primary roots was measured every 24 h. Measurements were carried out until the ninth day after irradiation, the day in which the normal non-irradiated control generally ceased to grow. Such short experimental duration enabled us to repeat and reproduce our results several times. Protective factors were derived from the rate of growth of irradiated roots pre-treated with one of those chemicals to that of roots irradiated in tap water, that is, irradiated control. This ratio was taken at a time when the normal non-irradiated control showed a maximum rate of growth.

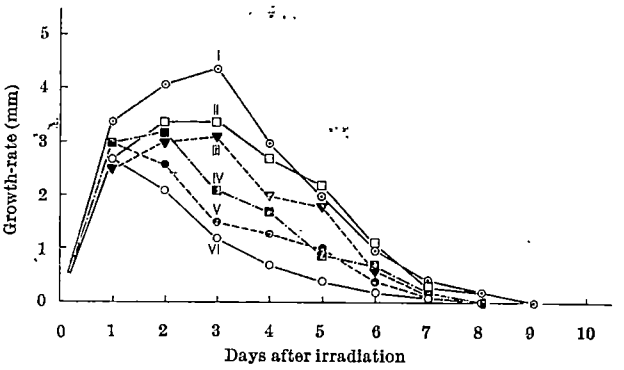


Fig. 1. Rate of growth of *Allium cepa* primary roots after different treatments. I, Normal, non-irradiated control; II, irradiated under anoxic conditions. Pretreated with and irradiated in cysteine (III), amino-thioacetamide hydrobromide (IV) and *N*-ethyl-aminoacetamide hydrobromide (V). VI, irradiated control

Among the eight compounds thus screened, two were found to offer some protection, the others being ineffective. Amino-thioacetamide hydrobromide was effective at 1×10^{-2} M concentration with the result that a protective factor of 1·7 was obtained, while *N*-ethyl-amino-thioacetamide hydrobromide at an optimum concentration of 5×10^{-3} M gave a factor of 1·25.

In comparison, roots pre-treated with a 5×10^{-2} M cysteine solution for 1 h and then irradiated in the same solution showed a protective factor 2·3, while roots irradiated in tap water deoxygenated by bubbling nitrogen for 45 min before irradiation (anoxic condition) showed a factor of 2·8.

Our results have thus shown that amino-thioamides, in which R = H or C_2H_5, were protective in *Allium*, though the degree of protection was lower than that shown by cysteine. The rest of the compounds having other substitutions were thoroughly ineffective.

It is of interest to note that thioacetamide was recently reported[5] as protecting *Allium cepa* cells against the action of alpha-rays and thermal neutrons, though the effect was limited in time.

I thank Prof. Z. Devidé for his advice, Prof. V. Hahn and Dr. K. Jakopčić for synthesis, supply of the chemicals and many other suggestions during this work, as well as the authorities of the Institute 'Rudjer Bošković', Zagreb, for facilities.

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Trophic Level Effect on the Accumulation of Cæsium-137 in Cougars feeding on Mule Deer

In aquatic ecosystems, cæsium-137 levels in predators exceed those of herbivores under conditions of equal contamination of the environment[1–3]. Examples of the step-wise increase in contamination-levels of cæsium-137 in four species of fish representing three trophic levels, and an explanation of the reason for the increase, will be published soon[4]. To our knowledge, no example of trophic level increase has been demonstrated in terrestrial wildlife, although an approximate 2- to 3-fold increase in cæsium-137 in man over his integrated food has been reported by several workers[5–7], and similar results were obtained by feeding cæsium-137 to animals[8,9].

An examination of the accumulation of fall-out nuclides in the tissues of mule deer (*Odocoileus h. hemionus*) (Raf.) has been carried out at this laboratory since September 1961. In the course of this work, a series of three cougars (*Felis concolor hippolestes* Merriam) was taken within a period of six weeks (December 1, 1962–January 16, 1963). Deer had been taken earlier, and additional samples were collected as soon as possible after the cougars were killed.

All the animals discussed in this communication were taken in the field with firearms[10], and most of the deer were collected from the same areas as were the cougars. The animals were skinned, and tissue samples were placed in plastic bags until counting was done with an 8-in. × 4-in. sodium iodide (Tl) crystal and a 400-channel γ-ray spectrometer. Flesh samples consisted of the hind leg from the hip joint to the knee (haunch). The femur was not removed.

Since deer flesh is the major source of food for the cougar, and the principal site of deposition of cæsium-137 in the body of the deer and cougars is muscle tissue, comparisons of accumulation between the two animals can be made using the skinned haunch as the sample (Table 1).

Table 1. CÆSIUM-137 IN FLESH OF MULE DEER AND COUGARS

Sample date	Deer		Cougars		^{137}Cs/kg flesh cougar/deer	^{137}Cs/gm K cougar/deer
	pc. ^{137}Cs/kg flesh	pc. ^{137}Cs/gm K	pc. ^{137}Cs/kg flesh	pc. ^{137}Cs/gm K		
12/1/62–1/5/63	425	107	1,080*	230	2·54	2·15
			2,270	526	3·89	5·11
1/15–16/63	583	103	1,860	424	3·20	4·12
Average	504 ($\bar{X}d$)	105	1,736 ($\bar{X}c$)	393	3·21	3·79

* A small, gravid female; this animal had been eating porcupines as well as deer. The other cougars were large males, and the gut contained few porcupine quills.

The average cæsium-137 content for the deer was 504 pc./kg and the average for the cougars was 1,736 pc./kg. Thus, 1,736/504 gives an increase ratio (*IR*) between deer and lions of 3·4. Although slightly higher, the Cs/K ratio shows the same relationship.

The results demonstrate a clear-cut increase of three-fold or better between the cæsium-137 level in deer flesh and cougar flesh and are in good agreement with the 3·4 increase that should be expected, according to Anderson *et al.*[5]. Implications of a trophic level (Λ) increase can best be demonstrated diagrammatically:

Λ^1 Photosynthetic plants (many species) Contamination level = X $\xrightarrow{IR = 3\cdot(?)}$ Λ^2 Primary consumers (herbivore-deer) Contamination level = $3X$ 504 pc. ^{137}Cs/kg $\xrightarrow{IR = 3\cdot4}$ Λ^3 Secondary consumers (carnivore-cougar) Contamination level = $9X$ 1,736 pc. ^{137}Cs/kg

Since man may vary in his trophic level relationship from a pure herbivore to virtually the level of a carnivore, the radiation dose from internally deposited cæsium-137 will be closely related to his dietary habits.

It is probable that the high levels of cæsium-137 in Alaskan Eskimos[11] and Laplanders from Northern Sweden[12] are a direct result of a trophic level increase relative to their predominantly animal diet. The animals on which they feed, in turn, subsist on plants which grow in a maximum accumulation environment for cæsium-137 and strontium-90; that is, a region characterized by climatic conditions conducive to the growth of lichens and other mat plants, very rapid plant growth during a short summer, usually wet conditions during the growth period, and cold, shallow soils that restrict the root systems to within a few inches of the surface. This combination of factors leads to a high accumulation in plants, herbivores and, ultimately, man.

Accordingly, any human population which is predominantly carnivorous should have higher body burdens of cæsium-137 than neighbouring populations which are omnivorous or herbivorous. Exceptions to this would be expected if the carnivorous population obtained its food from the ocean, and the neighbouring population obtained its plant foods from terrestrial sources, or if the meat eaters occupied a dry area and the vegetarians occupied wetlands. Oceanic foods are low in cæsium-137 (ref. 13), and plants grown in terrestrial wet lands contain higher quantities of cæsium-137 than those from otherwise equivalent dry lands[14,15].

If the dosage to bone from cæsium-137 is equal to the dosage from strontium-90, as reported by Anderson and Gustafson[16], further investigations of the accumulation of cæsium-137 in the biosphere assume greater importance. Evaluations of hazards resulting from cæsium-137 ingestion should incorporate provision for the trophic level increase.

The effect of this trophic level increase in cæsium-137, applied to conditions to be expected following extensive testing or atomic war, would be an increase in the hazard for predators—either animal or human. Terrestrial and aquatic mammalian predators would be subject to the greatest risk of all the biota, but local 'hot spots' resulting from differential deposition or ecological conditions could lead to exposures hazardous to the lower trophic levels. Predaceous species should be studied in greater detail to determine relative accumulation efficiency in other ecological situations—particularly the arctic and sub-arctic zones.

Investigations of human populations having essentially carnivorous food habits should be made in as many areas of the world as possible, since these people appear to be at greatest risk from cæsium-137 incorporated via the food web.

This work was supported by the Division of Radiological Health, Bureau of State Services, U.S. Public Health Service (*RH*–30), and the Utah State Department of Fish and Game provided personnel and transportation.

Addendum. Since this communication was written, we have learned that McNeill and Trojan[17] have shown the same relationship of cæsium-137 and potassium for human beings as we have found in cougars.

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BIOLOGY

Coding of the Colour Preference of the Gull Chick

GULL chicks (*Larus atricilla*) peck at their parent's red bill, an act which elicits regurgitation from the parent. In an investigation reported elsewhere[1], it was found that chicks peck preferentially in response to long and short wave-lengths of visible light (that is, they prefer red and blue to green and yellow). The following hypothesis attempts to account for this wave-length preference.

The bird's retina contains minute coloured oil droplets, a single droplet being contained in a single cone. Most avian species have three distinct colours[2]; the gull chick's eye appears to contain only red and yellow droplets[1]. The droplets are situated at the base of the outer segment of the cone, and thus filter incoming light before it strikes the visual pigment. The bleaching effect of the filtered light, and hence the response of the cone (C), for any given wave-length may be expressed as:

$$C = k_1 \log IOP \qquad (1)$$

where I is the intensity of the unfiltered light, O, the percentage transmission by the oil droplet, P, the percentage absorption of the visual pigment, and k_1, a constant of proportionality the value of which depends on the measurement of the cone response. The logarithmic transformation appears to be characteristic of visual receptors[3].

Assume that the red droplet-bearing cone is excitatory and the yellow one inhibitory. If inhibition is subtractive (as in lateral inhibition of the *Limulus* eye[3]), the pecking rate (R) at any given wave-length would be determined by:

$$R = k_2(C_r - C_y) = k_2 (\log IO_rP_r - \log IO_yP_y) \qquad (2)$$

which reduces to:

$$R = k_2 \log \frac{IO_rP_r}{IO_yP_y} \qquad (3)$$

where C_r and C_y are the responses of the red- and yellow-droplet-bearing cones, respectively, O_r and O_y are the transmissions of the droplets, P_r and P_y are the absorptions of the visual pigments, and k_2 is a proportionality constant.

Muntz[4] has suggested in another context that the interaction of visual receptors in wave-length coding is multiplicative (without logarithmic transformations), in this case:

$$R = k_3 \frac{IO_rP_r}{IO_yP_y} \qquad (4)$$

If the gull chick's eye contains but a single photopic pigment (as is probably true for the chicken[5] and the pigeon[6]), the P's cancel from equations (3) and (4), as must the I values, which are identical for a single wave-length. The pecking rate is thus proportional to the ratio of the transmissions of the oil droplets, or the logarithm of this ratio.

The chick's response rates were recorded automatically on a transilluminated pecking key for stimuli of selected wave-lengths between 450 and 650 mμ. The wave-length bands were obtained with interference filters, the energy transmissions of which were equated with a variable diaphragm using a thermopile and deflexion galvanometer. (The discrepancy between equal energy and the physiologically meaningful number of quanta at various wave-lengths thus introduces a small error.) Transmission spectra of the oil droplets were obtained using Strother's[1,2] method in his laboratory. The O_r/O_y ratios were computed for wave-lengths in the spectrum between 450 and 650 mμ, and are compared with the mean pecking rates in Fig. 1. Theoretical results from both forms of the hypothesis are in close agreement with the behavioural data.

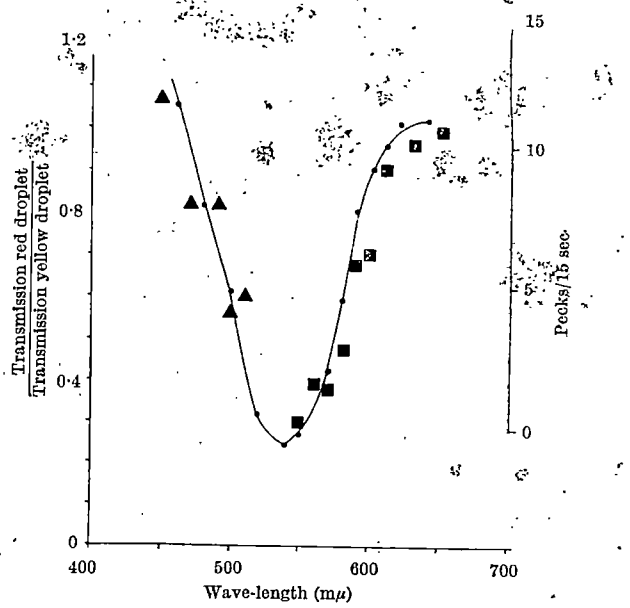


Fig. 1. The line and small dots represent calculated transmission ratios of the two oil droplet colours. The large triangles and the large squares represent mean pecking rates from groups of twelve and ten newly hatched *Larus atricilla* chicks, respectively

I thank Drs. D. Fluke, N. Guttman, P. Klopfer and G. K. Strother for their assistance. The work was done under the tenure of a U.S. Public Health Service (NIMH) pre-doctoral fellowship. I also thank Drs. K. Kirchfeld, P. Klopfer and J. Schwartzkopff for their advice.

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ENTOMOLOGY

Methyl Linolenate as an Essential Nutrient for the Cabbage Looper, *Trichoplusia ni* (Hübner)

A DIETARY requirement for polyunsaturated fatty acids has been demonstrated in several lepidopterous and orthopterous species. The most common expression of fatty acid deficiency was a slower larval growth-rate or faulty adult emergence with resulting wing deformity, or both[1–5]. In the lepidopterous species examined the requirement was fulfilled with either linoleic or linolenic acids[2,3,5]. In the genus *Ephestia*, the flour moths, linoleic or linolenic acids promoted larval growth and normal wing development[3]. Arachidonic acid improved the growth-rate of *Ephestia*, but had no positive effect on wing development[6]. Linoleic acid allowed normal wing development of the pink bollworm, *Pectinophora gossypiella* (Saund.), but, because lower levels were active, linolenic acid was considered more effective[5]. Normal wing development of the greater wax moth, *Galleria mellonella* (L.), was promoted with linoleic or linolenic acids, but not with arachidonic acid[2].

This communication reports on the fatty acid requirements of the cabbage looper, *Trichoplusia ni* (Hübner). A satisfactory meridic diet, a modification of that described for the bollworm, *Heliothis zea* (Boddie)[7], containing wheat germ (oil and solids) as the only plant

Table 1. Composition of the Basal Diet

Component	Amount (g)	Component	Amount (g)
Casein, vitamin-free*	3·5	Cholesterol	0·3
Dextrose	3·5	Sorbic acid	0·12
Cellulose powder	3·0	Choline chloride	0·10
Agar (Bacto)	2·5	Ascorbic acid	0·50
Salt mixture (Wesson's)*	1·0	Vitamin solution†	10·0 ml.
Sodium alginate	0·5	Distilled water	75·0 ml.

* Nutritional Biochemicals Corp. † Ref. 8

adjuvant and fatty acid source, has been developed for this insect. Table 1 shows the composition of the basal diet for *Trichoplusia*. Rearing procedures and fractionation investigations of the wheat germ have previously been reported[8]. The fractionation examinations revealed that wheat germ residue after chloroform–methanol 2 : 1 (v/v) extraction contained a factor required for normal larval growth, and the lipid fraction a material which promoted both larval growth and wing development. The latter was contained in the saponifiable fraction of wheat germ oil. Complete lack of this 'wing development factor' resulted in severe wing deformity and failure of the moths to emerge from the pupal case (Fig. 1). The condition shown in Fig. 1*B* was typical of the adults classified in the 'deformed wings' category in Table 2.

larvæ was very poor, being similar to that on the negative control diet (Table 2). This difficulty was obviated by the addition of methyl linoleate. A mixture of 200 mg of the linoleate plus 50 mg of methyl linolenate gave good growth (11·6 days to 50 per cent pupation), but of 38 adults produced, only four had normal wings. Thus the supplement of 100 mg of methyl linolenate was considered to be close to the threshold level.

The results from one assay of methyl oleate (> 99 per cent purity[10], 150 mg) tested as a mixture with 250 mg methyl linoleate indicated that methyl oleate did not promote normal wing development; of 31 adults produced all had deformed wings. Similar results were obtained when a mixture of 150 mg methyl arachidonate (> 90 per cent purity[10]) and 250 mg methyl linoleate was assayed.

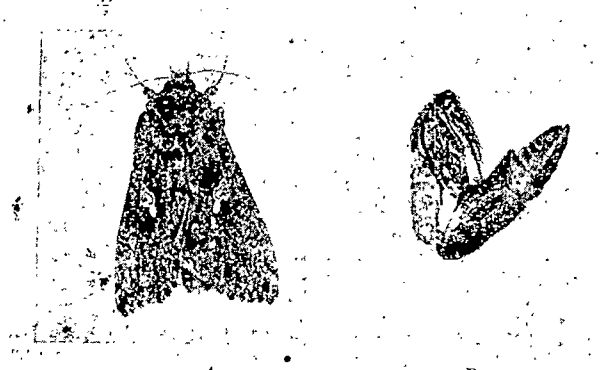
Dietary supplements of 126 mg methyl eicosa-5,8,11,14,17-pentaenoate (> 90 per cent purity[10]) and 126 mg methyl docosa-4,7,10,13,16,19-hexaenoate (> 90 per cent purity[10]) stimulated larval growth but did not permit normal wing development (Table 2). The interpretation of these results is uncertain because of the possibility of oxidative degradation of the supplements during the assay period. In view of the stimulation of larval growth, however, extensive degradation probably did not occur.

Table 2. Effect of Wheat Germ Substitutes on Growth Rate and Wing Development of *Trichoplusia**

Criteria	Positive control wheat germ 5·0 g, wheat germ oil 250 mg	Negative control wheat germ residue† 4·5 g	Methyl linoleate†‡ 500 mg	Methyl linolenate†‡ 500 mg	Methyl linolenate†‡ 250 mg	Methyl linolenate†‡ 100 mg	Methyl eicosa-pentaenoate†‡ 126 mg	Methyl docosa-hexaenoate†‡ 126 mg
Initial No. of larvæ§	40	40	30	30	40	40	40	40
Mean days to 50 per cent pupation at 30° C	11·2 ± 0·25	18·6 ± 0·25	11·7 ± 0·34	10·7 ± 0·34	11·6 ± 0·20	13·2 ± 0·26	12·5 ± 0·38	13·4 ± 0·21
Larval mortality (No.)	2	19	1	0	0	1	3	1
Pupal mortality (No.)	0	10	3	0	0	0	13	10
Normal wings (No.)	37	0	0	30	39	39	0	0
Deformed wings (No.)	1	11	26	0	1	0	24	29

* All groups received the basal diet plus the supplements indicated in the Table. The quantities of supplements listed are the amounts used per 100 g of basal diet (Table 1).
† Approximately 33 mg *dl*-α-tocopherol added to the negative control and to all fatty acid methyl esters assayed.
‡ These groups received an additional supplement of 4·5 g wheat germ residue after chloroform–methanol 2 : 1 (v/v) extraction.
§ Larvæ arranged in replicates of ten.

Several individual fatty acid methyl esters of a high degree of purity[9,10] obtained from the Hormel Institute, Austin, Minnesota, were tested for activity. A dietary supplement of 500 mg (and lower levels) methyl linoleate (> 99 per cent purity[10]) promoted larval growth and normal pupation, but did not promote normal wing development (Table 2)[8]. These results were contrary to the findings of the previous workers cited here, and prompted further tests on other fatty acids which are constituents of wheat germ oil. Table 2 shows that 500 mg methyl linolenate (methyl octadeca-9,12,15-trienoate, > 99 per cent purity[10]) allowed both good larval growth and normal wing development. Lower levels (250 and 100 mg) were equally effective except that for the 100 mg supplement the larval growth period was slightly prolonged (13·2 days to 50 per cent pupation). When still lower levels (50 and 25 mg) were tested, survival of the



A *B*

Fig. 1. *A*, Normal adult of *Trichoplusia ni*; *B*, adult partially emerged from pupal case showing extreme wing deformity

In many of the previous papers on insect fatty acid requirements, the purity of the unsaturated fatty acids used was not adequately documented. Some of the growth and wing development effects ascribed to linoleic acid may have been due to contaminating linolenic acid.

Our discovery that methyl linolenate is an essential nutrient for *Trichoplusia ni*, and that this requirement cannot be fulfilled by methyl linoleate, contrasts sharply with the reverse situation in the rat[11].

This work was supported in part by research grants *GM*–05093 and *AI*–04419 from the U.S. National Institutes of Health.

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MICROBIOLOGY

Patterns of Interferon Appearance in Mice injected with Bacteria or Bacterial Endotoxin

Previous work[1] has shown that the intravenous injection of large numbers of live *Brucella abortus* into chickens results in the appearance in the serum of a viral inhibitor with the properties of interferon. Inhibitor reached maximum levels about 12 h after inoculation. It was also reported that large numbers of Newcastle disease virus (NDV) particles produced maximum levels of interferon in chickens about 12 h after intravenous inoculation. Injection of large doses of other gram-positive and -negative bacteria or bacterial endotoxins failed to produce demonstrable inhibitors in the circulation of chickens at 6–8 h.

Since these results were reported, further work with chickens has shown that a barely detectable, but consistent, inhibitor activity appears in the serum at about 2 h after inoculation of large numbers of *Serratia marcescens* and *Salmonella typhimurium*, and after *Escherichia coli* endotoxin. In the case of *E. coli* endotoxin, it was necessary to inject birds intravenously with at least 40 mg in order to demonstrate inhibitor in serum at 2 h. Inhibitor activity, which usually was barely detectable at 2 h, had disappeared by 4–6 h after injection. The small and transient amounts in the serum made it too difficult to investigate the properties of the inhibitor produced at 2 h.

Table 1. Viral Inhibitor in Plasma of Mice at Different Times following Intravenous Injection of Various Organisms and Bacterial Lipopolysaccharide

Material injected	Dose	Inhibitor titres* of plasma pools (hours post-injection)									
		0·5	1	1·5	2	3	4	6	12	14	16
Newcastle disease virus	2×10^7 plaque-forming units		<16		16		128	512	2,048	1,024	1,024
Brucella abortus	1×10^9 viable units	<16	<16		16			96	192	256	96
Escherichia coli 0111 : *B4*	250 μg	<16	128	256	768	256		128	24		
lipopolysaccharide†	25 μg				64			<16	<16		
Salmonella typhimurium	7×10^8 viable units	<16	64		384		192	16	<16		
Serratia marcescens	3×10^7 viable units	<16	128		256		32	16	<16		

* Reciprocal of the highest dilution of plasma giving 50 per cent inhibition of plaques of VSV in *L* cells.
† Nucleic acid not detected spectrophotometrically.

In extending these studies to other species, it was found that the intravenous injection of mice[2] with *B. abortus* or NDV resulted in significantly higher levels of circulating inhibitor than those found in chickens. This permitted us to examine the time pattern of appearance of viral inhibitor in mice as well as to compare the properties of the inhibitors produced in response to stimulation by different bacteria, NDV and endotoxin.

Swiss Webster female mice weighing about 25 g were injected in the tail vein with the different materials in a 0·1-ml. volume of saline diluent. Blood samples were obtained at different times from groups of about 10 mice by cardiac puncture with heparin-rinsed syringes. Pooled plasmas were assayed for viral inhibitor by the plaque-reduction method[3] using an interferon-sensitive line of *L* cells. Cultures of *L* cells in 60-mm Petri dishes were exposed for 20 h to 3 ml. of two-fold serial dilutions of plasma and then challenged with 40–60 plaque-forming units of vesicular stomatitis virus (VSV). The titres of viral inhibitors were calculated from the dilution of plasma which reduced the plaque count to 50 per cent of that of the controls. The dose of NDV was based on plaque-forming units; numbers of bacteria were given as viable units but it should be noted that approximately the same mass of bacteria, determined by optical density measurements, was employed in each case. Clumping or differences in size explain variations in viable counts.

Table 1 shows the inhibitor titres of mouse plasmas obtained at different times after the intravenous inoculation of several gram-negative bacterial species, *E. coli* 0111 : *B4* endotoxin (Difco), and Newcastle disease virus. The results show that there were two distinct patterns of appearance of inhibitor in the circulation. The injection of NDV or *B. abortus* resulted in maximum inhibitor activity at 12–14 h. In contrast, the injection of *S. marcescens*, *S. typhimurium*, or endotoxin was followed by the early appearance of inhibitor in the plasma; maximum levels, far greater than any obtained in chickens, were observed at 2 h and these rapidly declined. Preliminary experiments have shown that direct exposure of *L* cells to 100 μg of endotoxin in serum-containing medium for 20 h did not reduce the subsequent plating efficiency of VSV, indicating that residual endotoxin in the plasma probably played no part in our experiments.

The characteristics of early-appearing inhibitor produced by endotoxin and the late-appearing inhibitors resulting from inoculation of *B. abortus* and Newcastle disease virus in mice were studied. The results showed that all three inhibitors prevented plaque formation by vesicular stomatitis virus only in mouse cells, not in chick embryo cells, and did not neutralize virus directly but acted on the host cells. In addition, all three inhibitors were stable to *p*H 3 treatment overnight but showed loss of activity when incubated with crystalline trypsin (50 μg/ml.) or when heated at 56° C for 1 h. These observations lead to the conclusion that the properties of the early-appearing inhibitor produced by endotoxin and the late-appearing inhibitors stimulated by *B. abortus* and NDV are similar to those of virus-induced mouse serum interferon as reported by Merigan[4].

The results with mice indicate that, as in chickens[1], *B. abortus* stimulated the appearance of interferon which reached peak titres in about 12 h. In mice, *S. typhimurium* *S. marcescens* and endotoxin produced inhibitor which appeared much earlier than inhibitor stimulated by *B. abortus* or NDV. This early-appearing inhibitor also resembled in properties and time of appearance the 'serum sparing substance' of Gledhill[5]. It is possible that the early-appearing interferon may be preformed and released from the cells with which the stimulating agent reacts, while the late-appearing interferon may represent inhibitor synthesized in response to *B. abortus* or NDV. Other explanations, including differences in the type of cell affected or differences in the rapidity of cell damage produced by the different agents, must be considered. However, it is known that all the agents used, including endotoxin[6,7], are rapidly sequestered in cells of the reticulo-endothelial system and it is possible that the inhibitors produced by the different agents are released in response to damage or stimulation of these cells.

This work was supported by grants *AI*–03414 and *AI*–03561 from the U.S. National Institutes of Health.

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, November 16

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 5 p.m.—Lieut. Comm. J. B. Dixon, R.N.: "Hydrographic Surveying in the Antarctic".

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Dr. R. C. Brian: "Electronics and the Mode of Action of Certain Herbicides".

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. P. M. S. Blackett, F.R.S.: "Britain and India's Development Plans" (Rickman Godlee Lecture).*

Monday, November 16—Thursday, November 19

INSTITUTION OF MINING AND METALLURGY, in co-operation with the Institution of Mining Engineers and the Institute of Quarrying (at Caxton Hall, Westminster, London, S.W.1)—Symposium on "Opencast Mining, Quarrying and Alluvial Mining".

Tuesday, November 17

BRITISH INTERPLANETARY SOCIETY, and the SOCIETY OF ENVIRONMENTAL ENGINEERS (at Northampton College of Advanced Technology, St. John Street, London, E.C.1), at 9.30 a.m.—Symposium on "Space Environment Simulators".

PHARMACEUTICAL SOCIETY OF GREAT BRITAIN AND THE SOCIETY OF COSMETIC CHEMISTS OF GREAT BRITAIN (at the Connaught Rooms, Great Queen Street, London, W.C.2), at 9.30 a.m.—Symposium on "Preservatives and Antioxidants".

SOCIETY OF CHEMICAL INDUSTRY, AGRICULTURE GROUP (at 14 Belgrave Square, London, S.W.1), at 10.30 a.m.—Meeting on "Soil Organic Matter".

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p.m.—Mr. L. B. Keeble: "The Future of London".*

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. D. A. Bradley, Mr. C. D. Clarke, Mr. R. M. Davis and Dr. D. A. Jones: "Adjustable Frequency Invertors and Their Application to Variable-Speed Drives".

PARLIAMENTARY AND SCIENTIFIC COMMITTEE, GENERAL COMMITTEE (in Committee Room 12, House of Commons, Westminster, London, S.W.1), at 5.30 p.m.—Discussion on "Population Control" initiated by Prof. A. S. Parkes, F.R.S., and Prof. Donald Reid.

INSTITUTION OF MECHANICAL ENGINEERS, AUTOMATIC CONTROL GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "One-Line Determination of Process Dynamics".

RESEARCH DEFENCE SOCIETY (in the Physiology Lecture Theatre, University College, Gower Street, London, W.C.1), at 6 p.m.—Prof. Alexander Haddow, F.R.S.: "The Contribution of Animal Experiment to Our Knowledge of the Causation and Therapy of Malignant Disease" (Thirty-third Stephen Paget Memorial Lecture); followed by the Annual General Meeting.

PLASTICS INSTITUTE, LONDON SECTION—REINFORCED PLASTICS SUB-GROUP (at the Coachmakers Arms, 88 Marylebone Lane, London, W.1), at 6.30 p.m.—Mr. G. T. Byast: "Plastics Equipment Fault Finding".

Tuesday, November 17—Wednesday, November 18

INSTITUTION OF GAS ENGINEERS (at Church House, Westminster, London, S.W.1), at 9.15 a.m. daily—30th Autumn Research Meeting.

Wednesday, November 18

BRITISH INSTITUTE OF RADIOLOGY, ISOTOPE STUDY SECTION (joint meeting with the Hospital Physicists' Association, in the Reid Knox Hall, 32 Welbeck Street, London, W.1), at 9.45 a.m.—Meeting on "Radio-isotope Scanning".

ROYAL INSTITUTION OF NAVAL ARCHITECTS (in the Memorial Building, 76 Mark Lane, London, E.C.3), at 10.30 a.m.—Discussion on "Ship Maintenance and Associated Design Problems".

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 2.30 p.m.—Dr. F. T. Perkins: "The Science and Practice of Immunology" (Dr. Aldred Memorial Lecture).

ROYAL METEOROLOGICAL SOCIETY (at 49 Cromwell Road, London, S.W.7), at 5 p.m.—Mr. J. Latham: "Change Transfer Associated with Temperature Gradients in Ice Crystals Grown in a Diffusion Chamber"; Mr. J. B. Matthews and Mr. B. J. Mason: "Electrification Produced by the Rupture of Large Water Drops in an Active Field".

INSTITUTE OF FUEL (at the Royal Institute of British Architects, 66 Portland Place, London, W.1), at 5.30 p.m.—Mr. K. Dixon, Mr. E. Skipsey and Dr. J. T. Watts: "The Distribution and Composition of Inorganic Matter in British Coals: Part 1—Initial Study of Seams from the East Midlands Division of the National Coal Board".*

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (joint meeting with the Science and General Division, at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "What is Measurement?" opened by Mr. L. Finkelstein, Mr. G. G. Gouriet, Dr. P. Vigoureux and Mr. A. C. Lynch.

WORSHIPFUL SOCIETY OF APOTHECARIES OF LONDON, FACULTY OF THE HISTORY OF MEDICINE AND PHARMACY (at Black Friars Lane, Queen Victoria Street, London, E.C.4), at 5.30 p.m.—Mr. R. R. Trail: "Sydenham's Influence on English Medicine" (Sydenham Lecture).

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, RADAR AND NAVIGATION AIDS GROUP (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Mr. R. L. Burr and Mr. J. Flounders: "An Air Surveillance Radar System".

INSTITUTION OF MECHANICAL ENGINEERS, AUTOMOBILE DIVISION (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "The Effect of Low Temperatures on the Operation of Diesel Vehicles".

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (joint meeting with the South-West Essex Technical College Chemical Society, at the South-West Essex Technical College, Forest Road, Walthamstow, London, E.17), at 7 p.m.—Dr. F. W. Gibbs: "Science in Soho—Scenes from the 18th Century".

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION, KENT SUB-SECTION (at the North-West Kent College of Technology, Liskin Road, Dartford, Kent) at 7 p.m.—Dr. S. R. Craxford: "The Removal of Sulphur from Flue Gases".

BRITISH INSTITUTE OF RADIOLOGY (at the National Hospital for Nervous Diseases, Queen Square, London, W.C.1), at 7.30 p.m.—Conversazione. Dr. Macdonald Critchley, C.B.E.: "The National Hospital—The Cradle of Neurology"; Dr. Richard Hunter and Dr. James Bull: "The Pineal Gland—a Radiological Landmark and its Place in the History of Psychiatry".

ROYAL AERONAUTICAL SOCIETY, GRADUATES' AND STUDENTS' SECTION (at 4 Hamilton Place, London, W.1), at 7.30 p.m.—Mr. G. Sage: "Structural Problems of Manned Space Flight".

Thursday, November 19

SOCIETY OF INSTRUMENT TECHNOLOGY (at the Connaught Rooms, Great Queen Street, London, W.C.2), at 2.30 p.m.—Symposium on "Performance Evaluation of Industrial Instruments".

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Mr. B. F. Skinner (Harvard University): "The Technology of Teaching —Its Nature and Some Problems Encountered in its Use" (Review Lecture).

LINNEAN SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. Philip H. Gregory, F.R.S., Prof. D. Müller and Mr. D. H. Bridger: "Mirror Yeasts".

LONDON MATHEMATICAL SOCIETY (at the Royal Astronomical Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Annual General Meeting, followed by Prof. G. Higman: "Bundles of Null-Spaces".

UNIVERSITY OF LONDON (in the Physiology Theatre, University College, Gower Street, London, W.C.1), at 5 p.m.—Dr. J. B. Chappell: "Mitochondria —Permeability and Function".*

UNIVERSITY OF LONDON (at Charing Cross Hospital Medical School, London, W.C.2), at 5.30 p.m.—Lord Todd, F.R.S.: "Organic Chemistry and the Progress of Medicine" (Huxley Lecture).*

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dame Honor Fell, F.R.S.: "Organ Culture in Medical Research". (Eighth of fourteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)*

CHEMICAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 6 p.m.—Discussion Meeting on "Free Radicals".

INSTITUTE OF REFRIGERATION (at the National College for Heating, Ventilating, Refrigeration and Fan Engineering, Southwark Bridge Road, London, S.E.1), at 6 p.m.—Informal Discussion on "Design in Relation to Service and Maintenance".

INSTITUTION OF MECHANICAL ENGINEERS, INTERNAL COMBUSTION ENGINES GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Fuel as an Engine Lubricant and/or Coolant".

SOCIETY OF CHEMICAL INDUSTRY, ROAD AND BUILDING MATERIALS GROUP (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Mr. D. H. Mathews, Mr. D. M. Colwill and Mr. R. Yuce: "Adhesion Tests for Bituminous Road Materials".

TELEVISION SOCIETY (in the Conference Suite, I.T.A., 70 Brompton Road, London, S.W.3), at 7 p.m.—Mr. J. L. E. Baldwin: "A British Video Tape Recorder".

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION, LEA VALLEY SUB-SECTION (at the College of Further Education, Hatfield Road, St. Albans), at 7.30 p.m.—Dr. R. A. Jeffreys: "Colour Photography".

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (at the Liverpool School of Tropical Medicine, Pembroke Place, Liverpool), at 7.30 p.m.—Laboratory Meeting.

Thursday, November 19—Friday, November 20

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C.2)—Conference on "Commutation in Rotating Machines".

Friday, November 20

BRITISH INSTITUTE OF RADIOLOGY (at 32 Welbeck Street, London, W.1), at 9.30 a.m.—Meeting on "Mammalian Radiobiology at the Cellular Level".

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Du Cane Road, London, W.12), at 4 p.m.—Prof. H. G. Miller: "Current Research in Multiple Sclerosis".*

INSTITUTE OF NAVIGATION (at the Royal Institution of Naval Architects, 10 Upper Belgrave Street, London, S.W.1), at 5.30 p.m.—Mr. S. Serebreny: "Meteorology and Supersonic Transport".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Prof. W. H. McCrea, F.R.S.: "Origin of the Solar System".

Saturday, November 21

SOCIETY FOR DEVELOPMENTAL BIOLOGY (at the National Institute for Medical Research, Mill Hill, London, N.W.7), at 10 a.m.—Dr. J. B. Solomon: "The Onset of Immunological Reactions in the Chicken"; Mr. D. James: "The Effects of Antigenic Differences on Placental Size in Mice"; Dr. J. A. Tata: "Amphibian Metamorphosis"; Dr. N. A. Mitchison: "Double Competence in the Response to Antigen".

LONDON COUNTY COUNCIL (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Dr. Stanley B. Hamilton: "Ancient Methods of Building".*

Monday, November 23

Institute of Actuaries (at Staple Inn Hall, High Holborn, London, W.C.1), at 5 p.m.—Mr. J. R. Ford and Mr. C. M. Stewart: "Population Trends in Great Britain".

Institution of Electrical Engineers, Electronics Division (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. R. F. Hoskins: "Loop Gain and Return Difference of Transistor Amplifiers".

Royal Institution of Naval Architects (at the North East Coast Institution of Engineers and Shipbuilders, Bolbec Hall, Newcastle upon Tyne), at 6 p.m.—Mr. W. D. Horsley: "The High Speed Generator—Eighty Years of Progress" (Sir Charles Parsons Memorial Lecture).

Institution of Mechanical Engineers, Manipulative and Mechanical Handling Machinery Group (in the Renold Building of the College of Science and Technology, Manchester), at 6.45 p.m.—Discussion on "The Mechanics of High-Speed Weaving".

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Research Assistant in the Geographical Research Division for work primarily in the field of economic geography—Dr. J. E. Martin, London School of Economics and Political Science, Houghton Street, London, W.C.2 (November 20).

Assistant Lecturer (honours graduate in pharmacy, preferably with a special interest in the physico-chemical aspects of pharmaceutical chemistry) in Pharmaceutical Chemistry—The Registrar, University of Strathclyde, George Street, Glasgow, C.1 (November 21).

Lecturer or Assistant Lecturer in the Department of Philosophy—The Registrar, The University, Leeds, 2 (November 23).

Senior Lecturer or Lecturer in Medical Statistics in the Departments of Mathematics and Public Health—The Secretary of the University Court, The University, Glasgow (November 27).

Lecturer or Assistant Lecturer (with interests in sensory processes and perception, or in the study of human learning) in Psychology—The Secretary and Registrar, Room 9, The University, Southampton (November 28).

Assistant Experimental Officer (with experience in biological or analytical laboratory work) in the Department of Biochemistry, to join a team investigating metabolic aberrations in cattle—The Secretary, Institute for Research on Animal Diseases, A.R.C., Compton, Near Newbury, Berkshire (November 30).

Junior Lecturer/Lecturer in Social Anthropology at Rhodes University, Grahamstown, South Africa—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, November 30).

Lecturer in Physiology and a Senior Lecturer in Physiology—The Secretary, The Royal Veterinary College (University of London), Royal College Street, London, N.W.1 (November 30).

Lecturer in the Department of Physics—The Secretary, University College, Gower Street, London, W.C.1 (November 30).

Lecturer (with a good honours degree in physiology and/or a veterinary qualification) in the Department of Veterinary Physiology in the Faculty of Veterinary Medicine—The Secretary, The University, Old College, South Bridge, Edinburgh, 8 (November 30).

Lecturers and Assistant Lecturers (honours graduates) in Chemistry, Botany and Zoology in the Faculty of Science, University College of Science Education, Cape Coast, Ghana—The Assistant Registrar, University College of Science Education, 15 Gordon Square, London, W.C.1 (November 30).

Research Assistant in the Department of Veterinary Physiology, to take part in an investigation, supported by the Agricultural Research Council, into the electrophysiology of the nervous control of gastrointestinal activity in sheep—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh, 8 (November 30).

Lecturer or Assistant Lecturer in Pure Mathematics—The Registrar, University of Kent at Canterbury, Westgate House, Canterbury, Kent (December 5).

Joseph Hunter Chair of Pathology—The Registrar, The University, Sheffield, 10 (December 12).

Lecturer in Mathematical Statistics at the University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, December 18).

Official Fellow and Lecturer in Philosophy—The Warden, Wadham College, Oxford (January 1).

Agricultural Superintendent (with a degree in agriculture) in Dominica, to be responsible to the Minister of Trade and Production for the administration of the Agricultural Department, for the agricultural development programme, including general supervision, and to act in an advisory capacity—The Appointments Officer, Room 301, Ministry of Overseas Development, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 213/52/01.

Head of the Chemistry Department—Grade VI; Head of the Mathematics Department—Grade IV; and Head of the Biology and Geology Department—Grade IV—Clerk to the Governing Body, Northern Polytechnic, Holloway, London, N.7.

Health Physicist (with an honours degree or equivalent in physics) to work on all aspects of safety and radiation protection and measurement—Recruitment Officer, U.K.A.E.A. Experimental Reactor Establishment, Dounreay, Thurso, Caithness, Scotland, quoting Ref. E.475/34.

Lecturer in Mathematics; a Lecturer in Computing; and a Lecturer in Statistics—The Academic Registrar, Northampton College of Advanced Technology, St. John Street, London, E.C.1.

Lecturer or Assistant Lecturer in Numerical Analysis—The Registrar (Room 22, O.R.B.), The University, Reading.

Physicist or Physical Chemist (with at least three years research experience in the field of semiconductor material behaviour, photochemistry, or polymer structure characterization)—The Director, Arthur D. Little Research Institute, Inveresk, Musselburgh, Midlothian, Scotland.

Senior Technician I for work in virology—Dr. H. B. E. Cadness-Graves, Public Health Laboratory, Peace Memorial Hospital, Watford, Hertfordshire.

Technician (preferably with experience with radioisotopes) for biochemical research on cerebral enzymes and proteins concerned in phosphorus metabolism—The Secretary, Institute of Psychiatry, The Maudsley Hospital, Denmark Hill, London, S.E.5.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

The British Drug Houses, Ltd. Industrial Applications of Panacide: Algicide, Bactericide, Fungicide. Pp. 10. Enzyme Substrates. Pp. 6. (Poole: British Drug Houses, Ltd., 1964.) [610

The Ramblers' Association. The Right to Roam: The Rambler and the Countryside. Pp. 32. (London: The Ramblers' Association, 1964.) 2s. [610

Imperial College of Science and Technology (University of London). Shorter Postgraduate Courses, Autumn Term, Session 1964–65. Pp. 16. (London: Imperial College of Science and Technology, 1964.) [610

Current Medical Research: a Reprint of Articles in the Report of the Medical Research Council for the year 1962–1963. Pp. iii+51+3 plates. (London: H.M. Stationery Office, 1964.) 4s. 6d. net. [610

Agricultural Research Council. Experimental Farm Buildings—Report No. 1: Cow Cubicles. By Dr. H. R. Livingston. Pp. 16. (London: Agricultural Research Council, 1964. Obtainable from H.M. Stationery Office, 1964.) 2s. 6d. [610

Electrical Research Association. Technical Report No. 5014: High Field Effects for Linear Chains of Dipoles or Limited Conducting Paths. By Dr. Vera Daniel and P. R. Vein. Pp. 13+3 figures. (Leatherhead: Electrical Research Association, 1963.) 12s. 6d. [610

Bulletin of the British Museum (Natural History). Botany. Vol. 3, No. 4: A Revision of the Genus *Petrorhagia*. By P. W. Ball and V. H. Heywood. Pp. 119–172+plates 13–15. 22s. Vol. 12, No. 1: Polyzoa from West Africa. Notes on the Genera *Hippoporina* Neviani, *Hippoporella* Canu, *Cleidochasma* Harmer and *Hippoporidra* Canu and Bassler (Cheilostomata, Ascophora). By P. L. Cook. Pp. 1–35+plates 1–3. 15s. (London: British Museum (Natural History), 1964.) [610

The University of Sussex. Prospectus 1964/65. Pp. 120. (Stanmer, Brighton: The University of Sussex, 1964.) [610

Institute of Seaweed Research. Annual Report for 1963. Pp. 23. (Inveresk, Midlothian: Institute of Seaweed Research, 1964.) [610

Other Countries

United States Department of the Interior: Geological Survey. Water-Supply Paper 1499–I: Water Resources of the Wichita Area, Kansas. By L. B. Petri, C. W. Lane and L. W. Furness. Pp. v+69+plate 1. Water-Supply Paper 1535–I: Sources of Mineral Constituents in Water from Granitic Rocks, Sierra Nevada, California and Nevada. By J. H. Feth, C. E. Robertson and W. L. Polzer. Pp. iv+70+plate 1. Water-Supply Paper 1535–J: Chemical Composition of Snow in the Northern Sierra Nevada and Other Areas. By J. H. Feth, S. M. Rogers and C. E. Robertson. Pp. iii+39+plate 1. Water-Supply Paper 1608–E: Hydrogeologic Reconnaissance of Poro Point and Vicinity, Luzon Island, Philippines. By G. F. Worts, Jr. Pp. iii+14+plate 1. (Washington, D.C.: Government Printing Office, 1964.) [510

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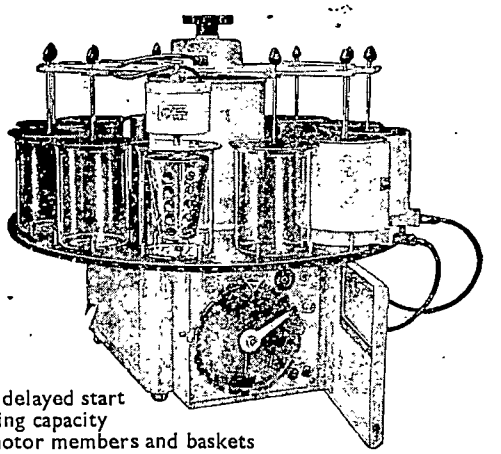
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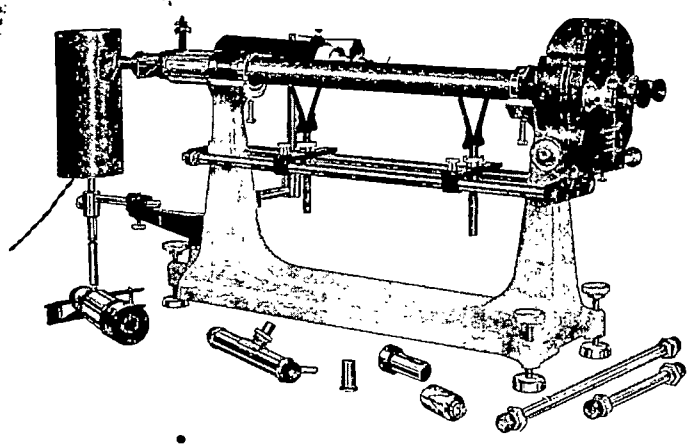
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BRITISH OVERSEAS RESEARCH AND DEVELOPMENT

THE latest pamphlet in the factual survey of *British Aid* which the Overseas Development Institute has published deals with technical assistance and is one of the most important the Institute has produced*. It is, moreover, fortunate in its timing, for it makes a fitting complement to the examination of the work of the Department of Technical Co-operation given in the tenth report from the Estimates Committee for the Session 1963–64†. It is no disparagement of the memorandum submitted on behalf of the Secretary for Technical Co-operation, in evidence before the Estimates Committee, to suggest that, factually, the Overseas Development Institute provides the more comprehensive account. At the critical level, the Institute's examination of the facts, however, is far more searching than that of the Estimates Committee and its comments and suggestions are more constructive and fundamental.

To the scientist, an important feature of both documents is the attention directed to overseas research. For the first time, we are given some idea of the extent of the effort that is being made, but there is a big difference between the two documents. This is illustrated by the simple fact that the Estimates Committee accepts without question the recommendation of the Trend Report for the dissolution of the Overseas Research Council. The Overseas Development Institute fully appreciates the ineffectiveness of the Research Council, but it sees a good deal further. The Overseas Research Council was deliberately made ineffective: the implication is that this was done to prepare the way for the recommendations from the Trend Committee, and if the Overseas Development Institute's pamphlet had done no more than bring the whole discreditable sequence into daylight it would have done sufficient public service.

It should be noted, of course, that the Estimates Committee was concerned primarily with the organization of the Department of Technical Co-operation and with the way in which financial control was exercised over its activities, and although some attention was directed to research, no specific recommendations were made in this field. It was stated in evidence that when the Department was established some 500 people were transferred from corresponding departments at the Colonial Office, including those for research and for communications and the whole of the Overseas Service Division. The Overseas Geological Survey with a staff of 193, the Directorate of Overseas Surveys with 419 and the Anti-Locust Research Centre with 48 were also transferred. The Department has some 30 advisers on technical subjects, most of whom come from the Colonial Office, and the duties of these advisers are described generally in the memorandum submitted for the Secretary for Technical Co-operation. Otherwise, while explaining the organization, the memorandum gives no information on the scale of overseas research.

In the evidence to the Committee the Department's expenditure on research is estimated at £1,961,000 apart from that on the Anti-Locust Research Centre, which brings the total to £2·2 million, of which about 40 per cent is spent in the United Kingdom and the remainder in the developing countries overseas, particularly in east and central Africa and in the Caribbean area. Of the £1 million spent in Britain, some £110,000 was spent on the Tropical Products Institute, £42,000 on the tropical section of the Road Research Laboratory, about another £40,000 each on the Tropical Stored Products Centre and the tropical division of the Building Research Station. About £45,000 is spent on two small units working on pesticides and other aspects of infestation, and some small projects at universities are also financed by the Department.

It was emphasized in evidence before the Committee that the purpose of these home-based research units is five-fold. First, they seek to continue the basic research which maintains a store of scientific capital. Next, they investigate specific problems, either in their own laboratories or by sending experts overseas as requested by overseas stations or Governments. Thirdly, they collect, collate, analyse and circulate the most recent information in their fields, thus keeping in touch the scientists working in isolation overseas. Fourthly, they assist in training overseas research workers, either in the institutes here or by sending training teams overseas, and fifthly, they serve as a home base for British scientists who can be seconded to overseas Governments, for short- or long-term investigations.

Besides stressing the importance of this continual traffic and contact between home-based scientists and overseas workers, particularly in formulating projects for investigation, and in avoiding duplication of effort, Mr. W. A. C. Mathieson, for the Department, emphasized the value of the work of the Anti-Locust Research Centre and its high international standing. He also pointed out that the work of the natural resources and research divisions of the Department was very closely linked, since the major part of the research effort was devoted to the application of the biological sciences to natural resources. He did not think that the recommendations of the Trend Report would significantly affect the research activities of the Department, and no questions were raised by the Estimates Committee concerning either the Overseas Geological Survey or the Directorate of Overseas (Geodetic and Topographic) Surveys.

The treatment of research in the study of the Overseas Development Institute is much fuller. First, in reviewing the development of British technical assistance up to 1961, it has a concise summary of the way in which colonial research developed and ramified, first under the Colonial Research Committee and later, when funds for research became available under the Colonial Development and Welfare Acts, under the Colonial Research Council: over the period 1946–62, about 7·5 per cent of expenditure under these Acts was on research schemes. Next, a chapter on the structure, finance and functions of the Department of Technical Co-operation considerably amplifies the information submitted in evidence to the Estimates Committee, and it also includes reference to the work of five Committees set up at various times to report on technical aid in specific fields: they include the Lord Bridges's Committee on Training in Public Administration;

* The Overseas Development Institute. *British Aid*—4: *Technical Assistance*. Pp. 196. (London: The Overseas Development Institute, 1964.) 7*s*. 6*d*.

† Tenth Report from the Estimates Committee together with part of the Minutes of the Evidence taken before Sub-Committee F and appendixes, Session 1963–64. Department of Technical Co-operation. Pp. xxix+243. (London: H.M. Stationery Office, 1964.) 16*s*. 6*d*.

Rawden Committee dealing with agriculture, animal health, forestry and fisheries; and the Brundrett Committee on overseas mining and geology.

The value of the survey should be sufficiently apparent from this, but before dealing with the chapter on research, it should be added that there are other chapters on the supply of personnel, on training, on regional programmes and Commonwealth Educational Co-operation, on multilateral technical assistance, and on co-ordination of technical and financial assistance. In one, on request procedures and priorities, it is noted that in research particularly most of the work supported from the Department's funds was started before independence with Colonial Development and Welfare Funds. It is probably easy to exaggerate the extent to which the pattern of assistance is determined by independent decisions in developing countries, especially in the field of research.

As regards the Department's sub-head for research, the survey explains that this is for "expenditure on the promotion of research for the benefit of developing countries" and that when it was first established its level was fixed for three years, from 1963–64, and it is intended each year to fix the provision for a further year ahead. This will enable the Department to give firmer assurances of support and facilitate forward planning. Professional advice on priorities is obtained from the Department's advisers and advisory committees, the research advisers being heads of the home-based research institutions supported by the Department, apart from the adviser on medical research and the agricultural research adviser. In this connexion the survey comments on the heavy concentration of research in agriculture and medicine and the relative neglect of economics, education and the social services. It is in the same connexion that the survey notes the demise of the Overseas Research Council. By implication the ineffectiveness of that Council in seeking to ensure the continuation of research units in former colonial territories after independence is attributed to the failure to supply the Research Council with funds of its own. Nevertheless, there is nothing in the account of the Department of Technical Co-operation's activities to suggest that the Overseas Research Council has become redundant or that, either within the Department or as an independent body, it could not strengthen the research effort overseas.

It might be observed in this same connexion that during the British Commonwealth Scientific Committee's third meeting in New Zealand (November 15–December 6), although the Agricultural Research Council and the Nature Conservancy are being represented as well as the Department of Technical Co-operation, there is no specific representation for overseas research such as the Colonial Research Council and the Overseas Research Council formerly supplied. The organizing agency for the Committee in Britain is the Department of Scientific and Industrial Research, which is also being represented at the meeting. Presumably it is too soon for the representation from Britain to reflect the changes in Government organization of civil science and overseas development, proposed for the new Cabinet, but such representation should receive the careful attention of the new Minister for Overseas Development and the Minister for Education and Science.

It is rather in the analysis of research expenditure that this survey by the Overseas Development Institute indicates the opportunities which confront an Overseas Research Council, and it is not easy to visualize an alternative method by which these opportunities can be seized and the concomitant responsibilities discharged. Of the £1·2 million in the 1963–64 budget for research in the developing countries, agriculture (including veterinary research and forestry) took £627,000 (£189,000 for East Africa, £144,000 for Central Africa and £133,000 for the Caribbean), and medical research £260,000 (East Africa, £91,000; West Africa, £67,000; Caribbean, £100,000). Most of this expenditure is in the form of grants towards the recurrent and capital costs of research institutions in the developing countries, normally on a 50–50 basis, but sometimes higher. The 800 or so research workers at these institutions include local as well as British nationals, and British support is still necessary in the countries that have recently become independent.

The mere list of some of the larger overseas institutions being supported in this way indicates the range of opportunity and the complexity of the task of securing adequate support for research. Of the ten East African institutions on the list some are known to be in difficulties, while those familiar with the reports of the Colonial Research Council will note that now only one is listed from West Africa, though there are three from the West Indies and one from Malacca. A further pertinent comment on this list is Dr. E. B. Worthington's remark in *Minerva* (Spring 1964) that while these and other institutions were vigorous before the break-up of the scientific edifice built by the colonial powers, many are but empty shells now. In refilling them the great need is for the marriage of the indigenous with the exotic. Here the rapid development of local universities gives most promise. Dr. Worthington was referring particularly to Dr. S. Dedijer's article on science in underdeveloped countries and to a subsequent article by Dr. M. Moravisik on "Technical Assistance and Fundamental Research in Underdeveloped Countries", and he also stressed the desirability of keeping the number of organizations in these countries to a minimum. Writing from Nairobi, Dr. D. Odhiambo in the same issue referred to the considerable lack of indigenous research workers in East Africa and to the absence of postgraduate schools capable of covering a broad spectrum of pure research. Technical assistance could help to meet both needs if foreign research workers were made available, if only for one to two years, to help create the appropriate atmosphere for research in the academic institutions, provided funds were made available for establishing postgraduate schools. It was in the hope of creating a scientific community in East Africa, as urged by Dr. Dedijer, that on independence the scientists in various East African regional research organizations had been organized into the East African Academy—from which Dr. Odhiambo wrote. Financial support is also given to government agricultural research stations in Sabah, Sarawak, Malawi and Swaziland, and the Department of Technical Co-operation is making a substantial grant to the reconstituted Agricultural Research Council of Central Africa over the three years from July 1, 1964. The Department also finances British participation in the work of the Natural Resources Research Council and the Medical Research Council in East Africa, and a new scheme is at present being considered for a few research fellowships to be offered by Britain each year for tenure at universities and other institutions in developing countries.

These fellowships are intended to attract those who have already had postgraduate research experience, and also to strengthen research at universities and other institutions, rather than to further the execution

of specific research projects. Both forms of support are essential, and under the organization that has existed since the Colonial Research Council was functioning it is the specific project that has tended to receive support. As the Overseas Development Institute comments pertinently: "The consideration that (overseas research institutions) are places where indigenous research workers could be trained and the foundations for a modern scientific community laid does not seem to play any significant part in British thinking". This, however, is probably the most urgent aspect of overseas research in the newly independent countries, and that in which British aid could have the most decisive effect. Fresh thinking and generous action at this point are as imperative from the point of view of the economic development and stability of these countries as they are from the point of view of research or of educational aid.

The home-based establishments which are wholly financed by the Department of Technical Co-operation include the Anti-Locust Research Centre, the Tropical Products Institute, the Tropical Section of the Road Research Laboratory, the Tropical Division of the Building Research Station, the Tropical Stored Products Centres of the Agricultural Research Council and the Tropical Pesticides Research Unit and Headquarters and Information Unit. Expenditure of £15,000 on the Central Pool of Scientists, which is administered by the Commonwealth Agricultural Bureau and the Department of Technical Co-operation, brings the total to £905,000 in 1963–64. Some £150,000 is spent by the Department on overseas research at universities and other independent institutions, of which £70,000 is spent equally on agricultural and medical research, £43,000 on trypanosomiasis and £29,000 on pesticides. This, however, represents only a part of the benefit which developing countries derive from research in British universities and institutes, and some of the work financed by the Department is extension of work on home products, while other work arises from contacts between universities and developing countries. The Tropical Products Institute, which has a staff of 160, estimates that about 40 per cent of its work is undertaken in response to requests and about 60 per cent on its own initiative.

Besides this, the Department of Technical Co-operation in 1963 spent about £115,000 on consultancies and about £1·1 million on geodetic, topographical and geological surveys—only about one-third of the surveys being carried out under regional programmes in 1963. The method of financing mapping surveys undertaken by the Directorate of Overseas (Geodetic and Topographic) Surveys, which employs a staff in Britain of some 500, has recently been changed to make regional programme funds cover virtually the whole cost of surveys in future. Its present budget is running at slightly more than £1 million a year and the developing country is usually expected to provide 15 per cent of the cost of aerial photography and the field survey work.

Overseas Geological Surveys has a staff of about 100, of whom normally 10 are overseas at any one time, and besides its major role of helping to build up local Geological Survey Departments in the colonies it offers specialist services, and its Geophysical Division conducts surveys in various countries. Expenditure in 1963–64 is estimated at £263,000, but it will be recalled that the Brundrett Report (see *Nature*, 203, 331; 1964) recommended that Overseas Geological Surveys should be amalgamated with the Geological Survey of Great Britain and the functions of the latter expanded to cover overseas work in geological and mineral assessment, the cost of the [illegible] being met by the Department of Technical Co-ope[illegible] Even the outlined budget is only of the order of £1 million, which, as already pointed out in these columns, seems a long way below what is desirable and compares poorly with expenditure in the same field by the United States or by France.

This recommendation has already been accepted by the Government, but the budget as well as the organization of the expanded Department could appropriately be reconsidered by the new Ministry of Overseas Development and the Ministry of Education and Science and probably also by the Ministry of Technology and the Ministry of Land and Natural Resources. The Overseas Development Institute in its present survey does not indeed discuss these particular recommendations, and it is silent on the further recommendation that the Extraction of Metals Group of the National Chemical Laboratory should be merged as soon as possible with the Mineral Processing Division of the Warren Spring Laboratory, which has also been accepted by the Government. This recommendation, however, which outwardly appears reasonable, has caused some concern among the scientific staff affected; apart from the actual merger there would appear to be room for further discussion about the new department, which clearly should be sufficiently strong to deal effectively with overseas work in this field.

The Brundrett Report could scarcely have stated better the case for expansion in overseas geology and mining, and while further consideration may well be required on the recommendation of the Trend Committee that the Geological Survey should itself be placed under the proposed Natural Resources Research Council, there appears to be no firm ground for concern as to the future of research generally in these fields. In particular, the Brundrett Committee's recommendation that a highly qualified Advisory Committee should be constituted to advise the Department of Technical Co-operation on problems of technical assistance in these fields may well be reconsidered. Even the Trend Committee, in recommending the dissolution of the Overseas Research Council, proposed that the Research Council should be replaced by a series of such advisory committees.

Even more concrete, however, are some of the conclusions and suggestions of the Overseas Development Institute in this field. First, it points out that 84 per cent of the Department of Technical Co-operation's bilateral expenditure in 1963 was in respect of parts of the Commonwealth which were still colonies at the beginning of 1960: Sarawak, for example, received more technical assistance than India, and virtually all the allocations to research mapping and geological surveys went to such countries, nor is it easy to see that Britain could have done less. Indeed, it is suggested that it would be a far-sighted act of statesmanship, leading to much happier relations, if the British Government assumed the complete cost of the compensation payments to British officials instead of the present 50 per cent.

Next, it is pointed out that on questions of technical assistance, the channels of communication are partly blocked, and are likely to remain so until Britain does more to influence and encourage requests and to identify the sort of help that developing countries need. The first step would be to arrange for permanent British representation overseas through technical advisers who have long acquaintance with the area concerned. Next, these divisions should be supported by a network of good aid

in all the major countries where Britain has large aid programmes. More frequent fact-finding and liaison visits by professional advisers or by full technical assistance missions are also desirable, and institutions in Britain should be encouraged to forge links with similar institutions in developing countries, and work out programmes of mutual co-operation. Arrangements to supply British staff to strengthen the Government departments in developing countries, responsible for requesting aid and planning its use, should be reviewed, and Britain should take more positive steps to let developing countries know what services she can make available.

The survey is particularly critical of the organization of Britain's resources for technical assistance. Too much research is on a hand-to-mouth basis with no certainty of continued employment. We need to build up institutions and a corps of people who will spend much of their working lives on overseas development. Only with such a nucleus of professionals and a framework of permanent institutions is it possible to draw fruitfully from time to time on the experience and expertise of research workers based in Britain. The establishment of a special institution for training and research in overseas development is being considered. This proposal, or the principle behind it, requires extension. A major recommendation of the survey is for the establishment of a career service, to provide a 'pool' of experts employed on a long-term basis.

With regard to training, integration of the supply of British university staff, and arrangements to give junior staff from developing countries administrative, teaching and research opportunities in Britain, are urged. As regards research, the main need is for more free resources to be available each year, thus permitting a more positive approach in applying British brains and ingenuity to tackle some of the technical problems of developing countries. In this field we cannot wait for requests. Developing countries are often unable to identify appropriate problems and it is important to ensure that activities are directed towards solving practical problems and that solutions, once found, are widely disseminated and applied.

Commenting on the present derisory allocations for economics, education and social sciences in the research budget of the Department of Technical Co-operation, the survey points out that these fields are crucial to development. It comments that research into birth control, which holds the key to the future of many developing countries, has as yet received no support. Even in agricultural and medical research there are scores of problems to which solutions could be found if resources were allocated. The geographical distribution of support for research is also uneven, and while the policy of housing units inside larger home organizations is sensible, in the absence of an effective Overseas Research Council it tends to aggravate the piecemeal development of research effort. Some such means is badly needed, to give a sense of direction to the whole research effort, to determine priorities and commission new research; the point should receive attention in any re-organization of the Department under the new Ministry.

Besides this, the survey stresses the need for more emphasis on the application of research findings and of other existing knowledge, and its reference to the importance of a close relation between research and capital aid seems to support the basic idea of the new Ministry of Overseas Development. The need for integrated administration and operation of capital and technical aid is regarded as a matter of high priority. Emphasis is placed on the urgent need for co-operation like that which already exists between the Directorate of Overseas Surveys and overseas survey departments, between the Anti-Locust Research Centre and the East African Locust Control Organization, and between British and overseas universities. Above, all, while generously acknowledging the magnitude of the British contribution to technical assistance, the survey shatters the idea of any complacency, either in research or in technical aid generally. There are now fewer British technical personnel overseas under official arrangements than there were seven years ago—and this is the vital factor.

The survey mentions the White Paper *Research Assistance for the Developing Countries* issued in July, 1964, just as the survey was being completed. That Paper* provides the first account of the research assistance given by the Department of Technical Co-operation and is the first attempt so far to fill in any measure the gap that was left when the report on *Colonial Research* was discontinued. It is clear that the general pattern is still the same; little is added in the very brief summaries of research to what is included in the survey of the Overseas Development Institute, though a figure of £150,000 a year is quoted for research grants to universities and research organizations in the United Kingdom for specific studies in various fields. Nevertheless, the White Paper is to be welcomed as a first step. With what is stressed by the Overseas Development Institute, and in the report of the Estimates Committee on the work of the Department of Technical Co-operation, it should at least help to keep the question of research to the forefront in the present re-assessment and re-organization of overseas development and of civil science. Taken together, the three documents could stimulate the constructive thinking that is sadly overdue in the whole field and without which neither re-organization nor more generous financial support will be fully effective.

* Department of Technical Co-operation. *Research Assistance for the Developing Countries.* (Cmnd. 2433.) Pp. 28. (London: H.M. Stationery Office, 1964.) 2*s.* net.

INDUSTRIAL ORGANIZATION AND SOCIOLOGY

Economics and Sociology of Industry

A Realistic Analysis of Development. By Prof. P. Sargant Florence. Pp. xiii + 258. (London: C. A. Watts and Co., Ltd., 1964.) 27*s.* 6*d.* net.

THE two main themes of Prof. Sargant Florence's work since his *Economics of Fatigue and Unrest* was published forty years ago both run through *Economics and Sociology of Industry*. First, there is his emphasis on the importance of sociological and social-psychological factors in industrial and economic problems and processes, and, secondly, there is his emphasis on the limitations of abstract, purely deductive model-building starting from assumptions of profit-maximization and over-simplified market forms, and his insistence on empirical and, where possible, quantitative, testing and measurement. This very wide-ranging book, composed of lectures delivered at Ruskin College, Johns Hopkins University, and the Royal University of Malta, amount to a review of Florence's leading interests and ideas in the fields of industrial organization and industrial sociology, extended, in the closing chapters, to the now absorbing problems of the development of the poorer countries.

Prof. Florence starts by setting out nine leading 'trends' or 'trains of events' in industrial development, such as the growing importance of industrial and service activities as against agriculture; the application of science; the growing importance of organizers, managers and administrators as against working operatives; urbanization; enlargement of scale in industry; the managerial revolution; and higher standards of living. This concept of a 'trend' that is something less than a 'law' seems somewhat vague but useful for the broad canvas on which Florence is engaged.

Prof. Florence then goes on to examine the mobility of labour geographically and occupationally, which always has been, and is to-day as much as ever, a key question in regional and national development policies, as to which analytical economists' assumptions have, since Ricardo, often been in terms of an unrealistic rapidity of movement in response to wage differentials. Prof. Florence's call for much more empirical investigation of the factors making for mobility and immobility can only be supported. The mobility of labour leads on to the economics of industrial location and urban development, and to problems of the selection of industries which can advantageously be stimulated in one way or another, towards the development of depressed or rural areas, and away from congested 'conurbations' (a Florentine term). Problems of the scale of industries come next, and of management and industrial relations and psychology, to which the trend to large scale gives rise. Here Prof. Florence is at his best. The last three chapters take us to the emergent countries, that is, those countries the economics of which have scarcely begun to follow out the 'trends' distinguished at the beginning of the book. The treatment here, though often lively and acute, becomes rather unsystematic, with disproportionate emphasis on one or two special enthusiasms, for example, the tourist trade. But Prof. Florence is never dull, and he manages to provide a just sufficient thread of argument to carry his readers through his wide-ranging variegated survey. Indeed, as regards some of the problems now recognized as central and critical, for example, population and birth control, Prof. Florence writes as a pioneer of long standing.

This would be a rousing, broadening book for students of economics to read alongside their narrower and more analytical text-books, setting before them in lively terms the realistic problems, for coping with which their analytical training is meant to prepare them.

T. W. Hutchison

REVIEW OF SOLID-STATE CHEMISTRY

Progress in Solid State Chemistry

Vol. 1. Edited by H. Reiss. Pp. vii+536. (London and New York: Pergamon Press, 1964.) 120*s*. net.

THE growth in the number of the various series "Advances in this . . .", "Progress in that . . .", is familiar to all practising chemists. The book under review is the first volume of yet another one, and consists of eleven chapters. These are entitled: "The Thermal Expansion of Ceramic Crystals" (H. P. Kirchner), "Lattice Energies and Related Topics" (M. F. C. Ladd and W. H. Lee), "Phases with the Nickel Arsenide and Closely-Related Structures" (A. Kjekshus and W. B. Pearson), "Lattice Imperfections and the Thermal Conductivity of Solids" (D. Greig), "The Relationship of Photoluminescence and Electroluminescence to Structure" (D. W. G. Ballentyne), "Ferrielectricity in Crystals" (C. F. Pulvari), "Alloy Semiconductors" (J. C. Woolley), "Physico-Chemical Aspects of Organic Semiconductors" (H. A. Pohl), "X-Ray Diffraction Studies of Crystal Perfection" (L. V. Azaroff), "Application of Nuclear Quadrupole Resonance" (G. A. Jeffrey and T. Sakurai), and "Use of Infra-red and Raman Spectroscopy in the [illegible] Organometallic Compounds" (Huggins and Ka[illegible]

The various 'Progress' and 'Advances' series now play an important part in the increasing volume of scientific literature, since they review the state of a limited area of subject matter at a given time. The value of a particular series, however, must depend to a considerable extent on whether it is up to date. Taking into account modern production techniques, it should be possible to reduce the time interval between submission of a manuscript to an editor and its appearance in a volume to less than one year. Volume 1 of *Progress in Solid State Chemistry* has evidently not met this requirement, because references to work published after 1961 are rare indeed. Clearly some of the authors deserve sympathy for the delay in appearance of their work. The book is unlikely to have a wide sale privately because of its high cost, and libraries which represent somewhat of a captive market for publishers of this kind of volume will have to judge whether the price of the book, coupled with the apparent two- or three-year delay in publishing some of its contents, makes purchase worth while.

Although the chapter on infra-red and Raman spectroscopy is excellent, one wonders why it is included at all in a book on solid-state chemistry, because the chapter is principally concerned with observations on liquids or solutions. To a lesser extent this criticism applies to the chapter on nuclear quadrupole resonance.

References in the book in general are not numbered, authors often being referred to by name within the cursive text with the year of their published work in parentheses. Since there are many hundreds of references this procedure has made the book longer than it need be. Even more important, in my opinion, because of the liberal scattering of names on most pages, the book has been made more difficult to read.

Production of the first volume of a series of books presents special problems. In view of this, perhaps the above comments are somewhat harsh, and a final judgement must await the publication of further volumes.

F. G. A. Stone

ASPECTS OF GEOLOGY

Les Volcans et leur Activité

Par Alfred Rittmann. Édition Française à Partir de la Deuxième Édition Originale Établie et Introduite par Haroun Tazieff. Pp. 461 (6 planches). (Paris: Masson et Cie., 1963.) 95 F.

The Study of Fossils

By Prof. J. F. Kirkaldy. (Hutchinson Biological Monographs.) Pp. 116. (London: Hutchinson Educations, Ltd., 1963.) 12*s*. 6*d*.

Minerals and Rocks

By Prof. J. F. Kirkaldy. Pp. 176+photographs. (London: Blandford Press, 1963.) 18*s*.

THE English translation of the second edition of Rittmann's important monograph, *Vulkane und ihre Tätigkeit* (*Nature*, 199, 1125; 1963), has now been followed by a translation into French made by J. Fridmann, and edited by H. Tazieff, of the University of Brussels. This version is noteworthy on account of the inclusion of 88 new text figures, additional to those originally used. Many of these are excellently reproduced photographs of volcanic phenomena, including a selection from the classic photographs taken by A. Lacroix in Martinique in 1902, and others that are recent and hitherto unpublished. Sixteen have been printed in colour, on four plates. A further addition, as an appendix, is an essay by Prof. Rittmann himself on the nomenclature and classification of volcanic rocks that have not been included in any previous edition. Libraries that do not already possess a

Rittmann's important work may find it worth while acquiring this attractively produced, though somewhat expensive, new edition.

Prof. Kirkaldy's *Study of Fossils* is a short discourse on palæontology intended specifically for biologists, though it may well serve others as a guide to the varied information which geologists derive from the examination of fossils. The first two chapters deal with the geological background, without which the significance of fossils cannot be appreciated. Two succeeding chapters discuss the nature and state of preservation of the organic remains, designated 'fossils', found in rocks, and the life succession which they illustrate. In the next three chapters the use of fossils as time markers, and as indicators of environmental conditions and palæoclimates, is dealt with. The final chapter discusses the evolutionary aspects of fossil remains. The book is adequately and clearly illustrated by a series of line diagrams.

Minerals and Rocks, in colour, is an elementary handbook on rocks and minerals, the chief merit of which is that 266 specimens are illustrated in colour. These were chosen, originally, to illustrate a book, *Stenar i färg* (Stones in Colour), by H. Lundegårdh, published in Stockholm in 1960, and were selected from Scandinavian museums. The colour photographs, made by Folke Johansson, have been used by Prof. Kirkaldy for his own book. On the whole, the colour reproductions are extremely good, and, no doubt, would serve to show a beginner the great variety in colour and texture that is encountered in rocks and minerals. They may serve to stimulate an interest in geology and mineralogy, but they cannot adequately take the place of actual specimens. The text that accompanies the plates has been entirely written by Prof. Kirkaldy. It attempts to compress within the compass of about 100 pages the essentials of the subject-matter of mineralogy, petrography and petrology. Unfortunately the book bears evidence of somewhat careless editing, the principal defect being that, in the index, the reference numbers given to specimens illustrated do not correspond with those given on the actual plates. V. A. Eyles

METAZOAN PARASITISM

The Nature of Parasitism

The Relationship of Some Metazoan Parasites to Their Hosts. By W. P. Rogers. (Theoretical and Experimental Biology: an International Series of Monographs, Vol. 2.) Pp. ix + 287. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1962.) 7.50 dollars.

MANY biologists would consider the nature of parasitism too complex to be discussed adequately in such a small book as this, even when attention is focused on helminths and Protozoa are excluded from consideration. Prof. W. P. Rogers has selected nematodes for special treatment for various reasons and not least because his own researches have long been concerned with their physiology. From the evolutionary point of view, the group has been remarkably successful and, while some thousands of species have become parasites of animals or plants, many more have retained a free-living mode of life. Yet all roundworms have a comparatively simple morphological organization which suits various types of environment; reproductive processes are somewhat standardized, life-cycles not unduly complex and there is little apparent physiological specialization. In many respects, therefore, nematodes lend themselves to the study of a kind of parasitism in which adaptation has often shown little modification of structure and function.

The book consists of four parts and eleven chapters, each of which has its own list of references to the original literature and most of which carry a short summary, and physiological interest is sustained throughout. This has been a handicap for the author, because physiological knowledge of the group is fragmentary and incomplete. Although deficiencies in our knowledge are evident, however, hypothesis has been used to guide the reader through the intricacies of functional speculations. Prof. Rogers's avowed aim has been to try to stimulate research rather than to summarize existing knowledge. The first two chapters are devoted to speculations on the host–parasite relationship and the point is soon made that it is important to know what is meant by 'infectiousness', that is, the capacity of the organism to infect the host and to furnish its parasitic existence with a system of metabolic processes which are distinct from, but in harmony with, those of the host. The earliest stage of infection often involves the removal of a protective outer covering such as the shell of the egg, the wall of the cyst or the sheath of the larva, and sometimes a ciliated epithelium which serves as a dispersal mechanism in trematode miracidia. The environmental components which facilitate the establishment of the infective agent as a true parasite need not be highly specific but must ensure that protective properties are not lost until entry has been made into an environment which will support a closer and more intimate relationship between host and parasite and may entail moulting, migration and metamorphosis.

Free-living roundworms undergo a series of moults during their life-cycle and infection by parasitic forms is preceded by a moult, after which development is suspended until an appropriate host has been acquired. The infective stage is thus a resting stage which forms a 'bridge' by which the parasite passes from the environment of one part of the life-cycle to a new environment in another part of the cycle. In considering a physiological basis for the nematode life-cycle, Prof. Rogers suggests that the action of the environment is always indirect and that metamorphosis is evoked by internal secretions which are produced as a result of stimuli from the environment acting on the receptor organs of the parasite. In arthropods, moulting is controlled by internal secretions and it is possible that similar control occurs in roundworms. Infection with eggs or larvæ of certain nematodes is 'triggered' by certain physical factors and chemical agents in the intestinal contents of the host, mainly dissolved carbon dioxide or undissociated carbonic acid but also *Eh*, *p*H and temperature. The consequent stimuli evoke the secretion of hatching or exsheathing liquids and, possibly, affect more cryptic developmental mechanisms. This is a theme which Prof. Rogers, in collaboration with Dr. Sommerville[1], has developed in greater detail since the book was published, mechanisms of moulting and metamorphosis being regarded as closely analogous in parasitic nematodes, insects and amphibians.

The Nature of Parasitism contains the groundwork of many other important considerations. In Part 2 the discussion of problems of infectiousness is continued in chapters on life-cycles and infection, the physiology of free-living stages and the physiology of infection. The free-living stages of nematodes are considered in relation to food reserves, oxygen requirements, metabolism and its end-products, and infection covers entry by way of the intestines or through the surface of the body. In Part 3 the author is concerned with the lives of parasites in separate chapters dealing with the physiology of the parasitic stages, including intermediary metabolism, muscle–nerve physiology and osmoregulation, and with the oxygen requirements and nutrition of parasites. Part 4 has separate chapters dealing with the propagation of parasites, their specificity in various stages and their evolution. So far as knowledge goes it has been discussed succinctly and well documented in this small book, which deserves to succeed in its object of stimulating research and bringing it into line with other developments in modern biology. Ben Dawes

[1] Rogers, W. P., and Sommerville, R. I., in *Advances in Parasitology*, edit. by Ben Dawes, 1 (Academic Press, 1963).

IN SITU DETECTION OF AN IONOSPHERIC ELECTRIC CURRENT

By Dr. K. BURROWS* and Dr. S. H. HALL

Department of Geophysics, Imperial College of Science and Technology, London, S.W.7

THE generally accepted basis for the explanation of the observed daily variations in the Earth's magnetic field is to be found in the dynamo theory, first postulated by Balfour Stewart in the 1882 edition of the *Encyclopædia Britannica*[1]. Schuster[2], Chapman[3] and other workers later elaborated the dynamo theory and, using magnetograms recorded at various observatories throughout the world, they were able to represent the complex and changing array of magnetic variations by a relatively simple system of horizontally stratified sheet currents, stationary relative to the Sun, but, relative to an observer on the Earth, continuously moving westward and varying in intensity with a predominantly 24-h period. The current system is identified by the symbol *Sq* to differentiate it from other proposed currents responsible for lunar (*L*) variations and storm-time (*D*) variations.

From an analysis of routine observatory magnetograms it is possible to estimate the integral, with respect to height of the postulated electric currents; a detailed analysis is, however, not possible from ground results alone. In particular it is difficult to assess such features as the height, vertical thickness and any possible stratification of the currents.

With the advent of high-altitude sounding rockets in 1946, direct *in situ* measurements of atmospheric properties became possible, and in 1947 Vestine *et al.*[4] first suggested that rocket-borne magnetometers could be used for investigating the distribution of these electric currents with height.

Assuming, for the moment, that the electric currents are located in the ionosphere, it follows that their horizontal dimensions must be large compared with their height and hence, to an observer on the ground, the *Sq* current system can be approximated to an infinite sheet current. The magnetic field associated with a current of this nature is $2\pi j$ œrsted, where j is the current density in absolute amps per centimetre, and it is independent of the height of the current sheet. Thus, if a rocket vehicle is used to carry a magnetometer, which is designed to measure F, the total scalar geomagnetic field, it should detect a change of $4\pi j . \cos I \sin \vartheta$ in passing through the current, where I is the inclination of the geomagnetic field below the horizontal, and ϑ is the angle between the direction of the current flow and the local magnetic north.

The much more intense current systems associated with auroræ have been detected[5] as also have the currents constituting the equatorial electrojet, a local intensified current which occurs, during the middle daylight period, in a narrow band (approximately 3° of latitude wide) along the magnetic equator[6,7]. In mid-latitudes, however, the expected 'discontinuity' due to the *Sq* current system is much less than at the magnetic equator, and this makes its detection correspondingly more difficult. Using the magnetograms from the Watheroo magnetic observatory in Western Australia (geomagnetic lat. −41·8°) it can be shown that the 'discontinuity' in F which might be observed by a rocket launched from Woomera (geomagnetic latitude −41·0°) is only of the order 10γ; furthermore, it is necessary to isolate this small effect from the 'background' field of the order 58,000γ, and as the sounding rocket ascends to its apogee, the ambient field it encounters will itself change by several thousand gammas[8].

Two *Skylark* rockets, *S.L.* 129 and 128, were fired from the Woomera rocket range (30° 56′ S., 136° 31′ E.) at 11.36 h c.s.t. on March 10, 1964, and at 11.37 h c.s.t. on March 12, 1964, respectively (c.s.t. = u.t. + 9½ h); each rocket carried a proton precession magnetometer as a secondary experiment. A description of the instruments and the data-processing techniques used is in course of publication[9].

In conjunction with the rocket flights a ground proton magnetometer[8] was operated, to record the diurnal variation in F, and a fluxgate instrument, kindly loaned by the Bureau of Mineral Resources, Melbourne, recorded

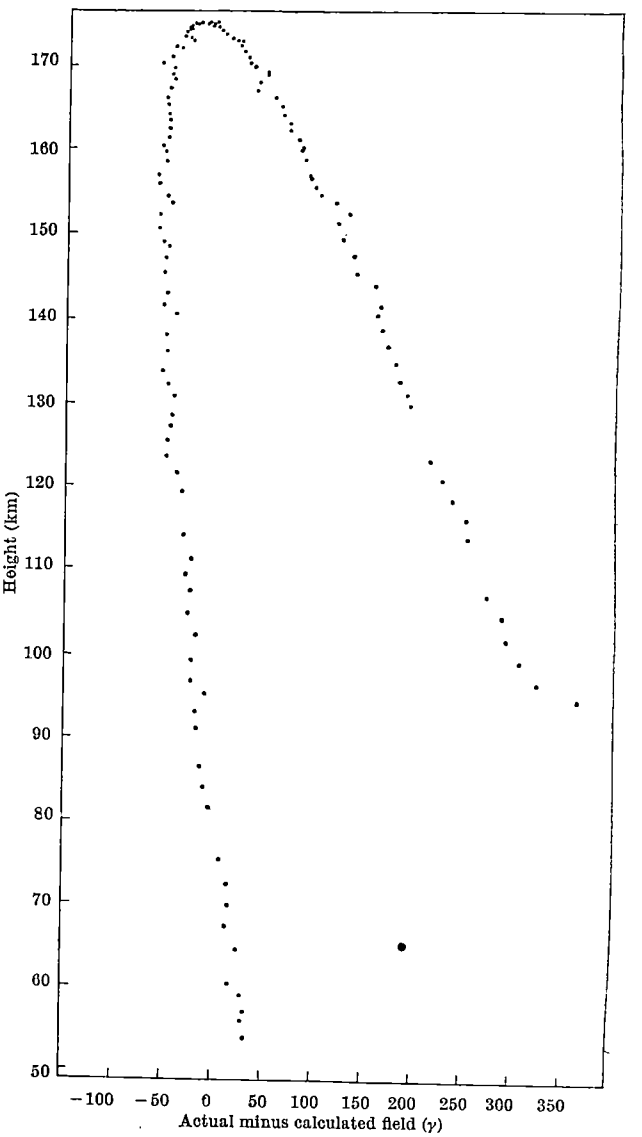


Fig. 1. Flight of *S.L.* 129

* Present address: Goddard Space Flight Center, Greenbelt, Maryland, U.S.A. (Fields and Particles Division).

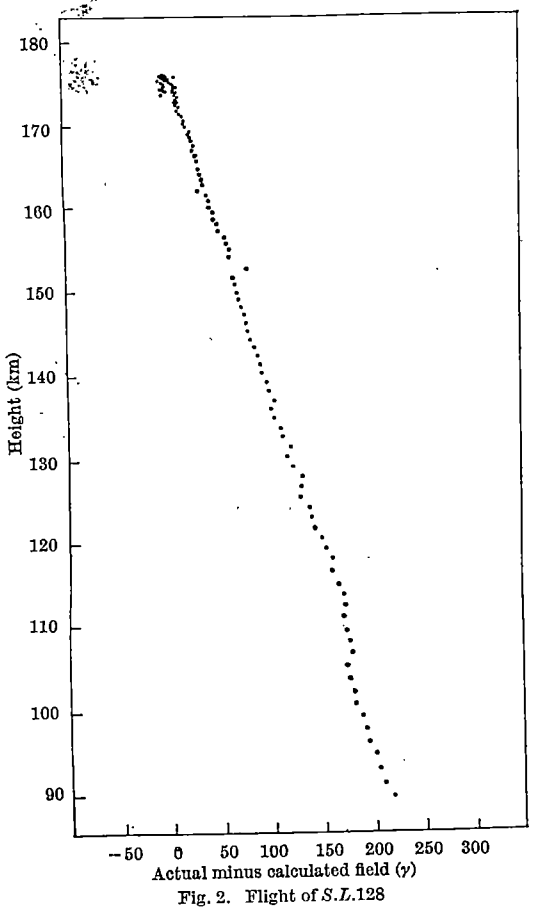


Fig. 2. Flight of *S.L.*128

H, the horizontal component. H was selected as being that component of the Sq variation field which is discontinuous in passing through the postulated current sheet.

Figs. 1 and 2 show the results of the flights on March 10 and 12, respectively, plotted in the form of differences between measured values of F and values computed by approximating the geomagnetic field to that of a geocentric dipole and neglecting the horizontal component of the rockets' trajectories. Only those readings obtained during the upward section of the second flight are presented; the data from the downward section have not yet been fully reduced, due to a shortage of computer time.

March 10 was an International Quiet Day[10] and the fluxgate record showed that, at the time of the flight, the value of H was close to the mean value for the day; it is not therefore surprising that no significant anomaly was recorded during the flight.

March 12, on the other hand, was a much more disturbed day. The fluxgate magnetometer recorded a significant departure from the undisturbed value at the time of the flight and the K_p index for the appropriate 3-h period was 4[10]. The reduced data from the rocket-borne magnetometer show an unmistakable change in slope at a height of about 104 km followed by a return to the original slope at about 112 km. The displacement of the upper section of the curve relative to the lower section is approximately 20γ. This indicates that, during this time, the rocket passed through an electric current sheet, flowing in a direction having a westerly component and of a nature similar to that which has been postulated to account for the daily magnetic variations recorded at ground-level. The absence of any similar 'discontinuity' during the rest of the flight up to apogee confirms that no other current system, of magnitude comparable with that already detected, was flowing in this height range.

The work is sponsored by the British National Committee on Space Research of the Royal Society; we thank Miss Helen Gibson, of the Weapons Research Establishment, South Australia, who did much of the routine data reduction.

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OXFORD EXPEDITION TO ASCENSION ISLAND, 1964

By F. B. ATKINS, Dr. P. E. BAKER, Dr. J. D. BELL and Dr. D. G. W. SMITH

Department of Geology and Mineralogy, University Museum, Oxford

ASCENSION ISLAND (lat. 7° 57′ S., long. 14° 22′ W.) is situated in the South Atlantic Ocean and rises from the crest of the Mid-Atlantic Ridge. The island is roughly triangular in outline and some 36 square miles in area. High temperatures, low rainfall and persistent trade winds characterize the climate, and these factors are reflected in low erosion rates, the distribution of pyroclastic deposits (in particular the marked asymmetry of the scoriæ and cinder cones), and the generally sparse vegetation on the lower ground. In recent years, however, the vegetation cover, considerable at higher altitudes, has extended widely over previously barren ground.

The position of Ascension Island on the Mid-Atlantic Ridge renders it important in present theories concerning the structure and evolution of the Atlantic basin. The objects of the recent expedition (led by one of us, J. D. B.) were to produce a detailed geological map of the Island, a preliminary version of which is given in Fig. 1, to elucidate its volcanic history, and to make a comprehensive collection of material for further petrological, mineralogical and geochemical investigation now in progress at Oxford. Concurrently with the geological survey a geophysical programme comprising gravity, magnetic and palæomagnetic investigations was carried out by members of the Geophysics Department of the Imperial College of Science and Technology, London. A more detailed, combined report including these investigations will be published at a later date. Further work on the mineralogy and chemistry of the acid and intermediate ejected blocks (see following) is in progress at the Department of Mineralogy and Petrology, Cambridge, and strontium isotope investigations will be carried out at Oxford.

Previous geological accounts of Ascension Island include those of Darwin[1], who restricted his discussion to some of the more striking features, and Daly[2], whose systematic treatment of both the volcanology and petrology is the most comprehensive up to the present time.

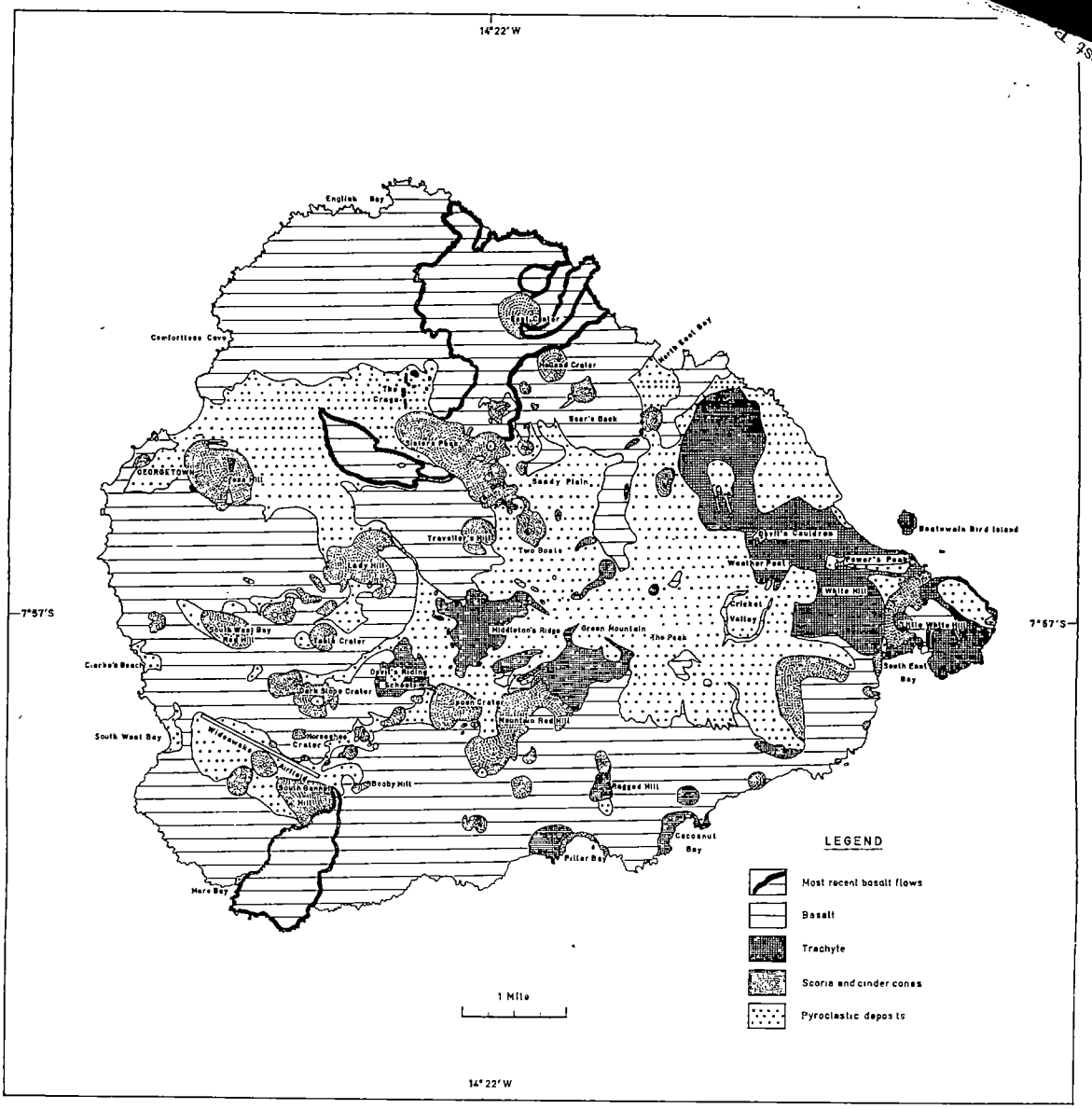


Fig. 1. Preliminary geological map of Ascension Island

Prof. D. S. Coombs, of the University of Otago, has also carried out certain mineralogical and chemical investigations of Ascension rocks (personal communication).

With the exception of thin local deposits of wind-blown sand and beach rock, Ascension Island is entirely volcanic in origin, being the uppermost part of a cone which rises from a depth of about 10,000 ft. below sea-level to the summit of Green Mountain at 2,817 ft. The main lava types are basalt and trachyte, with the principal trachyte masses occurring in the eastern part of the Island while basalt outcrops predominate in the west. Green Mountain itself is composed largely of trachyte, partly mantled by trachytic pumice and basaltic cinders, the latter forming most of the summit region.

Many of the trachyte hills are volcanic domes, for example, Weather Hill, the domes pass laterally into the thick flows. Daly described in some detail the structural features of the trachytes, such as carious weathering and silicified interfaces, and also directed attention to the occurrence of trachytic obsidian as a chilled phase at the base of some of the flows. Flow banding and the interbanding of trachyte and obsidian are well exposed at the base of the Middleton's Ridge trachyte.

The western part of the island is formed by a series of basaltic flows and parasitic cones which are generally asymmetrical with the steeper slopes on the windward, south-east, side. Many of the cones have been breached by lava flowing through their less substantial windward flanks. The basalts include aphyric types with or without vesicles and porphyritic types, the principal phenocrysts being plagioclase and/or olivine. Several of the flows have extensive encrustations of white fumarolic minerals which occur preferentially on the leeward side of any projections. The basalt flows, which are generally *aa* or blocky types, display a great variety of structural features including lava tunnels (for example, near Clarke's Beach), spatter cones (for example, Booby Hill), bocas (Horseshoe Crater), hornitos, and lava channels (for example, south of Spoon Crater). The channels or gutters referred to by Daly[1] as having been worn in older rocks by streams of hot lava are in reality the beds of lava streams which have constructed flanking levees by periodic overflowing similar to occurrences on Tristan da Cunha described by Baker and Harris[3]. Further features include lines of hillocks composed of scoriæ and bombs which may represent fragments of a disrupted cone transported on the surface of flowing lava (for example, east of South

...ay, Red Hill), and a small frozen lava lake preserved at a subsidiary centre west of Sister's Peak. There is no historical record of volcanic activity on Ascension, but some of the basaltic flows, for example that from Sister's Peak, are clearly very young, perhaps only a few hundred years old.

The pyroclastic deposits of Ascension Island have previously received little attention. On account of their large volume, extensive distribution and variety of depositional structures they merit detailed examination. Although much of the material is obviously associated with small parasitic centres, a substantial mantle of lapilli and ash covers large areas of Green Mountain and extends for considerable distances beyond. Whereas many of the pyroclastic deposits have resulted directly from subaerial falls or from re-working of such, on Sandy Plain thin beds of pumiceous dust and ash are so finely stratified and so regular over hundreds of square yards that they were probably deposited in an extensive body of water scarcely subject to current action. Local steepening of dips may be ascribed to limited post-depositional movements.

A probable pyroclastic flow is exposed at the roadside on Green Mountain at about 1,800 ft. It comprises at least three units, one of which, approximately 4 ft. thick, shows a gradation from unconsolidated pumice at the top, through lightly welded pumice with lenses of obsidian to a continuous obsidian base. Beneath this lies another unit, 20 ft. thick, consisting of poorly sorted unconsolidated pumice which lacks stratification. Laboratory investigation is required to confirm the origin of this deposit, but should it prove to be a flow it is of particular interest in view of the rarity of such phenomena on islands of the Mid-Atlantic Ridge.

The presence on Ascension of coarse-grained ejected blocks has long been known, and both Darwin and Daly described various types. Olivine-bearing and plagioclase-rich basic types are confined to Dark Slope Crater. These rocks are in many cases laminated and show signs of layering. New localities were discovered for other types of block which include dioritic, syenitic and granitic varieties. The majority of these occur in redistributed agglomeratic deposits.

Certain larger scale structures possibly involving considerable movements were noted by Daly. A problematic structure is Cricket Valley, an elongate depression measuring some 600 yards by 400 yards and about 450 ft. deep. Massive basalt outcrops in the lower part of the near-vertical walls and relatively fine pyroclastic deposits occur round the rim. A fine ash covers the flat floor. Daly ascribed its formation to a series of explosions which threw out the basaltic blocks now found in the immediate vicinity. These blocks are found only on the surface of the pyroclastic deposits south of Cricket Valley and their volume appears quite inadequate to account for the size of the depression. This fact, together with the steep walls of the depression, suggests that subsidence has played a part in the origin of Cricket Valley. The subsidence may have been connected with the protrusion of one or more of the trachytic masses in the area. The nearby Devil's Cauldron has probably had a similar origin. The deep valley between Power's Peak and Weather Post was also believed by Daly to have had an explosive origin, but it seems more likely that the valley remained as a strip of low land between two protruded trachyte masses.

Other structural effects connected with trachytic emplacement are found in Devil's Riding School and Bear's Back. Daly[1] interpreted the former as a basaltic lava cone, the large crater of which was filled by trachyte which flowed out over the rim to the north-east; the trachyte surface within the crater subsided and pyroclastic sediments, some claimed to be lacustrine, were deposited within the basin thus formed; the basining of the sediments was attributed to a continuation of axial subsidence. We consider that a series of near-horizontal basaltic flows was punctured and tilted to a high angle by an injection of trachyte, the surface of which later subsided as suggested by Daly. Bear's Back is probably another instance of a trachyte intrusion up-doming older basaltic rocks but, in this case, just failing to pierce them. A similar situation is found just to the south, where a small area of the upper, brecciated surface of a trachyte dome appears in a gully cut down through tilted basalt flows.

Regarding the general sequence of activity on Ascension it should be emphasized that less than a quarter of the volcanic pile extends above sea-level. The Island has been constructed within relatively recent periods of activity, during which time the volume of trachyte erupted has been notably large. There is no justification, however, for dividing the activity into an earlier trachytic and a later basaltic phase: both rock types have been produced at various periods in the Island's evolution.

We thank the Royal Society for a grant towards this expedition. We thank Prof. L. R. Wager for his advice, and Captain Roy W. Dixon, commanding officer of the U.S.A.A.F. Base on Ascension Island, for his constant and generous assistance. Thanks are also due to many other people who assisted us in a variety of ways.

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ENVIRONMENTAL STRESS AND BIOLOGICAL AGEING

By Dr. J. M. ORDY, C. ROLSTEN, Dr. T. SAMORAJSKI and Dr. R. L. COLLINS

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IT is generally accepted that genetic factors must play a key part in ageing and in determining the life-span of an organism. However, since the average life-span in a selected population can vary in accordance with some extreme environmental fluctuations, it has seemed equally plausible to assume that the external environment may also exert an influence on the rate of ageing and mortality. Although various biological theories of ageing differ in their emphasis on the relative role of genetic and environmental factors in determining the rate of ageing and mortality, numerous experimental observations with animal subjects have indicated that ultra-structural constituents, cells, tissues, organs and the organism itself may show various structural and functional alterations with increasing age[1–4]. However, while some morphological changes may become quite apparent with ageing, various physiological functions may not show alterations with age when observations on functional changes are made under steady-state environmental conditions since the most conspicuous alteration for the organism as a whole with increasing age is generally exemplified by a more gradual reduction in biochemical, physiological and behavioural

capacity to respond to repeated or sustained environmental fluctuations frequently characterized as environmental stress[5,6].

While it may seem obvious that old subjects are less able to withstand repeated exposure to environmental stress, few quantitative multi-disciplinary investigations covering the entire life-span of an organism have been undertaken to isolate and identify interrelationships among genetically determined influences in the rate of ageing and mortality in relation to the effects of specific environmental stressors. While definitive experimental findings based on longitudinal rather than cross-sectional observations are lacking, various experimental findings have suggested that even more subtle environmental influences may result in a decreasing physiological adaptation to stress, and also lead to a possible acceleration of the ageing process and increasing mortality[7]. Whereas various structural and functional alterations have been examined so far in various cell populations and organ systems, it is generally accepted that accumulated biochemical, physiological and behavioural impairments from repeated stress with ageing may be associated most closely with some breakdown in non-dividing neural cell populations in the central nervous system and in integrative neuroendocrine structures and functions of the organism[8].

Numerous previous laboratory investigations with animal subjects have also shown that more extreme changes in environmental temperature and exposure to various noxious stimuli can affect cellular processes, metabolic rate, physiological adaptation to stress, behaviour and mortality[9–12]. However, most previous investigations of ageing in animals have concentrated on general survival curves under more severe environmental changes without an examination of specific interrelationships among morphological, biochemical and behavioural changes in key organ systems. More recently, it has become apparent that appropriate multi-variate statistical designs and the availability of carefully controlled environmental chambers should permit a direct quantitative assessment of the effects of more subtle and specific environmental factors on decreasing adaptation to repeated stress in ageing in relation to morphological and biochemical changes in the central nervous system and neuroendocrines[13]. The multi-disciplinary investigation recorded here was undertaken in an environmental chamber with a pure strain of mice in order to determine some specific effects of repeated temperature, shock and combined temperature and shock exposure to behavioural adjustment to stress with increasing age in relation to concomitant structural and functional alterations in the central nervous system and the pituitary adrenocortical axis.

In order to decrease the genetic variability in the behavioural, biochemical and morphological observations, a highly inbred strain of *C57BL/10* male and female mice were used as subjects. All animals were born, raised and exposed to temperature and shock in the carefully regulated environmental chamber in accordance with a predetermined experimental design. At 100 days of age, groups *A*, *B*, *C*, *D* and *E*, containing 40 males and 40 females each, were subjected to the following stress schedule: all steady-state control groups designated subgroups (*a*) (10 males and 10 females) were placed in cages and maintained without disturbance in the larger chamber at 76° F and 52 per cent relative humidity until they were tested afterwards in a behavioural test battery and then were killed for biochemical and histological evaluations of brain and neuroendocrines.

Electric shock groups (*b*) were maintained in the larger chamber, received shocks 2 h each day for 7 days, followed by no shock for 21 days. All temperature stress groups (*c*) were maintained alternately for 21 days in the larger chamber and the following 7 days in a smaller chamber at 59° F and 52 per cent relative humidity until they were tested and killed. Temperature and shock stress groups (*d*) received alternately temperature and shock stress for two 7-day periods out of 28 days in accordance with a combined temperature and shock stress schedule. For attaining equal intervals in the stress schedules throughout the life-span, each stress cycle was divided into four 7-day intervals. Accordingly, group *A* was exposed to 4, group *B* to 8, group *C* to 12, and group *D* to 16 complete stress cycles. Group *E* was not killed but subjected to the stress cycles until natural death for an evaluation of differential mortality.

The experimental findings on the accumulation of intracellular lipofuscin or age pigments in the central nervous system with increasing age have been reported previously[14]. This article presents a statistical summary of the group *A* behavioural data as well as the stress-induced biochemical and morphological findings on the pituitary–adrenocortical system in group *A* exposed to 4 months of environmental stress. The results obtained with group *A* alone have significant theoretical implications for ageing since they cover 4 critical months of early maturity and presumably an optimum period of biological vigour to adopt to intermittent environmental stress.

Stress Effects on Behaviour

The theoretical basis for the selection of the behavioural tests was to obtain a quantitative assessment of the effects of repeated temperature and shock exposure during ageing on a relatively broad range of adaptive behaviour determined in a test battery consisting of both non-stress and stress conditions. In the standard rodent open-field exploration test, the response measures were: (1) latency in seconds to move from the middle square; (2) number of squares traversed in 2 min. Spontaneous locomotor activity was used as an index of non-stress reactivity and was determined in vertically revolving activity wheels. Wheel rotations were recorded photoelectrically for 1-h periods for 4 consecutive days. Stress reactivity under shock-elicited escape-avoidance conditions was determined in a conditioning apparatus which consisted of an enclosed longitudinally divided galvanized runway measuring 30 in. × 1 in. × 3 in.

An automatic programming unit controlled delivery of shock (*US*), paired light and sound warning signals (*CSs*) which preceded shock by 3 sec, and a timer. A microswitch located at the end of the runway and activated by the escape (*UR*) or conditioned avoidance (*CAR*) responses terminated the *CSs*, *US* and timer. Response measures were percentage of avoidance in 100 learning trials and speed of escape-avoidance running in seconds. Table 1 presents a statistical summary of the group *A* findings on the effects of 4 months of repeated environmental stress on behaviour, biochemistry and morphology. As shown in Table 1, the temperature and shock groups displayed significantly longer open-field latencies, more open-field exploration, decreased spontaneous locomotor activity, and impaired learning as well as running decrements in the shock-elicited escape-avoidance conditioning. In general, the behavioural findings with group *A* indicate that such repeated exposure to intermittent deviations from steady-state conditions as temperature and shock stress (covering even a relatively brief but optimum adaptive period of the entire life-span) resulted in various changes in adaptive behaviour under the non-stress and stress test conditions.

Stress Effects on Biochemistry

After 10 days of behavioural testing, blood was withdrawn from the mice by retrobulbar cavernous sinus punctures for an evaluation of stress-induced changes in serum enzyme- and cholesterol-levels. Enzyme activity was determined by standard assay procedures and transformed into international units (I.U.) for quantitative evaluation[15]. The animals were then killed for histological

Table 1. Summary of Group *A* Data on the Effects of Environmental Stress

	Experimental groups				Units	Probability
	Control *a*	Shock *b*	Temp. *c*	*S* and *T* *d*		
Behaviour						
Open-field latencies	6·27 (2)*	5·56 (1)	7·24 (3)	8·79 (4)	Sec.	0·05
Open-field sq. trav.	56·1 (1)	59·6 (2)	66·4 (3)	76·5 (4)	Sq./2m	0·005
Locomotor activity	1,925 (1)	1,717 (3)	1,766 (3)	1,667 (4)	Rev./h	0·025
Escape-avoid. (%)	30·7 (1)	12·1 (3)	22·1 (2)	6·0 (4)	100 t.	0·001
Escape-avoid. Lat.	3·66 (1)	4·99 (4)	4·15 (2)	4·96 (3)	Sec.	0·001
Biochemistry						
Serum LAP	22·3 (2)	22·5 (3)	21·2 (1)	24·0 (4)	I.U.	0·05
Serum Alk. P-ase	34·1 (1)	34·5 (2)	36·2 (4)	35·9 (3)	I.U.	*NS*
Serum cholesterol	115 (1)	121 (2)	123 (4)	122 (3)	Mg%	*NS*
Adrenal ascorbic acid	2·22 (2)	2·34 (3)	2·11 (1)	2·42 (4)	μg/mg	0·05
Morphology						
Animal weight	26·9 (1)	26·7 (2)	26·6 (3)	26·5 (4)	gm	*NS*
Pituitary weight	2·90 (1)	3·35 (3)	3·33 (2)	3·70 (4)	mg	0·01
Adrenal weight	2·12 (1)	2·13 (2)	2·13 (3)	2·22 (4)	mg	0·025
Adrenal histology						
Cortical area	0·951 (1)	0·932 (2)	0·873 (4)	0·891 (3)	mm^2	*NS*
X-zone area	0·098 (1)	0·110 (2)	0·110 (3)	0·119 (4)	mm^2	*NS*
Medullary area	0·215 (1)	0·317 (4)	0·228 (2)	0·292 (3)	mm^2	0·01
Total area	1·168 (1)	1·359 (4)	1·189 (2)	1·277 (3)	mm^2	*NS*
Rank sums	19·0	42·0	41·0	58·0		0·001

Kendall's coefficient of concordance for the behavioural, biochemical, and morphological data.
* Group ranks (brackets) in relation to deviation from control group. Rank sums differed significantly from a random expectation; $W = 0{\cdot}60$, $P < 0{\cdot}001$.

examination. The quantitative biochemical comparisons revealed that serum leucine amino-peptidase (LAP) enzyme activity and ascorbic acid-levels of the adrenals were significantly higher in the combined temperature and shock stress groups. However, alkaline phosphatase and serum cholesterol-levels did not differ among groups.

Stress Effects on Neuroendocrine Morphology

In the morphological evaluations, it is interesting to note that body-weights did not differ among the 4 groups. However, pituitary and adrenal weights of the 3 stressed groups were significantly higher than in the controls. In order to obtain some quantitative estimates of the structural changes in the adrenals in response to repeated stress, histological comparisons were made of the size and configuration of the cortex, *X*-zone and medulla in relation to total adrenal area. For these quantitative estimates, the different topographic areas were determined on histological sections of identical magnification by a camera lucida technique and then expressed as a percentage of total area of comparable sections of the adrenals in all groups. Although the cortical area and the *X*-zone did not differ as percentage of total area in the group comparisons, the medullary area was significantly enlarged in the shock- and the temperature-and-shock groups.

As already noted here, numerous circumscribed observations have been made previously on age-correlated alterations in behaviour in relation to some structural and functional changes in the nervous system and the neuroendocrines in response to short-term or acute stress. However, few comprehensive multi-disciplinary ageing investigations have been reported utilizing pure strains, an environmental chamber, as well as behavioural assessment, biochemical assays, histological evaluations, and a statistical analysis of the combined quantitative data in one unified experimental design. As shown in Table 1, in addition to performing a separate statistical analysis of variance procedure for each behavioural, biochemical and histological test, a composite multi-variate statistical ranking was made of the four experimental groups on all 16 variables to determine whether group profiles differed with respect to the effects of temperature, shock, and temperature and shock exposure.

Each group received ranks from 1 to 4 on each variable in relation to the degree of deviation from the steady-state environmental control groups. In all, 160 ranks were assigned. If the ranking was random among the groups, the rank sum expectation of any exposure group would be 40. The rank sums for the groups were as follows: *a*, 19·0; *b*, 42·0; *c*, 41·0; *d*, 58·0. Kendall's statistical coefficient of concordance revealed that these rank sums were significantly different from random expectation ($W = 0{\cdot}60$; P 0·001). Consequently, it may be concluded that repeated temperature, shock and combined temperature and shock exposure in group *A* covering even a 4-month biologically optimum period of the entire life-span resulted in significantly different structural and functional group profiles, and that the most deviant group in behaviour as well as the biochemical and histological findings was group (*d*), which was exposed to both shock and temperature fluctuations.

At the behavioural level it seems relevant to note that the temperature and shock exposure groups displayed various statistically significant differences in behaviour in contrast to the control group. These differences were apparent under both non-stress and stress behavioural test conditions. Previous findings have indicated that exploratory behaviour decreases with increasing age under normal environmental conditions[16]. However, in the work recorded here, open-field exploration was significantly higher in the temperature and shock exposure group. At the biochemical level, decreases in ascorbic acid- and cholesterol-levels have generally been observed in response to acute stress[17]. Various changes in enzymatic activity with increasing age under normal steady-state environmental conditions have been attributed to a possible gradual decrease in such intracellular elements as mitochondria. The stress-induced biochemical findings in this investigation suggest that changes in various enzymatic activities with age may be a sensitive index for biochemical comparisons between young and old subjects since it has also been observed that functional cells in key organs may be replaced by connective tissue in ageing[18].

It has been noted previously that the results of numerous previous investigations with animals have shown a variety of structural and cytological changes in the pituitary and adrenal glands in response to acute stress as well as with increasing age under normal environmental conditions[19–21]. The present morphological findings are in general accordance with those published results on structural changes with ageing. Although the adrenal cortical area and the *X*-zone did not differ as percentage of total area in the multiple group comparisons of group *A* of the present work, the medullary area was already significantly enlarged in the stressed groups. In general support of other reported findings, this morphological enlargement suggests a more direct and prompt activation of the adrenal medulla by repeated stress activation probably involving the hypothalamus and pituitary. Stress-induced morphological alterations in the cortex may be more gradual,

since direct pathways to the adrenal cortex are lacking[22]. In conclusion, it should be emphasized that this article deals only with a summary of the group *A* findings. These significant findings with group *A* make it apparent that large-scale experimental laboratory investigations on the effects of various specific environmental stressors on biological adaptation to repeated stress, rate of ageing and differential mortality have become feasible. When the complete experimental findings of groups *B*, *C*, *D* and *E* covering the entire life-span are completed and analysed, it will be possible to make a direct multi-variate statistical assessment of the relative contributions of specific environmental stressors on decreasing capacity to respond to repeated deviations from environmental steady-state conditions with increasing age, as well as establish a possible acceleration or even inhibition of the ageing process from an evaluation of specific and controlled environmental effects on differential mortality.

This work was supported by a research grant from the U.S. National Institutes of Health.

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OBITUARY

Prof. E. F. Freundlich

THE death at Wiesbaden of Prof. E. F. Freundlich on July 25 at the age of seventy-nine has deprived the astronomical world of an impressive personality, and one of the few remaining links with our science in bygone days before the First World War.

Erwin Finlay Freundlich was born at Wiesbaden-Biebrich on May 29, 1885, of mixed German–Scottish parentage, and of a large family in which scientific bent was not unknown; the renowned physical chemist, Herbert Freundlich, being one of his older brothers. His university studies in Göttingen led him first to a doctorate in mathematics as a student of Felix Klein; but it was Klein who deflected his career to astronomy by recommending his young scholar in 1910 to an assistantship at the Royal Observatory at Berlin.

The time when Freundlich joined the ranks of astronomers was a pregnant period in the outgrowth of our science, with the relativity theory approaching the prime of its life; and Freundlich with his mathematical background was particularly well prepared to follow its implications. Somewhat to the consternation of his more conservative contemporaries (including his director, Herrmann Struve), Freundlich joined with enthusiasm the early protagonists of relativity and eventually wrote one of the best expository text-books on it at that time (*Einstein's Relativitäts-Theorie*, Berlin, 1917), an achievement which earned him a lifelong friendship with Sir Arthur Eddington.

Not only did Freundlich set out to defend relativity on theoretical grounds, but he was also one of the first to undertake its experimental verification. To this end, with the support of the Prussian Academy of Sciences, he embarked (with Zurhellen) on the first solar eclipse expedition to measure the light deflexion in the gravitational field of the Sun in Russia, in August 1914. This effort unfortunately came to naught, for the outbreak of the First World War a few weeks before the date of the eclipse caused all equipment to be impounded and the astronomers interned. It was many months before the luckless travellers were eventually repatriated; and the actual detection of the relativistic light deflexion had to await the outcome of the Greenwich expeditions in 1919.

The first decade which followed the First World War marked the high noon in Freundlich's life. It brought him in close touch with Einstein, then himself at the peak of his powers (the large amount of unpublished *Einsteiniana* in Freundlich's possession should constitute invaluable material for future historians of science); and it was largely his efforts and close contacts with German industrial circles which led in 1921 to the foundation, in Potsdam, of the Einstein Institute with its solar tower (later renamed the Institute for Solar Research and amalgamated with the observatory), of which Freundlich became the first director. The work of the distinguished group of investigators (including von den Pahlen, von Klüber, Grotrian, Wurm and others) which Freundlich gathered around him is too well known to need elaboration here. In a study of the *K*-effect, von den Pahlen and Freundlich came (by their detection of the double-wave of the *K*-term in longitude) within an ace of the discovery of galactic rotation. The eclipse expedition to Sumatra of 1929 made up for the abortive one of 1914. and, as a result, Freundlich, von Klüber and von Brunn established the light deflexion at the limb of the Sun to be $2{\cdot}2'' \pm 0{\cdot}1''$—somewhat more than the theoretical prediction of $1{\cdot}75''$. Subsequent work has, on the whole, tended to cluster around the larger value; and this discrepancy (if finally established) may possibly play a similar part in the fate of the relativity theory in years to come as did the secular advance of Mercury's perihelion in Newtonian mechanics.

The advent of the Nazi Government to power in Germany in 1933 interrupted Freundlich's work; and because of his partly Jewish origin he left Potsdam to accept the professorship of astronomy in the University of Istanbul. He remained there for three years (the present Istanbul University Observatory was erected under his direction), and left in 1936 for a similar post in the German University of Prague. The Munich agreement and its aftermath uprooted Freundlich once again after little more than two years of residence in a congenial city and State. This time (declining an invitation to America), at Eddington's initiative he resorted to his Scottish motherland and the University of St. Andrews, where he spent the rest of his active life.

Shortly before that time, the University received a bequest from the late Prof. Gordon Lang for the estab-

lishment of a new university observatory; and Freundlich was entrusted with its erection as the observatory's first director. The outbreak of the Second World War interrupted this work almost immediately; and in the grim years that followed it took all of Freundlich's energy and determination to carry out his mission in the face of protracted material and man-power shortages—not to speak of perennial financial problems caused by the post-war inflation spiral. As the principal instruments for the new observatory—in keeping with St. Andrews's Gregory tradition—Freundlich wished to erect reflectors of the most modern type—the flat-field Schmidts. In collaboration with Linfoot and Waland, he succeeded in commissioning the 19-in. pilot model in regular service in 1949; but it was one of the disappointments of his life that he was unable to render the full-scale 39-in. flat-field Schmidt (the largest telescope of this type in the world) fully operational before his retirement.

On reaching the age of seventy in 1955, Freundlich retired from the Napier chair of astronomy at St. Andrews, but retained directorship of the observatory until 1959, when he finally left Scotland for his native Wiesbaden to spend his years in 'retirement'. If his middle age was often stormy, the evening of his life was calm. In a sense, however, Freundlich never really retired; for until he died he held an honorary professorship in the University of Mainz and his mind continued to remain active. His last book on *Celestial Mechanics* appeared in 1958. In the last decade of his life, Freundlich was much preoccupied with astronomical red shifts and, in particular, with the fate of the photons which may travel from distant galaxies for hundreds of millions of years through empty space to reach us. Do they retain their original energy unimpaired? His last paper on this subject appeared in the *Zeitschrift für Astrophysik* in February of this year.

Death came gently on July 25 to claim the life of a veteran of almost four score years; and his passing is mourned not only by his family but by two generations of grateful students to whom Freundlich gave generous help in various stages of their careers in Germany, Turkey, Czechoslovakia or Britain, and many of whom are scattered all over the world. They will indeed remember him long as a man of forthright character and wide culture (he was an accomplished musician), whose tall stature, unbent by age, and leonine head towered in any gathering over most of his contemporaries. He was always forthright in expressing his views—there was nothing of a politician in him—a true liberal of old vintage and of the type who instinctively evoked also strong feelings in others; very few people who ever met him at a close range remained indifferent to his personality. What greater tribute can a man be paid?

He is survived by his wife, Mrs. K. Freundlich, who shared with her husband more than half a century of common life through troubled times; and by his two children and many grandchildren who have made their home in Britain.

Zdeněk Kopal

NEWS and VIEWS

The Royal Society of London: Medal Awards

The following awards of medals have been made by the President and the Council of the Royal Society: *The Copley Medal*, to Prof. S. Chapman, advisory scientific director of the Geophysical Institute, University of Alaska, for his theoretical contributions to terrestrial and interplanetary magnetism, the ionosphere and the aurora borealis; *The Rumford Medal*, to Prof. H. C. van du Hulst, professor of astronomy in the University of Leiden, for his distinguished work on the scattering processes in the interplanetary medium and his prediction of the 21-cm spectral line from interstellar neutral hydrogen; *The Davy Medal*, to Prof. M. Calvin, professor of chemistry in the University of California and director of the bio-organic chemistry group in the radiation laboratory, for his pioneering work in chemistry and biology, particularly his elucidation of the photosynthetic pathway for the incorporation of carbon dioxide by plants; *The Darwin Medal*, to Prof. K. Mather, professor of genetics in the University of Birmingham and Vice-Chancellor elect of the University of Southampton, for his distinguished contributions to knowledge of cytology and genetics; *The Sylvester Medal*, to Dr. Mary L. Cartwright, Mistress of Girton College and University reader in the theory of functions in the University of Cambridge, for her distinguished contributions to analysis and the theory of functions of a real and complex variable; *The Hughes Medal*, to Prof. A. Salam, professor of theoretical physics at the Imperial College of Science and Technology in the University of London, for his distinguished contributions to quantum mechanics and the theory of fundamental particles.

Director of G.P.O. Research: Mr. R. J. Halsey, C.M.G.

Mr. R. J. Halsey, who retires from the post of director of research at the General Post Office on December 31, was born at Portsmouth in 1902, and was educated at the City and Guilds College on a Royal Scholarship. He took first-class honours degree and diplomas of both the City and Guilds and Imperial College of Science and Technology. He entered the Post Office Engineering Department in 1927, and, until his appointment as assistant engineer-in-chief in 1953, worked at the Post Office Research Station on line transmission. He was in charge of the work that led to the laying of the first-ever repeatered submarine cable, between Anglesey and the Isle of Man, in 1943. As assistant engineer-in-chief he led the Post Office project team on the first Transatlantic telephone cable. He became director of research in 1958, and in 1959 also a director of Cable and Wireless, Ltd. In 1957 he was made a Companion of the Order of St. Michael and St. George.

Dr. G. H. Metson

Dr. G. H. Metson, who has been appointed to succeed Mr. Halsey, was born in London in 1907 and joined the Post Office in 1925 as a youth-in-training. His early years with the Post Office were spent in the physics laboratory working on spectrography. Later he transferred to Northern Ireland, where he carried out research on magnetron oscillations and earned his M.Sc. and Ph.D. at Queen's University, Belfast. During the War he served with the Royal Corps of Signals, and won the M.C. for gallantry. In 1946 he was back at Dollis Hill as a principal scientific officer in charge of the Thermionics Group set up to study the causes of valve failure. Later his Division started to design and manufacture very-long-life valves. He was awarded a D.Sc. in 1958, and in 1962 received the Institution Premium, the highest award made by the Institution of Electrical Engineers, for a paper on an electrical engineering subject. He was appointed deputy director of research in 1962.

Plant Physiology at the University of Sussex: Prof. J. F. Sutcliffe

Prof. J. F. Sutcliffe, who has been appointed professor of plant physiology in the School of Biological Sciences in the University of Sussex, is at present professor of botany at King's College, University of London. Prof. Sutcliffe obtained first-class honours in botany in the University of Leeds in 1947, after his undergraduate career had been interrupted by war service in the Fleet Air Arm. His interests in the solute relationships of plants became evident in the work that he did for his doctorate on the effects of sugar and potassium on the extension growth of roots. Since his move to King's as an assistant lecturer in 1949, he has made the problems of salt uptake his own particular field, in which he is now established as a world authority. He has made many valuable and fundamental contributions to our knowledge of the mechanism of ion uptake, and has written a text-book for university students which is the best of its kind yet to appear. The calibre of his work is shown by the early award of the D.Sc. of the University of London in 1960. He spent one year in the laboratory of Prof. F. C. Steward at the University of Cornell as Rockefeller Foundation Fellow, and it was there that he turned his attention particularly to the coupling of salt uptake with protein metabolism. Prof. Sutcliffe has always been in the forefront of biological society activities. He has been the botanical secretary of the Society of Experimental Biology since 1960, and has served on the Membership Committee of the Institute of Biology since 1961. He was recorder of the Cell Physiology Section at the tenth International Botanical Congress at Edinburgh in August last. He is no narrow physiologist; his wide knowledge and interest in all aspects of plant biology are evident from the fact that he has been president of the Croydon Natural History Society since 1963. In addition to his reputation as a scientist, Prof. Sutcliffe will bring to Sussex a vigour and enthusiasm for both teaching and research which will be an invaluable asset to a new University, particularly in the early days of its development.

Inorganic Chemistry at Queen Mary College, University of London: Prof. D. C. Bradley

Prof. D. C. Bradley, senior professor of chemistry at the University of Western Ontario, Canada, has been appointed as from January 1, 1965, to the chair of inorganic chemistry left vacant by the resignation of Prof. J. Chatt, who has taken a post at the University of Sussex. Prof. Bradley graduated at Birkbeck College, London, where he started research with the late Prof. W. Wardlaw, and was successively lecture demonstrator, assistant lecturer and lecturer. In 1959 he was invited to his present post in Canada, where his responsibilities for the teaching of inorganic chemistry have included the reorganization of courses for the B.Sc. honours degree and the initiation of graduate courses for the M.Sc. and Ph.D. degrees. He has also taken part in general university administration and served on a committee of the Defence Research Board, Department of National Defence, Ottawa. Prof. Bradley's research has developed from his original work on zirconium alkoxides into a general study, by both synthetic and structural methods, of the alkoxides of a wide range of metals. Recently it has been extended to the dialkylamino derivatives of metals of groups IV, V and VI and to inorganic polymers containing transition metal atoms bonded to oxygen, sulphur, nitrogen or phosphorus. It is described in more than eighty scientific papers and many review articles, which have won him international recognition in his chosen field. He returns to the University of London at a time when long-awaited plans for the modernization and expansion of facilities for chemistry are beginning to materialize, and he should find considerable scope in the new buildings now being constructed at Queen Mary College.

The Colwyn Medal

The Colwyn Medal for 1964 is to be awarded to Dr. G. F. Bloomfield, international co-ordinator of Technical Advisory Service, Malayan Rubber Fund Board. The Medal, given by the late Lord Colwyn, a former president of the Institution of the Rubber Industry, is awarded yearly for services of a scientific or technical nature with a bearing on the rubber industry. Dr. Bloomfield began his career in rubber with the Rubber Growers' Association in 1932. After the War he was attached to the Rubber Research Institute of Malaya for some years, assisting in the introduction of the latest research techniques and in the Institute's rehabilitation after the Japanese occupation. He has published many contributions to the subject of rubber research and technology.

Indian Institute of Technology, Delhi

The Indian Institute of Technology at Delhi is the fifth of its kind to be set up in India with foreign aid. Already there is an Institute at Kharagpur which has been mainly equipped by American aid, one at Bombay which has been sponsored by the U.S.S.R., another at Madras sponsored by West Germany, and a fourth at Kanpur which is being equipped by the United States. When it became known six years ago that India was contemplating a College of Engineering and Technology at New Delhi, it was felt that here was an opportunity for H.M. Government to co-operate with British industry in a gesture which would be of long-term advantage to India and Great Britain. In order to meet the cost of British manufactured equipment required by the College, an appeal was launched by Sir Hugh Beaver, the then president of the Federation of British Industries, to which British industry responded with donations totalling more than £260,000. H.R.H. The Duke of Edinburgh laid the foundation stone in January 1959, and the College opened in 1961 with an initial intake of 150 students. In 1963 it was granted independence to confer its own degrees and was renamed the Indian Institute of Technology. It aims eventually to provide five-year residential courses for 1,250 undergraduates and research facilities for 300 postgraduates. At present the teaching staff includes eight British professors and further appointments from the United Kingdom will be made next year. With the attainment of Institute status the equipment programme has been extended. In response to a second appeal launched in December 1963 by Sir Cyril Harrison, chairman of the sponsors and immediate past-president of the Federation, contributions and promises of further support from British industry now exceed £55,000 and it is hoped that an additional £95,000 will be raised by the end of March 1965. (See also *Nature*, 200, 735; 1963.)

Loans of Scientific Apparatus to Schools

Dame Kathleen Lonsdale, professor of chemistry in University College, London, writes: "The Chemistry Department of University College, London, recently made it known, through the Association for Science Education, that certain surplus apparatus, which included potentiometers, resistance boxes, galvanometers and *p*H meters, could be applied for by school science teachers and would be made available on indefinite loan. The result was that nearly ninety applications were received from all over Great Britain, from grammar, technical, secondary modern and independent schools, and a great many teachers had to be disappointed. It seems probable that other colleges and industrial research departments may have, stored away in cupboards, apparatus that they would not like to destroy and cannot continue to store, but for which they have no immediately foreseeable use. The Association for Science Education is willing to act as a clearing house for offers of such apparatus, which

should be in reasonably good or repairable order. Those who are prepared to give or lend apparatus to schools should send a list of what is available to the General Secretary, Association for Science Education, 52 Bateman Street, Cambridge, and should specify what arrangements could be made for its direct handing-over or delivery to those schools which might apply to the Association for it as a result of subsequent publicity".

The Environmental Group

The inaugural meeting of the Environmental Group will be held at the Imperial College of Science and Technology on December 4. The Group has been formed to provide a forum for the discussion of generalized environmental problems. The discussions and meetings of the group are intended to disseminate information, to assist in providing 'feed-back' of information and to promote more fruitful co-operation between the various professions. Membership of the Environmental Group will be open to all those professionally interested in the environment created by buildings. The members of the provisional committee are: *Chairman*, P. Burberry; *Deputy Chairman*, J. S. McCulloch; *Secretary*, P. A. Jay; *Members*, A. Aldersey-Williams, A. Benjamin, T. A. Markus, D. Moizer, D. Poole. Further information can be obtained from Peter Jay, 16 Gloucester Place, London, W.1.

The Classification Society

At a meeting on April 17, 1964, a new society, the Classification Society, was founded, the aims of which are to promote co-operation and interchange of views between those interested in the principles and practice of classification in a wide range of disciplines. In order to carry out these aims it is proposed to produce a bulletin containing references to (and, if possible, summaries of) publications of interest to members, and to arrange meetings. The foundation of the Society follows the holding of a symposium, organized by 'Aslib' on April 6, 1962, entitled "Classification: an Inter-disciplinary Problem", at which it became clear that there are many aspects of classification common to such widely separated disciplines as biology, librarianship, soil science and anthropology, and that opportunities for joint discussion of these aspects would be of value to all the disciplines concerned. Copies of the report of the symposium (price 5*s*.) can be obtained from Aslib, 3 Belgrave Square, London, S.W.1. The annual subscription to the Society is £1, entitling members to receive the *Bulletin* free of charge and to attend and vote at meetings. Further particulars, and a membership application form, can be obtained from the secretary-treasurer, Mr. J. S. L. Gilmour, University Botanic Garden, Cambridge.

Geological Survey of Great Britain and the Museum of Practical Geology

The summary of progress of the Geological Survey of Great Britain and the Museum of Practical Geology for 1963 reports that an area of 1,091 square miles of field survey on the 6-in. scale was completed during the year (Pp. iii+99. London: H.M.S.O., 1964. 6*s*. 6*d*. net). Details are given of the many research activities, in field and laboratory, and of the practical application of the results to many economic and industrial problems. Preliminary research on the geochemistry of British shales shows that many of these rocks have an unusually high natural radioactivity. Since 1955 the Survey has carried out aeromagnetic surveys to investigate changes in the strength of the Earth's magnetic field; the results have provided much information concerning concealed geological structures. A new series of the very useful *Water Supply Reports* is being published to include recent research and records of underground water. Much revision work has been carried out in the Museum, and attendance at lectures and demonstrations reached a total of 15,665.

Institution of Civil Engineers

In his presidential address to the Institution of Civil Engineers on November 3, Mr. R. M. Wynne-Edwards was critical of the Trend Committee's proposals regarding the Department of Scientific and Industrial Research. He thought that the present independence of the Building Research Station and the Road Research Laboratory was an important factor in their value to the community, and that it would be a grave error to transfer them to executive ministries—a view which also found expression in the last report of the Road Research Laboratory. Mr. Wynne-Edwards also stressed the importance of determining the most effective way in which the Institution could make its views known to the Government, and expressed the opinion that one of the most urgent needs was to make full use of British civil engineering knowledge in the underdeveloped countries. To his knowledge, the Department of Technical Co-operation had never yet asked the Institution for its views on any subject. He also instanced the imaginative civil engineering schemes for Morecambe Bay and the Solway as examples of schemes which might fail, not on their merit but for lack of public support, because the community which had to provide the money was insufficiently informed. This demonstrated the need for the Institution to encourage independent thought in these and other topical matters and to find effective means of making them known. There was no room for petty-mindedness or rivalries and he regarded the federation of 13 major professional institutions in the Engineering Institution's Joint Council as a major step towards working together and thinking, speaking and acting as a united profession. Further, the productivity of the construction industries was an immediate challenge to the profession as well as to industry, for in this expenditure and its expansion the engineer and the architect held key positions and were the essential creative force. Whatever reasons might exist for divisions in the construction industry, the profession had a common task in producing the best possible design. He suggested that the engineering content in the construction of a building increased proportionately as its design was adapted to the special needs of its users.

Financial and Management Accounting

United Steel Co., Ltd., has published a lucid account of its financial and management accounting practices. The role of financial accounts in providing a record of the transactions of a business and as a means of conforming to statutory requirements is well presented, but the particular appeal of the booklet lies in the way financial accounts are used as the basis of management accounting in the Company. Current transactions are analysed and compared either with past performance or with predetermined estimates of current performance, in order to provide management with an instrument for the control of efficiency and for future planning. Costing, budgetary control and similar applications of accounting data for the control of industrial activities are well described. The publication would be of considerable use to all accountants who fail to use the information they produce for control purposes and invaluable to all managerial staffs who need to have more than a nodding acquaintance with financial and management accounting; no clearer presentation of this difficult subject has been made. Copies can be obtained, free of charge, from the Secretary, United Steel Co., Ltd., Sheffield.

Radioisotopes, Labelled Compounds and Radiation Sources

The recent edition of the catalogue of radioactive products, issued by the Radiochemical Centre, Amersham, is of greatly enlarged size and scope (*Catalogue of Radioactive Products: Radioisotopes—Labelled Compounds—Radiation Sources*. Pp. 164+13 plates. Amersham: The

Radiochemical Centre, 1964). Two thousand items are listed, and there is a new medical section comprising 76 radioactive chemicals, prepared and tested to pharmacopœial standards, which are in some measure of routine use in medical diagnosis or therapy. In the other sections the classification follows that of previous editions. The facilities are grouped under the headings: radioisotopes and labelled compounds; labelling, irradiation and other services; radiation sources; and radioactive material for instrument calibration. An index and some general information on ordering, registration, packaging and payment, are included. The Centre does not sell radium except in quantities of less than 5 mg, and in the United Kingdom all radium is issued on loan under the terms of a written agreement specifying an annual hire charge, payable six-monthly. The general section on radiochemicals has 1,125 entries, 390 more than in the 1962 edition. Fifty isotopes, mostly cyclotron-produced materials, are listed for the first time. Primary isotopes and labelled compounds are grouped together in a single alphabetical list, the primary isotopes in capital letters. A general trend to labelling at higher specific activities is evident, particularly with compounds with wide biochemical interest such as amino-acids and sugars. Some of the tritium-labelled compounds approach theoretical maxima of specific activity. The number and variety of radiation sources have increased substantially, and the section dealing with these sources consists of two parts—sources for clinical use and sources for the laboratory and factory. Each entry includes a summary of the relevant nuclear data, a description of the method of construction amplified by an illustration where appropriate, and the methods used for testing and measurement. The list of sources for radiotherapy includes new patterns of cobalt-60 ophthalmic applicators, cæsium-137 needles for interstitial use, and improved flexible β-emitting sheet material. For laboratory and industrial use there is an increased range of sources containing americium-241; sources of cæsium-137 for radiography, level gauging or laboratory instruments; and of strontium-90 for thickness gauging, in a ceramic form especially resistant to accidental damage and to high temperatures; and the recently developed cobalt-57 sources for Mössbauer experiments. Technical information on each item in the catalogue is of necessity brief, but more detailed information is given in *The Radiochemical Manual* and in the *Technical Bulletins* issued by the Centre.

X-ray Crystallography

An international summer school in "X-ray Crystallography" was held at Peterhouse College, Cambridge, during August 31—September 12. The course was arranged with twenty one-hour lectures and twenty two-hour periods of exercises involving only drawings and interpretations. Roughly half the work was devoted to the various types of X-ray diffraction photographs, their interpretation and proper use, and the second half to diffractometry and physical aids, other than X-rays, in determining crystal structures. The course was planned so that someone who had little knowledge of many aspects of X-ray crystallography would be able to follow most of the subject-matter. It was also arranged that the most recent developments in each of the matters dealt with were described. The students came from Belgium, Finland, France, Italy and Norway and they all had some knowledge of a part of the subject. Such a course seems appropriate for students of this kind because it enables them to obtain a more comprehensive view of X-ray crystallography. The lectures and demonstrations were given by Dr. W. A. Wooster.

National Institute of Oceanography

Volume 11 of the National Institute of Oceanography's Collected Reprints (published by the Institute, at Godalming, Surrey) contains 62 papers and articles. Classified according to subject, 30 are physical, 20 are biological, six are on the geology or geophysics of the sea-bed, two are chemical and four are general. There are no fewer than 14 papers on waves, providing evidence that this is still one of the main lines of research of the Institute and one in which its workers are keeping in the forefront of activity. Most of these papers deal with the observational and analytical techniques for studying the spectra of ocean waves, particularly the directional spectra. A number of other physical papers are concerned with long waves, internal waves, tides and mean sea level, including one on a comparison of the geodetic reference levels of England and France using oceanographic methods. Among the papers on biological subjects, including fisheries, there is a group of five on whales and whaling (a long-standing interest of the Institute), and six on particular species or groups of marine animals. Several papers describe improved apparatus and techniques, including nets and their closing mechanism. A further group of papers deals with the use of acoustic techniques for the study of various features of the sea bed and of the sediment and rocks below the bottom. The Institute's new research vessel, R.R.S. *Discovery*, was commissioned in December 1962, and the present volume includes descriptions of the ship and her scientific equipment.

Veterinary Non-proprietary Names

The *British Veterinary Codex* Revision Committee has adopted the following non-proprietary names for the veterinary substances indicated:

Non-proprietary name	Other names
Amlcarbalide	*NN'*-di(*m*-amidinophenyl)urea; 3,3'-diamidinocarbanilide. 'Diampron' is the isethionate.
Amprolium	1-(4-amino-2-propylpyrimidin-5-ylmethyl)-2-methylpyridinium chloride. The hydrochloride is an ingredient of 'Amprol', 'Amprolmix', and 'Pancoxin'.
Diminazene	*pp'*-diamidinodiazoaminobenzene. The aceturate is an ingredient of 'Berenil'.
Metriphonate	dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate; chlorofos; trichlorphon; Bayer *L*13/59; 'Dipterex'; 'Dyvon'; 'Neguvon'; 'Tugon'.
Naphthalophos	*N*-(diethoxyphosphinyloxy)naphthalimide; 'Rametin'.
Thenium	*NN*-dimethyl-*N*-(2-phenoxyethyl)-*N*-(2-thenyl)- ammonium. The closylate is an ingredient of 'Ancaris' and 'Canopar'.
Tylosin	An antibiotic derived from an actinomycete resembling *Streptomyces fradiae*. 'Tylan'.

The non-proprietary names are reported to be free from conflict with trade marks registered in Great Britain and Northern Ireland, and these names, or names resembling them, will not be registered as trade marks for pharmaceutical products or drugs in those countries. Some of the names, other than the chemical names, appearing in the second column above are registered trade marks. The adoption of a non-proprietary name does not necessarily imply that the *British Veterinary Codex* Revision Committee recommends the use of the substance in veterinary medicine or that the substance will be included in the *British Veterinary Codex*, although, if a substance is included, it is intended that the non-proprietary name shall be the title of the monograph. The *British Veterinary Codex* Revision Committee has undertaken, at the request of the Association of the British Pharmaceutical Industry, to provide non-proprietary names for veterinary products, and all requests from manufacturers and other interested persons for the provision of such names should be addressed to the Secretary, *British Veterinary Codex* Revision Committee, the Pharmaceutical Society of Great Britain, 17 Bloomsbury Square, London, W.C.1.

Amendment to List No. 1 (*Nature*, 202, 136; 1964):

Diaveridine	This is an ingredient of 'Darvisul'.

Kootenay National Park, Canada

Kootenay National Park is an area of exceptional scenic beauty consisting of 'rows' of mountains alternating with open valleys. It stretches for more than 60 miles parallel to the north-west-south-east trend of the Rocky Mountains, and, along its width, crosses various mountain

ranges and valleys in a series of 'jogs', from the divide leading to the Bow River valley on the north-east to Columbia River on the south-west. It lies wholly within the Province of British Columbia and consists of four different segments: the northern end comprises the drainage basin at the head of Vermilion River; the middle section is a belt at right angles to the first, following the Vermilion River across the general trend of Vermilion Range and extending into the Mitchell Range in the south-west; the main southern part of the Park is a strip along the Kootenay River valley; and a fourth part includes a 'jog' to westward, which takes in the valley of Sinclair Creek nearly to its junction with Columbia River. An attractive guide to the Park has recently been prepared by the Geological Survey of Canada Department of Mines and Technical Surveys, Ottawa, as Miscellaneous Report No. 9 (*Kootenay National Park—Wild Mountains and Great Valleys*, by David M. Baird. Pp. vi + 94. Ottawa: Queen's Printer, 1964. 1.50 dollars).

Irish Tubificidae

THE study of the Irish Tubificidae has been largely neglected since the publication of a check list by Southern in 1909. The only other person to have concerned himself with them was Friend, whose taxonomic investigations and identifications are in general considered not to be wholly reliable. A complete revision of the Irish tubificids has long been overdue and, in a paper by C. R. Kennedy, of the Department of Zoology, University of Liverpool, an attempt is made to bring knowledge of them up to date and into line with the European species (*Proceedings of the Royal Irish Academy*, 63, Section B, No. 13. Dublin: Hodges, Figgis and Co., Ltd., 1964. 2*s*. 6*d*.). The literature pertaining to the Irish tubificids is reviewed and, in addition, the results of an examination of Southern's specimens are presented together with information gained from visiting sites and making further collections. It has proved possible to confirm or correct the identity of most of the earlier records by an investigation of their descriptions, by examination of specimens or by both, and to bring the nomenclature up to date. As a result of this survey, seventeen species are now recognized as existing in Ireland, six of which are new Irish records. All of these are encountered, more or less commonly, in Britain.

New Medicines

ACCORDING to surveys conducted by Research Services, Ltd., 89 per cent of general practitioners consider that new medicines have been of great importance in recent medical advances in the control of disease, and 80 per cent of both general practitioners and the general public feel that it would be a serious matter if lower profits for pharmaceutical manufacturers were to reduce their work in developing these new medicines (*Attitudes towards the Pharmaceutical Industry amongst Family Doctors and the General Public*, 1962 and 1964). The public in general tended to overestimate the amount spent on National Health Service medicines. Of every £100 of National Health Service expenditure, about £13 went on medicines (in general practice and in hospital); two-thirds of the general public believed the figure to be more than £20, and two-fifths believed it to be more than £30. Moreover, although the proportion of total National Health Service costs spent on medicine has remained constant since 1959, 81 per cent of the general public and 67 per cent of general practitioners had the impression that it was increasing. General practitioners were asked what sources of information they relied on to keep up to date with pharmaceutical discoveries. 71 per cent named articles in medical journals; 36 per cent manufacturers' representatives; 25 per cent discussion with colleagues; 24 per cent manufacturers' literature through the post; 21 per cent the Government-sponsored publication, *Prescribers' Journal*; and 18 per cent advertisements in medical journals. Refresher courses, medical society meetings, the *Monthly Index of Medical Specialities*, discussions with pharmacists, hospitals and consultants were among other sources of information mentioned. The Association of the British Pharmaceutical Industry has a code which bans undesirable marketing practices among its member companies. This was revised recently after consultation with the British Medical Association. Two-thirds of the general practitioners interviewed could not provide examples of cases they felt should have been prevented by this code. The most commonly mentioned type of undesirable practice was price-cutting, mentioned by 5 per cent of doctors. Many of the individual instances related to events which occurred before the existence of the code. 89 per cent of the public said they had confidence in the safety of medicines prescribed for them by the doctor; only 4 per cent said they had not. The reasons given by the majority indicated an overwhelming confidence in their doctor, 71 per cent saying that their confidence was based on faith in their doctor and his prescribing. Copies of the survey are available from the Office of Health Economics, 62 Brompton Road, London, S.W.3.

Announcements

PROF. F. S. DAINTON, at present professor of physical chemistry in the University of Leeds, has been appointed to succeed Dr. B. L. Hallward as Vice-Chancellor of the University of Nottingham as from October 1965.

A MEETING of the Basic Science Section of the British Ceramic Society on "Physics and Chemistry of Ceramic Surfaces" will be held in London during December 15–17. Further information can be obtained from Dr. J. P. Roberts, Houldsworth School of Applied Science, the University, Leeds 2.

A CONFERENCE entitled "A Scientific Basis of Maximum Permissible Levels", arranged by the British Institute of Radiology, will be held in the Edward Lumley Hall, Royal College of Surgeons, London, W.C.2, on December 8. Further information can be obtained from the British Institute of Radiology, 32 Welbeck Street, London, W.1.

THE second annual conference on "Solid-State Physics", arranged by the Institute of Physics and the Physical Society, will be held at the H. H. Wills Physics Laboratory, University of Bristol, during January 5–8. Further information can be obtained from the Administration Assistant, Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

Two joint meetings of the Western Section of the Society for Analytical Chemistry and the Cardiff and District Section of the Royal Institute of Chemistry will be held at the College of Technology, Newport, and University College, Cardiff, on November 27 and December 11, respectively. At the first meeting, Dr. T. S. West (Imperial College of Science and Technology) will present a paper entitled "Complexometric Methods in Analytical Chemistry", and at the second, Dr. E. V. Truter (University of Leeds) will speak on "Thin-layer Chromatography". Further information can be obtained from the Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1.

A CONFERENCE on "Optics in Process Control", organized by the Optical Group of the Institute of Physics and the Physical Society, will be held at the Manchester College of Science and Technology during January 6–8. The main topics of discussion will include: optical monitoring and inspection; metrology and machine tool applications; optical methods of analysis; control of photographic processes; tracking and ranging devices; optics in the semiconductor industry; optics in computer technology. Further information can be obtained from the Administration Assistant, Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

UNIVERSITY FINANCE IN BRITAIN

IN issuing *Returns from Universities and University Colleges in receipt of Exchequer Grant, Academic Year 1962–1963**, the Universities Grants Committee, in contrast to the practice of the previous four years, has omitted a general survey of its activities: a separate publication is promised later in the year.

The number of full-time students at university in Britain increased to 119,004, compared with 113,143 in 1961–62, and of these 17,868 were in the Universities of Oxford and Cambridge, 23,338 were in the University of London, 49,464 in other English universities and colleges, 7,761 in the University of Wales and 20,573 in the Scottish universities. There were 13,375 students from overseas, 7,602 of whom were from within the Commonwealth (6,893 of whom were postgraduate); since 1953–54 the proportion of overseas students has risen from 9·9 per cent to 11·3 per cent. Of the full-time students, 29·1 per cent were in colleges or halls of residence, 51·6 per cent in lodgings and 19·3 per cent at home; these figures compare with 28·1, 40·9 and 31·0 per cent, respectively, in 1953–54. The proportions continue to vary widely in different universities and for men and women: in Scotland only 9·4 per cent of men and 18·5 per cent of women are in colleges or halls of residence, while 45·9 per cent of men and 42·1 per cent of women are at home.

Of the 87,654 full-time men students, 24·2 per cent were in arts and 11·8 per cent in social studies; 27·9 per cent in science; 19·0 per cent in technology and 10·9 per cent in medicine; for the 31,350 women students the corresponding figures are: 52·2; 10·4; 23·4; 1·1; and 9·7. Of the 20,793 postgraduate students, 3,755 were taking postgraduate courses in teacher training; the remainder were distributed as follows: arts, 3,691; social studies, 2,144; science, 6,323; technology, 3,027; medicine, 1,251; and agriculture, 429. Of 33,677 full-time students entered for the first time in 1962–63, 32,147 were reading for a first degree and 1,530 for a first diploma; 9,721 were women; 10,791 were in arts and 4,339 in social studies; 9,224 in science; 4,965 in technology and 2,756 in medicine. Over the past 10 years the increases in science and technology have been proportionally almost twice as great as in arts.

The proportion of State-assisted students was 88·4 per cent compared with 87·1 per cent in 1961–62 and 71·9 per cent in 1953–54, ranging from 93·2 per cent for Wales, 92·8 per cent at Cambridge, 90 per cent at Oxford, 92·2 per cent at other English universities and colleges (excluding London) to 82·8 per cent for Scotland. There were 16,424 part-time students (4,890 from overseas) and of those 12,768 were in English and 3,429 in Scottish universities. Of the 23,886 first degrees obtained, 16,435 were honours degrees; of the total 29·2 per cent were in science, 12·5 per cent in applied science, and 8·3 per cent in medicine. Full-time teaching and research staff, excluding Oxford and Cambridge, increased to 13,801 (12,786 in 1961–62) and 27·6 per cent are now in science, 14·3 per cent in technology, 16·2 per cent in medicine and 8·7 per cent in social studies; 11·7 per cent were professors, 6·6 per cent readers, 12·7 per cent senior lecturers and 46·7 per cent lecturers. Including Oxford and Cambridge the full-time academic staff totalled 14,132.

Of the recurrent income of £86,402,542 (£74,112,891 in 1961–62), £60,599,826 was from Treasury grants (70·1 per cent) and £655,150 from grants from Government departments; £8,575,430 (9·9 per cent) from fees; £2,087,756 (2·4 per cent) from endowments; £1,636,623 (1·9 per cent) from local authority grants; £506,820 from donations and subscriptions; £9,522,729 (11·0 per cent; £8,229,163 for 1961–62) from payments for research. For English, Welsh and Scottish institutions the respective totals are: £69,578,663; £4,547,139; and £12,276,940. Non-recurrent Treasury grants amounted to £36,481,214 (£28,638,256 in 1961–62) and of this £22,773,670 was in respect of building work, £3,795,906 professional fees, £8,493,852 furniture and equipment and £1,417,786 site and property purchases. Of the recurrent expenditure of £85,379,145 (£74,119,781 in 1961–62), 6·7 per cent was on administration, 41·9 per cent on salaries and superannuation of teaching staff, 12·0 per cent on departmental wages (technicians and laboratory assistants), 14·5 per cent on departmental and laboratory maintenance, 3·0 per cent on repairs and maintenance of buildings and 11·0 per cent on rates, insurance, heat, light, etc.

* University Grants Committee. *Returns from Universities and University Colleges in receipt of Exchequer Grant, Academic Year 1962–1963.* Pp. v+46. (Cmnd. 2456.) (London: H.M. Stationery Office, 1964.) 5*s*. 6*d*. net.

RHEOLOGICAL RESEARCH IN BRITAIN

THE science or engineering graduate who enters industry has some understanding of the processes of deformation and flow in solids and liquids, but this is usually restricted to the cases of ideally elastic solids, ideally plastic solids and Newtonian liquids. His knowledge of the creep and plastic flow in real solids and of the elastic and thixotropic effects displayed by non-Newtonian liquids is rudimentary. An understanding of these complex phenomena is essential, however, to the successful development of many industrial processes and to the manufacture of finished products with optimum mechanical characteristics. The situation would be improved by a greater emphasis on rheological topics in university courses, although it may be argued that the experimental techniques and mathematical theory involved are too specialized for extensive treatment in undergraduate teaching.

It cannot be said, however, that improvements in formal teaching will solve the problem entirely, because many of the problems confronting the industrial rheologist involve questions which are still matters of active research; and information on these, if published at all, appears scattered over an enormous range of technical, scientific and mathematical journals. In this connexion, the British Society of Rheology has for many years carried out a useful function by bringing together scientists from industry, the research associations and the universities in order to discuss problems of mutual interest in this field. It has, nevertheless, become apparent that in some directions research is being duplicated by different firms and institutions while in other directions it is almost entirely neglected. The Society, therefore, decided in 1963 to extend its activities by making information on active work more readily available, by ascertaining those directions in which further research is urgently required and by encouraging projects designed to fill the gaps in existing knowledge. It was a fortunate coincidence that at the same time the National Engineering Laboratory was considering the publication of a survey of rheological research in Britain, and the Society was able to co-operate in this work. The

survey has now been published as *Rheological Research in Britain, 1964**. It presents the information obtained by sending out nearly 600 copies of a questionnaire, and the replies give a clear picture of the very large amount of research in progress. Although only a few lines are devoted to each project, the survey runs to more than a hundred pages, being, in fact, the longest report so far published by the National Engineering Laboratory.

In this survey, a total of 226 establishments are listed, divided fairly evenly between universities, Government-owned and Government-aided institutions, and industrial laboratories. There is an index of these establishments and another of the individual research workers. A third index, devoted to materials under investigation, shows the extremely wide range of present-day rheology. It runs through from adhesives to zirconium, via bananas and barytes, coke and cream, earth and eggshells, glaciers and graphite, mud and muscle, plutonium and pesticides, semen and sewage, wheat and wood, and a hundred other substances. The tabulated information has been analysed, and a series of histograms shows the distribution of interest between materials, between phenomena, between methods of approach and between processes. The first of these shows, for example, the predominant interest of industry in polymers and multi-phase materials, while university interest is more generally distributed with some concentration on idealized materials. On the other hand, the interest in phenomena displayed by the universities runs nearly parallel with the industrial interest, although the latter is rather more concentrated on the non-Newtonian viscosity of liquids and the former on the plasticity of solids. Of the methods of approach, that of continuous shear viscometry and rheometry is the most generally used, but this is closely followed by theoretical methods and then by tensile and compressive testing. The extent of the industrial interest in the theoretical approach is quite remarkable, being equal to that shown by the universities. Another surprising feature is the smallness of the interest shown by industry in individual processes (such as extrusion, mixing, flow through pipes and channels) in comparison with the interest in fundamental phenomena.

The present survey is necessarily incomplete, and it would seem that the coverage is weakest in biology and in the study of metals. It is hoped that it will become a regular publication, and the compilers ask that anyone whose work has been overlooked should inform them so that it may be included in the next edition. In this way the survey should become a really comprehensive guide to those who may wish to seek specialized advice and to those who are concerned with the initiation of new research projects with industrial applications.

R. Roscoe

* Department of Scientific and Industrial Research: National Engineering Laboratory. Report No. 165: *Rheological Research in Britain, 1964*. (A Survey made jointly by the British Society of Rheology and the National Engineering Laboratory.) By A. Y. McLean and A. T. J. Hayward. Pp. i+103. (East Kilbride, Glasgow: National Engineering Laboratory, 1964.)

FAMILIAL TRENDS OF CANCER

SINCE January 1960, the New South Wales State Cancer Council has been conducting a survey of women treated for cancer of the breast in order to determine whether there is a higher incidence of cancer among the relatives of this group than among the relatives of a control group of the same size presumed not to be suffering from cancer. The survey is a sequel to many investigations of hereditary and familial trends of cancer which have been made in Europe, Britain and the United States during the twentieth century.

In the course of the survey carried out by Pamela Siddins*, each of 99 female patients with breast cancer and each of the 99 other women who formed the control group supplied a history of her family. The incidence of cancer among the siblings, parents, aunts, uncles, cousins and grandparents of the women in each group was determined from hospital records and from the cause of death entered on death certificates. A questionnaire was devised, which sought information concerning the age, marital status, geographical situation, socio-economic position, lactation, order of birth, and menstrual and fertility history of patients and control probands.

Incidence of cancer in family histories was taken as that existing at the time of interview. The ages of the patients included in the survey ranged from 33 years 6 months to 74 years 8 months; these consisted of 84 married women with children, 10 married women without children, and five unmarried women. Of the total number of women interviewed, only about 33 per cent were included in the survey. There were many reasons for unsuitability of probands, the chief reason being the fact that few families had lived in Australia for the required three generations. Many who thought that all their grandparents had died in Australia were later excluded from the survey when official records proved otherwise. It was soon realized that the original plan, which had been to locate all relatives back to great-grandparents, would be impracticable.

At first, the findings suggested that breast and other types of cancer occurred more often in the families of the patients with breast cancer than in those of the control probands. For analysis, the types of breast cancer encountered in the survey were divided into the following basic classes: scirrhous, encephaloid or medullary, mucous or colloid, adeno- and duct carcinoma, sarcoma, and Paget's disease of the breast. For 12 patients no histological reports were available, because of loss of records or because no biopsy (or other diagnostic surgical measure) had been performed. Adenocarcinoma and scirrhous carcinoma were the types most frequently encountered. The breast cancer probands included three twins, two belonging to a dizygotic pair and one to a monozygotic pair, but in none of the twins of these patients was there evidence of cancer. No case of cancer of the breast in males was encountered among the relatives of the probands. It occurred, however, in sisters of breast cancer probands in five families and in mothers of three probands.

Cancer occurred less frequently in similar relatives of the control probands. Controls interviewed at medical clinics were not aware that the survey was concerned with the incidence of any one disease. Patients and controls from cancer detection clinics, when asked for the illnesses and operations known in three generations of relatives, tended to emphasize the cancers rather than other diseases. Most frequently the incidence of cancer was incorrectly given by the proband—the number of cancers being exaggerated in those families where several cases were known, and underestimated in families whose attention had never been directed to it for any reason.

The information collected was analysed statistically by S. Chorlton, who found that the number of relations per cancer proband ranged from 18 to 190, with a mean of 70·5. Of these, the mean number for whom some reliable medical history was known was 50·8, or 72·1 per cent. For the controls, the average number of relations was 62·4 (range 14–127), of whom medical history was known for 48, or 77 per cent. The number of relatives with some documented medical history amounted to 5,042 (cancer group) and 4,753 (control group). Some form of cancer occurred in 87 out of the 99 families of patients with the disease—

* New South Wales State Cancer Council Publication No. 9.

breast cancer being found in 21 of these. For the controls, cancer occurred in a total of 80 families, with an incidence of breast cancer in 13.

There is a notable absence of any significant infant mortality rate due to cancer, attributable probably to poor diagnosis in earlier times. Mortality in the early age-groups, particularly in the 0–4 years age-group, is high, but, of 819 deaths of relations for both cancer and control groups, only one death from cancer is reported. This was reflected in a general reduction in the incidence of all cancers in both groups.

The mean age for detection of cancer of the breast in the proband is 47·5 years, and for those families with breast cancer in relatives it is 48·4 years. The mean ages for patients and control groups at the time of the survey were 50·1 ± 13·6 years (cancer group) and 50·4 ± 10·3 years (control group). This means that the cancer group has, on the average, lived 2·6 years since the diagnosis of their disease.

The number of cases of cancer and the types of cancer occurring in the two groups show a similar distribution, except for breast cancer. The highest rate for breast cancer among relatives occurs in maternal aunts. There is a higher incidence of breast cancers and all cancers in female relations of breast cancer patients. With regard to the effect of degree of relationship, there is a high incidence of breast cancer in persons with a coefficient of relationship of one-quarter in the patient group.

EPILEPSY IN AFRICANS

A STUDY of epilepsy in Africans in Southern Rhodesia has been undertaken by Dr. L. F. Levy, Dr. J. I. Forbes and Dr. T. S. Parirenyatwa of Harare Hospital, Salisbury, Southern Rhodesia (*Central African Journal of Medicine*, **10**, No. 7; July, 1964). In a field survey carried out in the Semokwe Reserve, 130 persons admitting to seizures were discovered in a population of approximately 17,500, and these sufferers were interviewed. A parallel group of 100 patients presenting at Harare Hospital with the complaint of seizures was also fully investigated.

The incidence of epilepsy in Africans is probably slightly higher than in Europeans, but African patients tend to present themselves for treatment only when their earning capacity becomes threatened by the disorder. Fits affecting women and children seem to be regarded by patient and parent alike as a misfortune to be accepted, and there must be large numbers of rural sufferers who have never sought medical help. Even allowing for this tolerance of fits, however, the discrepancy between the number of males (161) and females (69) in the combined study seems remarkable and contrasts strongly with the sex distribution reported by other authors.

The age of onset of the disease is similar to that found in other racial groups. Premonitory symptoms vary widely and have no special characteristics. The frequency of attacks in the rural group is interesting because it reflects the pattern of epilepsy which is uncontrolled and allowed to run its natural course. About 10 per cent of the sufferers in the Semokwe Reserve group had fits once a day or more; another 10 per cent had fits several times a month; more than half (56 per cent) had fits once or twice a month, and the remaining 24 per cent had them at intervals of once in two months or longer. A family history of fits was obtained from 65 per cent of the sufferers in the Semokwe Reserve group, but only from 20 per cent of those in the Harare Hospital group. The question of genetic factors in epilepsy should be re-evaluated in all races.

Accidents resulting from fits are common. Twenty-one per cent of the field group and 19 per cent of the hospital group had sustained burns at some time, and, in many cases, the deformity resulting from these was severe. Apart from the physical trauma, epilepsy has a marked effect on the life of the sufferer. In the Semokwe Reserve group 39 per cent of the children up to the age of 14 years were made the butt of humour, and 44 per cent of patients over the age of 15 years were made to feel outcasts by the rest of the community. Twelve male adults and one female had been dismissed from their jobs on account of their seizures. The effects of epilepsy on marital life seem to have no special peculiarities.

Enquiries into tribal beliefs about the ætiology of epilepsy reveal how widespread is superstition and to what extent belief in witchcraft persists. Fifty-nine per cent of Semokwe Reserve sufferers believed their epilepsy resulted from their being 'bewitched'.

The present treatment of epilepsy in such patients is frequently unsatisfactory. The standard anti-convulsant drugs are as effective in controlling their fits as in other races, but, in the first place, sufferers do not make themselves available for treatment; and, secondly, even if they do, the problem of achieving regular and conscientious administration of the selected drug remains. This was also the finding of Hurst *et al.* in the Meadowlands survey. Education and enlightenment of rural Africans will, no doubt, eventually change their present attitude of resignation. In the meantime, it seems that the only way to reach the sufferers would be to send mobile teams into the reserves to seek them out. Once the diagnosis has been made and curable underlying disease excluded, treatment with a long-acting injectionable anti-convulsant would appear to offer the best hope of adequate control.

RECENT REVIEWS OF FOSSIL FISHES

TWO publications of special interest have recently been published on fossil fish: Dr. R. H. Denison has reviewed the Cyathaspididae*, and Dr. C. Patterson† the Mesozoic Acanthopterygia.

Dr. Denison deals with the earliest known family of Agnatha, excluding isolated fragments from earlier strata. The material ranges from the base of the Middle Silurian (Wenlockian) to late Early Devonian (Dittonian). The author describes the anatomy, external and internal, of all forms, which comprise in his interpretation 63 species grouped into 19 genera. The classification adopted for the Cyathaspididae is essentially that of other recent writers, allowing for shifts in the hierarchical status of the taxa. Dr. Denison follows most scholars, Stensiö excepted, in dissociating the Heterostraci from close affinity with the cyclostomes, osteostraci and anaspids. He divides the class Agnatha into two sub-classes, the more primitive Diplorhina with Heterostraci and probably Coelolepida, and the more specialized Monorhina with Myxinoidea and

* Chicago Natural History Museum. *Fieldiana: Geology*. 13, No. 5: *The Cyathaspididae—a Family of Silurian and Devonian Jawless Vertebrates*. By Robert H. Denison. Pp. 309–473. (Chicago: Chicago Natural History Museum, 1964.) 5 dollars.

† *Philosophical Transactions of the Royal Society of London*. Series B: Biological Sciences. No. 739, 247 (July 2, 1964): *A Review of Mesozoic Acanthopterygian Fishes, with special reference to those of the English Chalk*. By C. Patterson. Pp. 212–482 + plates 2–5. (London: Royal Society, 1964.) 105*s*. 15.75 dollars.

with Petromyzonoidea, Osteostraci and Anaspida. All genera in the family are revised, with emphasis on the North American forms and *curricula vitae* given for all species, often with illustrations. In the latter part of his paper, Dr. Denison discusses the habitat, adaptation, evolution, growth and phylogeny of the family. He concludes that they were marine, not highly specialized for benthonic life but well adapted to moving in turbid and poorly lighted waters. He suggests that they were essentially bottom feeders, feeding in a manner similar to that described by Parrington[1] in 1958 for the anaspids. The primitive condition in the cyathaspids is considered to be a shield formed from the fusion of numerous small scales or tesseræ, with the development of large plates in later forms—a view which is consistent with the derivation of the Pteraspididae from the Cyathaspididae.

A review of Mesozoic fishes is long overdue, and Dr. Patterson's study admirably fills the hiatus. While little new material has been added to collections since Smith Woodward[2] produced his monograph on the acanthopterygians, Dr. Patterson has had the inestimable advantage of acid preparations for many of the specimens. Species are now known with a detail and precision impossible half a century ago. The emphasis in the work is on morphology rather than on systematics, and hence isolated scales, teeth and otoliths are omitted, the latter perhaps to be regretted on account of its stratigraphic potential. Dr. Patterson's lengthy review falls into three parts; the first deals with acanthopterygians and ctenothrissiforms of the English Chalk, their systematics and anatomy being discussed in detail; in the second part acanthopterygian faunas of all other Mesozoic deposits are briefly discussed and compared with the British fauna; in the third part the author discusses the morphological, systematic and evolutionary problems arising from his study.

The systematics and morphology of Parts 1 and 2 form the bulk of the work; careful and detailed anatomical descriptions are given accompanied by clear and well-labelled text-figures. The interpretations and deductions are collected into Part 3, which will be read by many not directly concerned with specific details. Dr. Patterson proposes a sub-division of the Beryciformes into three sub-orders; the distinctions are not clear cut and it is doubtful that this truly reflects phylogeny. The author discusses the evolution of the acanthopterygian upper jaw, the origin of the fin spines and the trigemino-facialis chamber. Examining the time span of the Beryciformes, the Cenomanian is seen to contain 19 species in 10 genera, while the Senonian has 22 species in 8 genera. These figures do not reflect the popular picture of rapid radiation of the group during the Mesozoic; they point rather to a pre-Cenomanian deployment, Patterson suggests from Ctenothrissiform stocks, with maintenance of diversity until Senonian times when the Beryciformes are largely replaced by the Perciformes.

In the evolution of the acanthopterygians, three functional trends are defined; increasing manœuvrability, reduction in the buccal dentition accompanied by increase in the pharyngeal dentition, and development of defence mechanisms. Dr. Patterson supports the concept of polyphyletic origin for the Beryciformes and implies the same for the Perciformes. Transitional forms link the two orders and the adaptive advantages of the changes involved—presence or absence of orbitosphenoid, number of pelvic and caudal fin rays—are not apparent. This treatise does not supply answers to all questions on acanthopterygian history; it presents that history clearly and authoritatively, thus enabling us to frame our questions more precisely.

R. J. G. Savage

[1] Parrington, in Westoll, T. S., *Studies in Fossil Vertebrates* (Athlone Press, University of London, 1958).
[2] Woodward, A. Smith, *Mon. Palaeont. Soc., London*, 1902–1912.

EARLY RECEPTOR POTENTIAL OF THE VERTEBRATE RETINA

A NEW receptor potential of the monkey retina has been reported recently by Brown and Murakami[1]. They observed this new response using an intra-retinal microelectrode and intense stimulus flashes. The latency of the response is very short (less than 0·1 msec), and the amplitude of the response is largest when the tip of the electrode is at the level of the ciliary connexion between the outer and inner segments of the receptors. Brown and Murakami's conclusion that this new response is generated by the receptors is of great significance for visual research; but some additional evidence is still needed to make the support for their conclusion fully convincing. For example, they observed this response in the cone retina of the cynomolgus monkey but could not observe it in the rod retinæ of the night monkey and cat. No attempt was made to directly identify the pigment on which this response depends.

In this communication I wish to report that a quite similar response occurs in the electroretinogram (ERG), and that this ERG response has been observed in every vertebrate eye so far investigated. Furthermore, several lines of evidence given here show that in the all-rod eye of the albino rat this response almost certainly depends on the visual pigment contained in the rod outer segments. Therefore, because the latency of the response is so short, this evidence provides strong and independent support for Brown and Murakami's conclusion that this new response is a receptor potential. Moreover, the amplitude of this new response is linearly proportional to the amount of pigment bleached by the stimulus flash. Thus this new response appears to be more closely linked to initial photochemical events than any other electrical response so far recorded in vertebrate eyes.

In the work recorded here, stimulus flashes were produced by a 65-W-sec photographic strobe lamp (Honewell Strobonar 65*C*) which had a flash duration shorter than 0·7 msec. To minimize electrical and photovoltaic artefacts, the strobe lamp was enclosed in a soft-steel box, and the electrodes, which consisted of saline-soaked cotton wicks leading to Ag-AgCl wires, were partially shielded from the light. With this arrangement, the amplitude of the stimulus artefact was less than 5 μV during the time-interval of interest. A series of simple lenses focused the light from the lamp on to the cornea, forming a roughly 110° Maxwellian view, and the flash energy was adjusted by interposing Wratten neutral density filters. In the rat, the effective flash energy was determined by observing the amplitude of the dark-adapted *b*-wave as a function of flash energy. The resulting curve of amplitude versus energy could then be compared with a calibrated curve previously described[2]. The effective flash energies are, therefore, given in terms of the number of quanta absorbed by the average rod per flash. This energy scale will be abbreviated to read 'quanta/rod'.

Oscilloscope traces of the ERG which exhibit the early response are shown in Fig. 1. Fig. 1*A* shows the ERG of a dark-adapted albino rat (Sprague–Dawley) for a flash with an effective energy of about $1{\cdot}5 \times 10^6$ quanta/rod. There are about 3×10^7 rhodopsin molecules/rod in the rat eye[2], so this flash bleached about 3–5 per cent of the visual pigment. The well-known *a*- and *b*-waves of the ERG are labelled in Fig. 1; at such high energies they have a somewhat unusual appearance. The new response can be seen at the start of the trace. The peak of this response occurs about 1 msec after the start of the flash, and most of the response is over before the *a*-wave begins to appear.

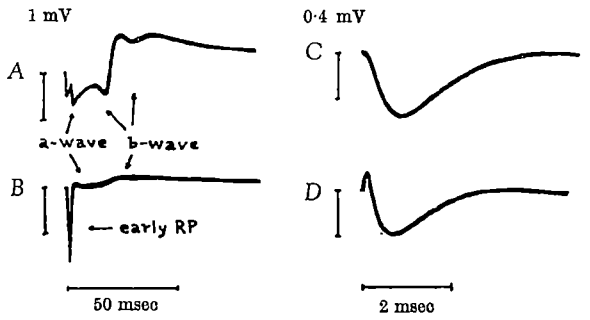


Fig. 1. ERG of the albino rat (*A*, *B*, and *C*), and frog (*D*). In (*A*), a flash with an effective energy of about $1{\cdot}5 \times 10^6$ quanta/rod was presented to the dark-adapted eye. One min later, in (*B*), a maximum energy flash was presented. In (*C*), after 1 more min, a second maximum energy flash was presented. This time the sweep speed and amplification were increased to show more clearly the time-course of the early *RP*. The same sequence of three flashes was presented to the excised eye of a frog, and the response to the third flash is shown in (*D*). Amplifier bandwidth: 0·8–10,000 c/s. Flash duration: 0·7 msec. White light presented in an approximately 110° Maxwellian view. Responses of positive polarity displayed as upward deflexions

Thus the time-course of this early ERG response is similar to that of the early intra-retinal response recorded by Brown and Murakami. In Fig. 1*B*, a flash with the maximum obtainable energy was presented. The eye was still quite light-adapted from the first flash, hence the *b*-wave was much reduced and the *a*-wave almost abolished. On the other hand, the amplitude of the early response considerably increased. This maximum energy flash probably bleached as much pigment as a short flash can bleach in the rat eye. According to recent work by Dowling and Hubbard, this is about 65 per cent of the pigment present[3]. In Fig. 1*C* a second maximum energy flash was then presented. Here, the sweep speed was increased to show more clearly the time-course of the early response. The amplification was also increased because the previous maximum energy flash reduced the amplitude of the early response. With higher sweep speeds the latency of the early response has been found to be definitely less than 60 μsec. The precise lower limit could not be determined with the present strobe lamp because the duration of its flash was much longer than this very short latent period. Even shorter latencies appear to occur in other animals. For example, Fig. 1*D* shows the ERG from an excised eye of a frog (*Rana pipiens*) for a maximum energy flash. Here, the large corneal negative component is preceded by a smaller corneal positive component. A similar wave-form has been observed in the ERG of the cat and the goldfish, and Brown and Murakami have found that the early receptor potential in the cynomolgus monkey has a bi-phasic wave-form (following communication). In every animal, the dominant phase of the early ERG response always has the same polarity as the *a*-wave. Therefore, both the time-course and polarity of the early ERG response are consistent with those of the early response recorded intra-retinally by Brown and Murakami. In the ERG experiments reported here, the early response has been observed in rat, goldfish and cat eyes for more than 3 h after the eyes were excised. This is in accord with the striking resistance to anoxia that Brown and Murakami report for the intra-retinal response[1]. Apparently, they did not detect the early response in the ERG because their maximum flash energy was too small to produce a detectable response. This might also explain why they did not detect the response in the cat or night monkey.

To summarize: the early ERG response is similar to the intra-retinal response recorded by Brown and Murakami because it has about the same very short latency and a similar time-course, its dominant phase has the same polarity as the *a*-wave, it can only be observed with very high flash energies, and it has the same striking resistance to anoxia. Moreover, the early ERG response has been observed in every animal so far investigated and, in particular, it has been observed in the essentially all-rod eye[4] of the rat. Since Brown and Wiesel[5] have already shown that the ERG reflects the same potential changes that are recorded intra-retinally in the local ERG, it is entirely reasonable to assume that the early ERG response is essentially equivalent to the early response described by Brown and Murakami. Accordingly, in the rest of this communication the early ERG response will be called the early *RP* (receptor potential), as suggested by Brown and Murakami[1]. From these results, then, it would appear that the early *RP* probably occurs in all vertebrate eyes, and that it is produced by rod as well as by cone retinæ.

Since the early *RP* has a latency shorter than 60 μsec, it seems clear that this response cannot be generated far from the pigment on which it depends. Consequently, if the early *RP* can be shown to depend on the visual pigment contained in the receptors, a strong argument, which is independent of microelectrode evidence, can be made for considering this response to be a receptor potential. Therefore, a number of critical experiments were performed in an attempt to make a positive identification of the pigment responsible for the early *RP*. In these experiments, the ERG of the albino rat was used because only one visual pigment need be considered, and a minimum number of other pigments are present.

The first experiment consisted of obtaining the approximate spectral sensitivity of the early *RP* by finding the flash energies needed to elicit equal amplitude responses at five different wave-lengths. For comparison, the spectral sensitivity of the dark-adapted *b*-wave was also obtained. Fig. 2 shows the results. The curve in Fig. 2 is redrawn from spectral sensitivity data on the dark-adapted *b*-wave obtained under somewhat better optical conditions by Dodt and Echte[6]. In the present experiment, the results were probably slightly distorted by the chromatic aberration of the simple lenses. Nevertheless, the data for the early *RP* match the curve well enough to indicate that the early *RP* probably depends on rhodopsin. Fortunately, this experiment could be arranged to make a comparison between the spectral sensitivities of the early *RP* and the *b*-wave with high precision. Such a comparison is inde-

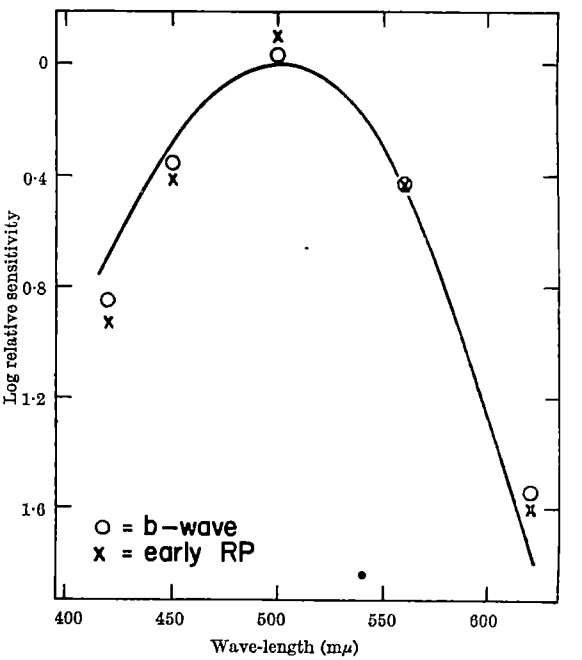


Fig. 2. Comparison of the spectral sensitivities of the early *RP* and the dark-adapted *b*-wave in the albino rat. The sensitivity is the reciprocal of the energy needed to produce a response of a given amplitude. The spectral width of the interference filters at 1/10 transmission was approximately ±6 mμ. Each point is the average of data obtained from four different rats. Data for the early *RP* were taken in a sequence which minimized the effects of pigment bleaching. The solid line is redrawn from data in Fig. 4 of ref. 6

pendent of chromatic aberrations, screening pigments, and changes in the absorption spectrum that occur when pigment is extracted. Furthermore, such a comparison becomes a quite rigorous test when applied to an eye that contains essentially only one visual pigment, such as the eye of the rat. In obtaining the data for Fig. 2, only the stacks of density filters had to be varied, and it is estimated that slight uncertainties in the calibrations of these filters (about $\pm 0{\cdot}7$ density unit for a total density of 6) introduced the limiting source of error. In Fig. 2 it can be seen that at each wave-length the separation between the points is never more than about 0·7 density unit. Thus, to within this experimental precision, these two ERG responses have identical spectral sensitivities. Therefore, the early *RP* in the rat almost certainly arises from the action of light on the visual pigment, rhodopsin.

The foregoing evidence does not necessarily imply that the rhodopsin responsible for the early *RP* is contained in the rods. For example, the early *RP* might be generated by rhodopsin located in the pigment epithelium. But Brown and Murakami have reported evidence which excludes the pigment epithelium as a possible source of the early *RP*[1]. Furthermore, Dowling[7] has shown that no rhodopsin is present in the pigment epithelium of the albino rat. Moreover, I have found that the early *RP* is easily observed in the excised retina of the rat (and also in the excised retina of the goldfish). Therefore, the sources of the early *RP* must reside in the retina, and most or all of the rhodopsin responsible for the early *RP* must be contained in the rod outer segments.

A number of other experiments may be performed on the early *RP* to verify further that it depends on the visual pigment. For example, since strong background light can drastically reduce the amount of visual pigment present in the retina, the amplitude of the early *RP* should be markedly reduced when a strong background light is present. This is, in fact, the case. The amplitude of the early *RP* in the ERG of the rat can be reduced to below the noise-level of the present experimental preparations by applying a sufficiently strong background light. When the background light is turned off, the early *RP* slowly recovers at a rate comparable to the rate of pigment regeneration. Results of another more quantitative experiment are shown in Fig. 1. Since Dowling and Hubbard have shown that a maximum energy flash bleaches about 65 per cent of the rhodopsin in the dark-adapted eye of the rat, the amplitude of the early *RP* for a succeeding flash should be reduced by at least a comparable amount. It can be seen in Figs. 1*B* and 1*C* that the amplitude for the second of two maximum energy flashes is, in fact, about 35 per cent as large as the amplitude for the first flash. This implies that, for a maximum energy flash, the amplitude of the early *RP* is probably linearly proportional to the fraction of pigment present in the eye.

This result suggested that the amplitude of the early *RP* might also be linearly proportional to the amount of pigment bleached by any flash. To check this, the amplitude of the early *RP* was obtained as a function of flash energy taking care to allow for losses of pigment caused by previous flashes. Fig. 3 shows the resulting data on a log log plot. The amplitude scale is labelled directly in millivolts; the flash-energy scale is given in log units of the beam energy, where log (flash energy) = 0 corresponds to the energy at which one quantum is absorbed by the average rod per flash. The light straight line in Fig. 3 has a slope of 1. This line fits the data to within the estimated experimental errors for the first 1·5 log units above threshold. Thus, in this range the amplitude of the early *RP* is linearly proportional to the flash energy. Evidently, the threshold for this linear response is determined by the experimental noise-level. At very high flash energies the amplitude does not increase linearly with flash energy, but this is to be expected because at these flash energies the fraction of pigment bleached by a flash is no longer a linear function of flash energy. Significantly, the shape or time-course of the early *RP* does not change with flash energy; only the amplitude changes.

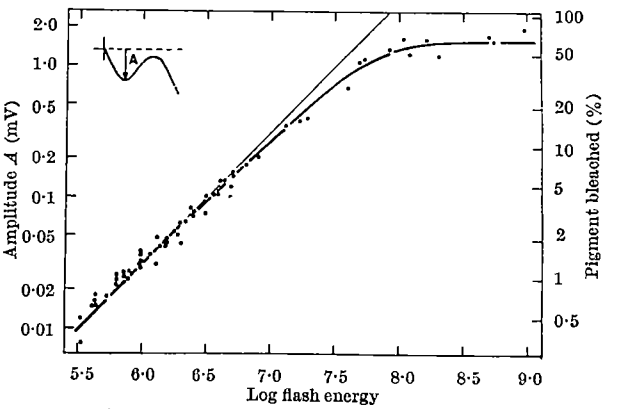


Fig. 3. Amplitude of the early *RP* in the albino rat as a function of flash energy on a log log plot. The flash energy is the energy of the stimulating beam, not the effective flash energy. However, it is calibrated by the method described in the text so that log (flash energy) = 0 is the beam energy at which one quantum is absorbed by the average rod/flash. The curved line shows the calculated fraction of pigment bleached. It is drawn relative to the theoretical scale of pigment bleached shown on the right. This scale is adjusted to make the fraction of pigment bleached by a maximum energy flash equal to 65 per cent. Because significant amounts of pigment must be bleached to obtain these data, in each experiment only five flashes were presented to the initially dark-adapted eye. Four of these flashes were in the range below log (flash energy) = 6·7, and together these four flashes bleached less than about 8 per cent of the pigment. The energy of the fifth flash was selected to obtain a good distribution of points in the high energy range. Thus each point above log (flash energy) = 6·7 was obtained in a separate experiment (15 experiments). Because the absolute amplitude of the response varies somewhat between preparations, the amplitudes of the responses to all five flashes were multiplied by a constant which made the four lowest points best fit the straight line drawn in the figure. All except the three highest-energy points were obtained with a small section of a ping-pong ball placed over the eye to provide uniform illumination of the entire retina[2]

The heavy line in Fig. 3 is a theoretical curve calculated by assuming that the amplitude of the early *RP* is proportional to the fraction of pigment bleached by the flash. The method used to obtain this curve is essentially the same as the method described in detail by Williams[8], who found that it satisfactorily accounts for the photoregeneration of pigment which occurs when intense flashes are applied to frog rhodopsin in solution. On a log log plot, neither the shape nor the slope of the theoretical curve can be altered, but the curve can slide along both axes until it best fits the data. As drawn in Fig. 3, the curve is within the experimental error of every point. Therefore, it appears reasonable to assume that over the entire flash energy-range examined the amplitude of the early *RP* is linearly proportional to the amount of rhodopsin bleached by a flash.

The theoretical scale of the fraction of pigment bleached, shown on the right side of Fig. 3, can be used to predict the number of rhodopsin molecules contained in a rod because the flash energy-scale is calibrated in terms of the number of quanta absorbed per rod. The predicted value depends on the quantum efficiency of bleaching[9], which is known to be between $\frac{1}{2}$ and 1. For this range of the quantum bleaching efficiency, the position of the theoretical scale implies that there are $4-8 \times 10^7$ rhodopsin molecules per rod in the rat eye. This result is in satisfactory agreement with an earlier value, 3×10^7 rhodopsin molecules per rod, obtained by a somewhat more direct method[2]. Therefore, the foregoing theoretical treatment not only adequately describes the amplitude dependence of the early *RP*, but also predicts satisfactorily the number of pigment molecules contained in a rod.

In summary, the foregoing experiments have shown that, in the essentially all-rod eye of the albino rat: (1) The early *RP* has the same spectral sensitivity as the dark-adapted *b*-wave. (2) The early *RP* is present in the excised retina. (3) For a maximum energy flash, the amplitude of the early *RP* is proportional to the rhodopsin present. (4) The amplitude of the early *RP* is linearly proportional to the rhodopsin bleached by a flash. Taken

together, these results provide strong evidence that the rhodopsin contained in the outer segments of the rods is the pigment responsible for the early *RP*. Therefore, on combining the foregoing results with those already reported by Brown and Murakami, it appears that a substantial case can be made for considering this early response to be a potential generated by the visual receptors. This new receptor potential can be expected to provide valuable information on the relation between the photochemical events in the outer segments and the later electrical potentials in the retina. Because the early *RP* appears to be so closely related to the visual pigment, it should provide a means for studying the earliest physicochemical stages of visual excitation, most of which have so far remained almost out of reach.

This work was supported by an Atomic Energy Commission contract with the University of Chicago. I wish to thank Mr. T. G. Ebrey for his assistance; Mr. W. K. Stell for providing goldfish eyes and excising their retinæ; Mr. I. Zucker for providing excised eyes of the cat; and Prof. John R. Platt for his advice.

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Biphasic Form of the Early Receptor Potential of the Monkey Retina

FOLLOWING our initial report of the early *RP* (receptor potential) of the monkey retina[1], a detailed paper is being prepared. In response to *Nature's* invitation, however, it is a pleasure to make a few points which relate to Cone's observations recorded in the preceding communication. One point especially should be made at this time, since it seems important for establishing that the response which Cone finds in the ERG by conventional recording methods is the same response which we have detected by intra-retinal recording.

It has been noted that the early *RP* of the monkey retina is biphasic in form, as illustrated in record *A* of Fig. 1. Following the stimulus artefact, there is a small negative phase of the early *RP* which precedes the larger positive phase. The negative phase was not shown in our first report, and it became visible following reduction of the stimulus artefact. The positive phase of the early *RP* is followed by the positive *a*-wave, which is the leading edge of the late *RP*[1,2]. Records *A* and *B* were obtained under the same conditions, except for higher sweep speed in record *B*; this resolves the complex shape of the stimulus artefact, followed by the negative phase of the early *RP*. Record *C* was then obtained after removing the light stimulus from the eye, and record *D* was obtained with the stimulus over the eye but with the electrode withdrawn from the retina; hence these records show only stimulus artefacts. Since both procedures abolish the initial negative phase of the response, it cannot be an electric or photoelectric artefact. It is part of the retinal response to light and may, therefore, be considered as part of the early *RP*.

It was noted in our first report that the latency of the early *RP* did not exceed about 100 μsec[1]. This value was measured to onset of the positive phase of the response. Fig. 1 shows that the negative phase begins immediately at termination of the stimulus artefact, and the latency of the negative phase in record *B* was 45 μsec. Since the

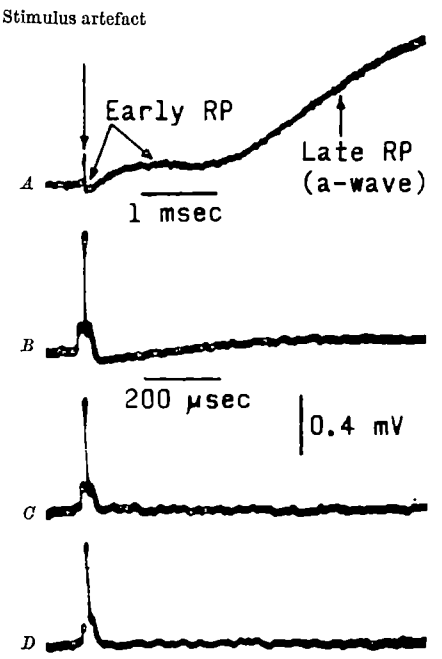


Fig. 1. Records showing the biphasic shape of the early *RP* when recorded by a tungsten micro-electrode in the fovea of the cynomolgus monkey at the retinal level of maximum response amplitude. Reference electrode in vitreous humour. Retinal circulation normal. Stimulus was 20 μsec flash produced by a condenser-discharge lamp (Grass photostimulator) and led to the eye by a fibre optics bundle[1]. Stimulus repeated every 10 sec. Signal amplification was condenser-coupled, with flat frequency response of 1·6–40,000 c/s. Positive signals displayed as upward deflexions. The voltage calibration is for all records; the upper time-scale is only for record *A*, and the lower time-scale is for records *B–D*. Record *A* shows the full time-course of the early *RP*, followed by the rising phase of the late *RP*. Record *B* shows the response under the same conditions but at higher sweep speed. In record *C* the electrode position was unchanged, but the fibre optics bundle was moved away from the eye. This shows only the electric artefact. In record *D* the stimulus was being delivered to the eye as in record *B*, but the electrode was pulled back into the vitreous humour. This procedure should abolish any retinal response, but should not abolish either electric or photoelectric artefacts. Since the initial negative phase of the response was abolished, it is a retinal response and may be considered as an initial phase of the early *RP*

negative response comes smoothly out of the positive stimulus artefact, it is evident that the true latency is less than this. In one experiment the artefact was considerably reduced, and latencies as short as 25 μsec were obtained. This is the shortest so far; but this is still a maximum value, and the artefact must be completely abolished before the true latency can be measured.

Since Cone's responses in the ERG are very similar to our intra-retinal records, in both form and time-course, it seems established that the same type of response is being recorded in the two cases. We have found that responses of small amplitude are more difficult to detect by conventional ERG recording than by intra-retinal recording; thus our failure to detect the early *RP* in the ERG is not surprising, since our intra-retinal signals have not exceeded about 200 μV. We agree with Cone's interpretation that his detection of the early *RP* in the ERG must be due to a more intense light stimulus. The fact that the early *RP* can now be recorded by both methods should prove of considerable value, since each recording method is well adapted to certain problems.

Comparison of Cone's records with ours shows that the early *RP* inverts polarity between the ERG and intra-retinal records. The late *RP* also shows a polarity inversion between the ERG and intra-retinal recording, and it has been shown that this is due to a radially oriented source and sink of the response which occur at different levels of the photoreceptor[2]. It therefore seems likely that the polarity inversion of the early *RP* has a similar basis. When stimulating intensities as high as that used by Cone are applied to intra-retinal recording, the greatly increased signal-levels may make it possible to investigate this problem.

When an electrode is withdrawn from the retina, starting from the retinal side of the pigment epithelium, both the negative and positive phases of the early *RP* increase in amplitude to a maximum which is distinctly removed from the pigment epithelium. Thus the negative phase of the early *RP*, like the positive phase, does not seem to be generated by the pigment epithelium but by the receptors. Since Cone has also observed the biphasic form of the early *RP* in excised retinæ, notably that of the goldfish, this conclusion seems well established. Detailed studies of the early *RP* as a function of electrode depth have now shown that the electrode depth of maximum response amplitude is the same for both the negative and positive phases of the early *RP*, as well as the positive response polarity of the late *RP*. Since all three responses may be plotted during a single electrode track, and since this experiment has been repeated many times, this finding seems secure. The absolute electrode depth of these maximum response amplitudes, however, is difficult to establish. Our initial observations indicated that the level of the photoreceptor at which this occurred was the connecting cilium[1]; but it now appears that the maximum response amplitudes are slightly more proximal, at the level of the inner segment. A hypothetical explanation of these findings has been developed and will be given in a more detailed paper.

Since the early *RP* has now been demonstrated in rods, as well as cones, it seems more likely that the early *RP* represents one of the links in the direct chain of excitatory events in vertebrate photoreceptors. Our comparisons between species indicate, however, that the early *RP* is more difficult to detect from rods than from cones[1]. It is also of interest that the initial phase of the biphasic early *RP*, which we have found larger in the pure cone fovea than in the peripheral retina of cynomolgus, has been noted by Cone only in animals which possess cones; this phase of the response was not found in the pure rod retina of the albino rat, although the response which was recorded had considerable amplitude. Hence these findings suggest that there may be both qualitative and quantitative differences between the early *RP*s of rods and cones; this requires further investigation.

The problems of how the early *RP* is generated, and its functional role in the photoreceptor, seem to be especially important. Cone's finding that the intervening photopigment is rhodopsin, in the rod case, excludes the possibility that the early *RP* may be dependent on some pigment of the photoreceptor other than the visual pigment (such as the chromatin of the cell soma, for example). Although such alternative possibilities seem remote, it remains to be proved that the initial phase of the biphasic early *RP*, in the cone case, is also dependent on the visual pigment. The latency of the second phase of the response is so short that it must be triggered by an isomerization of the photopigment, rather than by a bleaching reaction[1], and the latency of the initial phase is even shorter. In cynomolgus the early *RP* is undiminished 40 min after cessation of artificial respiration, and Cone has now found the response in eyes which have been excised as long as 3 h. It seems unlikely that a response with such high resistance to loss of blood supply, in the case of a mammalian photoreceptor, is due to an alteration of the resting potential of the outer membrane of the cell. This interpretation is also suggested by the fact that the late *RP* may be completely abolished, following cessation of artificial respiration, without affecting the early *RP*[1]. These observations are in agreement with our working hypothesis that the early *RP* is due to activity in the intracellular fibrils of the photoreceptor[1], but do not uniquely support this hypothesis. We have also suggested, on the basis of several striking similarities, that the early *RP* and the cochlear microphonic may be generated in fundamentally the same manner, although triggered by different adequate stimuli. The outer segment of the vertebrate photoreceptor is differentiated from a cilium, the primitive features of which are maintained at the connexion between inner and outer segments[3]; thus the early *RP* and cochlear microphonic may represent a specialized response mechanism of ciliated receptors. Cone's finding that the early *RP* is linearly proportional to light intensity, up to a limit, is of interest in this regard because "The cochlear microphonic is linearly proportional, up to a limit, to the displacement of the cochlear partition . . ." (ref. 3). Hence linearity of response amplitude with stimulus intensity provides a similarity, in addition to those already listed[1], between the early *RP* and the cochlear microphonic.

This work was supported by grant *B*–1903 from the U.S. National Institute of Neurological Diseases and Blindness.

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ISOLATION OF A SEROLOGICALLY ACTIVE, FUCOSE-CONTAINING, TRISACCHARIDE FROM HUMAN BLOOD-GROUP Le^a SUBSTANCE

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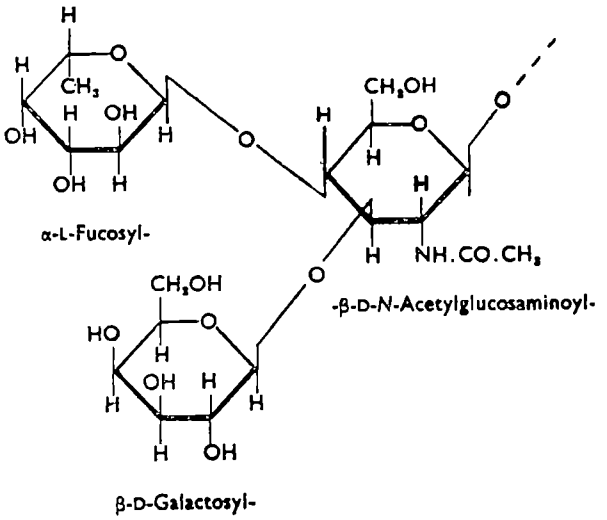
THE first clue to the nature of at least one important component of the Le^a group specific determinant structure was obtained when the inactivation of Le^a substance by an enzyme in *T. foetus* was specifically inhibited by L-fucose[1], one of four sugar components of the carbohydrate chains in the Le^a substance[2]. This result suggested that oligosaccharides containing L-fucose should be examined for their capacity to inhibit the agglutination of Le(a+) red-cells by human anti-Le^a serum, and a number of fucose-containing oligosaccharides, mostly of known structure, were investigated[1]. Of many compounds examined, only two, lacto-*N*-fucopentaose II (ref. 3) and lacto-*N*-difucohexaose II (ref. 4), were active.

α-Fuc
↓ (1→4)
β-Gal-(1→3)-β-GNAc-(1→3)-β-Gal-(1→4)-Glu
Lacto-*N*-fucopentaose II

α-Fuc α-Fuc
↓ (1→4) ↓ (1→3)
β-Gal-(1→3)-β-GNAc-(1→3)-β-Gal-(1→4)-Glu
Lacto-*N*-difucohexaose II

Abbreviations: Fuc = L-fucopyranosyl; Glu = D-glucose; Gal = D-galactopyranosyl; GNAc = *N*-acetyl-D-glucosaminopyranosyl.

Both compounds contained the branched trisaccharide structure:



which was not present in any of the other substances tested. On the basis of the inhibition results it was proposed that this trisaccharide was identical with, or closely related to, the specific determinant structure in the Le^a substance in human secretions. Additional evidence that a non-reducing L-fucose residue was joined at position 4 to *N*-acetyl-D-glucosamine in the Le^a determinant structure was obtained by studies of the specificity requirements of enzymes that destroy the activity of Le^a substance[5]. Thus, one such enzyme removed fucose from lacto-*N*-fucopentaose II, but not from oligosaccharides such as lacto-*N*-fucopentaose I (ref. 6) which contain an α-L-fucosyl residue glycosidically joined at position 2 to galactose.

Although four di- and three tri-saccharides were isolated from the partial acid-hydrolysis products of Le^a substance[7], none of these contained fucose and none was serologically active in the Le^a system. On the basis of the isolated oligosaccharides, however, it was proposed that Le^a substance contains two main types of carbohydrate chain:

(1) β-Gal-(1→3)-β-GNAc-(1→3)-β-Gal-(1→3)-GalNAc-
(2) β-Gal-(1→4)-β-GNAc-(1→3)-β-Gal-(1→3)-GalNAc-

Abbreviations: GalNAc = *N*-acetyl-D-galactosamine. Others, as given before.

The fucose residues were believed to be attached as branching units to these main chains. Evidence for the point of attachment of the fucose residues is difficult to obtain from a study of the partial acid-hydrolysis products owing to the acid-lability of the fucosidic linkages in the blood-group substances. Earlier results, and the isolation of fucose-containing oligosaccharides from *H* substance[8], showed, however, that this evidence is obtainable for the structures occurring at the non-reducing ends of the carbohydrate chains by examining the products of mild alkaline degradation, a procedure that does not readily eliminate fucose from these units. Several years ago, Dr. J. Thomas, at the Lister Institute, isolated from Le^a substance, by alkaline degradation, an Le^a-active trisaccharide that contained fucose, galactose and *N*-acetylglucosamine, but the exact structure of the compound was not determined[9]. The isolation and identification of this active trisaccharide from Le^a substance are now described.

The specific Le^a substance was isolated from a single ovarian cyst fluid[2]. It was treated with hot aqueous methanolic triethylamine, as already described for *H* substance[8], and from 4 g of substance, 2·4 g of dialysable products were obtained. This material was treated in aqueous solution with a mixture (40 ml.; 1 : 1 v/v) of 'Amberlite *IR*-120' (H^+ form) and '*IR*-45' (carbonate form) resins, to remove amino-acids and acidic degradation-products of sugars. The residual material (1·63 g) was adsorbed on a column (17 cm × 3 cm) of charcoal-'Celite' (1 : 1 w/w), and the column was eluted successively with water (5 l.) and aqueous ethanol (5 l. of 1·5 per cent v/v; 3 l. of 2·5 per cent v/v; 7 l. of 5 per cent v/v; 7 l. of 7·5 per cent v/v; 8 l. of 10 per cent v/v; and 9 l. of 15 per cent v/v).

The 5 per cent aqueous ethanolic effluent contained 160 mg of carbohydrate material which, after fractionation on paper chromatograms irrigated with ethyl acetate-pyridine-water (10 : 4 : 3 v/v; solvent *a*), afforded 24 mg of crystalline 4-*O*-β-D-galactopyranosyl-*N*-acetyl-D-glucosamine (*N*-acetyl-lactosamine).

The 15 per cent aqueous ethanolic effluent from the column yielded 155 mg of material which contained a component (I) having $R_{lactose} = 0{\cdot}68$ in solvent (*a*) and $M_G = 0{\cdot}22$ in borate buffer at *p*H 10 (ref. 10). After separation on a paper chromatogram and further purification on a small column (3 cm × 1 cm) of charcoal-'Celite' (1 : 1 w/w), about 10 mg of (I) was obtained as a chromatographically and ionophoretically homogeneous syrup, having $[\alpha]_D^{22} = -44° \pm 3°$ ($c = 0{\cdot}3$ in water). This compound (I) gave a negative indirect Ehrlich reaction[11], and reacted rather feebly on paper with silver nitrate-sodium hydroxide reagents[12]. With the benzidine-trichloroacetic acid reagent[13], (I) developed a dark greenish-brown colour similar to that given[14] by 3-*O*-β-D-galactopyranosyl-*N*-acetyl-D-glucosamine (lacto-*N*-biose-I)[15].

Chromatography of an acid-hydrolysate of (I) indicated the presence of galactose, fucose and glucosamine, and quantitative analysis[16-18] showed that these sugars were present in approximately equimolar amounts. However, after reduction of (I) with sodium borohydride, its hexosamine content was negligible, whereas the amount of galactose and fucose present was unchanged.

On partial hydrolysis in 0·2-N acetic acid at 85° for 4 h. the trisaccharide (I) yielded free fucose together with a disaccharide which was chromatographically and ionophoretically indistinguishable from lacto-*N*-biose-I. This disaccharide also resembled lacto-*N*-biose-I in giving a strong indirect Ehrlich reaction and a greenish-brown colour with the benzidine-trichloroacetic acid reagent; on alkaline degradation it yielded galactose and free chromogen[15]. Further hydrolysis of the disaccharide (in 0·02 *N*-polystyrene-sulphonic acid[19] at 86°) resulted in the gradual liberation of galactose and *N*-acetylglucosamine.

Oxidation of (I) (0·45 mg) in 0·01 M sodium meta-periodate (2·5 ml.) at 22° led to the consumption of 4·18 mol. of oxidant within 4 h, with the concomitant liberation of 1·98 mol. of formic acid and 0·23 mol. of formaldehyde. Oxidation under the same conditions after borohydride reduction[20] resulted in the uptake of 5·12 moles of periodate within 4 h, with liberation of 0·95 mole of formaldehyde; there was no over-oxidation.

On the basis of this evidence trisaccharide (I) is 3-*O*-β-D-galactopyranosyl-4-*O*-α-L-fucopyranosyl-*N*-acetyl-D-glucosamine, and is, therefore, identical with the structure proposed earlier for the specific determinant in Le^a substance.

From what is known at present about the alkaline degradation of polysaccharides, it appears that, in the absence of residues of uronic acids and sugars containing free keto-groups, degradation always proceeds in a stepwise manner from the reducing end of the chain[21]. Trisaccharide (I) and the *N*-acetyl-lactosamine isolated in the present work should, therefore, represent structures occurring at the non-reducing ends of the carbohydrate chains in Le^a substance. This agrees well with the results of earlier work on the partial acid-hydrolysis products of Le^a substance, on the basis of which the structures (1) and (2) were assigned to the main carbohydrate chains[7]. The structure of trisaccharide (I) indicates that at least some

of the chains of type (1) in the native Le^a substance carry an α-L-fucopyranosyl unit attached as a side-chain to position 4 of the *N*-acetyl-D-glucosamine residue. Similarly, the presence of free *N*-acetyl-lactosamine in the alkaline degradation products implies that at least some of the chains of type (2) do not carry fucose residues attached either to the non-reducing terminal D-galactose residue or to the penultimate *N*-acetyl-D-glucosamine residue. The *N*-acetyl-lactosamine structure is believed to be responsible for the cross-reactivity of the blood-group substances with horse anti-Type XIV pneumococcus serum[22] and, therefore, its occurrence as an unsubstituted non-reducing end unit in Le^a substance is in agreement with the comparatively strong Type XIV cross-reactivity usually observed for undegraded Le^a preparations.

The results in Table 1 show that trisaccharide (I) strongly inhibits the agglutination of Le^a erythrocytes by a human anti-Le^a serum, to a degree only slightly less than that of lacto-*N*-fucopentaose II. This lower inhibitory power may indicate that more than the terminal trisaccharide unit of the main chain of the Le^a determinant structure participates in the antigen-antibody binding reaction. The trisaccharide did not inhibit the agglutination of *O* cells by anti-*H* reagents.

Table 1. Inhibition of Agglutination of Le(a+) Red Cells by Human Anti-Le^a Serum with Fucose-containing Compounds

Inhibiting substances	Minimum amount (μg/0·1 ml.) of substace giving complete inhibition
Human Le^a substance	0·05
L-Fucose	> 1,000
Lacto-*N*-fucopentaose I	> 1,000
Lacto-*N*-fucopentaose II	1
Lacto-*N*-difucohexaose II	2
Trisaccharide (I) isolated from Le^a substance	2

The alkaline degradation products of Le^a substance also contained other oligosaccharides which have not yet been identified. Some of them had fucose residues, and were larger than trisaccharides; it is possible, therefore, that some of the carbohydrate chains in Le^a substance carry additional fucose residues, attached at positions more distant from the non-reducing ends. We were, however, unable to detect either of the *H*-active oligosaccharides, 2-*O*-α-L-fucopyranosyl-D-galactose and *O*-α-L-fucopyranosyl-(1→2)-*O*-β-D-galactopyranosyl-(1→4)-*N*-acetyl-D-glucosamine that were obtained earlier[8] by alkaline degradation of *H* substance. The present work thus provides the first direct chemical evidence for a structural difference between human *H* and Le^a blood-group substances, and confirms the suggestions made earlier[1,5,23] regarding the probable nature of this difference.

This work was supported in part by a grant from the Medical Research Council, to whom we express our thanks.

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PHOSPHOGLUCOMUTASE POLYMORPHISM IN MAN

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PHOSPHOGLUCOMUTASE (PGM) is widely distributed in mammalian tissues[1] and plays an important part in carbohydrate metabolism. It is a phosphotransferase (*EC* No. 2.7.5.1) which catalyses the transfer of a phosphate group between the 1- and 6-positions of glucose[2]. In the present article we report a procedure for the detection of the enzyme following starch-gel electrophoresis, and describe the results obtained using human red cell lysates and tissue extracts from different individuals. Several distinct PGM isoenzymes appear to occur in each individual, and clear-cut differences between individuals are also observed. These differences have been shown to be genetically determined and they represent a new human polymorphism.

Horizontal starch-gel electrophoresis using paper inserts (Whatman No. 3) was carried out at 5·5 V/cm for 17 h at +5° C and *p*H 7·4 (bridge buffer: 0·1 M *tris*, 0·1 M maleic acid, 0·01 M EDTA, and 0·01 M $MgCl_2$ adjusted to *p*H 7·4 with NaOH; gel buffer: 1 in 10 dilution of bridge buffer with water). The gel was sliced and then incubated at 37° C for 1 h in a reaction mixture consisting of $4{\cdot}6 \times 10^{-3}$ M glucose-1-phosphate (G1P), about 5×10^{-5} M glucose-1,6-diphosphate (G1,6diP), 10^{-2} M $MgCl_2$, $1{\cdot}2 \times 10^{-4}$ M NADP, about 0·04 unit/ml. glucose-6-phosphate dehydrogenase (G6PD), 0·1 mg/ml. phenazine methosulphate (PMS), 0·1 mg/ml. tetrazolium salt MTT (ref. 3), made up in 0·03 M *tris* buffer *p*H 8·0. This reaction mixture was designed to localize PGM according to the reaction sequence shown in Fig. 1. In the presence of G1P and small amounts of G1,6diP, G6P is formed. This is converted to 6-phosphogluconate (6PG) by G6PD with the concomitant reduction of NADP. The reduced NADP in the presence of PMS, which is thought to act as an electron carrier[4], causes the reduction of MTT to give a blue-coloured insoluble formazan which is deposited at the site of PGM activity.

G1,6diP, a critical ingredient of the reaction mixture, could not be obtained commercially. However, it was found to be present in sufficient amounts as an impurity in some but not all commercial preparations of G1P. It was determined in the various G1P preparations by two different methods. The first method (*A*) involved the enzymatic assay[2] of G6P formed from G1,6diP by brief acid hydrolysis. The G1P sample (10–100 μmoles) was hydrolysed in 0·5 ml. N sulphuric acid for 10 min at 100° C, cooled and then neutralized with 0·5 ml. N sodium hydroxide. To this was added 1·8 ml. 0·19 M *tris* buffer *p*H 8·0, 0·2 ml. 4×10^{-3} M NADP, and 1 unit G6PD (free

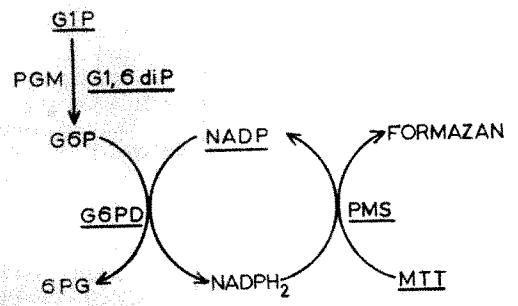


Fig. 1. Sequence of reactions for detection of PGM following starch-gel electrophoresis. The underlined components are contained in the reaction mixture applied to the gel

of 6PG dehydrogenase). The reaction was followed spectrophotometrically at 340 mμ, and the quantity of G6P present estimated from the maximal change in optical density produced by the addition of the enzyme. Correction for any free G6P present in the original G1P sample was made by carrying out a duplicate determination using the unhydrolysed G1P preparation. The second method (*B*) involved the separation of G1,6diP from G1P on an ion-exchange resin[5]. 45 mg of the G1P preparation was applied to a column (17·5 × 0·85 cm) of 'Deacidite *FF*' (2–3 per cent cross-linked) in the formate form. It was eluted with a linear gradient of ammonium formate (mixing flask contained 500 ml. water and donor flask 500 ml. 2 M ammonium formate) at a flow-rate of 20 ml./h; 10 ml. fractions were collected and analysed for total and acid labile phosphate[6]. G1P was eluted at about fractions 16–18, and G1,6diP at about fractions 28–32. Estimates obtained by the two methods of G1,6diP in various commercial preparations of G1P are given in Table 1. Those marked with an asterisk were found to be adequate for the present purpose. The presence of G1,6diP in the various preparations was also confirmed qualitatively by high-voltage filter paper electrophoresis. In general, the results obtained by the various methods were in good agreement.

Table 1. Estimates of G1,6diP in Various Commercial Preparations of G1P

Source	Salt	Batch No.	Estimate of μmoles G1,6diP per 100 μmoles G1P: Method *A* (enzymic assay)	Method *B* (column chromatography)	
British Drug Houses, Ltd.	Di.K	2302310	0·80	0·66	*
	Di.Na	2296570	2·87	2·45	*
C. F. Boehringer and Soehne, Mannheim	Di.K	6453513	None detected	None detected	
	Di.Na	6503514	,,	—	
	Di.K†	6154513	,,	—	
	Di.Na†	6234222	0·18	—	
L. Light and Co., Ltd.	Di.K	7075	1·10	1·06	*
	Di.Na	—	None detected	—	
Mann Research Labs., Inc.	Di.K	*L*2477	1·14	1·06	*
	Di.Na	*K*2122	2·00	1·85	*
Nutritional Biochemicals Corp.	Di.K	9786	1·60	—	*
	Di.Na	6208	0·02	—	
Schwarz Bio-Research, Inc.	Di.K	*GMK*6201	0·01	—	
Sigma Chemical Co.	Di.K	*G*122*B*–208	0·30	None detected	
	Di.Na	34*B*–1590	0·16	,,	

* Found to be satisfactory for detection and typing of human PGM in the conditions described.
† Obtained from Calbiochem.

When human red cell lysates, prepared either by brief sonication or by freezing and thawing washed red cells, are subjected to the procedure outlined above, at least seven zones of what appear to be PGM activity can be detected. Furthermore, hæmolysates from different individuals can be classified into three distinct types according to the pattern of zones present. These three types, which will be referred to as PGM 1, PGM 2 and PGM 2–1, are shown in Fig. 2. Zones *a* and *c* are present in type 1 and type 2–1 but absent in type 2. Zones *b* and *d* are present in type 2 and type 2–1 but absent in type 1. Zones *e*, *f* and *g* are present in all the types. The pattern of PGM zones observed in type 2–1 appears to include all the components present in both type 1 and type 2, and indeed it was found possible to obtain essentially the same 2–1 pattern by subjecting to electrophoresis an artificial mixture of approximately equal amounts of hæmolysates of type 1 and type 2. In some experiments, one or two rather weak zones with mobility slightly faster than zone *d* were observed in type 1 hæmolysates and it is possible, therefore, that the patterns may be more complex.

Evidence that the zones *a*–*g* shown in Fig. 2 do, indeed, represent PGM activity may be summarized as follows:

(1) If G1P is omitted from the reaction mixture, none of the zones develops.

(2) If G1,6diP is omitted from the reaction mixture, the zones appear only very slowly and are extremely weak. Reaction mixtures without G1,6diP were obtained either by using G1P which had been separated from G1,6diP on an ion-exchange column, or by using one of the preparations of G1P in which no G1,6diP could be detected. If to such a reaction mixture small quantities of G1,6diP are added, full development of the zones is obtained. The requirement for G1,6diP was found to be about 5×10^{-5} M.

(3) If G6PD is omitted from the reaction mixture, only zones *e* and *f* appear. This was found to be due to endogenous G6PD present in the hæmolysate, which migrates in this system as a diffuse zone overlapping zones *e* and *f*.

(4) If NADP or PMS is omitted from the reaction mixture, no zones develop.

(5) It has been shown that PGM activity can be considerably enhanced by Mg^{++} and imidazole or other metal binding agents[7]. In the work recorded here it was found that the inclusion of Mg^{++} and imidazole in the gel resulted in enhancement of the rate of development and intensity of the zones and that an even more striking effect was obtained by the inclusion of Mg^{++} and EDTA.

(6) PGM has been crystallized from rabbit muscle[8]. The procedure described here could therefore be critically tested by a comparison of the results obtained with a crude rabbit muscle extract and a crystalline preparation of purified rabbit muscle PGM. Rabbit muscle was homogenized in an equal volume of water and the supernatant

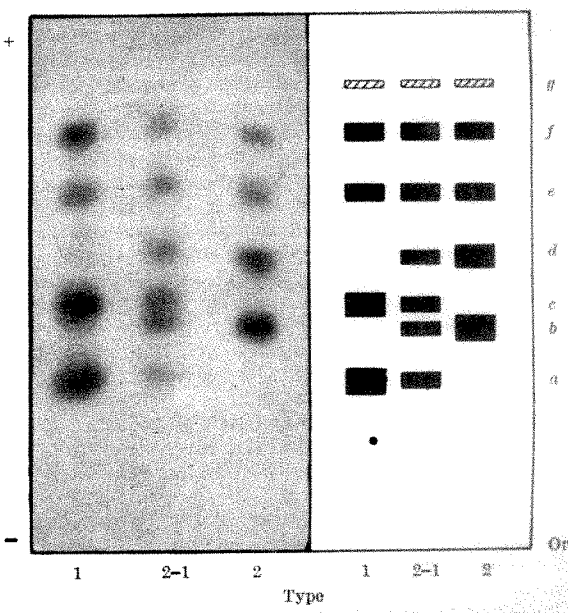


Fig. 2. Photograph and diagram of starch-gel showing components observed in hæmolysates from individuals of PGM types 1, 2–1 and 2

obtained after centrifugation diluted 1 in 50 with gel buffer. This was subjected to starch gel electrophoresis under the foregoing conditions side by side with a suitably diluted solution (about 0·04 mg/ml.) made from a crystalline rabbit muscle PGM preparation obtained commercially. Identical patterns of activity were observed (Fig. 3). There were at least three main zones, the most cathodal having the greatest intensity and the most anodal being much weaker than the others. The three zones had the same mobilities in the two preparations, and were different from those obtained with human material. Quite intense zones were observed with as little as 2γ of the crystalline preparation. Two other crystalline PGM preparations obtained commercially were also examined with the same result.

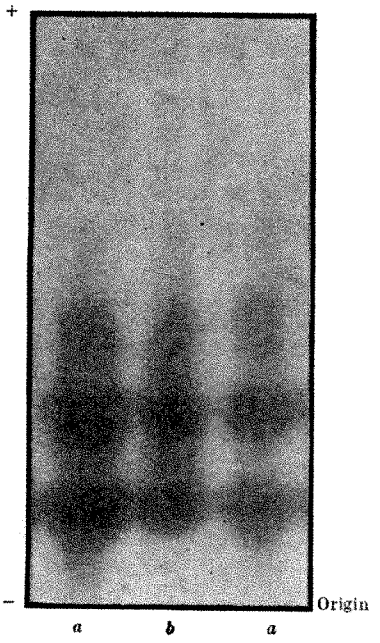


Fig. 3. Electrophoretic patterns of rabbit muscle PGM. (*a*) Solution of crystalline enzyme preparation; (*b*) crude aqueous muscle extract

The multiple zones of activity observed in the human red cell lysates could readily be demonstrated in aqueous extracts of a variety of other tissues. These included leucocytes, liver, kidney, muscle from heart and uterus, brain, skin and placenta. Except for the leucocytes and placenta, the tissue fragments were obtained either at post-mortem or by biopsy. The leucocytes were disrupted by sonication. The other tissues were homogenized in an equal volume of water. All the zones of activity (*a–g*) observed in the hæmolysates were found in the various tissue extracts. Furthermore, the different types of pattern could clearly be identified and, in general, all the tissues examined from any one individual showed the same type. In the case of the placenta, the type observed was in every instance the same as that found in the cord blood, and this was not necessarily the same as that of the mother. In some of the tissue extracts one or more weak zones migrating more rapidly than zone *g* were also observed, but these have not yet been characterized in detail. In general, in all the tissue extracts examined, the degree of activity of the zones was such as to exclude the possibility that they were derived from residual red cells in the tissues.

The characteristic PGM patterns were also demonstrated in extracts of human cells grown in tissue culture. The tissue cultures were established by Dr. M. Glen-Bott and Dr. R. G. Davidson from small skin biopsies. They were maintained in a medium made up of Eagle's[9] minimal essential medium 75 per cent, beef embryo extract ultrafiltrate 5 per cent, and human AB serum 20 per cent. The first passage was generally made after 3–5 weeks, and subsequent passages every 5–14 days when the Petri dishes (6 or 10 cm) were fully sheeted. At varying periods (up to 14 weeks and 10 passages) cells were gathered, washed and disrupted by sonication. The supernatant was subjected to electrophoresis under the standard conditions. A confluent single layer of cells from a small Petri dish (6 cm diameter) gave adequate amounts of material. All the zones observed in the hæmolysates were demonstrable in the tissue culture cell extracts, and the characteristic types were readily identified. In each instance the type of pattern (1, 2–1 or 2) observed in a particular tissue culture preparation corresponded to that found in hæmolysates from the donor whose skin had been used to start the culture.

These findings, and also the observation that repeated blood samples from single individuals over a period of several months were always of the same type, make it clear that the three types represent individual characteristics. The results with placental extracts and with cord bloods indicate that these characteristics are fully developed at birth.

Table 2. Incidence of Different PGM Phenotypes in 338 Unrelated English Adults

PGM phenotype	Males	Females	Totals	Frequency
1	98	88	186	0·550
2–1	65	62	127	0·376
2	19	6	25	0·074
Totals	182	156	338	1·000

Table 2 shows the numbers of each of the types found in 338 unrelated English adults. Table 3 summarizes the results obtained in 80 families in which both parents and at least one child were typed for PGM, and in 53 families in which only one of the parents was available for examination. The familial distribution is clearly non-random and there seems little doubt that the three PGM types are genetically determined. The results can most simply be explained if we suppose that the three types are determined by two autosomal allelic genes PGM^1 and PGM^2, such that homozygotes PGM^1 PGM^1 and PGM^2 PGM^2 are of type 1 and type 2, respectively, while type 2–1 represents the heterozygote PGM^1 PGM^2. All the 262 children in the 133 families shown in Table 3 are of PGM types predicted by the hypothesis, and the segregation ratios are consistent with Mendelian expectations.

Table 3. Distribution of PGM Phenotypes in 133 Families

Type of mating	No. of families	Children 1	2–1	2	Total
1 × 1	30	58	—	—	58
1 × 2–1	26	25	25	—	50
1 × 2	6	—	12	—	12
2–1 × 2–1	12	6	6	8	20
2–1 × 2	4	—	3	7	10
2 × 2	2	—	—	3	3
1 × ?	35	57	12	—	69
2–1 × ?	15	9	20	4	33
2 × ?	3	—	4	3	7
Totals	133	155	82	25	262

Estimates of the gene frequencies p and q of the postulated genes PGM^1 and PGM^2 may be obtained by counting from the population data given in Table 2. The values are $p = 0{\cdot}74$ and $q = 0{\cdot}26$. These estimates may be used to see whether the observed frequencies of the three types in the general population fit the Hardy–Weinberg expectation. The calculation is given in Table 4, and it is apparent that there is good agreement between the observed numbers of the three types in the population sample and the numbers expected assuming random mating and negligible selection.

If the hypothesis is correct, then one must suppose that the components *a* and *c* are determined by PGM^1 and that they are molecular alternatives of components *b* and *d* determined by PGM^2. Presumably a 'structural'

Table 4. EXPECTED AND OBSERVED NUMBERS OF DIFFERENT PGM PHENOTYPES TAKING GENE FREQUENCIES p, 0·74, q, 0·26

PGM genotype	PGM phenotype	Expected frequency		Expected numbers in population sample	Observed numbers in population sample
$PGM^1\ PGM^1$	1	p^2	0·548	185	186
$PGM^1\ PGM^2$	2–1	$2pq$	0·385	130	127
$PGM^2\ PGM^2$	2	q^2	0·068	23	25
Totals			1·001	338	338

locus is involved here, and it is of interest that it apparently does not affect, and may therefore not be concerned in, the determination of components *e*, *f* and *g*. Further elucidation of the molecular interrelationships of these components may be expected to emerge from structural and related investigations. Such investigations will also be of relevance in clarifying the relationship of the phospho- and dephospho-forms[2] of the enzyme to the various components described here.

Apart from the immediate biochemical problems posed by these findings, it is apparent that they will also be of interest for a variety of genetical investigations. In particular, the PGM polymorphism should prove a valuable new genetical marker in population and linkage investigations, and also in human cell genetics.

We thank Dr. J. W. Landells for providing post-mortem material, and Dr. Mary Glen-Bott for her help with the family investigations. Crystalline PGM preparations were obtained from C. F. Boehringer and Soehne and from Sigma Chemical Co. G6PD was obtained from C. F. Boehringer and Soehne, and from L. Light and Co., Ltd.

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STRUCTURE OF THE ALVEOLAR LINING FILM OF THE LUNGS

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IT has been shown[1] that the alveoli of the lungs are lined with a film formed from a substance of high molecular weight, which lowers the tension of alveolar surface. The alveolar lining film and its surface-active properties have recently been reviewed by Pattle[2]. In human pathology the importance of the lining film is shown in the respiratory distress syndrome of new-borns[3].

For detection of a lining film only indirect physical methods are used[2]. The surface-active material is not detectable in light microscopy, since there is not enough of it to detect histologically. Using the freezing and drying procedure and electron-microscope technique, Chase[4] described a homogeneous surface membrane instead of epithelial lining and postulated the non-cellular nature of the pulmonary alveolar lining. However, so far as we know, until now the alveolar lining film was not visualized directly in the electron microscope image as a separate layer on the epithelial surface.

In work recorded here we have tried to obtain some evidence of the existence of alveolar lining film by means of electron-microscope technique.

The lungs of three rabbits, 6 months to 2 years of age, were examined. The tissue samples taken from living animals were immersed in 0·5 per cent potassium permanganate buffered to *p*H 7·4 in veronal acetate buffer. The samples were fixed for 2 h at 4° C. The dehydration was effected by passing through a series of ethanol solutions of increasing strength (25 per cent, 15 min; 35 per cent, 15 min; 50 per cent, 15 min; 60 per cent, 15 min; 70 per cent, 15 min; 80 per cent, 15 min; 90 per cent, 15 min; 96 per cent, 30 min; 100 per cent, 30 min; 100 per cent, 2 h; 100 per cent, 30 min). Except for the last change, done in room temperature, the dehydration was performed at 4° C. For embedding, 'Araldite *D*' (Ciba) with hardener '964 *B*', dibutyl phthalate and accelerator '964 *C*' were used. Three mixtures were prepared as follows: I, 'Araldite' 15·0, hardener 5·0, dibutyl phthalate 0·5; II, 'Araldite' 10·0, hardener 10·0, dibutyl phthalate 0·5; III, 'Araldite' 10·0, hardener 10·0, dibutyl phthalate 0·5, accelerator 0·5. After dehydration the samples were transferred from absolute ethanol to a 50 : 50 absolute ethanol with the first 'Araldite' mixture (I) for 10 h at 48° C. It was followed by three changes (24 h each) in the first 'Araldite' mixture (I) at 48° C, then by three changes (24 h each) in the second 'Araldite' mixture (II) at 48° C, and finally in the third 'Araldite' mixture (III) at room temperature (24 h). The samples transferred to gelatin capsules condensed at 58° C. Also used for fixing was 1·0 per cent osmium tetroxide in veronal buffer (*p*H 7·4) containing sucrose, while for embedding a mixture of methylmethacrylate and *n*-butylmethacrylate was used.

The ultra-thin sections revealed the well-known pattern of the air-blood barrier consisting of epithelial lining, basement membrane complex and endothelial lining. In sections derived from tissue blocks fixed in potassium permanganate and embedded in 'Araldite' the plasma membrane of epithelial cell of alveoli frequently was closely attached by an irregular layer of diffuse material (Fig. 1). It consisted of more-or-less compact granules facing the lumen of the alveoli. The alveolar side of the layer has no sharp border. However, sometimes it showed a membranous appearance. The thickness of the layer varies greatly: usually it was about 200 Å, but it has reached more than 1000 Å. The discontinuity of the layer was rather a constant event. Often some remnants of the layer were situated more or less perpendicular to the plasma membrane of epithelial cells. The last event, as well as the aforementioned discontinuity of the layer, seems to be artefact. More compact granular material was observed filling some infoldings between the cells of alveolar lining. In the cytoplasm of the epithelial lining, no lamellar inclusions[5] were discerned: instead, some globular agglomerations of granules were observed. The granules were arranged in arrows concentrically or randomly distributed in rather compact clumps. The aforementioned structures showed some resemblances to inclusions described by Campiche[6].

The results presented here were observed in each of the animals examined. The detection of the material, forming here and there a distinct layer on the surface of epithelial lining, was possible only in tissues fixed in potassium permanganate and embedded in 'Araldite'. It seems quite possible that the granular layer on the surface of alveoli described here corresponds in some way to the alveolar lining film as revealed by indirect physical methods. Perhaps the observed granules correspond to a macromolecular component of the surface-tension lowering substance. It should be emphasized that there is some similarity between the granules of the layer attached to the epithelial lining and those observed in

globular structures in epithelial cytoplasm. This suggests some relationship between both structures.

In a further examination of the electron-microscope evidence of the alveolar lining film we have used an adaptation of Mowry's modification of the Hale staining method to electron microscopy, as described recently by Gasic and Berwick[7]. The lungs of five healthy rabbits, aged between 2 weeks and 1 year, were examined. Fresh tissue samples taken from peripheral parts of the lungs were fixed overnight at room temperature in 10 per cent neutral phosphate-buffered formalin. After washing with water, the samples were rinsed in 12 per cent acetic acid, exposed to Müller's colloidal iron solution (*p*H 1·3) for 60 min, rinsed in 12 per cent acetic acid, treated for 20 min in a mixture of 2 per cent hydrochloric acid and 2 per cent potassium ferrocyanide, and finally rinsed in distilled water. Tissue samples which were treated in the same way, except for exposure to colloidal iron, were used as a control. In addition, samples, taken from each lung, were fixed in 1·0 per cent osmium tetroxide in veronal buffer containing sucrose. Methacrylate embedding was used.

In ultra-thin sections the Hale-positive material consists of electron-opaque ferric ferrocyanide crystalline aggregates or coarse mass with no structural details. The Hale-positive material appears as a continuous surface coat of the alveoli, closely attached to the epithelial lining (Figs. 2 and 3), including the alveolar side of the so-called 'granular' or dust cells as well (Fig. 4). Even the microvilli were covered by the Hale-positive material. Some detachment of the opaque layer of the alveoli was only infrequently observed and was interpreted as an artefact. The thickness of the layer varied; usually it was about 300 Å. In the work described here no ultra-structural component belonging to the air-blood barrier, or other structural forms observed, revealed any distinct amount of the Hale-positive material.

The method used gives a good assessment of the existence of lining film by means of the electron-microscope technique. As regards the chemical composition of the lining film, however, the data presented have to be interpreted rather carefully. There is evidence to show that the surface active substance of the alveolar lining is a lipoprotein[8]. The positive Hale reaction, however,

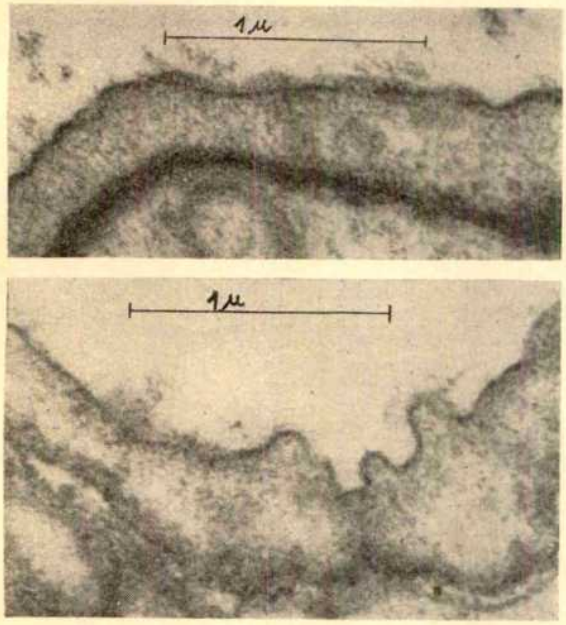


Fig. 1. Epithelial lining after fixation with $KMnO_4$ and embedding in 'Araldite'. At the alveolar side (upper side) some diffuse, granular material attached to cell membrane is observed (*a*). Here and there it presented a membranous appearance (*b*)

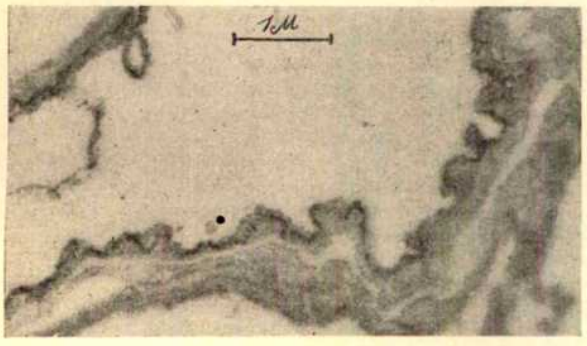


Fig. 2. Section of rabbit lung fixed in formalin and stained by the modified Hale technique. Aggregates of crystals of ferric ferrocyanide coat the alveolar space. Hale-positive material attached closely to the epithelial lining. Basement membrane complex and endothelial lining are also visible

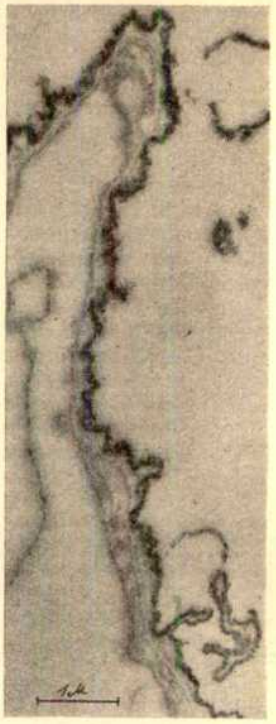


Fig. 3. Prepared as Fig. 2: epithelial lining of alveoli covered by a continuous layer of Hale-positive material. Crystals of ferric ferrocyanide outline the processes of epithelial lining

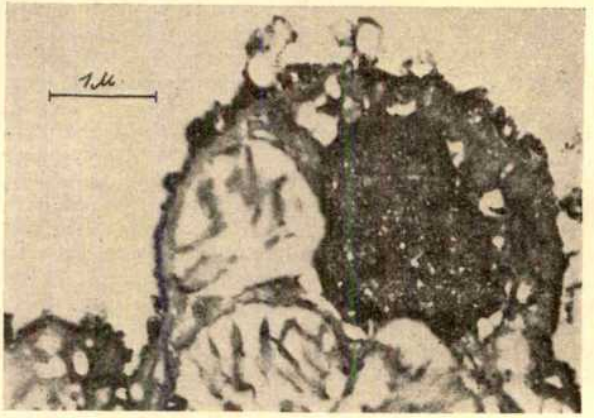


Fig. 4. Prepared as in Fig. 2: a portion of so-called 'granular' cell of the alveolar lining. This cell has a Hale-positive coat similar to that in Fig. 3

could indicate the existence of another component of the acidic mucopolysaccharide nature in the lining film. The comparison of the results obtained from tissue blocks fixed in potassium permanganate, embedded in 'Araldite', and results revealed by means of Hale's method could support the idea of the existence of various chemical components in the alveolar lining film. The granular material observed by us in the alveolar layer had some counterpart in the globular agglomerations of the epithelial cells of alveoli, while the Hale-positive material was found only on the alveolar surface of the cells.

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LUNG ALVEOLAR SURFACTANT, LUNG ELASTICITY, AND LUNG STABILITY

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EVIDENCE exists to show that a highly surface-active mixture of materials lines the alveoli of mammalian lung[1–4]. It is thought to form a 'bimolecular' layer there which is related intimately to the 'unit membrane' of plant and animal cells[5,6]. It is capable of reducing the surface tension markedly at the interface between water or some aqueous solutions and air when its area is diminished steadily[2]. This fact has been used to develop the concept that lung alveolar surfactant confers stability on lung alveoli by adjusting capillary pressure through adjustment of surface tension at their surfaces as their radii of curvature change with breathing movements[2]. In addition, such action is thought to produce a substantial part of the elastic properties of whole lungs[7]. A cogent point in favour of this hypothesis consists in the similar appearance between surface tension-area hysteresis loops obtained from alveolar surfactant on the surface balance and pressure-volume hysteresis loops obtained from excised lungs of various mammals[2,8]. The latter hysteresis loops are largely unaffected when pressures are held constant at the corresponding volumes for periods of up to 40 min, that is to say, under static conditions which are necessary for measurement of elastic properties of matter. The former hysteresis loops, however, have not been observed heretofore under static conditions, leaving the above comparison open to question until suitable evidence is at hand.

Surface tension-area hysteresis loops have been obtained with a specially designed surface balance which permits examination of lung alveolar surfactant at rates of change of area which might be considered similar to those existing in the alveoli of intact lung[3,9]. Under these conditions, the surface tension produced by the lung alveolar surfactant tended to proceed rapidly toward some intermediate value before maximum or minimum area of the surface was attained. Further anomalous behaviour of lung alveolar surfactant was observed when its area on a surface balance was reduced slowly to one-fifth of its initial area, then held constant while surface tension was measured against time. The surface tension rose from about 6 to about 27 dynes/cm in 7 min[10]. This observation indicates that relaxation of 'elastic' properties is slow, but is still much too rapid to agree with the 40 min of substantially unaffected results obtained with excised lungs. It suggests a lack of elasticity in the surface-tension behaviour produced by lung alveolar surfactant.

The area of the lung alveoli has been considered to remain virtually constant during breathing[11]. Under these conditions the lung alveolar surfactant might maintain stability of lung alveoli by producing very low and almost constant surface tensions and so would have little influence on elasticity of the lung. Adequate ventilation of the lungs certainly can be provided without change in their volume or surface area[12]. If the surface tension produced by lung alveolar surfactant *in situ*, under these static conditions, reached the values produced by it under static conditions on the surface balance, then alveolar instability might be expected, but does not result. Stability and elasticity of the lungs, on the other hand, might be due to tissue and not surface forces, or to geometric changes of alveolar surfaces during breathing movements[7,13].

From these observations, divergent hypotheses have arisen regarding the cause of stability and elasticity of lung tissue. It would be well to note that these disparate views have resulted from studies of different model systems, the application of which to events occurring in the living lung *in situ* may be subject to question until direct observations are obtained.

With this understanding of the limitations of model systems, a surface balance was used to re-examine the contribution of lung alveolar surfactant to the elasticity of lung, and to the spacial stability of lung alveoli.

Equipment and methods have been reported, with the exception that the hypophase in the present experiments contained no rinsings of mammalian lung[8,9]. The hysteresis loops presented here are not so smooth as those previously attained with the equipment used[9]. This is due mainly to replacement of the gear drive for control of movement of the barriers of the surface balance with a very slightly erratic chain drive and to the fact that the equipment had been removed to a less stable environment. The significance of the results, however, is believed not to be impaired by these minor artefacts.

Fig. 1 is a photograph of the experimental results from which the least maculate results were chosen for presentation. To obtain data for surface tension-area hysteresis, cycling rates of 14 and 7 cycles/min were used throughout the experiments shown in the top and bottom rows of the figure, respectively. Each of the 10 uninterrupted hysteresis loops shown in Fig. 1 was recorded from the maximum to the minimum and back to the maximum area. At this moment the barriers were arrested virtually instantaneously by shorting the driving electric motor. A large clock which operated at 4 beats/sec was strapped to one of the observer's ears to permit estimation of the duration of each arrest of the barriers. After waiting the number of seconds shown in the lower right corner of each recording, motion of the barriers was resumed until the area designated by each recorded vertical line ('spike') was reached. At this moment the barriers were arrested again for the same period shown. All recordings proceed in a clockwise direction. At each arrest of motion of the barriers, surface tension proceeded abruptly toward what appeared to be some constant value. This value appears to be about 20 dynes/cm for the upper row of data and was attained within 6 sec under static conditions. For the lower row the corresponding values are 27·5 dynes/cm and 8 sec, respectively. The discrepancy between 20 and

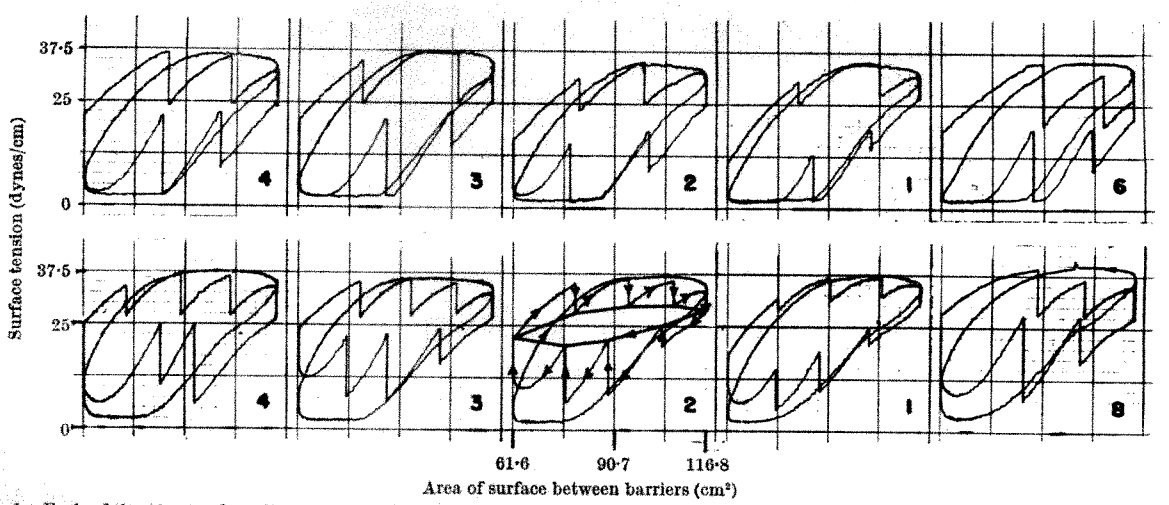


Fig. 1. Each of the 10 sets of results was obtained by first recording one complete hysteresis loop starting from and returning to maximum area clockwise. At this moment the barriers were arrested for the seconds shown at the lower right corner of each set. The same period of arrest of the barriers was repeated at each vertical line in each set. The upper row of sets was recorded at a free cycling rate of 14 cycles/min; the lower at 7/min. One set is labelled to indicate for each set; direction of motion, area bounded by the terminations of the members of each family of vertical lines, area swept by the barriers. The ratio of maximum to minimum area for all sets is the same and is approximately 1·9 : 1

27·5 dynes/cm was produced largely by the greater rate at which surfactant was added to the surface in the former case. This forces the equilibrium position to lower values.

The tips of the spikes formed under static conditions (2 sec) in the central set of data of the bottom row have been connected by straight lines to illustrate the static hysteresis under these conditions. When the free rate of cycling of the barriers was 14 or 7 cycles/min, this static hysteresis virtually disappeared at the end of 6 or 8 sec, respectively.

It would appear from these experiments that lung alveolar surfactant produces little, if any, elasticity of the surface on the surface balance. To the extent that such data can be applied to the mammalian lung, it might be said that elasticity at an aqueous surface produced by lung alveolar surfactant contributes little or nothing to elasticity of the lung. The equilibrium surface tension was also sufficiently great to suggest that lung alveolar surfactant contributes little to the spatial stability of mammalian lung through a mechanism involving a low and constant surface tension, although an increase in stability over that which would be provided by a surface of pure water would be expected.

The concept that lung alveolar surfactant produces stability of lung alveoli by producing a very low and relatively constant surface tension can be only touched on here. Results of examination of such material in the form of bubbles and the present investigations of it with a surface balance appear to be compatible when lung elasticity is considered, but mutually exclusive with regard to stability of the lung. Resolution of this conflicting evidence might depend on future ability to produce very low static surface tensions with lung alveolar surfactant on a surface balance. On the other hand, the question might be resolved by learning what effects the chemical composition and architecture of lung alveolar surfactant have on physical properties and behaviour of bubbles derived from lung alveoli.

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THIN-LAYER CHROMATOGRAPHY ON POLYAMIDE LAYERS: SEPARATION OF FAT ANTIOXIDANTS

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THE use of polyamide powder in column chromatography of phenolic compounds has been introduced, for example, by Carelli[1] and by Grassmann[2]. The work of Hörhammer *et al.*[3–5] especially has shown that this type of chromatographic analysis is very suitable for the separation and identification of many groups of phenolic compounds, flavonoids, etc., occurring in natural products.

Polyamide powders have recently been introduced as support in thin-layer chromatography by Davídek[6,7] and by Egger[8]. This technique has also been applied successfully to the analysis of flavonoids, anthocyanins, glycosides, fat-soluble vitamins, etc.

In column chromatography on polyamide the separation process was found to be highly affected by hydrogen bonding forces between the hydroxyl groups and the CONH groupings in the polyamide molecules[5]. It has been generally assumed that in thin-layer chromatography on polyamide layers hydrogen bonding likewise would play a predominant part.

My investigations concerning polyamide thin-layer chromatography of lipophilic compounds such as antioxidants, fatty acids, esters, however, gave circumstantial evidence as to the conception that in these instances hydrogen bonding is not of great importance, but that the

Table 1. R_F VALUES OF ANTIOXIDANTS WITH THIN-LAYER CHROMATOGRAPHY ON POLYAMIDE LAYERS

	A	*B*	*C*	*D*	*E*
Support:	Polyamide (MN)	Polyamide (Woelm)	Polyamide (Woelm)	'Silica gel *G*' (Merck)	Paper (Whatman 1)
Binder:	Starch	Starch	'Mowilith'	Plaster of Paris	—
Solvent:	Methanol–acetone–water (60 : 20 : 20)	Light petroleum–benzene–acetic acid–dimethylformamide (10 : 10 : 5 : 0·25)	Light petroleum–benzene–acetic acid–dimethylformamide (10 : 10 : 5 : 0·25)	Light petroleum–benzene–acetic acid– (10 : 10 : 5)	Chloroform–acetic acid (99 : 1), according to Sedláček[17]
Methyl gallate	0·68	0·09	0·06	0·06	0·03
Ethyl gallate	0·55	0·12	0·08	0·09	0·14
Propyl gallate	0·52	0·16	0·10	0·11	0·38
Butyl gallate	0·48	0·21	0·14	0·16	—
Octyl gallate	0·18	0·44	0·29	0·23	0·71
Dodecyl gallate	0·06	0·68	0·47	0·32	0·88
BHA	0·30	0·90	0·67	0·63	0·96
BHT	0·24	—	0·99	0·94	0·96
Sesamol	—	0·67	0·42	0·50	—
NDGA	0·26	0·10	0·08	0·09	0·47
Ascorbyl palmitate	0·04	—	—	0·08	—

polyamide layer will act as an apolar support or alternatively in some special cases as a kind of polar stationary phase. The type of stationary phase involved will mainly depend on the composition of the mobile solvent.

On 'loose layers' of polyamide, as were used by Davídek[6], the cross-sections of the spots were about 2 cm, which is somewhat large for obtaining distinct separations (see also ref. 9). I therefore found loose polyamide layers impracticable. Polyamide layers, prepared by mixing the powder with methanol, are also very thin and fragile. The use of an appropriate binding agent like starch or polyvinyl acetate was preferred. Starch-bound polyamide layers were prepared according to the procedure described by Machery and Nagel, by admixing 5 ml. of starch solution (10 per cent) to a slurry of 4·5 g polyamide in 45 ml. methanol–chloroform (3 : 2). Alternatively, I succeeded in preparing firmly bound polyamide layers by adding synthetic glues like polyvinyl acetate as binder. As an example 'Mowilith *CT* 5*a*' (from Messrs. Hoechst) was applied for this purpose. Forty-five millilitres of a 10 per cent solution of this 'Mowilith' in methanol was mixed with 7 g of polyamide powder and this slurry was spread on the glass plates by Stahl's spreader.

Various fat antioxidants, namely, the series of gallic acid esters, BHA, BHT and sesamol, etc., were analysed on starch-bound polyamide layers in the solvent methanol–acetone–water (60 : 20 : 20), according to Davídek[10–12].

As is shown in Table 1, series *A*, and Fig. 2, line *A*, the R_F values of the gallic acid esters decrease with increasing length of the carbon chain. The sterically hindered phenolic compounds BHT and BHA, having strong lipophilic properties, exhibit low R_F values of respectively 0·24 and 0·30. This separation pattern is indeed analogous to that found when analysing these antioxidants with reversed-phase paper chromatography on papers impregnated with vegetable oils or paraffin oil[13–15]. The same correlation between R_F and chain-length was found when analysing the higher fatty acids and their esters on polyamide layers, using the solvent mixtures methanol–acetone–water (40 : 40 : 20) and (30 : 10 : 10) respectively (see Fig. 1, *A* and *B*). These separations are analogous to those achieved in normal reversed-phase systems, for example, by Kaufmann and co-workers[16]. I therefore suppose that in these circumstances the polyamide layer will act as an 'apolar stationary phase'.

A quite different pattern of R_F values was found, however, when using non-aqueous solvent mixtures which contained acetic acid, for example, a mixture of light petroleum–benzene–acetic acid (10 : 10 : 5) (preferably with the addition of 0·25 dimethyl formamide). As is shown in Table 1, series *B*, *C*, and Fig. 2, line *C*, the gallic acid esters with long chain-lengths now display higher R_F values than the lower members of the series. There is a nearly linear correlation between R_M value and the length of the alkyl chain of these esters. In this solvent the lipophilic compounds BHT and BHA have high migration rates. The pattern of the spots is somewhat similar to that obtained in paper chromatographic analysis, for example, in the solvent chloroform–acetic acid (99 : 1) (refs. 17 and 18), where the stationary phase is built up by a cellulose–water complex[19]. This agreement was demonstrated by comparison of the R_F sequences in the series *B*, *C* and *E* in Table 1.

In the case of those solvents containing acetic acid, one should assume a type of partition process between a 'polar stationary phase', built up by acetic acid-imbibed polyamide particles, and the apolar mobile solvent. The

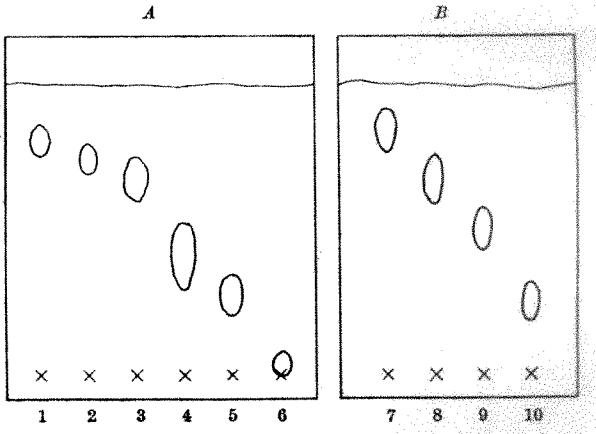


Fig. 1. Separation of lipophilic compounds by thin-layer chromatography on polyamide layers. Polyamide powder (Messrs. Woelm), starch bound. *A*, Separation of the higher fatty acids. Mobile solvent: methanol–acetone–water (40 : 40 : 20). Detection: spraying with 4 N sulphuric acid, eventually followed by a spray with 0·05 per cent rhodamine *B*. Spot 1, capric acid; spot 2, lauric acid; spot 3, myristic acid; spot 4, palmitic acid; spot 5, stearic acid; spot 6, arachinic acid. *B*, Separation of the higher fatty acid esters. Mobile solvent: methanol–acetone–water (30 : 10 : 10). Detection: 2·7-dichlorofluorescein under ultra-violet light. Spot 7, methyl laurate; spot 8, methyl myristate; spot 9, methyl palmitate; spot 10, methyl stearate

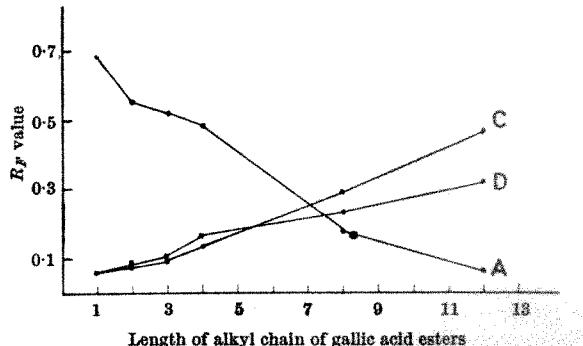


Fig. 2. R_F values of a series gallic acid esters in the systems *A*, polyamide/methanol–acetone–water (60 : 20 : 20); *C*, polyamide/light petroleum–benzene–acetic acid-dimethylformamide (10 : 10 : 5 : 0·25); *D*, 'Silica gel *G*'/light petroleum–benzene–acetic acid (10 : 10 : 5), as given in Table 1

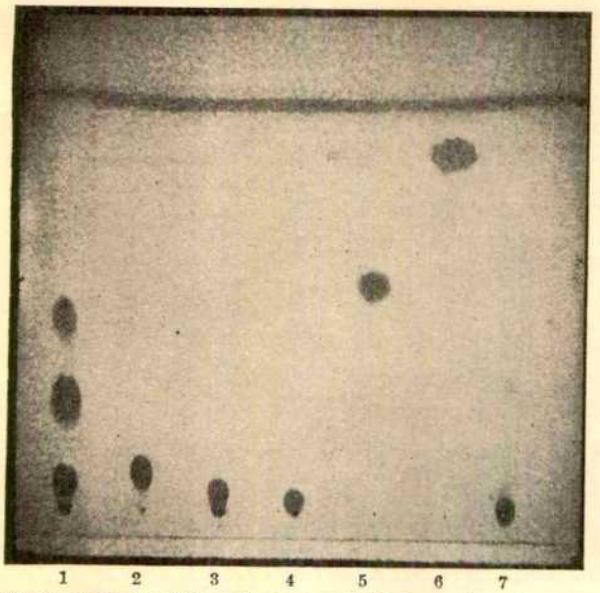


Fig. 3. Separation of fat antioxidants with thin-layer chromatography on polyamide layers. Binding agent: starch. Mobile solvent: light petroleum–benzene–acetic acid–dimethylformamide (10 : 10 : 5 : 0·25). Detection: ferric (III) sulphate–potassium ferricyanide reagent. Spot 1, dodecyl-, octyl-, propyl gallate; spot 2, butyl gallate; spot 3, ethyl gallate; spot 4, methyl gallate; spot 5, sesamol; spot 6, BHA; spot 7, NDGA

polyamide–acetic acid complex is comparable to the cellulose–water complex in paper chromatography. Further evidence of this concept is given by the agreement of the separation pattern of antioxidants on polyamide layers with that on 'Silica gel *G*' layers (compare Table 1, series *C* and *D*). The addition of a small quantity of a non-volatile polar organic liquid like dimethylformamide, ethyl lactate, phenyl cellosolve, etc., will help in building up this complex polar stationary phase and thus prevent the occurrence of tailing effects. The spots of the antioxidants now have a reasonable small cross-section.

The systems *C* and *D* (Table 1) were applied in practice to the separation and identification of common fat antioxidants, namely, gallates, BHA, BHT, NDGA, etc. In margarine and animal fats small amounts of gallates, as low as 0·005 per cent, were determined by using this technique (preferably in system *D*). The estimation of small amounts of BHT in fats with classical methods of analysis previously had encountered great difficulties. On silica gel layers a spot of about 1 μg of BHT could be clearly visualized with a ferric (III) sulphate–potassium ferricyanide colour reagent. By using an appropriate extraction procedure, I could thus determine the presence of quantities of BHT present at as low as 0·0001 per cent in food products like milk powder.

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CHARGE-TRANSFER COMPLEX FORMATION, CARCINOGENICITY AND PHOTODYNAMIC ACTIVITY IN POLYCYCLIC COMPOUNDS

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CHARGE-transfer complex formation is a short-range intermolecular interaction involving the transfer of an unpaired electron from donor (*D*) to acceptor (*A*) molecules, yielding a complex which may later dissociate into two free radicals: $D + A \rightleftarrows D \cdot A \cdot \rightleftarrows D \cdot + A \cdot$. An intense new absorption band, characteristic of the complex but of neither component alone, can be detected by spectral difference[1–3], or sometimes by the formation of coloured complexes when donor and acceptor molecules are forced into apposition[4–6]. The concept of involvement of charge transfer from the π pool of carcinogens to intracellular acceptor sites was derived from investigations, on relatively small numbers of compounds *in vitro*, relating carcinogenicity to the formation of certain types of charge-transfer complex[4–6]. An opportunity to determine the general applicability of this theory of charge transfer in carcinogenesis was made possible by the availability of a large series of polycyclic compounds of known carcinogenicity. It was also considered of interest to establish whether complex formation could account for the positive association between carcinogenic and photodynamic properties, recently demonstrated in the same series of compounds[7].

The ability of 107 polycyclic compounds to produce coloured complexes with iodine, chloranil, trinitrobenzene or acridine was determined. Whether the latter affords a true test for the formation of charge transfer, as opposed to other complexes, has been disputed[6,8]. Solutions of all compounds were tested in organic solvents at 0·1 M, using previously described techniques[4–6], briefly presented here.

Iodine test. Strips of Whatman No. 1 paper were moistened with chloroform solutions of the compounds under investigation and exposed to iodine vapour in a stoppered tube containing iodine crystals. In the absence of immediate coloration, observations were extended to 3 min. In addition, this test was also performed by dropping a 0·1-M iodine solution in chloroform on paper previously treated with compound solutions.

Table 1. Results of Colour Reactions with Iodine, Chloranil, Trinitrobenzene and Acridine

The 107 polycyclic compounds tested are classified by grade of carcinogenic activity, and data on photodynamic activity are also given

	Compound and source*	Colour reactions				Photodynamic activity $1/C_{30}$
		Iodine	Chloranil	Trinitrobenzene	Acridine	
	+ + + Carcinogens					
1	7-Methylbenz[*a*]anthracene (1)	Dark brown	Grey	Dark orange	Light yellow	12·50
2	12-Methylbenz[*a*]anthracene (1)	Light brown	Beige	Orange	Light yellow	14·29
3	7,12-Dimethylbenz[*a*]anthracene	Dark brown with black spots	Green-grey	Dark red	Dark yellow	5·88
4	6-Fluoro-7-methylbenz[*a*]anthracene (1)	Light brown	Blue-grey	Dark orange	Light yellow	16·67
5	5,6-Dimethylbenz[*c*]acridine (2)	Light brown	Red-brown	Yellow	Dark yellow	6·25
6	7,9-Dimethylbenz[*c*]acridine (3)	Dark brown	Red	Orange	None	50·00
7	7,10-Dimethylbenz[*c*]acridine	Dark brown	Brown	Yellow	None	5·56
8	Benzo[*a*]pyrene	Black	Green	Red	Dark yellow	> 100·00
9	6-Methylbenzo[*a*]pyrene	Black	Light yellow	Dark red	Dark yellow	8·33
10	3-Methylcholanthrene	Dark brown with black edges	Purple	Dark red	Dark yellow	6·25
11	5,6-Benzopyrido[2′,3′ : 1,2]carbazole (3)	Dark brown	Grey-blue	Dark orange	None	< 0·10
12	5,6-Benzopyrido[3′,2′ : 1,2]carbazole (3)	Light brown	None	Yellow	None	< 0·10
13	3,4,8,9-Dibenzpyrene (4)	Dark brown	Beige	Dark red	Dark yellow	< 0·10
14	3,4,9,10-Dibenzpyrene	Dark brown	Beige	Dark red	Light yellow	0·20
	+ + Carcinogens					
15	Benzo[*c*]phenanthrene (1)	Light brown	Purple-red	Yellow	None	0·29
16	5-Methylbenzo[*c*]phenanthrene (1)	Light brown	Purple	Orange	None	0·23
17	6-Methylbenzo[*c*]phenanthrene (1)	Light brown	Purple	Yellow	None	0·23
18	5-Methylbenz[*a*]anthracene (1)	Light brown	Grey	Orange	Light yellow	50·00
19	8-Methylbenz[*a*]anthracene (1)	Light brown	Grey	Orange	None	> 100·00
20	3-Fluoro-7-methylbenz[*a*]anthracene (1)	Light brown	Blue	Dark orange	Light yellow	33·33
21	9-Fluoro-7-methylbenz[*a*]anthracene (1)	Light brown	Grey-blue	Orange	Light yellow	10·00
22	10-Fluoro-7-methylbenz[*a*]anthracene (1)	Light brown	Black-grey	Orange	Light yellow	25·00
23	Dibenz[*a,h*]anthracene (5)	Light brown	Beige	Yellow	Light yellow	< 0·10
24	Benzo[*c*]fluoranthene	Light brown	Beige	Yellow	None	5·26
25	Dibenz[*a,h*]acridine (2)	Light brown	None	Yellow	None	0·18
26	1,2,3,4-Dibenzpyrene	Light brown	None	Dark orange	None	< 0·10
27	1,2,4,5-Dibenzpyrene	Dark brown	None	Orange	None	< 0·10
28	1,2,3,4,5,8,9-Tribenzpyrene	Light brown	None	Red	Light yellow	< 0·10
29	Naphtho[2′,1′*a*]perylene (6)	Dark brown	Orange	Brown	Light yellow	< 0·10
	+ Carcinogens					
30	2-Aminoanthracene	Dark brown	Yellow	Black	Dark yellow	< 0·10
31	2-Methylbenzo[*c*]phenanthrene (1)	Light brown	Purple	Yellow	None	0·48
32	3-Methylbenzo[*c*]phenanthrene (1)	Light brown	Purple	Yellow	None	0·56
33	Chrysene	None	None	Yellow	Light yellow	< 0·10
34	Benzanthrone (7)	Light brown	None	Yellow	None	4·76
35	Benz[*a*]anthracene	Light brown	None	Orange	Light yellow	33·33
36	6-Methylbenz[*a*]anthracene (1)	Light brown	Grey	Orange	None	25·00
37	7-Aminobenz[*a*]anthracene (7)	Dark brown	Green-grey	Black	Dark yellow	2·00
38	9-Methylbenz[*a*]anthracene (1)	Light brown	Green-blue	Pink	Light yellow	100·00
39	10-Methylbenz[*a*]anthracene (1)	Light brown	Beige	Orange	Light yellow	33·33
40	12-[2′-pyridyl]benz[*a*]anthracene (6)	Dark brown with black spots	Grey	Orange	Light yellow	3·33
41	7-Chloro-5,6-dimethylbenz[*c*]acridine (2)	Light brown	None	Dark yellow	None	20·00
42	9-Chloro-5,6-dimethylbenz[*c*]acridine (2)	Light brown	None	Orange	None	0·56
43	10-Chloro-5,6-dimethylbenz[*c*]acridine (2)	Light brown	None	Yellow	None	2·70
44	11-Chloro-5,6-dimethylbenz[*c*]acridine (2)	Light brown	None	Orange	None	0·56
45	Dibenz[*a,j*]anthracene	Light brown	Light brown	Yellow	None	< 0·10
	Non-carcinogens					
46	Phenanthrene	Light brown	Beige	Yellow	None	< 0·10
47	3-Methylphenanthrene	Light brown	Red	Yellow	None	< 0·10
48	9,10-Quinonephenanthrene (9)	Light brown	Orange	Orange	Light yellow	< 0·10
49	9,10-Dihydrophenanthrene	Light brown	Red-brown	Yellow	None	0·10
50	Anthracene	Light brown	None	Orange	None	0·29
51	1-Amino-6-chloro-9,10-quinoneanthracene	Dark brown	None	Dark red	None	< 0·10
52	2-Sulphonylchloride-9,10-quinoneanthracene (10)	Light brown	None	Light yellow	Dark yellow	< 0·10
53	9,10-Dihydroanthracene	None	Light yellow	Yellow	None	< 0·10
54	Fluorene	None	Beige	Yellow	None	< 0·10
55	Carbazole	Light brown	None	Orange	None	< 0·10
56	Acridine	Light brown	Red	Yellow	—	0·23
57	Pyrene	Light brown	Blue-grey	Orange	None	0·23
58	1-Methylpyrene	Dark brown with black edges	Green-grey	Orange	Light yellow	0·63
59	3-Methylpyrene	Dark brown with black spots	Green-grey	Red	Light yellow	0·77
60	6-Aminochrysene (7)	Dark brown with black spots	Green	Purple	Dark yellow	9·09
61	Fluoranthene	Light brown	Red	Yellow	None	0·24
62	Benzo[*a*]fluorene	Light brown	Black-grey	Orange	None	< 0·10
63	Benzo[*b*]fluorene	Light brown	None	Yellow	None	< 0·10
64	Benzo[*c*]fluorene	Light brown	Black-grey	Orange	None	< 0·10
65	Triphenylene	None	Pink	Yellow	None	< 0·10
66	Naphthacene	Light brown	Beige	None	Light yellow	1·41
67	1-Methylbenz[*a*]anthracene (1)	Light brown	Light grey	Dark orange	Light yellow	25·00
68	2-Methylbenz[*a*]anthracene (1)	Light brown	Beige	Orange	None	16·67
69	3-Methylbenz[*a*]anthracene (1)	Light brown	Beige	Orange	None	100·00
70	4-Methylbenz[*a*]anthracene (1)	Light brown	Beige	Pink	None	> 100·00
71	7-[1′-Naphthyl]benz[*a*]anthracene (6)	Light brown	None	Red orange	Light yellow	< 0·10
72	7-[2′,4′-Dimethylphenyl]benz[*a*]anthracene (6)	Light brown	Grey	Red orange	None	0·10
73	7-[4′-Dimethylaminophenyl]benz[*a*]anthracene (6)	Dark brown	Green-grey	Brown orange	Light yellow	< 0·10
74	7-[4′-Carbomethoxy]benz[*a*]anthracene (6)	Light brown	Grey	Orange	Light yellow	0·24
75	7-[4′-Hydroxyphenyl]benz[*a*]anthracene (6)	Light brown	Black-grey	Red orange	Light yellow	1·00
76	12-[3′-Pyridyl]benz[*a*]anthracene (6)	Dark brown	Grey	Orange	Light yellow	2·38
77	12-[4′-Pyridyl]benz[*a*]anthracene (6)	Dark brown	Beige	Yellow orange	None	< 0·10
78	12-[2′,3′-Dimethylphenyl]benz[*a*]anthracene (6)	Light brown	Beige	Red orange	None	0·18
79	12-[3′-Methylphenyl]benz[*a*]anthracene (6)	Light brown	Grey	Red orange	None	0·50
80	12-[4′-Methylphenyl]benz[*a*]anthracene (6)	Light brown	Grey	Red orange	None	0·24
81	7,12-Dionebenz[*a*]anthracene (11)	Light brown	Beige	None	None	25·00
82	4-Chloro-7-bromobenz[*a*]anthracene (8)	Light brown	Beige	Yellow	Light yellow	14·29
83	5-Fluoro-7-methylbenz[*a*]anthracene (1)	Light brown	Grey	Orange	Light yellow	33·33
84	Benzo[*b*]diphenyleneoxide	Light brown	Blue-grey	Yellow	None	< 0·10
85	5*H*-Benzo[*b*]carbazole	Light brown	None	Red	None	< 0·10
86	6,6-Dimethyl-6,11-dihydrobenz[*b*]acridine (2)	Light brown	Light yellow	Light yellow	None	< 0·10
87	6,6-Dimethyl-11-keto-6,11-dihydrobenz[*b*]acridine (2)	Light brown	None	None	None	0·38
88	6,6-Dimethyl-12-carboxy-6,11,dihydrobenz[*b*]acridine (2)	Light brown	None	None	Light yellow	< 0·10
89	6,6,11-Trimethyl-11-hydroxy-6,11-dihydrobenz[*b*]acridine(2)	Light brown	None	Light yellow	Light yellow	< 0·10

[*continued overleaf*

Table 1.—*Continued*

	Compound and source*	Colour reactions: Iodine	Chloranil	Trinitrobenzene	Acridine	Photodynamic activity $1/C_{30}$
90	Benz[*c*]acridine (2)	Dark brown	Red-brown	Yellow	None	14·29
91	5,6-Dimethylbenz[*c*]acridone (2)	Light brown	None	Dark orange	Light yellow	< 0·10
92	Benzo[*e*]pyrene	Dark brown	Grey	Dark orange	Light yellow	2·08
93	Dibenz[*a,c*]anthracene (5)	Light brown	Beige	Orange	None	< 0·10
94	Benzo[*k*]fluoranthene	Light brown	Beige	Orange	Light yellow	0·45
95	Benzo[*ghi*]fluoranthene	Light brown	Pink	Yellow	Light yellow	2·00
96	Benzo[*a*]chrysene	None	None	Yellow	None	< 0·10
97	Benzo[*b*]chrysene	None	None	Orange	None	< 0·10
98	Perylene	Dark brown	None	Dark red	Dark yellow	< 0·10
99	Dibenz[*a,j*]acridine	Dark brown with black edges	Light yellow	Yellow	None	0·11
100	1,2,6,7-Dibenzpyrene (4)	Light brown	None	Yellow	Light yellow	< 0·10
101	Benzo[*ghi*]perylene	Dark brown	Beige	Pink	Light yellow	50·00
102	Anthanthrene	Dark brown	None	Dark purple	Dark yellow	0·11
103	Anthanthrone (7)	Light brown	None	None	Light yellow	0·16
104	Coronene	Light brown	None	Orange	Light yellow	0·29
105	7,16-Quinonedibenzo[*a,o*]perylene (7)	Light brown	None	Orange	None	0·14
106	1,2,8,9-Dibenzpentacene	Light brown	None	None	Light yellow	0·22
107	Pyranthrene	Light brown	None	None	Light yellow	0·15

* Source: (1) Dr. M. S. Newman (Ohio State University, Columbus); (2) Dr. N. H. Cromwell (University of Nebraska, Lincoln); (3) Dr. N. P. Buu-Hoï (Institut du Radium, Paris); (4) Dr. G. S. Fell (Royal Beatson Memorial Hospital, Glasgow); (5) Dr. C. Heidelberger (McArdle Memorial Laboratory, University of Wisconsin, Madison); (6) Dr. F. A. Vingiello (Virginia Polytechnic Institute, Blacksburg); (7) Dr. E. Sawicki (Robert A. Taft Sanitary Engineering Center, Cincinnati); (8) Dr. M. C. Kloetzel (University of Southern California, Los Angeles); (9) Dr. C. J. Kensler (Arthur D. Little, Inc., Massachusetts); (10) Dr. T. C. Oakwood (Pennsylvania State University); (11) Dr. E. Moriconi (Fordham University, New York)—all of whom we thank for specimens.

Table 2. Complex Formation in 107 Polycyclic Compounds as determined by the Four Colour Tests
Compounds classified by carcinogenic and photodynamic activities

	No. of compounds	Iodine test +	Iodine test −	Chloranil test +	Chloranil test −	Trinitrobenzene test +	Trinitrobenzene test −	Acridine test* +	Acridine test* −
Carcinogens									
With strong photodynamic activity†	25	9	16	21	4	19	6	17	8
With weak photodynamic activity	20	6	14	12	8	11	9	7	13
Total	45	15	30	33	12	30	15	24	21
Non-carcinogens									
With strong photodynamic activity	13	5	8	13	0	9	4	8	5
With weak photodynamic activity	49	8	41	28	21	27	22	20	28
Total	62	13	49	41	21	36	26	28	33
Total	107	28	79	74	33	66	41	52	54

* Results for acridine not shown in table under acridine test. † $1/C_{30} \geq 2{\cdot}0$.

Table 3. Relative Odds Method for Analysis of Associations

Property II \ Property I	Positive	Negative	Total
Positive	A	B	$A+B$
Negative	C	D	$C+D$
Total	$A+C$	$B+D$	$A+B+C+D$

Relative odds ratio = AD/BC
$AD/BC > 1$ = Positive association
$AD/BC < 1$ = Negative association
$AD/BC \sim 1$ = No association

Chloranil test. Acetonitrile solutions of polycyclics were tested on paper with 0·1-M solution of chloranil in acetonitrile, as described for the iodine paper test.

Trinitrobenzene test. Equal volumes of trinitrobenzene and 0·1-M polycyclic solutions in tetrahydrofuran were mixed on depression slides. Aliquots of water were then added and the colour of any resulting precipitates noted. In addition, this test was also performed in the same way as the iodine paper test.

Acridine test. Acridine solutions in chloroform at 0·1-M were dropped on to filter paper which was then moistened with polycyclic solutions in the same solvent.

The results of the four colour tests are recorded in Table 1, where the compounds are grouped by grade of carcinogenic and photodynamic activities. Photodynamic activity is expressed as $1/C_{30}$, the reciprocal of the concentration, in μg/ml., producing 90 per cent lethality of *Paramecium caudatum* by 30 min, compounds being regarded as having strong activity when $1/C_{30} \geq 2{\cdot}0$ (ref. 8). Under standard conditions, complete reproducibility was found with all the colour tests, irrespective of which technique was used. Erratic results did occur with the iodine test if the moistened paper was allowed to dry before exposure to iodine vapour. Colour production was immediate with the trinitrobenzene slide and all paper tests, but not with the iodine vapour test.

For purposes of statistical analysis, a positive result was scored when a black or dark brown colour was given with iodine, any colour with chloranil, any colour other than yellow with trinitrobenzene, and yellow with acridine[4–6]. The number of compounds positive and negative for each colour test are classified by carcinogenic and photodynamic status in Table 2. The 'relative odds technique'[9] is the method of analysis used to define the associations considered here. Table 3 shows that for T compounds $(A + B + C + D)$ the association of two particular properties, I and II, can be measured by the relative odds ratio AD/BC. Where these properties are not associated, this ratio will approach unity. Positive associations are indicated by ratios greater than unity, while negative associations are indicated by values less than unity. With large numbers of compounds, the relative odds ratio can be considered a reliable measure of the association. However, for smaller samples, the degree of reliability may be lower, necessitating tests for significance. In analyses of the association between any two properties, adjustments may be made for a third property by grouping the compounds into those positive and negative for the third property. Adjustment of this type, perforce, reduces the numbers in each group and results in reduced significance, even if the odds ratio remains unchanged.

Table 4. Inter-association of Four-colour Tests for Complex Formation in 107 Polycyclic Compounds

Colour test A	Colour test B	Test results, No. compounds A+B+	A+B−	A−B+	A−B−	Association Relative odds	χ^2	P
Iodine test	Chloranil test	24	4	50	29	3·5	3·9	< 0·05
	Trinitrobenzene test	25	3	41	38	7·7	8·1	< 0·005
	Acridine test*	20	8	32	46	3·6	6·5	< 0·02
Chloranil test	Trinitrobenzene test	49	25	17	16	1·8	1·5	*N.S.* (> 0·2)†
	Acridine test*	37	36	15	18	1·2	0·1	*N.S.* (> 0·2)†
Trinitrobenzene test	Acridine test*	39	27	13	27	3·0	6·0	< 0·02

* Results for acridine not shown or used in calculations. † *N.S.* = Not significant.

The four colour tests reported here make available individual measures of particular types of charge-transfer complex formation and their results are not strictly comparable. This, in all probability, accounts for the imperfect nature of the positive inter-associations between the results of these tests (Table 4). The inter-associations involving the iodine test are stronger than are those for the other colour tests, suggesting that the iodine test, with relatively few positive results, provided a more stringent measure of strong charge-transfer complex formation. Corresponding stringencies for the other tests might also increase their apparent validity. It is of interest, in this connexion, to note that dark brown, besides black, was scored here as positive with the iodine test (Prof. A. Szent-Györgyi, personal communication), in contrast to the original report on this test which restricted positives to black[5].

Table 5. Association of Carcinogenic and Photodynamic Activities

Associations are shown for all 107 polycyclic compounds, and separately for compounds positive or negative on each of four tests for complex formation

	Relative odds	χ^2	P
All compounds (107)	4·7	12·2	< 0·0005
Compounds positive by:			
Iodine test (28)	2·4	0·6	*N.S.* (> 0·2)
Chloranil test (74)	3·8	6·3	< 0·02
Trinitrobenzene test (66)	5·2	8·3	< 0·005
Acridine test (52)*	6·1	7·6	< 0·01
Compounds negative by:			
Iodine test (79)	5·9	10·4	< 0·002
Chloranil test (33)	∞	5·1	< 0·025
Trinitrobenzene test (41)	3·7	1·9	< 0·2
Acridine test (54)	3·4	2·5	< 0·2

* Results for acridine not used in calculations.

Table 6. Association of Photodynamic Activity and Complex Formation as indicated by Four Colour Tests

Associations are shown for all 107 polycyclic compounds, and separately for carcinogens and for non-carcinogens

	Relative odds	χ^2	P
All compounds (107)			
Iodine test	2·3	2·7	0·1
Chloranil test	6·2	10·0	< 0·002
Trinitrobenzene test	2·3	2·8	< 0·1
Acridine test*	2·9	5·6	< 0·02
Carcinogens (45)			
Iodine test	1·3	0·0	*N.S.* (> 0·2)
Chloranil test	3·5	2·2	< 0·2
Trinitrobenzene test	2·6	1·4	*N.S.* (> 0·2)
Acridine test	3·9	3·6	< 0·1
Non-carcinogens (62)			
Iodine test	3·2	1·9	< 0·2
Chloranil test	∞	6·6	0·01
Trinitrobenzene test	1·8	0·4	*N.S.* (> 0·2)
Acridine test*	2·2	0·9	*N.S.* (> 0·2)

* Results for acridine not used in calculations.

Table 7. Association of Carcinogenic Activity and Complex Formation as indicated by Four Colour Tests

Associations are shown for all 107 polycyclic compounds, and separately for compounds with strong or weak photodynamic activity

	Relative odds	χ^2	P
All compounds (107)			
Iodine test	1·9	1·5	*N.S.* (> 0·2)
Chloranil test	1·4	0·3	*N.S.* (> 0·2)
Trinitrobenzene test	1·4	0·5	*N.S.* (> 0·2)
Acridine test*	1·3	0·3	*N.S* (> 0·2)
Strong photodynamic agents (38)			
Iodine test	0·9	0·0	*N.S.* (> 0·2)
Chloranil test	0·0	0·9	*N.S.* (> 0·2)
Trinitrobenzene test	1·4	0·0	*N.S.* (> 0·2)
Acridine test	1·3	0·0	*N.S.* (> 0·2)
Weak photodynamic agents (60)			
Iodine test	2·2	0·9	*N.S.* (> 0·2)
Chloranil test	1·1	0·0	*N.S.* (> 0·2)
Trinitrobenzene test	1·0	0·0	*N.S.* (> 0·2)
Acridine test*	0·8	0·1	*N.S.* (> 0·2)

* Results for acridine not used in calculations.

The positive association between carcinogenic and photodynamic activities reported previously[7] cannot be accounted for by the possibility that both activities are associated with complex formation, as the association obtains equally for compounds with and without complex formation (Table 5). A positive but imperfect association between photodynamic activity and each measure of complex formation is expressed in Table 6. There is no systematic indication that this association differs from carcinogens to non-carcinogens. The possible involvement of charge transfer in photodynamic activity has been suggested by the observation that tryptophan gave protection against the photodynamic toxicity of benzo(*a*)-pyrene[10]. In view of recent evidence on the photo-induction of free radicals from charge-transfer complexes[11,12], photodynamic injury might result from the effects of free radicals photo-induced from charge-transfer complexes of photosensitizing polycyclic electron donors and intracellular electron acceptors[7]. The production of free radicals from black complexes of benzo[*a*]pyrene and iodine has been reported[5], but the possibility of photo-induction of these radicals does not appear to have been considered. As can be seen from Table 7, there is no significant association between carcinogenicity and charge-transfer complex formations as indicated by any colour test. The positive association between complex formation and photodynamic activity demonstrated here, together with the photodynamic-carcinogenic association, could induce an apparent statistical association between carcinogenicity and complex formation. In the present data, based on 107 polycyclic compounds, this induced association between complex formation and carcinogenic activity is not significant, but could appear stronger[4–7] in a smaller and more selected group of compounds. For example, restriction of consideration to the results of the iodine test for the + + + carcinogens in Table 1 suggests a degree of association between complex formation and carcinogenicity which, however, disappears when investigations are extended to non-carcinogens and carcinogens of lesser potency.

Application of techniques which provided the original *in vitro* evidence[4–6] for involvement of charge-transfer complex formation in carcinogenesis to a large series of polycyclic compounds has revealed no particular association between complex formation and carcinogenicity, and does not support the recommendation[7] that charge-transfer tests be used to screen for carcinogenicity. On the basis of available evidence, it is now considered unlikely that charge-transfer complex formation plays a significant part in determining the carcinogenicity of polycyclic compounds.

This investigation was supported by U.S. Public Health Service research grant *C*–6516 from the National Cancer Institute.

We thank Prof. A. Szent-Györgyi, Dr. I. Isenberg and Profs. A. and B. Pullman for their helpful comments.

Note added in proof. Our attention has just been directed to an article by N. P. Buu-Hoi and P. Jacquignon (*Experientia*, 13, 375; 1957), who have reached similar conclusions to those reported here.

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EFFECT OF ELECTROCONVULSIVE THERAPY ON PLASMA CORTISOL-LEVELS

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A NUMBER of investigations of the activity of the adrenal cortex in subjects undergoing emotional and physical stress have been made. Early workers used various indirect measures of adrenal activity, but most recent work has been concerned with the plasma concentration of cortisol, and it has been shown that emotional distress is frequently accompanied by a raised plasma cortisol-level. Raised levels of about 18–22 μg/100 ml. have been reported[1] in relatives accompanying casualties to hospital and in patients awaiting surgery[2]. These levels compare with means of between 12 and 15 μg/100 ml. found in subjects examined under less stressful conditions[3]. Very high values have also been reported[4] in medical students before examinations. The elevation of plasma cortisol-levels has also been ascribed to emotional distress when this is a presumed part of a psychiatric illness such as acute schizophrenia[5], anxiety state[6] or depressive illness[7].

Since almost by definition mental illness implies an abnormal response to emotional stresses, interest in this field has tended to focus on abnormalities of responsiveness. In depressive illness, clinical reports suggest reduced responsiveness to both psychological and physical stress, and many workers consider that one type of depressive illness, called variously endogenous or physiological depression, reflects a failure of hypothalamic centres concerned with instinctive drives and adrenal stress mechanisms, and it has been frequently suggested that E.C.T. acts by stimulating these mid-brain centres. However, early work using various indices of adrenocortical responses to electroconvulsive therapy (E.C.T.) suggested that stress responses to E.C.T. were essentially non-specific.

In some preliminary experiments we made assays of circulating corticotrophin. While it was possible to demonstrate the release of corticotrophin following E.C.T., the method was both cumbersome and expensive. In the work recorded here, therefore, we have examined plasma cortisol-levels before and after E.C.T. in patients diagnosed as suffering from either schizophrenia or depression.

The work was carried out at a large psychiatric hospital in London where a number of patients were receiving E.C.T. The patients examined were those having this treatment with a definite diagnosis of schizophrenia and those who were confidently regarded as not schizophrenic. Each patient was interviewed by one of us (P.B.) and the diagnoses were made on the basis of this interview together with help from the hospital notes and, where necessary, discussion with the consultant in charge of the case. In this way, 23 schizophrenic patients and 23 depressed patients were selected for examination.

The latter group consisted almost entirely of cases of severe depression, but one patient in whom anxiety predominated over depression was included. Cases showing mixed depressive and schizophrenic features were excluded, as were patients in poor physical health and those over 65 years of age.

The mean age of the schizophrenic group was 30·2 years (range 20–53) and of the depressive group 48·0 years (range 21–65). All the schizophrenic patients were having either chlorpromazine or trifluoperazine, and 5 were also having anti-depressant medication. In the depressive group, 13 patients were having phenothiazines and 12 were having anti-depressants; in the case of 7 patients, both drugs were being taken together.

E.C.T. treatments were given between 9.30 a.m. and 11 a.m. Atropine was used as premedication in all cases, and the anæsthetic consisted of intravenous sodium thiopentone. Suxamethonium chloride was used routinely as a relaxant. An initial specimen of 4 ml. of venous blood was collected after the administration of anæsthetic and relaxant, and immediately before the shock was applied. Three subsequent specimens were collected 0·5, 1 and 2 h after the shock. The blood was heparinized and each specimen was centrifuged shortly after collection. The plasma was kept at −20° C until assayed. Plasma cortisol was determined by the spectro-fluorometric method of Zenker and Bernstein[8], the fluorescence being measured 20 min after addition of the fluorescent reagent.

A single set of observations was made on each patient, and these were made at different treatments in the course. Differences in the treatment number were neither marked nor significant for the two groups and, though this variable does influence the effect of E.C.T., it does not affect the issues discussed here. A separate examination of serial observations will be reported later.

The mean cortisol values for the two groups of patients are given in Table 1. The mean change between the pre-treatment value and the 0·5 h reading for the depressed group alone is significant ($P < 0·05$) but not that for the schizophrenic group. The 1 h and 2 h readings are not significantly different from the pre-treatment values for the combined group or for either group taken separately.

Although the depressives have a lower pre-operative mean value and show both a larger absolute and even greater proportionate increase in plasma cortisol following treatment, the differences between the depressive and

Table 1. Mean Plasma Cortisol-levels (μg/100 ml. Plasma) before and after E.C.T.

	Resting	½ h	1 h	2 h
Depressed patients (n, 23)	15·0 ± 7·1	21·0 ± 11·8	17·7 ± 8·2	14·9 ± 7·4
Schizophrenic patients (n, 23)	16·5 ± 8·1	19·2 ± 7·1	17·3 ± 6·1	14·8 ± 5·6

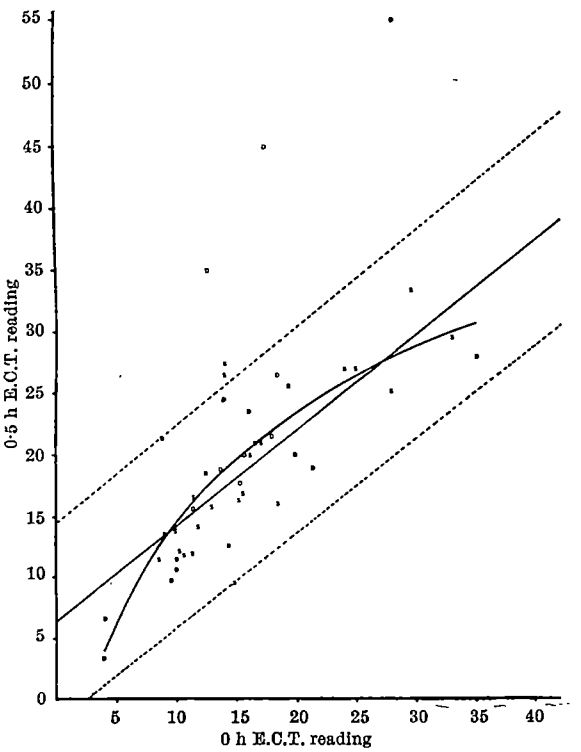


Fig. 1. Scattergram to illustrate the relationship between diagnostic status, pre-E.C.T. and 0·5 h levels of plasma cortisol. Using Chauvenet's criteria, the three aberrant observations have been excluded from these calculations and the straight lines give the resulting linear regression together with its confidence limits. The excluded values are all more than 4 *S.D.* from the regression. The curvilinear regression has the form $y = -16{\cdot}73 - 0{\cdot}632\,x + 11{\cdot}69\sqrt{x}$

schizophrenic groups are not significant using either parametric or non-parametric tests. The differences do not appear to be related to the difference in age between the two groups nor to differences in drug treatment or treatment number.

Inspection of the individual observations (Fig. 1) suggests that three of the depressive patients made markedly aberrant responses to E.C.T. while the remainder formed a homogeneous group with the schizophrenic patients. Using Chauvenet's criterion and Bartlett's test of homogeneity, it is possible to show that these three subjects have normal pre-treatment values and atypical treatment responses, and that the remaining patients, regardless of diagnosis, form a homogeneous group. For this group the linear regression equation $y = 6{\cdot}22 + 0{\cdot}785x$ gives a satisfactory fit for the relationship between the pre- and post-treatment levels. However, since it would be physiologically likely that the responsiveness of the adrenal glands would have an upper limit, it is of interest that the negatively accelerating curvilinear relationship $y = a - bx + c\sqrt{x}$ provides a significantly better fit for the data ($P < 0{\cdot}01$).

The close similarity of the results obtained with schizophrenic and depressive patients is of considerable interest. Pincus and Hoagland[9] reported that in schizophrenia the pituitary-adrenocortical system was relatively unresponsive compared with normal controls. The more recent work of Bliss[5], however, has shown that in schizophrenia the resting cortisol values and the adrenocortical responses as measured by increase in circulating plasma cortisol are essentially similar to those of normal controls. There is less work on stress response in depressive illness, although the observations of Broad *et al.*[10] and those of Gibbons and McHugh[7] are agreed that resting cortisol-levels are high in depression and fall as clinical improvement occurs.

There are two principal differences between the findings of the English and the American workers. First, the Americans found that treatment with E.C.T. produced a sustained rise in plasma cortisol in spite of clinical improvement. Secondly, the final values for the English workers are well below the normal control values reported by the Americans, except that some of the final observations were high because the patients remained depressed. Our own observations throw little light on these two points, but the finding that depressive patients are homogeneous with schizophrenics so far as both resting and post-E.C.T. cortisol-levels are concerned suggests that the sustained elevations observed by other workers reflect a normal hypothalamic-adrenocortical response to the stress of the illness. If this were the case, then it might well be postulated that the physiological stress of E.C.T. is relatively invariate and independent of the psychological stresses of the illness, that the increase in circulating cortisol should be fairly constant, and that the patients who were more stressed by the illness would tend to show a relatively smaller physiological response following E.C.T. The reverse relationship is, in fact, found, and this means that for E.C.T. the resting level of cortisol is a measure of the responsiveness of the patient as well as a possible measure of the degree of subjective stress of the illness. Physiologically it would appear unlikely that responsiveness would increase rectilinearly with resting level if the latter reflected in any way a stress response, that is to say, a ceiling effect should be postulated and the relationship between resting and post-E.C.T. levels should be curvilinear and negatively accelerating. As already mentioned, the data fit a relationship of this type better than a simple linear regression. These findings appear compatible with the findings after traumatic surgery reported by Sandberg *et al.*[11].

The foregoing discussion of the physiological effects of E.C.T. largely ignores the fact that this treatment is a psychological stress as well as a physiological one. It remains possible that a peak of emotional tension is reached immediately prior to the treatment and that this leads to a peak in plasma cortisol between half and one hour later. That this explanation is unlikely to be correct is supported by the preliminary observations carried out on blood corticotrophin. On 7 out of 11 occasions this was found to be increased immediately after treatment compared with the pre-treatment values. The maximum release of corticotrophin has been shown by Hodges and Vernikos[12] to occur within minutes of the imposition of a stress, and these observations suggest that the major stimulus occurs at the time the shock is applied.

With regard to the three atypical subjects, it is clearly very unlikely indeed that these three observations are due to chance variations. That all three are diagnosed as suffering from depressive illness could easily be a chance finding, but is perhaps of some interest. Careful re-examination of the records provides no suggestion of any technical error, and this, together with the fact that the values obtained for the resting pre-treatment cortisol-levels are all within the range for the rest of the subjects, makes it almost certain that these three patients are reacting to E.C.T. in a physiologically different way. This difference bears no discernible relationship to treatment number, outcome of treatment or drug régime. The three patients are at the upper end of the age-group, but previous work shows no marked effect of age on adrenal responsiveness and the correlations of resting plasma cortisol-levels with age in the present study are small and not significant ($r = -0{\cdot}171$ $P < 0{\cdot}3 > 0{\cdot}2$). The correlation with age for the 0·5 h values is even smaller ($r = 0{\cdot}066$ $P < 0{\cdot}7 > 0{\cdot}6$). Although there was no evidence of physical ill-health in any of these patients, it remains possible that this effect could be due to subclinical liver damage—one patient was receiving 'Parstelin', the other two 'Amitriptyline HCl'. In the series reported by Gibbons and McHugh[7], only two patients showed sharp increases in plasma cortisol following treatment. Only one of these received E.C.T., but their observations, as well as

those presented here, support the thesis that depressive illnesses are not physiologically homogeneous[13].

We thank Dr. I. Sutton and the staff of Friern Hospital for their assistance and Mrs. Myfanwy Kerr for help with the statistical analyses. One of us (P.B.) was supported by the Mental Health Research Fund; the others, by the Medical Research Council.

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ETHYLENE OXIDE AN ANTAGONIST OF ETHYLENE IN METABOLISM

By MORRIS LIEBERMAN, SAM ASEN and Dr. L. W. MAPSON

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RECOGNITION of ethylene oxide as an inhibitor of ethylene production in fruit[1] presents new possibilities for exploring both the mode of action of ethylene and its biosynthetic pathway. This report summarizes recent experiments with ethylene oxide, in which reversible antagonism to senescence was observed in a number of different tissues.

When a proper concentration of ethylene oxide is applied to some fruit during the pre-climacteric period, ethylene production is suppressed and ripening is often retarded. Ethylene production and ripening are inhibited (Fig. 1, bottom) only so long as excess ethylene oxide remains in the tissues (Fig. 1, top). After dissipation of the absorbed ethylene oxide, either by expulsion or by reaction within the tissues, the fruit resume production of ethylene and ripening in the normal manner. In this type of inhibitory action, which has been demonstrated with plums and tomatoes, ethylene oxide may be considered a metabolic antagonist to ethylene.

The foregoing statements are based on results of more than two dozen experiments. However, sensitivity to ethylene oxide varies considerably among different fruit tissues, and many tissues are irreversibly injured. The greater the production of ethylene at the time of treatment, the higher the dosage of ethylene oxide required to suppress ethylene and ripening. With high-ethylene-producing fruit, such as the avocado and apple, ethylene production has not, as yet, been suppressed without permanent damage to the tissues.

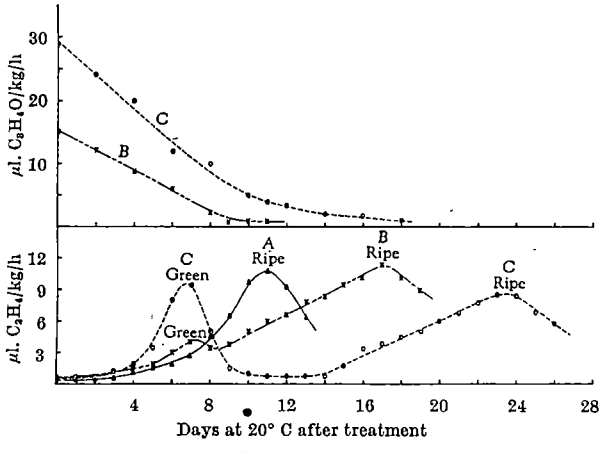


Fig. 1. Bottom, course of ethylene production in tomatoes (var. 'Potentate') at 20° C; (*A*) control; (*B*) ethylene oxide, 0·35 per cent v/v for 20 h; (*C*) ethylene oxide, 0·65 per cent v/v for 20 h. Fruit were mature green at time of treatment. Rise in ethylene production in (*B*) and (*C*) during 5, 6 and 7 days is due to initial stimulation by ethylene oxide. Stage of ripeness shown on ethylene curves was determined by visual observation. Top, course of expulsion of ethylene oxide from tomatoes during holding in enclosed atmosphere. Fruit were removed and atmosphere flushed with air each day before return of fruit to enclosure in air. Ethylene and ethylene oxide were determined by gas chromatography as previously described (ref. 1)

After treatment with relatively high concentrations of ethylene oxide (above 0·5 per cent), some fruit show an initial sharp transitory rise in ethylene production, which may be much greater than the highest point normally reached at the climacteric peak. An extreme form of this phenomenon was found to be associated with subsequent inability of the fruit to ripen properly, and may be related to cellular damage. The result of ethylene stimulation by ethylene oxide may be seen in Fig. 1 (*C*) and (*B*) (bottom) during the 6th and 7th day. Stimulation of this magnitude is comparatively mild. These fruit were not injured, and ripened normally. It appears, therefore, that ethylene oxide inhibits directly at the site of ethylene production, and may also act at the site of ethylene action, judging by its suppression of ripening in fruit already producing ethylene.

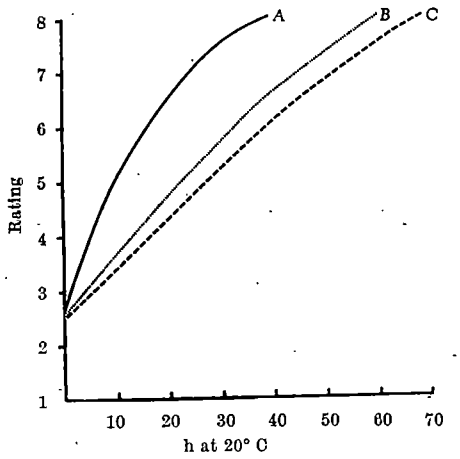
The anti-ageing effect of ethylene oxide has also been observed in recent months, in experiments with flowers. Senescence in flowers is associated with ethylene production[2] and may be considered to be a ripening process. Roses (cv. 'Better Times') subjected to 0·25 per cent ethylene oxide (v/v) in an enclosed atmosphere for 20 h at 15° C showed considerably increased longevity after removal to an open environment at 20° C. The buds opened more slowly, and reached full bloom about 30 h later than controls (Fig. 2). The delay in ageing results from absorption of ethylene oxide and lasts as long as ethylene oxide is being released from the tissue. Loss of absorbed ethylene oxide from the floral tissue occurs very rapidly (Fig. 3). However, the rate of release from the rose buds can be retarded further by enclosure, in air, of the ethylene oxide-treated roses, beyond the initial 20-h treatment.

Because roses produce extremely minute amounts of ethylene (less than 0·1 p.p.m.), inhibition of ethylene production in roses treated with ethylene oxide was not clearly demonstrated. With carnations, however, antagonism between ethylene and ethylene oxide was observed. Carnations are extremely sensitive to ethylene and, when exposed, exhibit symptoms known as 'sleepiness'. Sleepiness is characterized by incurving of the petals and closing of fully opened flowers. Exposure to ethylene concentrations of 1 p.p.m. will cause 'sleepiness'[2].

When carnations were exposed to both ethylene (1 p.p.m.) and ethylene oxide (0·1 per cent) simultaneously, 'sleepiness' did not occur (Fig. 4). Ethylene oxide also antagonized loss of water caused by treating carnations

with ethylene (Fig. 5). Loss of water was diminished in the presence of ethylene oxide even when compared to untreated controls (Fig. 5). These and other published results[3–5] suggest that one physiological effect of ethylene is to increase loss of water, by altering permeability of cell membranes. Ethylene oxide appears to counteract this effect. Therefore, the inhibitory action of ethylene oxide appears to strike not only at the source of biosynthesis of ethylene but also at a site of its action.

We have thus observed, in a number of experiments, that different physiological responses to ethylene are antagonized by ethylene oxide. Ripening of fruit, 'sleepiness' and senescence in flowers, and loss of water due to increased cell permeability have all been delayed or



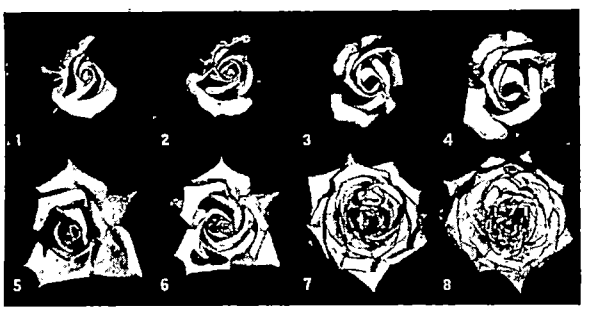


Fig. 2. Rating of roses during holding period at 20° C after treatment; (*A*) control; (*B*) treated with 0·25 per cent ethylene oxide for 20 h at 15° C; (*C*) as (*B*) but held in enclosed atmosphere, after removal from initial 0·25 per cent ethylene oxide atmosphere. Bottom, basis for ratings in Fig. 2

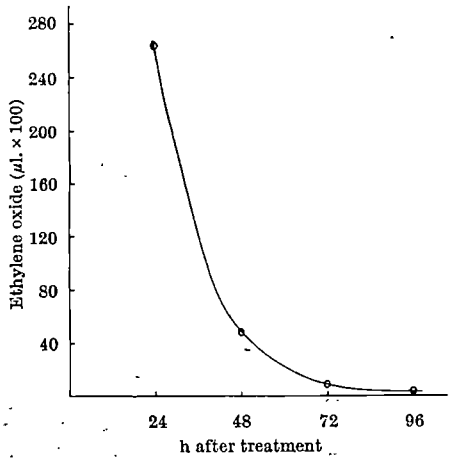


Fig. 3. Expulsion, by roses, of absorbed ethylene oxide into closed atmosphere after initial 20-h exposure to 0·25 per cent ethylene oxide. Chambers were flushed with air each day after assay

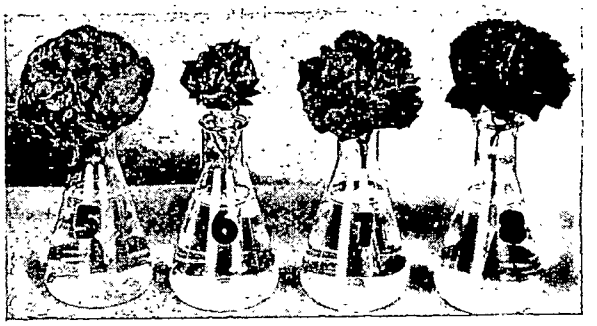


Fig. 4. Effect of ethylene, ethylene oxide, and ethylene and ethylene oxide applied simultaneously, on carnations. Figure shows condition of flowers 2 days after treatment (20 h at 20° C). (5) Control, (6) 1 p.p.m. ethylene, (7) 1 p.p.m. ethylene and 0·1 per cent ethylene oxide, (8) 0·1 per cent ethylene oxide

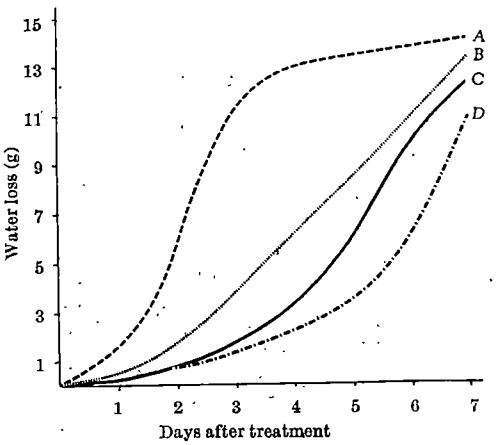


Fig. 5. Acceleration and retardation of water loss by carnations after treatment with ethylene and ethylene oxide respectively. (*A*) 1 p.p.m. ethylene; (*B*) 1 p.p.m. ethylene + 0·1 per cent ethylene oxide; (*C*) control; (*D*) 0·1 per cent ethylene oxide

neutralized by exposure to ethylene oxide. Even the ethylene-induced triple response of etiolated pea seedlings was partially prevented when both ethylene and ethylene oxide were applied simultaneously. Although the etiolated pea seedlings were stunted, they did not show epinasty or swollen growing tips. All these observations, taken together, point to a generalized antagonism of ethylene and ethylene oxide in plant metabolism. The fact that ethylene and ethylene oxide have related structures may be pertinent to their interaction.

If ethylene is a ripening hormone[2], speculation suggests that ethylene oxide may be an anti-ripening or anti-ageing hormone. At any stage in the life of a fruit or flower, the level of ethylene production may be controlled by ethylene oxide. Perhaps minute quantities of ethylene control metabolic events which are important to the young plant. Ethylene oxide may regulate the levels of ethylene in the young plant, thereby preventing excess ethylene and associated catabolic reactions. At the pre-climacteric stage in development of fruit, ethylene metabolism may be released from control by ethylene oxide, and thus allow ageing reactions to go forward at full pace. This may account for the climacteric rise in respiration in fruit metabolism. Suppression of ethylene production by ethylene oxide can be explained by current work which suggests that biosynthesis of ethylene may occur in a reaction sequence, one step of which could be inhibited competitively by ethylene oxide.

Although in earlier work[1] we tentatively identified ethylene oxide in volatiles from cytoplasmic particles from apples, and in volatiles emanating from green tomatoes, this could not be repeated in recent experiments. Therefore, ethylene oxide has not been conclusively identified

as a product of normal metabolism, and, on that account, these speculations on the role of ethylene oxide are not supported. The inability to recognize ethylene oxide in volatiles produced by fruit may be due to its extreme reactivity and solubility, which allow no excess to escape from the fruit tissue. Alternatively, ethylene oxide, produced in the cells, may be immediately converted to acetaldehyde, its less-reactive isomer. Whether or not ethylene oxide is a natural component of plant metabolism, the fact remains that it can act as a reversible anti-ripening or anti-ageing factor. This effect is brought about by suppressing ethylene production and also by directly antagonizing reactions which are stimulated by ethylene.

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PRELIMINARY INVESTIGATION OF THE INFLUENCE OF CONTROLLED ATMOSPHERE STORAGE ON THE ORGANIC ACIDS OF APPLES

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IN the procedure described here, apple extract from 'McIntosh' pulp tissue containing 3 m.equiv. of total free acid was separated on 23 cm³ of 'Dowex-1' anion exchange resin into 12 major acid fractions.

The fruit was harvested from a mature tree on two different dates, September 17 and October 5, 1961. The best harvest date, for storage, during this season was September 27. Fruit was put into two commercial-size storage rooms. One was a conventional cold storage room held at 32° F; the other was gas-tight and maintained at 38° F with an atmosphere of 3 per cent oxygen and 5 per cent carbon dioxide. Fruit was analysed at the end of six months' storage in each room. On removal from storage the fruit was frozen at −5° F until the extraction procedure was begun. The frozen fruit was peeled and wedges were cut to the carpels, diced, and dropped (50 g from each of four apples) into 450 ml. of boiling absolute ethanol. This was extracted in a Waring blender and filtered through cheese-cloth; the pulp was re-blended in 250 ml. of 85 per cent ethanol. The filtered juice from the second extraction was combined with the first. Clean-up procedure consisted of evaporation of the alcohol under vacuum at 35° C, centrifugation, and passage over 'Dowex-50' cation exchange resin to remove amino-acids. The use of 'Darco G-60' charcoal, partially deactivated with acetic acid, resulted in the removal of 10 per cent of the total acid from the McIntosh pulp extract. Charcoal treatment was, therefore, not used in these analyses; no problem of resin clogging resulted.

The final column was a shortened 50-ml. burette of 10·5 mm inside diameter, containing 23 cm³ 'Dowex-1' anion exchange resin (200–400 mesh, acetate form) to a height of 25 cm. Sugars were rinsed from the column with 125-ml. distilled, deionized water. Acids were removed from the column by gradient elution with acetic and formic acid. The gradient elution apparatus comprised two polyethylene flasks connected at the bottom by a one-way check valve between the outlets. The principle of the operation of this gradient mixer was similar to one described by Bock and Ling[1]. The system produced a straight-line gradient of acid concentration increase versus volume of eluent run through the column. A flow rate of 1 ml./min was maintained by a pressure head of water and an automatic levelling device[2]. A number of gradient schemes were tested in an effort to obtain good separation of the acids. The scheme that gave the best results is shown in Table 1. Five ml. fractions from the resin column were dried of eluting acid in a vacuum shaker evaporator (Buchler rotary 'Evapo-mix') at 35° C, then dissolved in 5 ml. water and titrated with 0·01 N NaOH.

Table 1. Gradient for 3·0 m.equiv. 'McIntosh' pulp acid

	Mixer flask	Feeder flask	Volume
I	Water	3·0 N acetic	165 ml. each
II	3·0 N acetic	2·3 N formic	145 ml. each
III	4·0 N formic	6·2 N formic	114 ml. each
IV	8·0 N formic		200 ml.

The results, expressed as m.equiv. acid per 100 g fresh tissue, are presented in Table 2. The figures listed are the averages from duplicate samples. Recovery of known acids by this procedure included high values of 120 per cent for quinic and shikimic acids, low values of 58 and 66 per cent for lactic and phosphoric acids, respectively, and values of 91, 93, 95, 100 and 104 per cent for fumaric, glycolic, succinic, citric and malic acids, respectively.

Table 2. Effect of Storage Treatment on Acid Metabolism

Peak	September 17, 1961, pick		October 5, 1961, pick	
	Controlled atmosphere	Air	Controlled atmosphere	Air
	m.equiv. per 100 g fresh weight		m.equiv. per 100 g fresh weight	
1	0·0228	0·0182	0·0452	0·0176*
2	0·0180	0·0100*	0·0148	0·0105*
3	0·1730	0·1550*	0·1450	0·1350*
4	0·0089	0·0061	0·0093	0·0045*
5	0·0335	0·0206*	0·0291	0·0199*
6	0·0248	0·0151*	0·0213	0·0114*
7	0·0012	0·0068	0·0076	0·0085
8	7·3700	4·1300*	7·6300	4·6400*
9	0·0094	0·0093	0·0132	0·0078*
10	0·1060	0·1450*	0·1270	0·1490*
11	0·0251	0·0277	0·0289	0·0303
12	0·3310	0·3780	0·3480	0·4370*

* Deemed to be significantly different because both the values of the treatment replicates were higher or lower than both the values of the other treatment replicates.

The peak fraction numbers, 1, 2, 3, etc., in Table 2 results indicate the order in which the acids were displaced from the column. Overlapping of adjacent peaks occurred only between peaks 1 and 2 and peaks 3 and 4. However, from the results of one-way paper chromatography it appeared that all the acid peak fractions contained at least two different acids.

The peak fractions of Table 1 were tentatively identified by their R values on the resin column and R_F values on paper chromatograms. It appeared that peaks 2, 3, 7, 8, 10 and 12 included shikimic, quinic, citramalic, malic, citric and phosphoric acids, in that order. Chlorogenic acid was thought to be present and to be eluted from the column between the maxima of peaks 11 and 12. Paper chromatograms of acids from that region produced brilliant blue, yellow, and green colours under ultra-violet light. The sample treated with charcoal did not retain these acids. Similar behaviour was reported for chlorogenic-type acids by Hulmè[3].

Total acid concentration was much greater in fruit from controlled atmosphere storage than in that from air storage. This agrees with earlier reports for total acidity in modified atmospheres[4]. Malic acid, peak 8, accounted for most of the difference in total acid between the two treatments, but there were seven other acid fractions which were found to be greater in fruit stored in the

controlled atmosphere. Quinic and shikimic acids were probably among them. Three acid peak fractions were higher in fruit out of air storage than from 'controlled' storage. These included citric and phosphoric acids.

The differences in the various acid concentrations between treatments could be accounted for by either, or a combination of both, of two ways: either by supposing a difference in the rate of depletion of the acids, or by supposing a greater production of the acids in one storage atmosphere than in the other. Information about the amounts of the acids present at gathering time was not obtained.

If controlled atmosphere conditions simply slowed down the normal processes that occurred in air, one would expect malic acid and quinic acid to be more abundant in the controlled atmosphere, and citric acid and shikimic acid to be lower in the controlled atmosphere. These results would correspond to earlier reports[5-7] that, in air-storage, malic acid decreased at a steady rate, quinic acid decreased during the second half of the storage period after increasing during the first half, citric acid increased slightly in pulp tissue over the storage period, and shikimic acid increased during storage. The actual results reported here agree with these expectations for malic, quinic and citric acid, but not for shikimic acid, since the shikimic peak fraction (peak 2) was greater in the fruit stored in a controlled atmosphere than in air-stored fruit. This peak contained other unidentified acids, however, the relative quantities of which were not determined. It is possible that differences in the unknown acids masked the difference in shikimic acid.

It seems unlikely that the higher malic acid content in fruit stored in a controlled atmosphere could be due to its limited oxidation in low oxygen tensions, since its loss in stored apples has been shown to be independent of oxygen. It was the same in nitrogen as in air[8]. There is evidence that 'McIntosh' apples in air containing 5 per cent carbon dioxide produce malic acid at significant rates by fixation of CO_2 (ref. 9). Thus the higher malic content in applies stored in a controlled atmosphere may have resulted from CO_2 fixation.

The production of citric acid in air-stored fruit[5-7] may be oxygen-dependent. This might account for its higher content in air-stored fruit. Hulme and Arthington[10] have demonstrated that, on oxidation, quinic acid was converted to citric acid and some other acids. Direct evidence for oxidation of quinic acid to citric acid *in vivo* is lacking.

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INTERACTION OF PHOSPHORUS-32- AND CARBON-14-LABELLED TRITOLYLPHOSPHATE WITH GLASS SURFACES

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DEPLETION of phosphorus-32 from radioactive phosphate salts of high specific activity has been attributed to adsorption on glass walls[1]. Hensley[1,2] has investigated the adsorption of various anions to glass surfaces. He found that adsorption of these ions occurred only from basic solution under certain conditions. While phosphate ions behaved like the others at room temperature, at moderately high temperatures they exhibited an unexplained unique affinity to the glass surfaces. This indicates a possibility that the phosphate group is capable of forming a stable chemical bond, covalent in nature, with the surface of glass. Since organophosphorus compounds are readily synthesized, chemisorption via the phosphorus functional group may provide a new means of attaching organic groups to glass surfaces. These are useful for various applications, such as coupling agents for glass reinforced plastics or water-repellent coatings. The nature of the interaction of phosphates with glass surfaces is also of importance in the synthesis of radioactive organophosphorus compounds where wall reactions are a serious problem in the use of high specific activity materials.

In the work recorded here an investigation was made of the chemical reactivity of tritolylphosphate (TTP) on the surface of 'Pyrex' and fused silica glasses. In the first series of experiments the TTP was labelled with phosphorus-32, and applied to the surface of 'Pyrex' glass disks from a $1{\cdot}8 \times 10^{-4}$ molar benzene solution. One or more drops of solution were applied to the surface of each disk, the benzene allowed to evaporate, and the radioactivity on the surface counted. The disks were then heated in an oven for various periods of time at various temperatures. Subsequent to determining the radioactivity remaining after heat treatment, the surface layer was extracted with benzene, water, and N hydrochloric acid, successively, and the radioactivity remaining on the surface determined again after each rinsing. In the second series of experiments, the procedure was repeated utilizing carbon-14-labelled TTP. Finally, the procedure was repeated on a pure fused silica surface, at selected times and temperatures, utilizing both phosphorus-32- and carbon-14-labelled TTP.

The 'Pyrex' disks used had a geometric surface area of 2·0 cm^2 and were abraded with dry 120 'Carborundum' paper. To attain optimum reproducibility of adsorption measurements, the abraded surfaces were not polished[1]. The fused silica disks, with a geometric surface area of 3·1 cm^2, were similarly prepared. The phosphorus-32-labelled TTP was a mixture of *ortho*- and *para*-isomers, yielding 420×10^3 c.p.m./μmole in an end window Geiger–Müller counter. The carbon-14-labelled TTP was the *para*-isomer, yielding 228×10^3 c.p.m./μmole in a windowless flow counter.

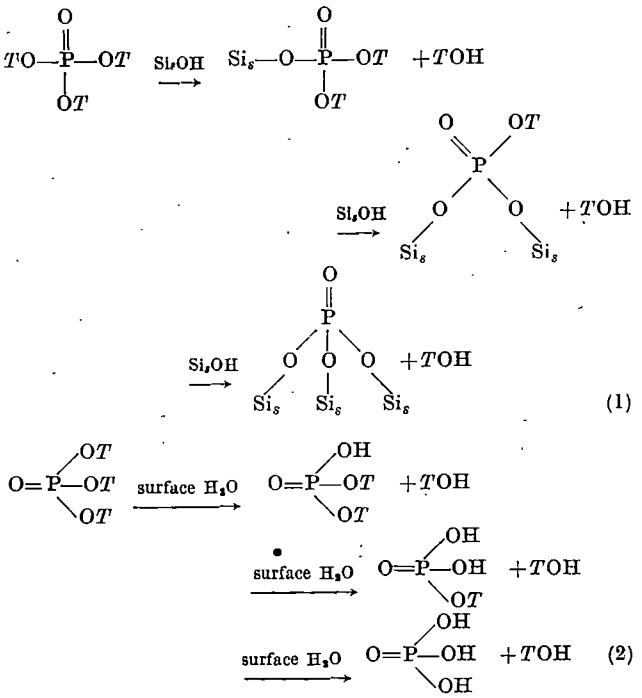
The radioactivity remaining on the 'Pyrex' disk surfaces after the various treatments is given in Table 1 for phosphorus-32-labelled TTP and in Table 2 for carbon-14-labelled TTP. In both Tables, column 2 gives the total amount of activity applied to the surface as determined after allowing the benzene solvent to evaporate. Column 1 gives the temperature and time of contact of this applied layer with the glass surface. Column 3 gives the activity remaining after contact at the given time and temperature. Columns 4, 5 and 6 show the residual activity on the surface after each of successive rinses in benzene, water, and hydrochloric acid. Table 3 gives these results, at the indicated temperature and time of treatment, for TTP applied to a fused silica surface.

Table 1. Residual Radioactivity on 'Pyrex' Glass Surfaces treated with Phosphorus-32-labelled TTP at Various Temperatures

1 Temperature and time of treatment (°C)	(h)	2 Total activity applied (c.p.m.)	3 Activity remaining after treatment: No rinse	4 Benzene rinse	5 Water rinse after benzene rinse	6 HCl(N) rinse after water rinse
25		3,841		632	14	4
105	0·5	38×10^3	3,549	678	72	36
105	1·0	38×10^3	3,550	777	104	48
105	2·0	38×10^3	2,807	805	183	109
105	3·0	38×10^3	2,321	938	157	68
105	4·0	38×10^3	2,149	1,000	224	109
155	1·0	5,899	2,980	1,785	625	322
155	2·0	6,028	2,576	1,730	691	367
155	3·0	6,340	2,664	2,004	844	396
155	4·0	7,060	2,724	2,110	844	427
200	1·0	6,355	2,328	2,120	1,040	565
200	2·0	6,414	2,452	2,353	1,218	451
200	3·0	6,810	2,481	2,417	1,446	456
200	4·0	7,211	2,863	2,759	1,476	475

The phosphorus-32 data, taken separately, can be explained according to two general hypotheses, which will henceforth be called I and II. According to I, the TTP which is applied to the surface is partly chemisorbed, with the chemisorbed molecules remaining intact, and the amount of chemisorption increasing with temperature and time of heating. The activity in column 4 represents this chemisorption, while the difference between columns 3 and 4 represents deposited or physically adsorbed material, which is removed with benzene. The action of the water rinse in removing some of the chemisorbed material would then be due to its own hydrolytic action on the chemisorbed TTP to produce phosphoric acid and cresol, followed by removal of the water soluble radioactive phosphoric acid. The phosphorus-32 residue after water extraction would then be that portion of the chemisorbed TTP not hydrolysed by the water wash.

Hypothesis II, on the other hand, assumes that the retention of phosphorus-32 on the glass surface, which is observed to increase with temperature and time of contact, is due to a chemical reaction resulting in fission of the molecule to organic and inorganic fragments. This can result from either of the following two types of reaction:



(1)

(2)

where Si_s is a surface silicon atom, and T is the tolyl radical.

Reaction 1 involves chemical combination of the TTP with a surface silanol group to yield free cresol and a surface silyl phosphate. Reaction 2 consists of hydrolysis of the TTP by surface adsorbed water molecules to yield free cresol and phosphoric acid. The plausibility of reaction 1 is supported by measurements on the adsorption of triethylphosphate to vacuum baked silica powder and *E* glass fibres[3], which gave evidence of the formation of a surface silyl phosphate. Indirect evidence for reaction 2 has been obtained from the wetting properties of smooth silica surfaces treated with tritolylphosphate and other esters[4]. According to hypothesis II, then, the activity in column 4 represents either phosphoric acid (benzene-insoluble) formed according to reaction 2, and/or the surface silyl phosphate formed according to reaction 1. The difference in the values of columns 3 and 4 represents unreacted TTP (benzene-soluble). The water-extraction procedure removes that portion of the activity in column 4 due to phosphoric acid, so that the activity in column 5 represents the surface silyl phosphate formed in reaction 1. The hydrochloric acid hydrolyses part of this surface compound, with the remaining portion accounting for the activity in column 6. It can be seen from column 4, then, that as the temperature or time of heating is increased, the amount of TTP reacting to form either H_3PO_4 or $(Si_sO)_3PO$ increases. From column 5 it is evident that the amount of $(Si_sO)_3PO$ formed increases with temperature or time of heating. A comparison of columns 4 and 5 shows that the ratio of $(Si_sO)_3PO$ to H_3PO_4 increases with increasing temperature. It is interesting to note, at this point, that hypothesis I, unlike II, does not provide an explanation for this increasing ratio of column 5 to column 4 with increasing temperature. Since only one chemisorbed species is postulated in I, there is no particular reason for the amount retained after water extraction to change with increasing temperature of reaction.

The chemical forms of phosphorus represented by the radioactivity values in some of the columns in Table 1 are given in Table 4 for each hypothesis. For simplicity, all product molecules are represented as having undergone reaction at all three functional groups. It can be seen that, according to I, the phosphorus in column 4 remains chemically combined with the tolyl groups. According to II, however, the tolyl groups have split off

Table 2. Residual Radioactivity on 'Pyrex' Glass Surfaces treated with Carbon-14-labelled TTP at Various Temperatures

1 Temperature and time of treatment (°C)	(h)	2 Total activity applied (c.p.m.)	3 Activity remaining after treatment: No rinse	4 Benzene rinse	5 Water rinse after benzene rinse	6 HCl(N) rinse after water rinse
25		2,893		20	15	12
105	0·5	3,194	2,787	75	55	42
105	1·0	2,839	1,981	168	154	107
105	2·0	2,990	1,708	197	148	89
105	3·0	3,222	1,538	259	188	116
105	4·0	3,397	1,186	307	144	98
155	1·0	2,518	558	224	38	26
155	2·0	2,829	426	293	53	33
155	3·0	2,921	354	286	39	24
155	4·0	3,055	343	320	29	13
200	1·0	2,743	251	251	36	11
200	2·0	2,893	230	230	56	39
200	3·0	3,288	197	197	47	25
200	4·0	3,347	151	142	44	10

Table 3. Residual Radioactivity on Fused Silica Surfaces treated with Labelled TTP

1 Radio-isotope	2 Temperature and time of treatment (°C)	(h)	3 Total activity applied (c.p.m.)	4 Activity remaining after treatment: No rinse	5 Benzene rinse	6 Water rinse after benzene rinse	7 HCl(N) rinse after water rinse
^{32}P	25		3,269		798	44	19
^{32}P	200	1	7,114	2,274	2,192	779	456
^{14}C	25		3,555		0	0	0
^{14}C	200	1	2,657	21	18	10	5

from the phosphorus portion of the molecule to form cresol, which will no longer be present on the surface due to its solubility in benzene. In column 5, the phosphorus once again retains the organic tolyl groups in the molecule according to I. According to II, however, the tolyl groups are not present. It is apparent, then, that if the experiment wore repeated with carbon-14 labelling, in which case the radioactivity would emanate from the tolyl group, the relative activities in the various columns would remain exactly the same according to I. According to II, however, substantial differences in the relative activities of the various columns must be observed for the two different types of labelling.

Table 4

Column	Chemical forms of phosphorus: Hypothesis I	Hypothesis II
3	TTP + chemisorbed TTP	TTP + H_3PO_4 + $(Si_sO)_3PO$
4	Chemisorbed TTP	H_3PO_4 + $(Si_sO)_3PO$
5	Chemisorbed TTP	$(Si_sO)_3PO$

Examination of the data obtained from carbon-14-labelled TTP, given in Table 2, shows that substantial differences in the activity ratios are indeed obtained. The nature of these differences, furthermore, is in excellent accord with the assumptions of II. For example, a comparison of column 3 with column 2 shows that the amounts of phosphorus-32 activity lost by evaporation increase continually from 8 per cent at 0·5 h and 105° C to approximately 62 per cent for 1 h or more at 200° C. The compound evaporating must be TTP, which has a vapour pressure of 1–10 mm in the range 155–198° C. The increase in evaporation tapers off at higher temperatures and longer times due to the increasing residue of H_3PO_4 and $(Si_sO)_3PO$. The carbon-14 activity in column 3 is lost in equal amounts to phosphorus-32 at low temperatures and short times, where the loss is due essentially to TTP. At higher temperatures and longer times, however, where according to II increasing hydrolysis takes place, the rate of loss of activity increases, in contrast to that of phosphorus-32, which decreases. This is due to the fact that while hydrolysis produces non-volatile phosphorus compounds, resulting in retention on the surface of phosphorus-32, it produces a carbon compound (cresol) more volatile than the original TTP. Consequently, at 200° C where all the TTP is hydrolysed, the carbon-14 activity remaining is only approximately 7 per cent, as compared to 38 per cent for the phosphorus-32 activity. Again, examination of column 4 shows a fairly constant residue of about 5–10 per cent of the activity applied (column 2) at all temperatures. Tentatively taking the residue to be negligible, this is exactly in accordance with II, which assumes the desorption by benzene of all the unhydrolysed TTP, as well as any TOH (cresol) resulting from hydrolysis, which did not already evaporate. The extent to which the surface reaction has taken place does not affect the nearly complete removal by benzene of all remaining carbon-14 activity. According to hypothesis I, of course, the carbon-14 activity would have been present throughout in the same proportions as the phosphorus-32 activity.

The results in Table 3 justify the assumption that reaction of the TTP on 'Pyrex' glass occurs chiefly with the silica network. It is furthermore interesting to note that the residual carbon-14 activity in columns 4 and 5 of Table 2 is no longer present when the reaction occurs on pure silica. This indicates that this activity is due to a small amount of reaction with non-siliceous components of the 'Pyrex' surface.

The simplified representation of hypothesis II in which all product molecules have undergone reaction at all three functional groups is confirmed by the results of the carbon-14 labelling. For example, no more than small amounts, if any, of the compounds $(Si_sO)PO(OT)_2$, or $(Si_sO)_2PO(OT)$, are formed. The results, however, do not preclude formation of the mixed acid-esters $(Si_sO)PO(OH)_2$ and $(Si_sO)_2PO(OH)$ along with, or in place of, $(Si_sO)_3PO$. The formula for the monomeric surface ester postulated is therefore $(Si_sO)_nPO(OH)_m$, where $n=1$, 2, or 3, $m=0$, 1, or 2, and $n+m=3$. The results also do not preclude the possibility of polymerization, which can occur, for example, as follows:

$$(Si_sO)PO(OH)_2 + PO(OT)_2OH \longrightarrow (Si_sO)PO(OH)\text{—}O\text{—}PO(OT)(OH) + TOH$$

where TOH is split off as a result of reaction between a cresyl (OT) group originally present and an acid group present as a result of prior hydrolysis. The same result is also possible, of course, from the splitting off of water between two acid groups.

The reactions of TTP on a 'Pyrex' or fused silica surface may consequently be classified into two overall categories. One involves the formation of a chemisorbed inorganic phosphorus compound joined to the surface by means of an Si_s-O-P linkage. The other consists of hydrolysis of the TTP by water molecules adsorbed at the surface to produce free phosphoric acid and cresol. Ability to form the Si_s-O-P linkage probably accounts for the unusual reactivity of various phosphates with the surface of glass.

The opinions or assertions contained in this article are the private ones of the authors and are not to be construed as official or reflecting the views of the U.S. Naval Service.

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HYPERPOLARIZATION OF A NEURONE MEMBRANE BY BARIUM

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The Effect of Barium on the Membrane Potentials of a Neurone Soma

AMONG the divalent cations, barium and strontium are specific in causing some excitable cells to produce an action potential resembling that of cardiac muscle either in the presence or absence of extracellular sodium ions[1–4]. The frog's spinal ganglion cell is such a cell[5], and its action potential is similar in sodium-free barium solution and in solutions of nitrogen compounds of restorative type[6–8]. Tasaki[5] reported that ganglion cells in isotonic barium chloride solution showed the reversed resting potential of +20 to +40 mV or the ordinary resting potential of −40 to −70 mV, and that these potentials could be switched back and forth between the two stable levels by delivering anodal and cathodal pulses alternately. This communication deals with the membrane potentials of the toad's spinal ganglion cells perfused with barium solutions of varying ionic composition. The perfusion was performed in a small chamber in which the solution could be changed in a fraction of a second by a multiple tap. Membrane potentials were

recorded between an ordinary intracellular microelectrode and an indifferent agar–3M KCl electrode.

By substituting the isotonic barium solution ($BaCl_2$, 80 mM; KCl, 2·0 mM; $CaCl_2$, 1·8 mM; and $KHCO_3$, 0·5 mM) for Ringer's solution (Fig. 1, *A*), the resting potential increased approximately 20 mV in less than 15 sec. The increased potential level was maintained almost constant for 2 min, then tended toward a progressive decrease with a slope of 6–10 mV/min, so that the phase of hyperpolarization usually lasted for 5 min. The action potential induced by antidromic stimulation was initially heightened and suddenly fractionated into a smaller spike which eventually disappeared as time advanced. When the succeeding slow depolarization reached the level of about −10 mV, there appeared a sudden reversal of membrane potential (spontaneous firing of the membrane) reaching a level of +80 mV. The reversed potential declined very slowly down to approximately +20 mV, forming a plateau lasting 1–5 min, then abruptly returned to the level of −10 to −20 mV. This level was maintained so long as the flow of barium solution was continued or sometimes a similar potential reversal was repeated. When Ringer's solution was restored the decreased membrane potential returned to the original value with a slope of 3–7 mV/min and the antidromic action potential which had disappeared also recovered.

The reduction of barium concentration down to 40 mM by replacing barium chloride with an osmotically equivalent quantity of sodium chloride did not appreciably change the initial hyperpolarization. On the other hand, the slope of the succeeding depolarization and the height of spontaneous firing were considerably reduced. Further reduction of barium concentration decreased the hyperpolarization and almost none was observable in barium solutions less than 24 mM. The lowering of the peak level of spontaneous firings was fairly logarithmic against the reduction in barium concentration and showed a slope of 45 mV per ten-fold change of barium concentration. A similar relation was also observed when barium chloride was replaced stepwise with an equimolar amount of sucrose.

An increase of potassium concentration up to 10 mM, by replacing barium chloride with potassium chloride, did not alter the hyperpolarization. Further increase in potassium concentration reduced the hyperpolarization and eventually changed it to a depolarization. However, the resulting depolarizations during the first several min in potassium-rich barium solutions were always much smaller than those in potassium-rich Ringer's solutions.

The electrotonic potentials of both hyperpolarizing and depolarizing directions were greatly augmented in barium solutions. The augmentation was usually completed within the first 15 sec of barium flow. The augmented tonic potentials thereafter maintained the same magnitude regardless of the progressive decrease in the resting potential. During the spontaneous firing, however, the magnitude of tonic potentials was greatly reduced and was always smaller than that in Ringer's solution. The tonic potentials in 32, 56 and 80 mM barium solutions were respectively about 2·5, 3·7 and 4 times the initial values in Ringer's solution. The V–I curves obtained from the tonic potentials extended almost in a straight line up to the absolute membrane potential of −20 mV, indicating a marked decrease of the rectifying property of the membrane to outward currents. Thus, barium produced a marked reduction of membrane conductance which might be largely brought about by the decrease of membrane permeability to potassium ions. This is in keeping with the experimental fact that the barium-treated cells became remarkably insensitive to the change in the external potassium concentration.

These results indicate that the diffusion potentials of potassium and sodium ions are not directly involved in the initiation of Ba-hyperpolarization, since the hyperpolarization was independent of external sodium concentration and was unaffected by an increase of potassium concentration. It appears therefore that if the hyperpolarization is entirely due to the change in diffusion potentials across the cell membrane, chloride ion alone would then be responsible for Ba-hyperpolarization. In fact, E_{Cl} across the cell membrane is theoretically increased in parallel with the increase of barium concentration.

To check this hypothesis the preparation was first perfused with chloride-free sodium glutamate solution (Na-glutamate, 112 mM; Ca-glutamate, 1·8 mM; K-glutamate, 2·0 mM; $KHCO_3$, 0·5 mM), and then with the isotonic barium glutamate solution (Ba-glutamate, 80 mM; K-glutamate, 2·0 mM; Ca-glutamate, 1·8 mM; $KHCO_3$, 0·5 mM). In spite of the complete depletion of chloride ion with such a large anion, the cell membrane in this barium solution was hyperpolarized to the same extent as in the isotonic barium chloride solution. The only difference noticed was that the succeeding depolarization in barium glutamate solution appeared slightly earlier, and its slope was greater. Furthermore, the perfusion of the preparation, which was pre-incubated in 10 mM K-Ringer's solution having the same [K] [Cl] product as that of ordinary Ringer's solution, with 10 mM K and 32 mM Ba solution also having the same [K] [Cl] product ($BaCl_2$, 8·2 mM; Ba-glutamate, 23·8 mM; Na-glutamate, 16·0 mM; KCl, 9·5 mM; $CaCl_2$, 1·8 mM; sucrose, 85·0 mM; $KHCO_3$, 0·5 mM) produced a hyperpolarization as large as in the isotonic barium chloride solution (Fig. 1, *B*). The hyperpolarization obtained after pre-incubation in potassium-rich media was thus always larger than that induced without pre-incubation. In fact, pre-incubation of the preparation into potassium-free Ringer's solution markedly suppressed or even nullified the hyperpolarization.

The Mechanism of the Hyperpolarization Effect

It can be concluded from these results that so far as the establishment of Ba-hyperpolarization is concerned the diffusion potential of ions across the cell membrane is not directly involved. Some other process, possibly an active extrusion of some cation or an active intrusion of some anion, is evidently responsible for the hyperpolarization. The most probable mechanism, on the basis of present information, is the active extrusion of sodium ion, since acceleration of the hyperpolarization by pre-incubation in potassium-rich Ringer's solution and its depression by pre-incubation in potassium-free Ringer's solution are well in accord with the property of active sodium extrusion[9,10]. The succeeding slow depolarization and the spontaneous potential reversal were quite dependent on barium concentration, and the latter showed a specific logarithmic relation with barium concentration. The spontaneous potential reversal, therefore, seems to be due to the sudden inrush of barium ion into the cell and its peak level may be determined by the electrochemical potential difference of this ion across the membrane. The succeeding depolarization might be brought about mainly by the decrease in P_K and the passive inward movement of barium ion.

We have now to present more direct evidence in support of this interpretation. First, the relation between the temperature of the perfusing barium solution and the membrane potential was investigated. The preparation was perfused with Ringer's solution, at the same temperature as the barium solution, for more than 10 min before the application of barium solution. The results obtained with isotonic barium chloride solution (Fig. 2) may be summarized as follows: At around 0° C, hyperpolarization was not visible and a slow progressive depolarization occurred immediately after the beginning of barium flow. At around 10° C, hyperpolarization was usually induced, but its magnitude was less than 5 mV. Typical

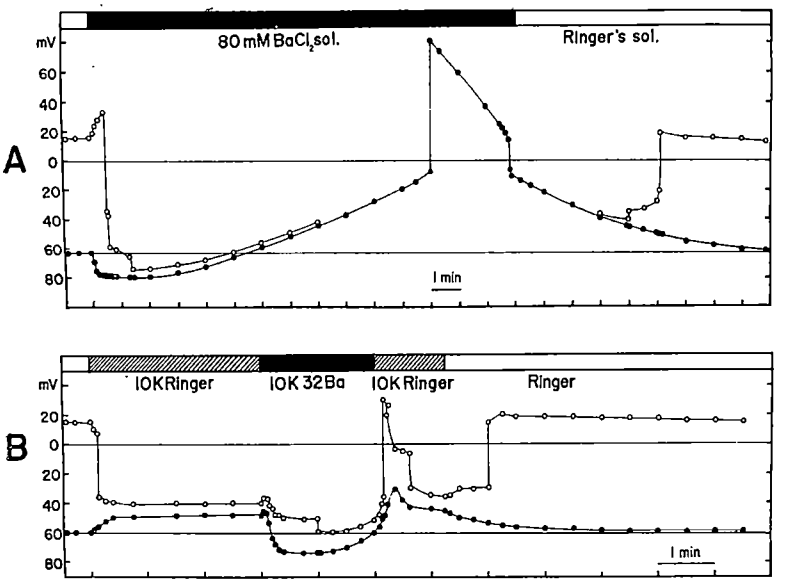


Fig. 1. Alterations in resting potential (solid circles) and the height of antidromic action potentials (open circles) of the toad's spinal ganglion cells perfused with barium solutions. In *A* an isotonic barium chloride solution was substituted for Ringer's solution. In *B* the preparation was perfused with a potassium-rich Ringer's solution before perfusion with a potassium-rich barium solution. The [K][Cl] product of these two solutions was kept the same as that of Ringer's solution

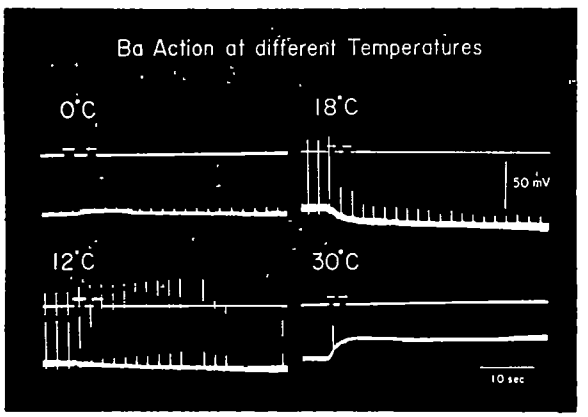


Fig. 2. The effect of isotonic barium solution at different temperatures on the resting potential of the toad's spinal ganglion cells. Barium solution was substituted for Ringer's solution at the beginning of the first rectangular pulse on the zero potential line. Antidromic responses were evoked every 2 sec except under perfusion with barium solution at 30° C. With solution at 0° C the antidromic conduction was blocked at M–NM junction in that particular case (left upper), so that merely M spikes are seen

hyperpolarization was observed in a temperature range between 18° and 22° C. When the temperature of the barium solution was raised to 30° C or more, there was usually no sign of hyperpolarization and the cell membrane was immediately depolarized. The rate of slow depolarization was considerably reduced at a temperature below 5° C and was appreciably increased at 30° C. At all temperatures we have examined, however, the changes in the succeeding depolarization, and the spontaneous firing, were apparently smaller than the alteration in the hyperpolarization. Actually, the Q_{10} of the latter was approximately 3·0 in a temperature range between 0° and 20° C. Such a dependency on the temperature clearly indicates that some chemical process is involved in the initiation of barium hyperpolarization. The conversion of the hyperpolarizing action of barium at around 30° C is difficult to explain at present. However, at such a high temperature the physicochemical nature of the membrane might be made more sensitive to the depolarizing action of barium, or possibly the destruction of some enzyme reduces the chemical force.

Next, the effect of a metabolic inhibitor on the barium action was examined by perfusing the preparation with Ringer's solution containing 2,4-dinitrophenol before applying the barium solution. The membrane potential of the cells perfused with 1 mM dinitrophenol Ringer's solution for 5–10 min usually showed a decrease of up to 5 mV at 20° C. The application to such a cell of barium at the same temperature immediately resulted in a further depolarization. Thus, pre-incubation in a dinitrophenol solution always caused a complete disappearance of hyperpolarization even at the optimal temperature. At around 30° C, similar pre-incubation accelerated the slow depolarization, but the threshold for spontaneous firing was considerably raised. These potential changes were completely reversible when the normal Ringer's solution was substituted for barium solution. The disappearance of the hyperpolarization with the metabolic inhibitor also supports the suggestion that the process underlying the Ba-hyperpolarization has some direct relation to cell metabolism.

It has been reported that the active sodium efflux is roughly proportional to the intracellular concentration of sodium ion[10]. Increase of the intracellular sodium ions must augment Ba-hyperpolarization, if it is associated with the active extrusion of sodium ions. Sodium ions were therefore electrophoretically injected into the cell through the recording microelectrode[7] filled with 3M NaCl before the perfusion of the cell with barium solution. The injection of sodium ions with an outward current of approximately 1×10^{-8} amp for 1–2 min depolarized the cell membrane by 5–40 mV during perfusion with Ringer's solution at around 20° C. By applying isotonic barium solution at the same temperature immediately after the injection, the membrane potential was usually increased by 30 mV or sometimes more than 40 mV. As previously mentioned, the cell membrane is depolarized by the isotonic barium solution at around 30° C. Even at such a high temperature, the membrane potential of the cells which received the sodium injection was increased as much as 20 mV or more by perfusion with barium solution (Fig. 3). The magnitude of the potential increase induced at such a high temperature was usually smaller than at 20° C, but the slope of the succeeding depolarization was markedly reduced in comparison with those induced without sodium injection. These observations may sufficiently prove that Ba-hyperpolarization is accompanied by the active efflux of the intracellular sodium ions.

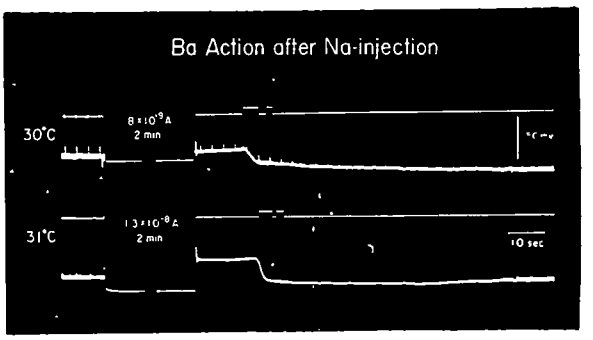


Fig. 3. The effect of barium solution at a high temperature on the cells which received electrophoretic injection of sodium ions through the recording microelectrode. The temperature of perfusing solutions, and the intensity and duration of outward currents used for sodium injection, were indicated on each record. Barium solution was substituted for Ringer's solution at the beginning of the first rectangular pulse on the zero potential line

In the normal Ringer's solution, the potential associated with the active efflux of sodium ions may be totally or partially neutralized by the coupled active uptake of potassium ions[10]. Even if it is not perfectly neutralized its contribution to the resting potential may be negligibly small, since the diffusion potentials of potassium and chloride ions will entirely cover such a potential. However, in barium solution, membrane permeability to normal diffusible ions seemed to be markedly reduced. Moreover, the marked reduction of delayed rectification and the remarkable prolongation of action potentials in barium solution suggest that the potassium-carrying system is markedly suppressed in barium solutions. This again seems to indicate that in barium solution there might occur an uncoupling of sodium–potassium pumping by the suppression of the inward movement of potassium ions. In such a state, the potential accompanied by the active efflux of sodium ions may be unmasked and become large enough to be recorded.

This work was supported by the Institutes of Neurological Diseases and Blindness, U.S. National Institutes of Health grant *NB* 03141–02.

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SHOCK-INDUCED FREEZING OF 'ACTIVATED' SUPERCOOLED WATER

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PROF. G. PICCARDI has reported that a mercury bulb (a hollow glass sphere containing a drop of mercury in neon at reduced pressure) "activates" water[1]. Water so treated has properties subtly different from untreated water. The mercury bulb when agitated emits very small quantities of electromagnetic radiation. An examination of this radiation has been reported by Halla and Nowotny[2], and further investigations are being carried out here.

Work on the shock-induced freezing of supercooled water is also under way at this laboratory. It was decided to investigate the effect of 'activation' of water on shock-induced freezing. The cooled shock-tube has been described elsewhere[3] so that full details will not be given here. Suffice it to state that the shock-tube was maintained at −10° C, that the 0·5-c.c. samples were held in glass tubes 2 in. long by 1/4 in. outer and 1/8 in. internal diameter and that a 1·1 lb./in.^{2} over pressure shock wave lasting 2 milliseconds was administered by bursting a 'Cellophane' diaphragm with 15 lb./in.^{2} air pressure.

The water samples consisted of three types. The de-ionized water samples received no treatment. The water had been de-ionized, distilled, and stored in polyethylene carboys. It was withdrawn into a covered beaker and submitted to test.

Blank water samples were subjected to agitation with a bulb containing no mercury. Such a bulb does not emit radiation.

'Activated' water samples were subjected to agitation with a mercury bulb. The agitation in both cases consisted of making a fixed number of revolutions with the bulb in the beaker in a fixed length of time.

The samples were submitted blind so that the shock-tube operator did not know which type of water sample he was handling until after the daily tests were completed. Freezing was carried out within half an hour after agitation.

Table 1 lists the experimental results. Examining the freezing temperature of those samples not receiving a shock and those receiving a shock at one temperature but freezing at a lower temperature (shock ineffective), it is apparent that the recorded natural freezing temperatures were originally in the neighbourhood of −7° C, then rose to about −3° C, and then sharply dropped back to about the original freezing temperature, as shown in Fig. 1 (the line was drawn by eye and should not be regarded as quantitative, but only as indicating the time-temperature trend). The reason for this behaviour is not known, but, with one exception which will be given here, it does not prevent useful analysis of the data.

If a shock is effective in inducing freezing, the sample freezes instantaneously on administration of the shock and the freezing temperature is that at which the shock is administered. If the shock is ineffective, the sample continues to cool in the shocktube until it spontaneously freezes. Fig. 2 is a plot of the temperature at which the shock was administered versus the freezing temperature of the sample. The line is the locus of the points at which shock temperature equals freezing temperature. If the shock is ineffective, the freezing temperature will be below the shock temperature and the plotted point will fall below the line.

Two de-ionized water samples were shocked at −5·5° C, but both froze at colder temperatures, indicating that the shock was not effective despite the relatively low temperature at which it was administered. Other work[3] has shown that there is a threshold temperature, about 2° C above the spontaneous freezing temperature, above which

Table 1. Experimental Results

Day	Sample	Shock temperature	Freezing temperature
1	Blank	—	−6·5
		—	−7·0
		−5·0	−5·0
	Active	—	−7·5
		—	−7·0
		−5·5	−5·5
	De-ionized	—	−6·5
		—	−7·5
		−5·5	−7·0
2	Blank	−4·5	−5·0
	De-ionized	−5·5	−6·5
	Active	−3·5	−3·5
6	Active	−3·0	−5·5
	Blank	−3·5	−3·5
		−3·5	−3·5
7	Active	−3·5	−3·5
	Blank	−3·5	−5·5
		−3·5	−5·5
	Active	−3·5	−3·5
8	Active	−3·5	−3·5
		—	−2·5
		−3·5	−3·5
	Blank	—	−3·5
	Active	—	−5·5
	De-ionized	—	−3·5
	Blank	—	−4·5
	Active	—	−2·5
9	Blank	—	−4·0
	De-ionized	—	−4·5
		—	−4·0
	Active	—	−6·0
	Blank	—	−6·5
	De-ionized	—	−6·5
	Blank	—	−6·5
	Active	—	−6·5

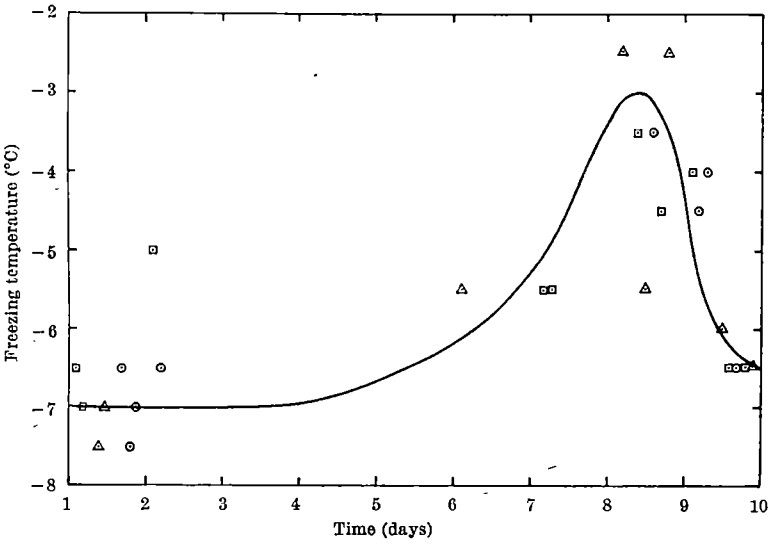


Fig. 1. Time trend of spontaneous freezing temperature of treated water. ○, De-ionized; □, blank; △, activated

shocks are always ineffective. Possibly −5·5° C is in the neighbourhood of this threshold temperature for de-ionized water.

Six blank water samples were shocked. Three exhibited shock-induced freezing, two at the high temperature of −3·5° C. However, another two were not affected at this temperature. The final blank sample was shocked at the intermediate temperature of −4·5° C but was not affected or only slightly affected, freezing at −5° C. These results indicate that agitation alone sensitizes the water to freezing at elevated temperatures induced by administering a shock.

Seven 'activated' water samples were shocked. Only one was shock insensitive—the one shocked at −3° C, the highest shock temperature tried. Possibly −3° C is above the previously mentioned threshold temperature for the 'activated' water. A second 'activated' sample was shocked at −5·5° C and froze at that temperature. The remaining 'activated' samples were all found to be affected by the shock at −3·5° C, a temperature where only 0·5 of the blank samples were affected. These results indicate that the activation process also sensitizes the water to shock-induced freezing and is even more sensitizing than the agitation alone. Water is probably

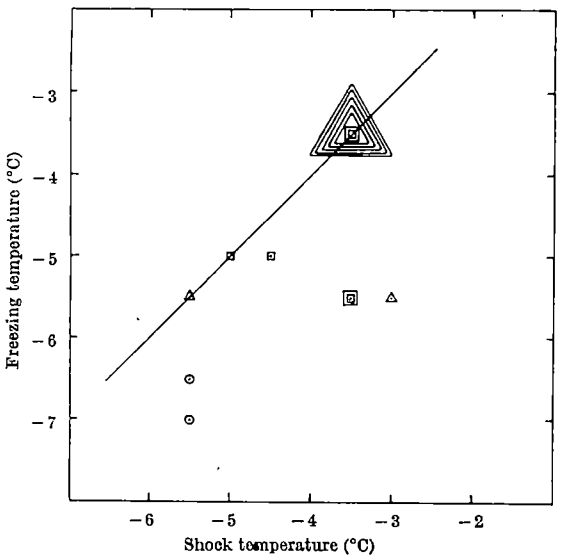


Fig. 2. Effect of shock on freezing temperature of treated water. ○, De-ionized; □, blank; △, activated

sensitized by agitation because air and dust motes are beaten into it, and these then presumably act as freezing nuclei in the presence of shock. The activation sensitization must include this process but also must have an additional effect, functioning at still higher shock administration temperatures, due to the electro-magnetic radiation. At present, the second effect is not understood in a mechanistic sense.

The distribution of the samples is given in Table 2. The paucity of shocked samples of the de-ionized water is due to the extensive study of shock-induced freezing of water to be reported on by others[3]. Comparison of the number of blank and 'activated' water samples affected by shock versus those subjected to shock indicates that 'activation' has a greater effect than agitation alone, in that a higher proportion of 'activated' samples are affected by the shocks.

The actual mean temperatures involved are given in Table 3. The natural freezing temperature in the absence of shock is practically identical for all three samples, indicating that the treatments do not introduce freezing nuclei that are active in the absence of shock at temperatures above those at which nuclei originally present in the de-ionized water samples are active.

Table 2. Distribution of Samples

Sample	No shock	Shocked	Shock effective
De-ionized	6	2	0
Blank	7	6	3
Active	7	7	6

Table 3. Effect of Shock on Freezing Temperature of Supercooled Treated Water

Sample	Freezing temperature (° C) No. shock	Shocked	Shock effective
De-ionized	−5·4	−6·8	—
Blank	−5·5	−4·7	−4·0
Activated	−5·4	−4·1	−3·8

The treatments caused the water to freeze at higher temperatures when subjected to shock. For the blank samples this is probably explainable on the basis of introducing (additional ?) air and dust motes as previously noted. Again, however, the 'activated' water samples are more shock sensitive as shown by their even higher shock-induced freezing temperatures. The lowering of the shock-induced freezing temperatures of the shocked de-ionized water samples must be disregarded as previously mentioned in the discussion of Fig. 1, since this result is based on only two samples, both tested on day 1 when the natural freezing temperature was low and thus does not include samples from subsequent days when the natural freezing temperature was some 4° C higher (Fig. 1). All the other data in Table 3 contain samples obtained throughout the test period and thus reflect an average over all the various natural freezing temperatures.

The last column of Table 3 gives the average freezing temperatures of only those samples whose freezing was induced by shock. It indicates that activation is somewhat more effective than agitation.

This work shows that the electromagnetic radiation from a mercury bulb raises the temperature at which shock-induced freezing occurs in supercooled water.

We wish to thank Dr. G. G. Goyer for making the shock-tube available for this work.

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LETTERS TO THE EDITOR

ASTROPHYSICS

Detection of an Interstellar Flux of Gamma-Rays

The intensity and energy distribution of radiation produced by astrophysical processes is important to an understanding of stellar evolution and the mechanism of cosmic ray production and acceleration. Previous efforts to detect a γ-ray flux have been confined to balloon and rocket flights, close to extraneous sources of radiation in the atmosphere and the radiation belts. Although measurable fluxes have been observed in such experiments, only γ-ray fluxes accompanying solar flares have been identified as extraterrestrial in origin[1]. In this communication, we report γ-ray measurements at distances of 7×10^4 km to 4×10^5 km from the Earth, far enough to make contributions from the Earth negligible. The experiment involved operation of a spectrometer on two flights of the *Ranger* spacecraft with the primary purpose of monitoring surface radiation from the Moon. Results obtained in cislunar space point to the detection of a substantial interstellar flux of γ-rays.

The scintillation spectrometer consisted of a 2·75-in. bevelled cæsium iodide (Tl) scintillation crystal, with a plastic scintillator phoswich for the rejection of charged particles[2]. The mass surrounding the inorganic scintillator crystal has a mean value of approximately 2·5 gm/cm², the half-thickness for radiation of 60 keV. Output pulses from the phototube were amplified and analysed in a kicksorter of 32 channels, the last of which counted all pulses above a given level. The detector system was housed in a thin aluminium shell at the end of an extensible 6-ft. boom. The boom was provided to reduce the background of spacecraft-generated secondary γ-rays, and was programmed to extend after operation for 12 h in a stowed position. Data transmission from the earlier flight spanned a period of 40 h; 5 h of results were received from the second flight with the boom in the stowed position only. The combined energy spectra of the two flights cover a range of 70 keV–4·4 MeV.

An initial report on the flux observed between 0·4 and 2 MeV on the earlier flight has appeared previously[3]. The results from this flight have been refined and extended to include the full energy range scanned, as well as the stowed position mode of operation. The contributions from calibration sources of cobalt-57 and mercury-203, and of two extraneous radiation sources have been removed. Data from the second flight have been similarly reduced. In the second flight, the spacecraft was passing through the fringe of the outer radiation belt when the spectrometer began to operate. Therefore, only results received during the last hour of flight, when the reproducibility of successive readouts indicates that the belts had been passed, are considered here.

The observed fluxes from *Ranger* 3 with the detector stowed and extended, and *Ranger* 5 stowed, are shown in Fig. 1. Extension of the boom reduces the contribution of cosmic-ray-induced secondary counts by a geometrical factor of 13. Fig. 1 shows that the actual reduction was much less. From this it is deduced that the contribution of the interstellar flux predominates in the extended position.

The spectra of the two flights in the stowed position are seen to be in reasonably good agreement, although the absolute values differ by about 20 per cent. This difference may or may not be significant.

The upper channels of the *Ranger* 3 extended spectrum appear high since the cross-section for the production of secondary γ-rays is not expected to decrease abruptly above 1 MeV. These count rates were also accompanied by an anomalously high count rate in the overflow channel which appears to have been due to a partial malfunction in the instrument. The differential spectrum varies roughly as $E^{-2.3}$ for the extended case and $E^{-1.7}$ for the stowed. The instrumental response has not yet been unfolded.

Small peaks at 0·51 MeV in both stowed spectra can be attributed to secondary production. The absence of peaks at 0·51 MeV in the extended position, and at 2·23 MeV in the stowed position, of both *Ranger* 3 and *Ranger* 5 sets upper limits for these lines which are generated by pair production and neutron–proton reactions respectively. Based on the *Ranger* 3 results, where long periods of stable performance were measured, these become 0·014 γ/cm² sec for the 0·51-MeV line and 0·005 γ/cm² sec for the 2·23-MeV line.

The integrated fluxes observed are 3·1 counts/cm² sec for 0·09–4·3 MeV on *Ranger* 5, 3·2 counts/cm² sec for 0·12–2·5 MeV on *Ranger* 3 (detector stowed), and 3·1 counts/cm² sec between 0·07 and 1·2 MeV on *Ranger* 3 (detector extended).

The Sun has been eliminated as the source of the observed flux by the lack of any significant change in count-rate response after the *Ranger* 3 spacecraft performed an orientation manœuvre which carried the detector through an angle of 145° with respect to the Sun. Laboratory tests have shown that the detector possesses sufficient anisotropy of response to have produced detect-

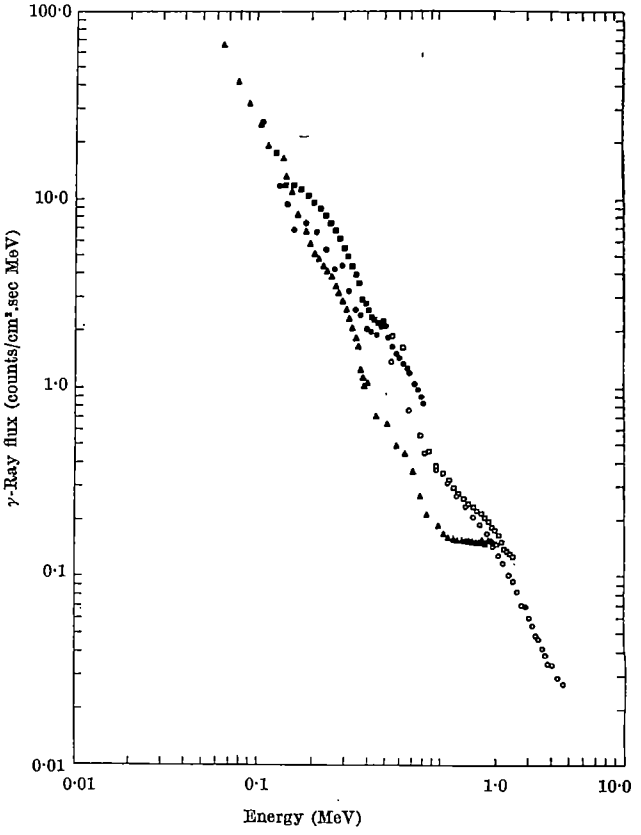


Fig. 1. Energy spectra obtained in cislunar space by the γ-ray spectrometers on *Ranger* 3 and *Ranger* 5. *Ranger* 3, detector stowed; ■, high gain; □, low gain. *Ranger* 3, detector extended; ▲, high gain; △, low gain. *Ranger* 5, detector stowed; ●, high gain; ○, low gain

able changes in count rates at the low-energy end if 25 per cent or more of the flux were solar in origin.

The primary γ-ray flux detected in these experiments is evidently an interstellar flux because of: (1) the distance of the instrument from the Earth and the constancy of count rate with change in distance once the radiation belts are left behind; (2) the comparatively small reduction in counting rate with extension of the detector from the spacecraft, much less than if this were entirely the result of cosmic-ray secondary production; (3) the ability to eliminate the Sun as the prime source.

The intensity of the flux makes a synchrotron origin very unlikely, while the absence of a line at 0·51 MeV makes it unlikely that the flux has been produced by nuclear interactions of high-energy particles. The mechanisms of bremsstrahlung and inverse Compton scattering which have been suggested recently are more promising[4].

Extrapolation of these results to the mean energy of the X-ray flux detected in rocket flights[5] indicates a general agreement. If the agreement is meaningful, certain mechanisms for the production of this X-ray flux such as line transitions and synchrotron emission can be eliminated.

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On Intergalactic Gas as a Possible Absorber of Extragalactic Radio Noise

IT has recently been pointed out by Smith[1] that most of the background radiation at low frequencies (< 10 Mc/s) is probably of extragalactic origin. The intensity of this radiation increases with decreasing frequency until about 3 Mc/s where it turns over sharply[1–3]. This turnover is probably due to absorption by ionized hydrogen, the required emission measure being about 4 cm^{-6} pc (for an electron temperature of 10^4 °K). It was suggested by Hoyle and Ellis[4] that the ionized hydrogen lies in a slab parallel to the galactic plane, with an electron concentration $n_e \sim 0{\cdot}15$ cm^{-3} and a thickness ~200 pc. This model has been ruled out by the rocket and satellite observations of Walsh *et al.*[3] and Smith[1], which show that even when integrated over a hemisphere the radiation has a very sharp turnover in its spectrum, corresponding to the emission measure being nearly isotropic around us. These authors suggest[5] that we are surrounded by a more or less spherical *H* II region with, say, $n_e \sim 1$ cm^{-3} and radius ~4 pc (parameters which would not lead to unreasonably large Faraday rotation effects).

This explanation has the unattractive feature that we are required to be near the centre of this region, since otherwise again the turnover would not be sharp. We would like to direct attention to an alternative explanation which avoids this difficulty. This explanation becomes possible if most of the radiation at these low frequencies is indeed extragalactic. According to some cosmological theories extragalactic space is filled with ionized hydrogen of electron density in the range 10^{-5} to 10^{-4} g cm^{-3}. Indirect arguments suggest[6] that the temperature of this gas is in the neighbourhood of 10^5 °K, but these arguments may not be correct, and the temperature may in fact be close to 10^4 °K. In this case the emission measure of the gas out to a Hubble radius lies in the range 0·3 to 30 cm^{-6} pc. Since most of the extragalactic radiation has a path-length of about a Hubble radius the intergalactic gas might be responsible for the absorption. This suggestion has the advantage of providing an immediate explanation for the isotropy of the absorbing region around us.

A detailed calculation of the spectrum expected on various cosmological models leads to rather awkward integrals, and until the observations are more reliable it does not seem worth attempting to fit them closely. However, we can easily make the estimate of the last paragraph somewhat more precise. For simplicity we shall consider only the Einstein–de Sitter and steady state models. The density of the gas in the Einstein–de Sitter model is 2×10^{-29} g cm^{-3} for a Hubble constant τ of 10^{10} years. Its temperature is usually considered to be low (say, a few degrees absolute), but the heating effects of galaxies[6] could raise it to 10^4 °K so that it would then be ionized (which would also avoid difficulties with the 21 cm observations[7–10]). Now in this model 5 per cent of the extragalactic radiation (assumed to have a constant source strength) was emitted at epochs before 10^9 years (on the usual Robertson–Walker time-scale). At that epoch the electron concentration was greater than it is to-day (because of the expansion of the universe). When allowance is made for this, and for the red shift, the effective path-length for this part of the radiation is about 20 $c\tau$. Since the present electron concentration in the Einstein–de Sitter model is 10^{-5} cm^{-3}, this part of the radiation reaches us through a region with emission measure ~6 cm^{-6} pc (on the favourable assumption that the temperature was 10^4 °K over the whole path-length). The corresponding optical depth is unity at about 2 Mc/s. Thus we would expect the intensity to drop by about 5 per cent in the region of 2 Mc/s, as is in fact observed. Owing to the rapidity with which absorption sets in once it has started, this crude calculation suffices to show that the intergalactic gas of the Einstein–de Sitter model could have the required opacity.

In the steady-state model the temperature of the gas is constant, by hypothesis, and again we take it to be 10^4 °K. In this model 5 per cent of the extragalactic radiation was emitted more than 10^{10} years ago. The electron concentration n_e is now constant and because of the red shift the effective path-length for this part of the radiation is less than 10^{10} light years, and is in fact half this quantity. The theoretical value of n_e is uncertain, but if we take the value favoured by me[6], formerly (due to Pirani[11]) of 5×10^{-5} cm^{-3}, the corresponding emission measure is 4 cm^{-6} pc, which again is in satisfactory agreement with observation.

Further observations may make it possible to distinguish between the local and the cosmological explanation for the absorption. Measurements with greater angular resolution would enable tighter limits to be placed on the degree of isotropy of the absorbing region. If the galactic contribution to the background could be detected by its anisotropy it would show the same absorption behaviour as the extragalactic background on the local explanation but not on the cosmological one. A more difficult but more decisive method would be to observe the low-frequency spectra of discrete radio sources, which again should reveal whether the absorbing *H* II region is local or universal. Whichever explanation turns out to be correct these considerations provide

valuable constraints on the possible density and physical state of the intergalactic gas.

Note added in proof. Since this letter was written some new ground-based observations of the low-frequency background have been published: Ellis, G. R. A., *Nature*, 204, 171 (1964); Ellis, G. R. A., and Hamilton, P. A., *Nature*, 204, 272 (1964). These observations appear to support the local explanation of the absorption. However, a final decision awaits confirmation by observations from above the ionosphere.

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PHYSICS

Standard Date Periods with Declination Limits

A system of dividing the year into 16 periods, each defined by certain solar declination values having numerically equal positive and negative magnitudes, has been discussed by me in an earlier paper[1]. Research has been continued on the selection of the most suitable declination values and the corresponding dates by examination of ephemerides for the Sun during 1952–64 (ref. 2), resulting in slight modifications of the original selections. Such a system would have many advantages in solar engineering research, where bright Sun conditions are of predominant importance, the computation of solar angles of incidence on variously inclined surfaces being greatly facilitated by the limitation to the five standard numerical declination values shown in the fourth column of Table 1.

The ideal system for sub-division of the year would comply with the following conditions: (1) limiting declination values having numerically equal positive and negative magnitudes; (2) a uniform system of dates for all years in the cycle of four years between successive leap years; (3) the same number of days in each period.

The yearly cycle of the Sun's apparent motion is asymmetrical because of the eccentricity of the Earth's orbit and the inclination of the ecliptic, there being 186·4 days from the spring equinox through summer to the autumnal equinox and 178·8 days from the autumnal equinox through winter to the spring equinox. Hence conditions (1) and (3) are mutually incompatible and condition (3) is quite impossible to fulfil if condition (1) is retained. Conditions (1) and (2) can be closely approximated for non-leap years, but for strict correlation a different sequence of dates should be used for leap years, though this would constitute an undesirable complication of the system. It was decided that condition (2) should be the dominating one for all years, even though this involved deviations of a few minutes of angle on certain dates from the declination values set out in Table 1, which shows the proposed standard dates, limiting declination values and mean declinations for the 16 periods. Declination values are at local apparent noon and the dates are for the meridian of Greenwich (0° long.). No significant change is needed over the range ±3 h International Time Zones, but beyond this range there are a few modifications. The length of period 4*A* must be increased by one day in leap years.

Table 1. System of Dates for Standard Declination Periods

Period	Limiting dates	No. of days	Decln. limits	Mean decln.	No. of days	Limiting dates	Period
1*A*	Dec. 22 Jan. 13[2]	23	−23°27′ −21°30′	−22°47′	22	Dec. 21 Nov. 30[1]	1*B*
2*A*	Jan. 14[2] Feb. 4[1]	22	−21°30′ −16°15′	−19°05′	23	Nov. 29[1] Nov. 7[3]	2*B*
3*A*	Feb. 5[1] Feb. 26	22	−16°15′ − 8°45′	−12°34′	22	Nov. 6[3] Oct. 16[1]	3*B*
4*A*	Feb. 27 Mar. 20	22[5]	− 8°45′ 0	− 4°23′	23	Oct. 15[4] Sept. 23[3]	4*B*
5*A*	Mar. 21 Apr. 12[2]	23	0 8°45′	4°23′	23	Sept. 22[3] Aug. 31[3]	5*B*
6*A*	Apr. 13[2] May 5[2]	23	8°45′ 16°15′	12°34′	23	Aug. 30[3] Aug. 8[3]	6*B*
7*A*	May 6[2] May 28	23	16°15′ 21°30′	19°05′	23	Aug. 7[3] July 16	7*B*
8*A*	May 29 Jun. 21	24	21°30′ 23°27′	22°47′	24	July 15 Jun. 22	8*B*

The above dates are for International Time Zones +3 to −3 h (45°W.–45°E. long.). (1) Retard date by one day for Int. Time Zones +9 to +12 h (135°W.–180°W. long.). (2) Retard date by one day for Int. Time Zones +3 to +12 h (45°W.–180°W. long.). (3) Advance date by one day for Int. Time Zones −3 to −12 h (45°E.–180°E. long.). (4) Advance date by one day for Int. Time Zones −9 to −12 h (135°E.–180°E. long.). (5.) Add one day to period for leap years

The *A* and *B* periods of corresponding number may usually be combined for the purpose of collating data on solar energy utilization, especially as periods of bright sunshine are mainly concerned. This does not imply that the total sunshine hours are equal in the *A* and *B* periods and allowance must be made separately for the relative duration of bright Sun conditions. The system has been found satisfactory for evaluating research on solar engineering applications, and if more widely adopted would facilitate comparisons between different research work as well as simplifying any computation involved.

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Propagation of Stress Waves in Liquids

The underlying assumption implicit in work concerned with the propagation of stress waves in liquids is that any pressure disturbance originating at some point in the liquid travels unchanged in form with a velocity $c = \sqrt{(dp/d\rho)}$, which is independent of the density. Such a wave is an 'acoustic' wave, and it is well known that this assumption is amply justified for the small variations of density and pressure usually developed by sound sources under water. But is it justified when the pressure at a point in a liquid rises suddenly by 200 atmospheres (say) due to the passage of a transient stress pulse? The purpose of this communication is to consider this question on the basis of the type of conditions prevailing in previous experiments described by me and other workers[1–3].

Consider unit mass of water at a temperature of 15° C. The initial pressure before the arrival of the stress wave is 1 atmosphere and we require to find the fractional decrease in volume occurring when the pressure rises rapidly by 200 atmospheres. Strictly speaking, we should treat the change as an adiabatic one; but since the adiabatic and isothermal bulk moduli for water differ by so little, we shall obtain a sufficiently good approximation if we assume that the change is isothermal and use the equation:

$$K = V_0 \frac{\Delta p}{\Delta V}$$

Here K, the bulk modulus, is taken to be $2{\cdot}13 \times 10^{10}$ dynes cm^{-2}; V_0 is the volume at 15° C, that is, 1 cm^3; Δp is 200 atmospheres and ΔV the decrease in volume of the unit mass under consideration. This gives us $\Delta V/V_0 = 9{\cdot}46 \times 10^{-3}$, that is, a decrease in volume of about 1 per cent. If ρ_0 and ρ are respectively the initial and final densities then we have, with sufficient accuracy,

$\Delta\rho/\rho_0 = \Delta V/V_0$ and $\rho = 1{\cdot}00946\ \rho_0$. Thus the sudden rise in pressure is accompanied by a density increase of about 1 per cent.

The velocity of propagation U at this higher pressure may, as a first approximation, be estimated by assuming that the pressure changes from its initial value to its final value in an extremely short time, that is, the pressure change approximates to a step-function such as we have in a shock wave. In such a case the value of U is given by the well-known equation of Rankine and Hugoniot, namely:

$$U^2 = \frac{\rho}{\rho_0}\left[\frac{p - p_0}{\rho - \rho_0}\right]$$

In the present case this gives:

$$U^2 = 1{\cdot}00946\left[\frac{p - p_0}{\rho - \rho_0}\right]$$
$$= 1{\cdot}00946\ c^2$$

where c is the acoustic velocity. Thus $(U-c)/c = 0{\cdot}0047$, which is a fractional increase of a little less than 0·5 per cent.

We therefore see that the velocity of propagation U in the regions of higher pressure is only about a half per cent greater than the velocity c of the acoustic waves. Thus the usual assumption that stress wave propagation in a liquid is of the acoustic type appears to be justified and represents a very close approximation to what actually happens.

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Fast Flux Reversal in Laminated Nickel–Iron Thin Films

This communication deals with fast flux reversal in 80–20 nickel–iron films laminated with silicon monoxide. The magnetization curves of such films were shown by Clow[1] to be characterized by very low coercive forces. The laminated film used in this measurement consisted of ten laminations of nickel–iron, each 200 Å thick, separated by silicon monoxide of about half the nickel–iron thickness. The silicon monoxide and nickel–iron layers were deposited alternately using a differential shutter. A control film was deposited at the same time but screened from the silicon monoxide, and therefore was essentially a solid film of nickel–iron 2000 Å thick. The films were 1 cm diameter spots evaporated on to glass substrates at 320° C in a pressure of about 1×10^{-5} mm mercury. The nickel–iron deposition rate was 25 Å/sec. The laminated films had an easy direction coercivity, $H_c = 0{\cdot}17$ œrsteds, and an anisotropy field, $H_K = 3{\cdot}9$ œrsteds, while the control films had $H_c = 0{\cdot}76$ œrsteds and $H_K = 3{\cdot}4$ œrsteds.

The flux reversal measurements were made in a 50 Ω strip line section with a centre member 2·5 cm wide to ensure a uniform field at the sample. The current pulse was obtained by discharging a cable through a coaxial relay with mercury wet contacts. The field pulse had a rise of less than 0·5 nsec with a duration of 1 μsec and was continuously variable in amplitude. The flux change was observed with a single turn loop around the centre of the film arranged as a bridge in conjunction with another loop, with provision to balance the inductive voltage in both amplitude and time. The flux change was observed by integrating the induced voltage with an active integrator with a response time of 1·25 nsec, a decay time of 12 μsec and an effective gain of 1 V-μsec for 0·5 V output.

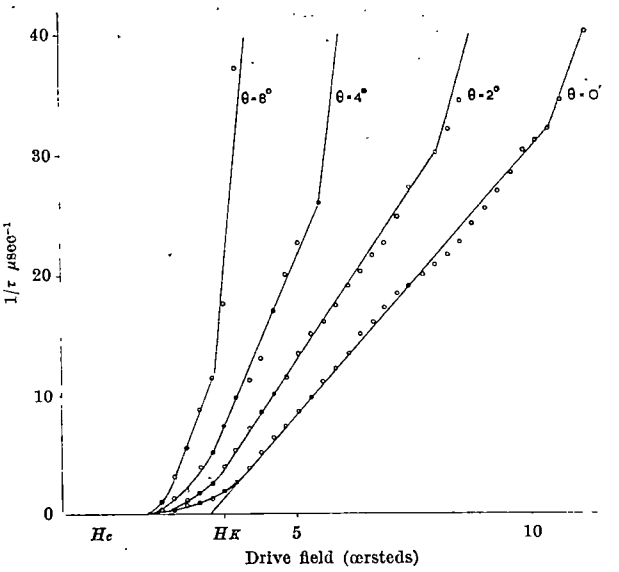


Fig. 1. Inverse reversal time as a function of drive field, with the initial angle as a parameter, for a thin nickel–iron film used as a control when the laminated film was made

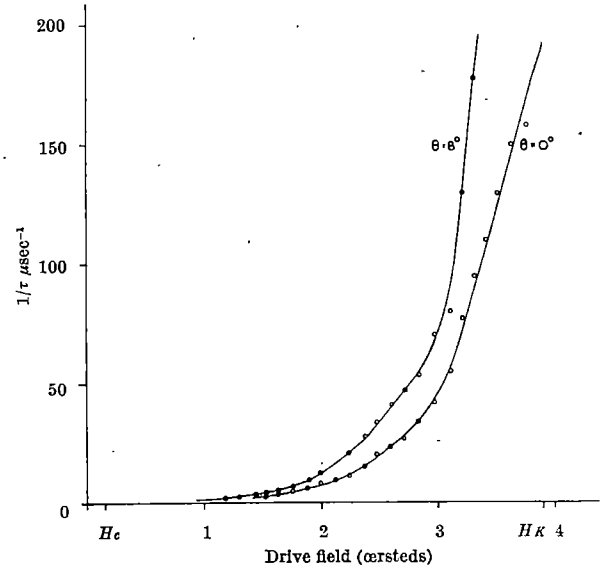


Fig. 2. Inverse reversal time as a function of drive field, with the initial angle as a parameter, for a laminated nickel–iron film.

The output of the integrator was observed using a conventional sampling oscilloscope with a response time of 0·6 nsec. The reversal time, τ, was measured as the time from 10–90 per cent of the integrated flux. Various coils were arranged around the sample to provide the reset field, transverse field and Earth's field bucking.

Fig. 1 is a plot of the inverse of the 180° reversal time as a function of the pulsed driving field in the presence of a static transverse field $H_\perp$. The parameter shown is the initial angle, θ, calculated as $\sin^{-1} H_\perp/H_K$, assuming single domain behaviour. This result is typical of films where three characteristic regions[2] are observed. The intermediate drive region (for $\theta = 0$) is linear and has a slope of about 5 (μsec œrsted)$^{-1}$ with an extrapolated intercept for infinite reversal time of about H_K. The sensitivity to initial angle is also typical.

Fig. 2 gives the results for the layered film (note the change of scale from Fig. 1). To preserve the single domain character of the film when the demagnetizing field was large compared with the coercive force, a constant field of 0·2 œrsteds was applied parallel to the easy

axis in opposition to the pulsed driving field. All results have been corrected for this field. It can be seen that the linear portions of the plot have disappeared, that the sensitivity to initial angle has disappeared and that reversal times as short as 2 nsec were obtained for fields less than H_K. This character can be ascribed to the layered nature of the films since other films of essentially the same low coercive force, and single films with thicknesses about 200 Å, exhibit reversal character similar to Fig. 1.

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Single Sweep Oscillograph Traces of Sonoluminescence

It has been reported[1–7] that sonoluminescence, which is a weak light emission accompanying ultrasonic cavitation in certain liquids, occurs as a discrete flash once every cycle of the stimulating acoustic field. However, I have recently obtained photographs of single-sweep oscillograph traces of the anode current of a photomultiplier used to observe sonoluminescence, and these photographs show that whereas sonoluminescence does occur as pulses always at roughly the same phase of the sound field, the pulses do not occur with every cycle (Figs. 1 and 2. The upper member of each of the pairs of traces shown in these photographs is the sound field).

Cavitation was produced in tap-water with a magnetostrictive window type transducer coupled to a double quarter wave velocity transformer, the system being capable of delivering a total of about 10-W acoustic power at a frequency of 21·2 kc/s over an area of about 0·3 cm². The sonoluminescence trace was obtained by inserting a 5 k ohm resistor in the anode lead of a 15-stage E.M.I. photomultiplier and displaying on a 'Tektronix' oscilloscope. Figs. 1 and 2 were photographed on 'Polaroid' film of 3,000 A.S.A. rating.

It might appear that the probability of a sonoluminescence pulse arising during a given cycle of the sound field (about one-fifth in this investigation) is linearly related to the probability of a suitable nucleus being present in the volume of water where the sound pressure exceeds the threshold value and during the interval that this condition is fulfilled. It would then be reasonable to expect

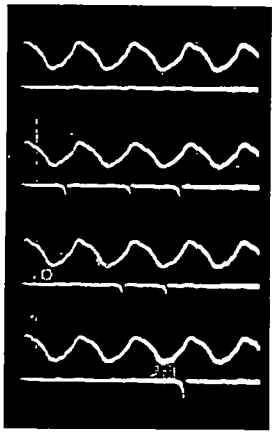


Fig. 1. Oscillograph traces of pick-up voltage and photomultiplier anode voltage (single sweep)

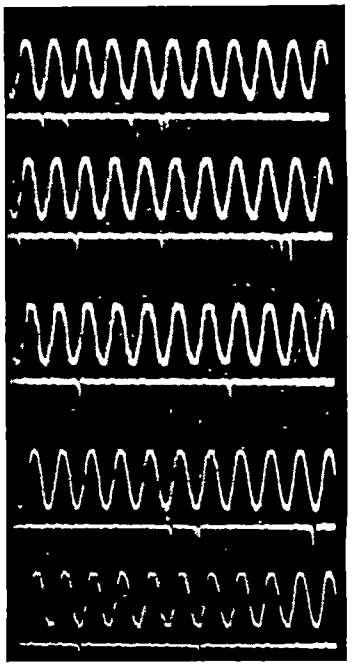


Fig. 2. Oscillograph traces of pick-up voltage and photomultiplier anode voltage (single sweep)

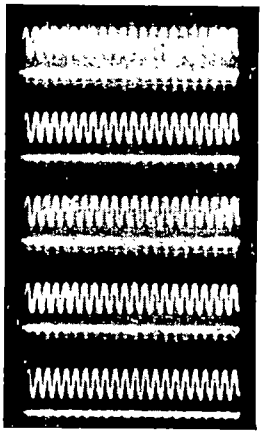


Fig. 3. Oscillograph traces of pick-up voltage and photomultiplier anode voltage (many sweep)

many sweep oscillograms to show an averaging effect whereby the sonoluminescence seems to occur as a flash with every sound cycle (Fig. 3).

Fig. 2 shows that occasionally more than one pulse occurs at about the same time. The frequency of such events is consistent with the chances of two nuclei being present almost simultaneously in the sensitive region during the prescribed interval (about 1/25). The possibility of two pulses arising from the rebound or disintegration of a single nucleus must also be admitted, however.

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Variation in the Ratio of Strontium-89 to Strontium-90 in Precipitation Samples with Sampling Height

It was shown that during March–December 1959 (ref. 1) the specific activities of fission products from fall-out in precipitation increased with sampling height. Some evidence was also found for an increase in the ratio $^{89}Sr/^{90}Sr$ with sampling height, but this increase was not significant ($P \sim 90$ per cent).

(where y is the transformed $^{89}Sr/^{90}Sr$ ratio, and x is the sampling height).

An analysis of variance showed significant differences between the slopes for dry and wet months for both group I ($P \sim 99$ per cent) and group II ($P > 99$ per cent) (Tables 2 and 3).

Hence, it is evident that in 'dry' months the slope was significantly steeper than in the 'wet' months, that is, the $^{89}Sr/^{90}Sr$ ratios in 'dry' months were significantly higher in the upper precipitation collectors than in the lower. An

Table 1. Ratio of Strontium-89 to Strontium-90 in precipitation collected at Eight Different Heights in the Period October, 1961–September, 1963

Height (m) Month	1	7	23	39	56	72	96	123	Mean	Mm precipitation and group
Oct. 61	127·0	93·9	117·0	75·3	73·2	90·8	82·7	72·0	78·3	70 II*w*
Nov. 61	112·0	106·0	85·9	97·1	106·0	112·0	120·0	108·0	105·9	48 II*w*
Dec. 61	65·3	64·0	59·5	42·5	29·1	46·6	71·5	61·7	55·0	59 I*w*
Jan. 62	57·5	44·3	53·2	50·6	52·9	54·4	16·2	51·8	47·6	70 I*w*
Feb. 62	36·9	25·9	30·7	36·3	(29·5)	28·8	31·1	33·5	31·6	56 II*w*
Mar. 62	8·9	10·7	14·9	11·3	15·2	11·3	14·4	(10·7)	12·2	24 I*d*
Apr. 62	8·5	11·0	11·9	9·1	13·1	12·7	27·2	13·8	13·4	20 I*d*
May 62	9·4	10·2	(10·1)	8·2	9·5	8·7	10·4	8·7	9·4	57 II*w*
June 62	4·1	6·4	3·7	1·3	6·1	7·2	8·6	2·1	4·9	28 I*w*
July 62	4·2	5·1	5·5	3·8	4·8	6·5	5·5	4·2	5·0	112 II*w*
Aug. 62	7·9	5·6	6·4	3·9	3·1	6·8	4·7	3·6	5·3	111 I*w*
Sep. 62	19·0	17·5	18·6	14·2	14·1	16·7	20·1	14·3	16·8	36 II*w*
Oct. 62	22·2	22·1	17·6	17·3	16·6	18·2	20·2	19·7	19·2	25 II*w*
Nov. 62	45·3	29·3	41·2	38·4	42·1	36·2	41·2	36·1	38·7	30 II*w*
Dec. 62	20·6	10·9	14·2	24·9	19·6	(19·1)	16·3	(18·2)	18·0	20 I*d*
Jan. 63 / Feb. 63	12·1	11·4	13·3	15·6	19·5	20·8	20·1	19·3	16·5	4 II*d* / 7
Mar. 63	16·0	14·4	17·3	15·9	16·4	17·6	15·7	16·5	16·2	15 II*d*
Apr. 63	11·9	10·2	11·6	11·9	10·5	11·4	11·0	10·4	11·1	37 II*w*
May 63	7·0	7·5	7·2	7·6	6·7	7·8	7·4	7·8	7·4	22 II*d*
June 63	5·2	5·3	5·5	5·4	5·2	5·4	6·6	(5·8)	5·6	35 II*w*
July 63	3·8	4·3	4·4	4·0	4·1	4·6	4·3	4·1	4·2	73 II*w*
Aug. 63	2·6	1·8	2·7	2·5	2·5	2·5	2·9	2·5	2·5	123 II*w*
Sep. 63	1·3	0·9	0·8	1·3	1·4	1·8	1·4	1·5	1·3	24 I*d*

Figures in brackets are missing values calculated by the method of least squares. These figures are omitted in the regression analysis.

Table 2. Comparison of the Lines in Group I Analysis of Variance

Variation	Sum of squares of deviations	Degrees of freedom	Variance	Test
about lines	0·4147	57	$0·7275 \times 10^{-2}$	
between slopes	$0·5045 \times 10^{-1}$	1	$0·5045 \times 10^{-1}$	6·94

Table 3. Comparison of the Lines in Group II. Analysis of Variance

Variation	Sum of squares of deviations	Degrees of freedom	Variance	Test
about lines	$0·7378 \times 10^{-1}$	114	$0·6472 \times 10^{-3}$	
between slopes	$0·4952 \times 10^{-3}$	1	$0·4952 \times 10^{-3}$	7·65

After the resumption of large-scale nuclear testing in September 1961, it became possible to repeat the experiment over a longer period. The $^{89}Sr/^{90}Sr$ ratios were determined in precipitation collected at eight different heights in the meteorological mast at Risö after the principles used in 1959 (ref. 1).

Table 1 shows the results for the 24-month period: October 1961–September 1963.

The $^{89}Sr/^{90}Sr$ ratios in Table 1 were treated as follows. The ratios were converted to the logarithms, because the figures were logarithmically distributed. The converted ratios for each month were divided by the corresponding (converted) mean ratio for the month. The purpose of this operation was to eliminate the effect of the time variation of the $^{89}Sr/^{90}Sr$ ratios. The months were separated into two groups, one with high variance (I) and one with low (II). This was necessary for the performance of a regression analysis, where the differences in the variances have to be insignificant. The two groups of months were each sub-divided into two sub-groups, one representing 'dry' month (*d*), that is, months with less than 25 mm precipitation and another representing 'wet' month (*w*), that is, months with 25 mm and more precipitation (Table 1).

The following regression lines were calculated:

$$y = 1 + 0·1040 \times 10^{-2} (x - 46·55) \quad (Id)$$

$$y = 1 - 0·4452 \times 10^{-3} (x - 52·13) \quad (Iw)$$

$$y = 1 + 0·4050 \times 10^{-3} (x - 52·13) \quad (IId)$$

$$y = 1 + 0·6857 \times 10^{-5} (x - 52·39) \quad (IIw)$$

explanation for this phenomenon has been proposed earlier[1]: it is assumed that the dry deposit, which yields the higher concentrations in the upper precipitation collectors, is of greater particle-size than the deposit from rain. Larger and heavier particles will be deposited more readily than fine particles, and consequently the coarse particles will consist of younger fission products (greater $^{89}Sr/^{90}Sr$ ratio) than the fine ones.

I thank Mr. Heinz Hansen for his advice concerning the statistics.

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Equivalence of the Schroedinger and Heisenberg Pictures

In a recent re-examination[1] of the foundations of quantum mechanics, Dirac asserts, *inter alia*, that the Heisenberg and Schroedinger pictures in quantum electrodynamics are not equivalent because the unitary operator which connects both pictures, namely $e^{iHt/\hbar}$, where $H = H_{\text{Dirac field}} + H_{\text{Maxwell field}} + H_{\text{interaction}}$, is the total Hamiltonian in the Schroedinger picture, does not exist. It is the purpose of this communication to question this assertion.

It is assumed from the outset that H is, in common with all observables in quantum mechanics, self-adjoint; that is, $H = H\dagger$ and $\mathfrak{D}_H = \mathfrak{D}_{H\dagger}$, where $\mathfrak{D}_H$ and $\mathfrak{D}_{H\dagger}$ are the domains of H and its adjoint in the Hilbert space $\mathfrak{H}$ of the system. Accordingly, if $\{E(\lambda)\}$ are the projectors comprising the resolution of the identity belonging to H and if $F(\lambda)$ is an arbitrary complex-valued function of λ, then the operator $F(H)$, with the domain $\mathfrak{D}_F$, is defined[2] by the Radon–Stieltjes integral:

$$< \Phi|F(H)|\Psi > = \int_{-\infty}^{+} F(\lambda)\mathrm{d}(< \Phi|E(\lambda)|\Psi >)$$

for all $|\Phi>$ in $\mathfrak{H}$ and all $|\Psi>$ in the set $\mathfrak{D}_F$ of all vectors in $\mathfrak{H}$ such that $\int_{-\infty}^{+\infty} |F(\lambda)|^2\mathrm{d}(||E(\lambda)|\Psi>||^2)$ exists. Hence, if $F(\lambda) \equiv e^{i\lambda t/\hbar}$, then $\mathfrak{D}_F$ is the set of all vectors $|\Psi>$ in $\mathfrak{H}$ such that $\int_{-\infty}^{+\infty} \mathrm{d}(||E(\lambda)|\Psi>||^2)$ exists. And since $\lim_{\lambda\to-\infty} E(\lambda)|\Psi> = |0>$ and $\lim_{\lambda\to+\infty} E(\lambda)|\Psi> = |\Psi>$ for all $|\Psi>$ in $\mathfrak{H}$, it is clear that $\mathfrak{D}_F = \mathfrak{H} \supset \mathfrak{D}_H$, so that $e^{iHt/\hbar}$ certainly exists. Also, using[2]

$$< \Phi|G\dagger(H)F(H)|\Psi > = \int_{-\infty}^{+\infty} G^*(\lambda)F(\lambda)\mathrm{d}(< \Phi|E(\lambda)|\Psi >)$$

it is evident that $e^{iHt/\hbar}$ is unitary and that H is identical in both pictures.

Thus the Schroedinger and Heisenberg pictures are equivalent, provided merely that H is self-adjoint.

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H. S. Perlman assumes that H is a self-adjoint operator, operating in Hilbert space. This assumption is not valid in quantum electrodynamics. H, like the other dynamical variables, then operates on vectors in some kind of space, larger than a Hilbert space, the nature of which is unknown.

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GEOLOGY

Kink-bands and Related Geological Structures

Dr T. B. Anderson[1] considers that kink-bands are symmetrically distributed about a planar anisotropy caused by cleavage, and that this is indicative of the major principal pressure acting within the cleavage. This is not justified since in rocks with strong planar anisotropism there is no basis for interpreting "a component parallel to the strike of the *s*-surface"[1,2] as the maximum principal pressure. Indeed, according to Hoeppener[3], the view that the maximum principal stress bisects the obtuse dihedral angle may be fallacious.

However, permitting Anderson's assumption, it is apparent from his values that the kink-bands are not symmetrically arrayed about the modal cleavage. This asymmetry may be explained in terms of a small discrepancy in the modal cleavage value; but it probably reflects true variations in the orientation of the kink-bands since, although the dextral kink-bands are very consistent in their orientation, it would appear from Anderson's statement that the sinistral ones are less so. Further, the two kink-bands in Anderson's Fig. 2, because they do not intersect, are not truly conjugate and may be explained in terms of a first-order shear on which a second-order shear has developed. One must, therefore, question that Anderson's kink-band system is the product of irrotational strain as would be the case where P_{max} is contained within the cleavage. This is critical, for should P_{max} not be contained within the cleavage of strongly planar-anisotropic rocks the strain will be rotational, P_{max} will not bisect the obtuse dihedral angle and the kink-bands will not be symmetrically disposed about the *s*-surface.

It is important to Anderson's theory that, as stated by him, "The orientation of the kink-band itself does not change". However, in a summary of work from the Rhenish Schiefergebirge, Hoeppener[4] concluded that relative rotation between the rock and the shear-planes must occur. Similarly, in the Start Point area[5], South Devon, where steeply dipping kink-bands cross a sub-vertical cleavage, I observed in plan view and on the cleavage face kink-bands of the same movement-sense crossing each other, bifurcating and converging.

For the orientation of the kink-bands to be consequential on the rotational movements on the foliation surfaces between the kink-planes, as suggested by Anderson, it is implicit that the direction of shear paralleling the kink-bands is also consequential. In south Devon, however, I have traced a kink-band along its strike into a single kink-plane with associated pinnate tension joints[6], in turn passing into a strip of tension gashes *en echelon* (Fig. 1). Similar associations are figured by Engels[7], and in all cases the movement-sense is consistent throughout the association. Thus, the direction of shear paralleling the kink-band must be the primary displacement, while rotational movement on the foliation surfaces between the kink-planes is secondary.

Although greater variation in the angle of internal friction (φ) might occur, it is generally thought to lie between 25° and 35° (ref. 8). According to Anderson's theory this would place limiting values of 115° and 125° on the dihedral angle between conjugate kink-bands containing P_{max}. However, Hoeppener[3] recorded values ranging between 105° and 140°, Ramsay[9] reported values of less than 90°, and I have noted values of less than 90°

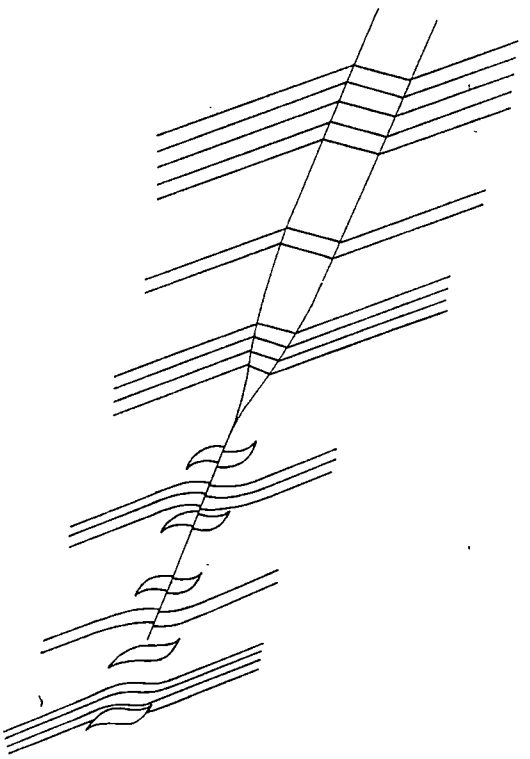


Fig. 1. Diagrammatic association of kink-band, pinnate tension joints, and tension gashes

ranging up to 140°. Therefore, although an extreme value of φ (= 50°) might be advocated to account for the 140° dihedral angle, the less than 90° values are inexplicable by Anderson's hypothesis.

Where regional data are evaluated statistically, the angular spreads of the kink-bands and cleavage about their modal values should be given. This will permit a fuller understanding of the behaviour of the kink-bands with respect to the cleavage. For example, given that the modal values indicate an approximately symmetrical distribution of kink-bands about the cleavage, should the dextral kink-band exhibit a wide angular spread while the cleavage and sinistral one have narrow values, it will be apparent that the regional relationship between the kink-bands and the cleavage has a degree of asymmetry.

In conclusion, I fully support Anderson's view that kink-banding is an incipient strain-slip cleavage, but would not exclude other geneses of this structure.

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MARSHALL's statement that I considered "that kink-bands are symmetrically distributed about a planar anisotropy caused by cleavage" is scarcely a fair synopsis of the sentences: "In any one area the kink-bands may all be roughly parallel and all may have the same sense of displacement. However, conjugate sets, composed of kink-bands in two different orientations with complementary displacements, are not uncommon"[1]. Clearly I fully realized that kink-band systems are not all symmetrical about the foliation and that the symmetrically arranged conjugate set is a special case.

The clause "there is no basis for interpreting 'a component parallel to the strike of the *s*-surface' as the maximum principal pressure" is vague and uses its quotation out of context. In fact, I followed Flinn[2] in stating that kink-bands are "the expression of a movement which can have a component parallel to the strike of the *s*-surface"[1]. I also said that "kink-bands effect a shortening parallel to the original foliation" and may be "interpreted as a response to a major principal stress acting in a direction contained in, *or close to*, the original foliation"[1]. This obviously does not imply that kink-bands are necessarily produced by a maximum principal pressure acting exactly along the foliation.

Marshall goes on to allege that the kink-bands I described from the Ards Peninsula[1] were "not symmetrically arrayed about the modal cleavage". The modal values given[1] were: dextral kink-bands, N.15° E.; sinistral kink-bands, E.40° S.; modal cleavage plane, N.70° E., all planes being approximately vertical. The modal cleavage plane was therefore within 2·5 degrees of exactly bisecting the obtuse angle between the kink-bands. This, as Marshall must know, is very well within the limits of experimental error in the measurement of the orientation of small planar structures in a structurally complex area. The deviation from perfect symmetry is negligibly small.

Marshall also alleges that the two kink-bands shown in my Fig. 2 "because they do not intersect, are not truly conjugate". In his paper on conjugate folds Ramsay[3] states "conjugate folds may be defined as sets of paired reversed folds whose axial planes are inclined toward one another". In this accepted sense the kink-bands shown are conjugate. Marshall clearly has his own definition.

Marshall contends that the two kink-bands in Fig. 2 "may be explained in terms of a first-order shear on which a second-order shear has developed". In fact neither kink-band is a shear plane, nor is it parallel to a shear plane. However, treating the two structures as shear planes of opposite sense, one first-order, the other second-order, measuring the angle between and using the formula in McKinstry's paper[4], to which Marshall refers, a value for φ of either 9° or 51° is obtained. Both of these are well outside the range of possible φ values Marshall himself quotes.

Marshall's observation of kink-bands of the same movement-sense crossing each other, bifurcating and converging does not conflict with my view that during the formation of a single kink-band the orientation of the band does not change. The sentence he quotes is again taken out of context. Taking the simplest analogy, the fact that faults may cross-cut, bifurcate and converge does not mean that each fault-plane changed its orientation during faulting.

I fail to see how a kink-band, defined essentially by two kink-planes, can pass "along its strike into a single kink-plane", the axial surface of a single fold. Certainly Marshall's Fig. 1 does not show this phenomenon. The two kink-planes of the kink-band illustrated converge and pass into a shear plane, not a kink-plane. Unfortunately, the critical transition point is not adequately illustrated.

Marshall argues from Fig. 1: "the direction of shear paralleling the kink-band must be the primary displacement, while rotational movement on the foliation surfaces between the kink-planes is secondary". Since the displacement effected by the kink-band is a product of the shear movements on the foliation and the one cannot exist without the other this is a circular argument. In many kink-bands there is no "shear paralleling the kink-band". The displacement across the band is effected by folding alone and the foliation planes are continuous. Without a primary shear plane the movement on the foliation planes can hardly be described as secondary.

Marshall states that the angle of internal friction (φ) is "generally thought to lie between 25° and 35°" and refers to a paper by Hubbert[8]. In fact, Hubbert[8] (p. 364) states: "Thus from fault data . . . the angle of internal friction would lie between 10° and 50°". Substituting these values for those quoted by Marshall neatly accounts for the range of 'dihedral angles' reported by Hoeppener[6], and, in fact, the mid-point of Hoeppener's range (122°) coincides almost exactly with the prediction (120°) obtained by taking the mid-point (30°) of either of the φ ranges quoted as a basis for calculation. I would accept this as a support rather than a contradiction of the angular relationship I outlined.

While conjugate faults usually enclose an angle of less than 90° about the direction of maximum principal pressure[7], field geologists occasionally observe pairs of conjugate faults which are apparently exceptions to this rule, implying negative values of φ, though these are comparatively rare and do not affect the correctness of E. M. Anderson's[7] conclusions when modal orientations calculated from adequate data are considered. A very similar situation seems to obtain in the geometry of conjugate pairs of kink-bands. Here values of less than 90° for the same angle are as exceptional as values of more than 90° among conjugate fault systems.

Finally, it appears that Marshall has not considered the recent experimental work of Paterson and Weiss[9]. These authors produced kink-bands by compressing cylinders of phyllite under high confining pressure. "In the specimens compressed parallel to the foliation, the kink-bands are in symmetrically oriented conjugate sets". Individual bands "appear at approximately 50° to the direction of shortening", that is, making in conjugate

pairs an angle of 100° about the maximum principal pressure. In cylinders inclined at small angles (less than 25°) to the foliation asymmetrical conjugate sets developed. At angles of 25°–45° only one set of kink-bands appeared and at greater inclinations of the foliation to the cylinder axis, and hence to the maximum principal pressure, the deformation took other forms. No mention is made of any change in orientation of the kink-bands during growth.

These experimental results are in good agreement with the geometrical relationships and mechanisms of formation I outlined, particularly with the statement that kink-bands "may be interpreted as a response to a major principal stress acting in a direction contained in, or close to, the foliation".

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Live and Dead Foraminifera between the Sarns, Cardigan Bay

The Sarns of Cardigan Bay are submarine ridges of boulders, cobbles and pebbles, running out to sea at right angles to the coast. The longest, Sarn Badrig or St. Patrick's Causeway, is more than 13 miles long. These striking features, and the areas of finer sediment between them, are being investigated. During the summer of 1961 samples were collected from sand areas in shallow, near-shore waters above the five fathom line, off Aberystwyth, off Clarach and off Borth in Cardigan Bay. These samples were surface skims of sediment obtained by use of 'Normalair' aqualung equipment from a rubber boat. Dr. T. D. Adams and Mr. W. Barrett assisted with this work. Analysis of the fauna in these samples by the standard rose bengal staining technique[1] revealed that all the foraminifera were dead, and many of the shells were abraded and worn.

In late 1962 the Department acquired the research vessel, *Antur*, and systematic bottom sampling of the whole area between Sarn Cynfelyn and Sarn Badrig was undertaken. This work was done in co-operation with Dr. H. A. Jones and Mr. J. R. Moore, III, the object being to map recent sediment distribution and its relation to living and dead foraminifera, and to other forms of marine life. Most of the samples were collected with the Holmes vacuum grab, others with the Van Veen grab. Boat operations were continued through the winter of 1962/63 and an area of about 200 square miles was sampled at approximately one-mile intervals. This survey covered the area between the Sarns down to the 10-fathom line and made it possible to compare samples collected at different seasons.

Petrological work by Mr. Moore shows that the predominant sediments in this area are fine sands ($\frac{1}{4}$–$\frac{1}{8}$ mm) with subsidiary patches of coarse sands and sandy gravels. Living specimens of foraminifera are absent from these sediments—not only from samples collected by the Van Veen grab, where it could be argued that surface sediment was swamped by subsurface material, but also in vacuum grab samples of less than 50 ml. where the sediment included a high proportion of surface material.

As was shown by Herdman and Lomas[2] the net current movement, and thus transport of sediment, in the shallow parts of the eastern Irish Sea, is landwards. Admiralty charts show that currents up to 1 knot obtain in the area between the Sarns. These speeds are quite sufficient to transport smaller foraminifera, average size between 0·1 and 0·5 mm (ref. 3). This indication that the dead foraminifera are moving with the sediment is strongly supported by their marked size sorting. In the fine sand areas they fall mainly on the 100 mesh (152 microns) sieve. In the coarse sand and gravel areas the majority are found in the 60 mesh (251 microns) sieve with some well-grown forms on the 30 mesh (500 microns) sieve.

Clues to the origin of at least some of the species found dead in the current-swept sand of the bay are provided by samples from the Sarns themselves. Parts of the Sarns are awash at low spring tides. They are thus shallow banks providing convenient anchorage for many kinds of marine algae and associated life forms. Cobbles and boulders obtained with the Van Veen grab, particularly from Sarn Bwch, carry a rich assemblage of algae, hydroids, tunicates and bryozoans with associated molluscs and foraminifera. Most of the foraminifera are living, and include not only fixed forms, such as *Cibicides*, but also numerous members of the Miliolidae. When they die these forms are undoubtedly swept into the sands. Foraminifera also live on the surface of the relatively small areas of mud deposition in the Bay, such as in the 'trawling grounds' south of Sarn Cynfelyn. It is unlikely that specimens from these quiet areas are worked into the sands (though the muds include specimens which may have been winnowed from the sands). There are, however, many forms in the dead assemblage that occur neither on the Sarns nor, living, on the mud. These include *Bolivina* and *Lagena* as well as *Globigerina*, and are presumably swept in from the deeper parts of St. George's Channel, or beyond.

Carter[4], in his work on the East Anglian crags, has shown that the foraminifera are size-sorted. The sands between the Sarns are likewise the product of a rapidly advancing transgressive sea. Identification of such current-swept microfaunas as occur in these sands may thus assist in the identification of similar sedimentary bodies, above unconformities, in the sub-surface.

The work described here is part of a research project concerning the geological history of Cardigan Bay, directed by Prof. Alan Wood. The diving equipment was supplied by the College and the research vessel financed by the Department of Scientific and Industrial Research.

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M_2 Component at Hyderabad

In an earlier paper[1] the authors reported the recording and analysis of the gravity variation with time in the month of December, 1961, at Hyderabad (long. 78° 27′ E and lat. 17° 26′ N). The record was continued for another six months in 1962 and the data were analysed only for the main lunar semidiurnal tidal component M_2, as it was found that this was the most consistent and dependable component. It is found desirable to check the calibration of the photographic recording paper (in milligals) frequently. The theoretical amplitude is 0·069 milligals for Hyderabad.

In Table 1 the gravimetric factor G is the ratio of the observed amplitude (A) in milligals to the theoretical amplitude. From this factor the characteristic numbers Love (h, k,) and Shida (l) are calculated.

Table 1

Month	A	G	h	k	l
Jan.	0·0815	1·18	0·64	0·31	0·08
Feb.	0·0807	1·17	0·61	0·29	0·08
Apr.	0·0830	1·20	0·71	0·34	0·09
May	0·0839	1·21	0·75	0·36	0·10
June	0·0812	1·18	0·64	0·31	0·08

Taking the average values of f from the equation $h=\frac{5f}{2f+1}$ for different months as 0·180, the rigidity value of the Earth $\mu=gd\rho/19f$ (where g is 978·5 dynes/sec², d, density 5·52 g/c.c. and ρ = distance from the centre of the Earth to the observation point = 6375·567 km) is found to be $1{\cdot}1\times10^{12}$ dynes/cm², which value is in general agreement with those obtained by other workers.

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CRYSTALLOGRAPHY

New Representatives of the $NbAs_2$ and $ZrAs_2$ Structures

The monoclinic unit cell and the space group of $NbAs_2$ and isomorphous $TaAs_2$ have recently been determined by Saini *et al.*[1]. In the course of an examination of transition-element polycompounds I observed this structure also in VP_2, VAs_2, NbP_2, TaP_2, $MoAs_2$, α–WP_2 (low-temperature modification) and WAs_2. The titanium-group phosphides TiP_2, ZrP_2 and HfP_2, on the other hand, were found to be isostructural with the orthorhombic $ZrAs_2$ (ref. 2) and $HfAs_2$ (ref. 3). This structure is referred to as $PbCl_2$ (C23) type. However, in these titanium-group pnictides anion–anion bonds seem to be important so that I prefer to distinguish this variety from the ionic $PbCl_2$ type by using a different name. The lattice constants, as derived from powder patterns, are listed in Table 1.

Table 1. Lattice Constants of Orthorhombic $ZrAs_2$-type and Monoclinic $NbAs_2$-type Phases

	a (Å)	b (Å)	c (Å)	β
TiP_2	6·181 ± 3	8·256 ± 3	3·346 ± 3	
ZrP_2	6·494 ± 3	8·744 ± 3	3·513 ± 3	
HfP_2	6·467 ± 3	8·646 ± 3	3·497 ± 3	
VP_2	8·466 ± 9	3·106 ± 2	7·170 ± 8	119° 16′ ± 4′
VAs_2	9·059 ± 7	3·272 ± 2	7·481 ± 7	119° 47′ ± 4′
NbP_2	8·878 ± 9	3·266 ± 2	7·529 ± 9	119° 8′ ± 5′
TaP_2	8·870 ± 8	3·267 ± 2	7·497 ± 7	119° 24′ ± 4′
$MoAs_2$	9·064 ± 6	3·295 ± 2	7·715 ± 8	119° 22′ ± 3′
α–WP_2	8·501 ± 9	3·167 ± 2	7·471 ± 8	119° 20′ ± 4′
WAs_2	9·079 ± 5	3·318 ± 2	7·692 ± 5	119° 26′ ± 3′

My samples all showed metallic conductivity and a positive thermoelectric power of 10–25 μV/° C. I do not know at present whether the metallic conductivity is an intrinsic property of all these compounds or if it is due only to impurities or to a deviation from stoichiometry. In the $ZrAs_2$-type compounds the tendency to saturate all bonds is obvious. All anions form pairs which would suggest an ionic formula $Zr^{4+}(As_2)^{4-}$. The probability for the occurrence of semiconducting properties should be greatest in the 5d compound HfP_2. If a parallelism between corresponding $d\varepsilon^6$ and d^0 compounds

$$\begin{array}{ccc} PtS_2 & ----- & PtP_2 \\ \downarrow & & \downarrow \\ HfS_2 & ----- & HfP_2 \end{array}$$

is postulated we would expect a rather large energy gap in the case of HfP_2. The As–As distance 2·58 Å (ref. 3) reported for $HfAs_2$, on the other hand, is somewhat large for a saturated anion–anion bond (As–As single-bond distance 2·42 Å (ref. 4)), indicating the possibility that a small fraction of the cation electrons are left in a metallic d band.

Though the detailed structure of $NbAs_2$ is not known*, we suppose that the anions also form pairs in this type of structure. In the vanadium-group phases one then expects to find a similar metallic d band as in the corresponding dichalcogenides VSe_2, NbS_2, TaS_2, etc. It is interesting that $MoAs_2$, α–WP_2 and WAs_2 also crystallize in the $NbAs_2$ structure. In these latter compounds two extra d electrons of the cation would be sufficient to fill a d^2 band supposing the cation to have a trigonal prismatic environment. In this case, then, diamagnetism and non-metallic properties would be possible as in MoS_2, WS_2, etc. In the high-temperature modification β-WP_2, which crystallizes in the MoP_2 structure[5], anion pairs are still present (P – P = 2·17 Å, corresponding to a single bond). The cation has in fact six P neighbours forming a trigonal prism but a seventh W–P bond occurs and this additional bond may explain the observed metallic behaviour of MoP_2 and β–WP_2.

I thank Miss Elfriede Rittershaus and Mr. H. U. Boelsterli for assistance in sample preparation and X-ray work.

* *Note added in proof.* Meanwhile, the structure of $NbAs_2$ and of isomorphous $NbSb_2$ has been determined[6]. Since only half of the As atoms form pairs (As_{II} – As_{II} = 2·45 Å, whereas As_I – As_I = 2·98 Å) the structure would allow $NbAs_2$ to be a diamagnetic semiconductor with ionic formula $Nb_2^{5+}(As_2)^{4-}As_2^{3-}$. The observed metallic properties, therefore, are due to an accidental overlapping of bands as occurs in $TiTe_2$ and $PdSb_2$. $TaSb_2$ is isomorphous with $NbSb_2$ and has the following lattice constants: a = 10·22 ± 2 Å; b = 3·644 ± 4 Å; c = 8·29 ± 2 Å; β = 120° 24 ± 8′.

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CHEMISTRY

Water Structure around Silver Iodide Sol Particles

In order to explain lower values of differential capacity at the negative silver iodide/solution interface than at the mercury/solution interface, Lyklema[1] proposed that the Stern layer in the former may be of icelike (tetrahedral) structure, and pointed out that this would also explain the greater specificity of cations in the silver iodide case. More recently Levine and Bell[2] have commented that such structure would tend to remove the distinction between inner and outer Helmholtz planes so that the fluctuation potential would become small, this making the maximum in the outer Helmholtz plane potential much less marked. This effect would then explain the relative constancy of the flocculation concentration for silver iodide sols at high (negative) potentials[3,4] compared with those of silver bromide and silver chloride sols, which have been reported[5] to decrease at high negative potentials. The hypothesis is supported by the well-known epitaxis of ice on silver iodide which is more effective near the zero point of charge[6].

The purpose of this note is to report further evidence of such structure both from pulsed proton nuclear magnetic resonance (spin-echo[7]) measurements on negative silver iodide sols and from studies on the rates of rapid flocculation of these sols at varying surface potential by excess electrolyte.

For the spin-echo measurements (using a spectrometer constructed by Mr. W. E. Porter of this department) silver iodide sols were prepared by the addition of 0·1 M silver nitrate solution to 0·11 M potassium iodide solution followed by dialysis in 'Cellophane' tubing against constantly renewed distilled water at 25° C for about 10 days. This procedure reduces the excess iodide concentration to about 10^{-5} M (pI 5) or below. For measurements at

pI 3, the sols were made 10^{-3} M with respect to iodide ions by the addition of the appropriate small quantity of concentrated potassium iodide solution from an 'Agla' syringe. In order that sols at different pI could be compared at the same sol concentration an identical quantity of distilled water was added to the sol as dialysed. In one experiment, in order to increase the magnitude of the observed effect, the sol concentration was increased by light centrifugation before division into two parts and adjustment of pI.

Table 1. Relaxation Times from Spin-Echo Measurements on Silver Iodide Sols

		T_2 (sec)			T_1 (sec)		
	sol conc.	pI ~ 5	pI 3	Water	pI ~ 5	pI 3	Water
Sol *A*	0·05 M	2·21	2·30	2·43	2·38	2·61	2·69
Sol *B*	0·05 M	2·18	2·53	2·61			
Sol *C*	0·05 M	2·09	2·35	2·44			
Sol *D*	*	1·07	2·18	2·61			

* Sol initially at 0·05 M concentrated by light centrifugation.

Table 1 shows the values of the transverse relaxation time T_2 and the longitudinal (spin-lattice) relaxation time T_1, obtained from spin-echo measurements on sols as dialysed (pI ~ 5) and at pI 3. The relaxation times for pure water, measured at the same time as those for the sol, are included in each case.

It can be seen from Table 1 that both T_2 and T_1 reduce in the order water > pI 3 > pI ~ 5 with the T_1/T_2 ratio equal to 1·1. The reduction is due to an increase in the correlation time for molecular motion[8] which is indicative of a corresponding increase of water structure.

The effect cannot be due simply to a change in iodide ion concentration since even at pI 3 it is too small to have an appreciable effect and, moreover, the iodide ion is a known structure breaker so that any effect would be in the opposite sense. A reasonable interpretation of these results is that silver iodide sol particles are surrounded by a layer of tetrahedral water structure which tends to break down at high surface potentials where water dipoles become orientated as around ions.

Supporting evidence for this view has been obtained from measurements of the rate of rapid flocculation of negative silver iodide sols in excess electrolyte. As shown by Smoluchowski[9], the bimolecular rate constant k_0 in the expression $-dN/dt = k_0N^2/2$, where N is the number of particles at any time t, should, in the absence of a force field, be given by $k_0 = 8\,kT/3\eta$, where k is the Boltzmann constant and η the viscosity of the dispersion medium. For particles of radius corresponding to the Rayleigh region k_0 can be measured turbidimetrically[10,11] by taking the initial slopes $(d\tau/dt)_{t\to 0}$ of plots of turbidity τ versus time, to give k_0 from:

$$\left(\frac{d\tau}{dt}\right)_{t\to 0} = \frac{AC_s^2k_0}{\rho^2} \qquad (1)$$

where C_s is the sol concentration (gm ml.$^{-1}$), ρ the particle density and A the Rayleigh constant. For these experiments sols of concentration 5×10^{-4} M were prepared by direct mixing of silver nitrate and potassium iodide solutions. The mean particle radius was found to be 70–100 Å by electron microscopy. Flocculation experiments using aged sols of appreciably larger particle radius gave, within the limits of experimental error, identical results to those obtained for the smaller particles.

Table 2. Bimolecular Rate Constants for Rapid Flocculation of Silver Iodide Sols

pI	k_0(obs.) ml. sec^{-1} × 10^{12}	k_0(obs.)/k_0(calc.)
3	8·7	0·70
4	7·3	0·59
5–13	4·7	0·38

Table 2 shows the results for the rapid flocculation of sols at various values of pI, and a comparison between the observed k_0 and that calculated from the Smoluchowski equation k_0(calc.). For pI values greater than 5 an appropriate amount of silver nitrate was included in the flocculating electrolyte and the combined solution added to a sol initially at pI 5. The results shown are average values for potassium, magnesium and lanthanum nitrates as flocculating electrolytes, differences between these electrolytes being insignificant compared with the estimated experimental error (~10 per cent).

Although the absolute values of k_0(obs.) may be somewhat in error because of either defects in the model used to derive equation (1) or uncertainties in the determination of the initial slopes of the curved τ versus t plots, the increase in rate from pI 5 to pI 3 seems significant. This increase corresponds with the increase in relaxation time towards that of pure water shown in Table 1 and could therefore be due to the dissolution of tetrahedral water structure which acts as a small energy barrier to flocculation at pI 5 and above. The asymmetry of the zero point of charge (pI 10·5) precludes investigation of a similar effect for positive sols.

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Reversible Photochemical Bleaching in Frozen Aqueous Systems at 77° K

It is known that some colourless glasses containing certain impurity centres become coloured on exposure to visible light at room temperature and get colourless again in the dark, evidently due to some thermal back-reaction. We have found some systems which show a similar behaviour at temperatures of about 77° K. The systems referred to are transparent matrices of ice containing certain solutes such as sulphuric or phosphoric acid at concentrations of 1–10 mole per cent. If these glassy ice matrices are irradiated with ionizing radiations (γ-rays, X-rays, etc.) they become coloured, deep yellow in the case of sulphuric acid and pink in the case of phosphoric acid, respectively. If now these coloured glasses are exposed to a source of visible light for several minutes, for example, a 500-W tungsten lamp, this colour disappears. However, on annealing these glasses in the absence of light the colour is again restored, for example, at a temperature of about 120° K the original colour is very rapidly and completely restored in a very short time. These processes can be followed by both spectrophotometric as well as electron spin resonance techniques since the colour centres formed on irradiation as well as the species formed on optical bleaching of the colour centres show characteristic absorption and electron-spin resonance spectra. The results of these investigations, which will be published more fully elsewhere, have led to the following conclusions.

It is known that absorption of, for example, γ-radiation by the ice matrices leads, in the first instance, to the formation of positive and negative polarons in the ice designated by $(H_2O)^+$ and $(H_2O)^-$, respectively[1,2]. The negative polarons react with the protons in the acid matrix to give hydrogen atoms, whereas the positive

polarons react with, for example, the sulphate ions present according to:

$$HSO_4^- + (H_2O)^+ \rightarrow HSO_4.(H_2O) \rightarrow SO_4^-.(H_3O)^+ \quad (1a)$$

$$SO_4^{2-} + (H_2O)^+ \rightarrow SO_4^-.(H_2O) \quad (1b)$$

giving the radical ion SO_4^- which shows a characteristic electron spin resonance spectrum and absorbs light in the visible region with absorption maximum at 446 mμ. A corresponding process occurs in the phosphate matrices where it leads to the formation of the HPO_4^- radical ion, which shows maximum absorption at 525 mμ.

In the photochemical bleaching with light which is absorbed by these coloured radical ions an electron is transferred from the hydration shell to the radical ions, for example, according to:

$$SO_4^-.(H_2O) + h\nu \rightarrow SO_4^{2-}.(H_2O)^+ \quad (2)$$

leading to the formation of a positive hole trapped in the hydration shell of the respective ions. The presence of a positive hole can be inferred from an examination of the electron-spin resonance spectra. The latter were found to be identical after bleaching of both the γ-irradiated sulphate and phosphate ice matrices. If now these colourless matrices are warmed up to a temperature of about 120° K. the original colour is practically immediately restored. This procedure, that is, of photochemical bleaching and thermal restoration, corresponding to reaction (1*b*), can be repeated through many cycles with practically no change in the concentrations of the intermediate species involved in these processes.

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Molecular Structure of π-allyl-palladium Acetate

The structures of π-allyl complexes of transition metal ions are of interest because the allyl ligand can be regarded as intermediate in behaviour between olefines and conjugated aromatic systems. π-Allyl palladium complexes have, in particular, assumed importance as catalytic agents in carbonylation and other reactions involving unsaturated organic halides[1,2]. The only structural analysis of an allyl complex so far reported is that of the dimeric π-allyl palladium chloride[3], the reaction of this complex with silver acetate yielding π-allyl palladium acetate[4]. A three-dimensional X-ray analysis of the structure of this molecule has now been completed, the molecular stereochemistry being shown in Fig. 1; estimated standard deviations of the observed bond lengths are 0·003 Å (Pd–Pd), 0·02 Å (Pd–O), 0·035 Å (Pd–C), 0·04 Å (O–C) and 0·05 Å (C–C) and correspond to a discrepancy index of 0·068 for 1,065 reflexions.

The π-allyl palladium residues are bridged by the two acetate groups, the overall arrangement showing considerable similarity to that of the binuclear cupric acetate dihydrate[5] in which a metal–metal bond is evidenced largely by magnetic and spectroscopic criteria (see, for example, ref. 6). The Cu–Cu bond-length of 2·64 Å is equivalent to that of 2·94 Å for the Pd–Pd distance in the present complex; the difference between the mean Cu–O (1·97 Å) and Pd–O (2·11 Å) bond-lengths can be taken as the difference between the metal radii so that judged by these data the Cu–Cu and Pd–Pd bond-lengths should differ by 0·28 Å (observed 0·30 Å). Formally, with the allyl anion acting as a four-electron donor, the present results offer a further example of the formation of a metal–metal bond between two d^8 ions, but the relation between the cupric acetate structure and the present one

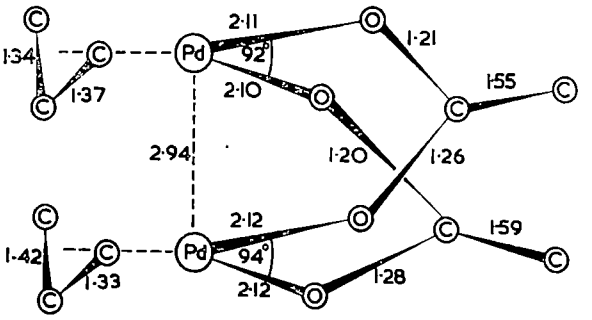


Fig. 1

is perhaps made clearer if we regard the palladium ion as interacting with the π-allylic radical since metal–metal bonds are then formed between d^9 ions in both examples. The Pd–Pd distance is 0·3–0·4 Å shorter than those reported for Pd (dimethylglyoxime)$_2$ (3·26 Å) (ref. 7), $[Pd(NH_3)_4].[PdCl_4]$ – 3·25 Å (ref. 8), $[Pd(NH_3)_4].[Pd(Br_4)]$ – 3·33 Å and $[Pd(NH_3)_4]$ $[Pd(SCN)_4]$ – 3·35 Å (ref. 9), in which complexes there is no ligand bridging of the d^8 palladium ions.

The palladium–allyl bonding is such that the planes of the allyl ligands make dihedral angles of 125° and 110° (± 5°) with those defined by the respective O–Pd–O groups. A re-examination of the structure of $(\pi\text{-}C_3H_5PdCl)_2$ (ref. 10) shows the allyl plane in this complex to be similarly oriented at an angle of 118° with respect to the plane of the Pd_2Cl_2 system with the Pd–C (allyl) bond-lengths varying from 2·15 ± 0·02 Å in the case of the terminal carbon atoms to 2·01 ± 0·04 Å for the central atom of the allyl group. An independent examination[11] of the structure of $(\pi\text{-}C_3H_5PdCl)_2$ at −140° C does not, however, confirm this difference, and indeed while the mean values for these bonds in the present structure are 2·08 Å and 2·05 Å, respectively, no significance can be attached to this result, or to the observed differences in the carbon–carbon bond distances in the allyl ligands.

The configuration of the allyl ligand with respect to the palladium ion differs from that which might have been anticipated on the basis of a molecular orbital description of the electron distribution in the free ligand. As such the same conditions may apply, *mutatis mutandis*, to those which have been discussed elsewhere for the bonding of cyclic ligands to transition metal ions[12–14].

We thank Dr. B. L. Shaw for providing us with a sample of the complex, and the Department of Scientific and Industrial Research for a studentship (M. R. C.).

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Phosphate-mediated Dehydrogenation of Alcohol in the Photochemistry of Ferrocyanide in Aqueous Solution

In a series of investigations it was shown[1] that the excited states of negative ions in aqueous solution may dissociate thermally to yield a solvated electron. Thus in the case of the halide ions[1] (chloride, bromide and iodide), the hydroxyl ion[1], thiocyanate[2] and thiosulphate[3] the reactions of solvated electrons could be investigated. The application of flash photolysis techniques[2] permitted the detection of the absorption spectrum of the solvated electron in such systems, including solutions of ferrocyanide. In the case of organic solutes in aqueous solution (phenolate and β-naphtholate[4]), the formation of solvated electrons was shown to occur from an excited state resulting from an internal transition not involving charge transfer to the solvent, as was the case with other ions.

It was shown that solvated electrons photochemically produced could be converted to hydrogen atoms, for example, by hydrogen ions and also by a series of reagents acting as Bronsted acids. Of these the role of phosphate ions was of particular interest since they occurred in neutral solutions[5].

Solvated electrons act as reducing agents, but hydrogen atoms may act not only as reducing agents but also as oxidizing agents by virtue of the high bond energy of the H–H bond[6]. Accordingly, hydrogen atoms resulting from such conversion of solvated electrons may dehydrogenate, for example, alcohols and oxidize them by stages to aldehydes or ketones[1a]. The full sequence is then, for example:

$$X^- + h\nu \longrightarrow (X^-)^* \longrightarrow X\cdot + e^-_{aq} \quad (1)$$

$$e^-_{aq} + H_2PO_4^- \longrightarrow H\cdot + HPO_4^{=} \quad (2)$$

$$H^\circ + CH_3CH_2OH \longrightarrow H_2 + CH_3\dot{C}HOH \quad (3)$$

$$X + CH_3\dot{C}HOH \longrightarrow X^- + H^+ + CH_3CHO \quad (4)$$

This sequence is of particular interest for the photo and radiation chemistry of biological systems, since at near neutral *p*H the main primary product will be the solvated electron. However, if suitable converters, phosphate ions or others, are present in the biological system then even at the biological *p*H the reactions of H atoms may be decisive for the results observed. For example, phosphate-mediated oxidations may be obtained from processes which are initiated in general as electron transfer processes—not necessarily initiated always by light or radiation.

We have now investigated the absorption spectrum and the photochemistry of ferrocyanide ion in aqueous solution. Of interest was the correlation between the absorption spectrum, which exhibits bands due to internal ligand field transitions, and the photochemistry of this ion which is a model for some biologically important substances. Photochemical experiments were carried out at 214, 229, 254 and 265 mμ as well as above 300 mμ.

The effect of *p*H on the absorption spectrum was examined. Using the technique of environmental effects on the absorption spectrum[3,7] a charge transfer to solvent band was found centring around 260 mμ, obscured underneath two ligand field transitions. At neutral *p*H the quantum yield for the formation of solvated electrons reaches a maximum of unity when irradiations are carried out in the charge transfer band at 254 mμ and is lower at quantum energies higher than that. Illumination in the absorption band above 300 mμ does not result in the formation of solvated electrons. The maximum quantum yields, φ, shown in Table 1 were determined using N_2O as the electron scavenger[8], which was shown to react rapidly with solvated electrons produced photochemically[1]. Experiments were carried out in the presence of added ethanol to capture O^- and OH radicals formed according to:

$$e^-_{aq} + N_2O \longrightarrow N_2 + O^- \xrightarrow{+H^+} \cdot OH \quad (5)$$

$$\cdot OH + C_2H_5OH \longrightarrow H_2O + \cdot C_2H_4OH \quad (6)$$

followed by

$$Fe(CN)_6^{3-} + \cdot C_2H_4OH \longrightarrow Fe(CN)_5^{4-} + C_2H_4O \quad (7)$$

Thus aldehyde is formed, but ferricyanide does not accumulate.

When using phosphate ions instead of N_2O as the electron scavenger and measuring the hydrogen evolved due to the dehydrogenation of the alcohol by the hydrogen atoms formed according to reaction 4, the results shown in Fig. 1 were obtained. When ferrocyanide is irradiated in the presence of alcohol but without phosphate being present, there is no hydrogen evolution at all. In the presence of phosphate dehydrogenation, thus oxidation, of the alcohol occurs and the kinetics represented by the half-power plot in Fig. 1 correspond formally to that expected[1] when phosphate competes with the recombination of the solvated electron with its parent ferricyanide ion. Using phosphate, the peak limiting quantum yields obtained with N_2O as the electron scavenger could not be reached. With increasing concentration phosphate became a limiting factor. Similar observations were obtained[9] in studies of the oxidizing action of H atoms produced from hydrogen gas[10]. The results there showed that phosphate ions may form complexes with atomic hydrogen, competing with other acceptors.

Table 1. Dependence of the Photochemical Parameters on the Wavelength of the Exciting Light

λ mμ	φ(N_2), from experiments with N_2O	α, arbitrary units
214	0·88	1·5
229	0·89	1·2
254	1·00	0·96
265	0·40	1·1
> 300	0·0	—

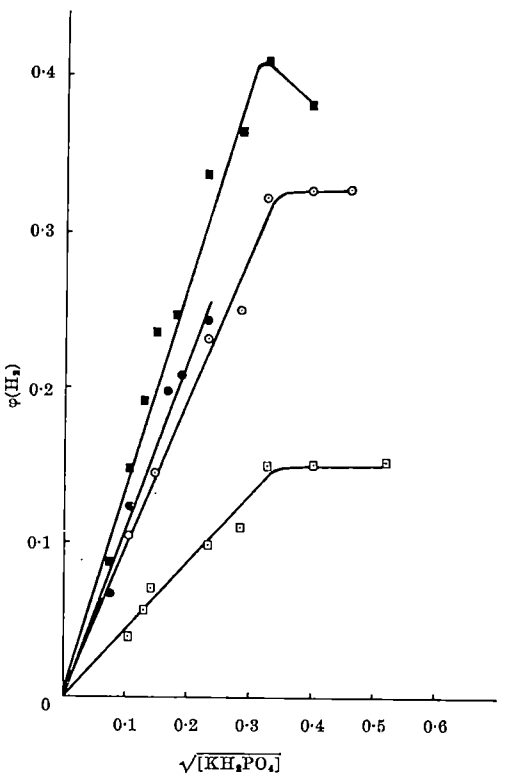


Fig. 1. Dependence of the quantum yield of hydrogen evolution on the square root of the concentration of phosphate. Solutions illuminated with light at ■ 214, ● 229, ○ 254 and □ 265 mμ. Concentration of ethanol = 1·0 M, of ferrocyanide 1 × 10⁻³ M. Note that at 214 mμ some of the incident light is absorbed by phosphate itself at high concentrations

The formal treatment of the photochemical scavenger kinetics results in the dependence of the quantum yield, φ, on scavenger concentration $[S]$, over a range of concentrations according to:

$$\varphi = \varphi_r + a\Phi \sqrt{[S]} \quad (8)$$

where, as shown in Fig. 1, in the present case the residual quantum yield in the absence of scavenger, φ_r, equals zero. a is a parameter representing the probability of recombination between the solvated electron and its parent ion. Increasing a points to an increased probability for the solvated electron to escape and react with the scavenger. The slopes of the straight lines in Fig. 1, obtained from experiments with phosphate, together with the values of the maximum quantum yield, Φ, obtained from experiments with N_2O, show that at 254 mμ in the charge transfer band the maximum quantum yield reaches its highest value; but at the same time the probability of recombination between the solvated electron thus formed and the parent ion is also at its highest. At higher quantum energies the maximum quantum yield for solvated electron formation decreases somewhat, but the probability that the solvated electron once formed escapes recombination with its geminate partner increases somewhat. These results point to the possibility of investigating the characteristics of the photochemical 'cage'.

It is to be emphasized that in the sequence of reactions 1–4 the absorbing species, ferrocyanide in the present case, is not consumed and serves as a catalytic mediator for the light-induced dehydrogenation of the alcohol.

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BIOCHEMISTRY

Heat-yellowing of Wool and Silk

Previous work on the heat-yellowing of wool has been summarized by Howitt[1]. Neither the amino-acids responsible for the phenomenon, nor the reactions involved, have been elucidated, and an attempt has, therefore, been made to solve these problems by studying the action of heat on untreated and chemically modified wools. The general principle was to modify different side-chains and cross-linkages in turn, and then to compare the extent of yellowing of the untreated and chemically modified wools after heating for 24 h in a slow stream of dry air or nitrogen at 150° C, the yellowness indices of the samples being determined by a procedure similar to that of Norton and Nicholls[2]. Using iodinated wool and wool methylated with diazomethane it was shown that the tyrosine side-chains of wool play little part in heat-yellowing under the foregoing conditions; but no satisfactory way of discovering the part played by the hydroxylic side-chains of serine and threonine could be devised. As silk is rich in these amino-acids, and as methylation with dimethyl sulphate and alkali is permissible with this material[3], the action of heat on untreated and methylated silk was examined. The results are given in Table 1.

Table 1

Sample	Yellow index: Before heating	After heating in: Air	After heating in: Nitrogen
Untreated	1	44	11
Methylated	12	18	17

It is clear that the hydroxylic side-chains of either serine or threonine, or both, play an important part in the heat-yellowing of silk, and, presumably, wool. Recent work by Norton and Nicholls[4] suggests that threonine plays little part in heat-yellowing, but the importance of serine is confirmed by the fact that it promotes the N → O peptidyl shift, which appears to be crucial in the only method of acetylation[5], among the many we have examined, that is capable of minimizing the heat-discoloration of wool.

We thank the International Wool Secretariat for grants in aid of this investigation.

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Scopoletin, Scopolin and Chlorogenic Acid in Tumours of Interspecific *Nicotiana* Hybrids

Phenol and certain substituted phenolic compounds were reported recently to be co-carcinogenic in animal tissue[1]. The investigation of phenolic compounds in tumorous plant tissue of *Nicotiana* hybrids is of great academic interest in that much is known of the genetic background[2,3], cytology[4], chemical composition[5] and the effects of radiation[6] and of certain anti-tumour chemicals[7] on the tumorous plant. Some of these compounds are known to take part in alkaloid metabolism[8].

Certain interspecific combinations of *Nicotiana* are known to produce tumours spontaneously[2]. Materials used in the work recorded here were *N. glauca* Grah., *N. langsdorffii* Weinm., *N. suaveolens* Lehm., *2n* and *4n* (*N. glauca* × *N. langsdorffii*) and *4n* (*N. suaveolens* × *N. langsdorffii*). New tumours were green or white in colour, and when older they gradually turned brown or grey.

Composite material from the same group of test plants was used for analysis. Tumours were separated by the location of their growth, and by their age. Extractions were made immediately after gathering in a Waring blender at room temperature (22 ± 2) Each 30 g of fresh material was extracted twice with 120 ml. of 70 per cent ethanol, allowing 7 min for each extraction. Additional ethanol was used for washing. Combined ethanol fractions were diluted with an equal volume of water and extracted with ethyl ether to remove pigments.

The ethanol fraction was filtered, concentrated *in vacuo*, and then made up to a 30 per cent methanol solution. This solution was fractionated with a polyamide resin 'Ultramidpulver' column. Three main zones were detected with ultra-violet light (3660 Å) and were eluted separately: fraction 1, a blue-fluorescing front zone containing scopolin; fraction 2, a non-fluorescing zone; and fraction 3, an intense blue-fluorescing zone containing scopoletin.

Identification of scopoletin. The scopoletin (6-methoxy-7-hydroxycoumarin) fraction was first concentrated *in vacuo* to dryness at room temperature, using a rotary evaporator, by azeotroping the water with isopropyl

alcohol. The solid was then sublimed, and the sublimed scopoletin was re-crystallized from methanol (m.p. 207°–209° uncorr.; authentic scopoletin[9], m.p. 208°–209°). The ultra-violet absorption spectrum of the isolated scopoletin was identical with that of synthetic scopoletin (max. 344, 297, 252 and 228 mμ, min. 306, 270 and 248 mμ). R_F values of the crystalline scopoletin agreed with those of the synthetic scopoletin from four different solvent systems by paper chromatography. Results are shown in Table 1.

Table 1. R_F VALUES OF SCOPOLETIN, SCOPOLIN, CHLOROGENIC ACID AND RELATED COMPOUNDS

Compound	R_F values in solvent systems*					
	1	2	3	4	5	6
Scopoletin, synthetic	0·92	0·47	0·48	0·42	—	—
Scopoletin, isolated	0·91	0·47	0·48	0·42	—	—
Scopolin, synthetic	0·56	0·73	—	0·44	0·74	—
Scopolin, isolated	0·57	0·74	—	0·44	0·72	—
Isolated and authentic chlorogenic acid	0·68	0·60 0·80	—	—	0·48	0·75 0·82
Caffeic acid	—	0·35	0·10	—	—	—
Quinic acid	0·30	—	—	—	0·09	0·51

* Solvent systems used are: (1) *n*-butyl alcohol–acetic acid–water (6 : 1 : 2, v/v/v); (2) isopropyl alcohol–acetic acid–water (5 : 0·1 : 95, v/v/v); (3) benzene–acetic acid–water (6 : 7 : 3, v/v/v, upper layer); (4) *n*-butyl alcohol saturated with 2 N ammonium; (5) *n*-butyl alcohol–benzene–pyridine–water (5 : 1 : 3 : 3, v/v/v/v, upper phase); (6) 15 per cent aqueous acetic acid.

Identification of scopolin. The scopolin (7-glucoside of scopoletin) containing fraction 1 obtained from the 'Ultramidpulver' column was purified through a cellulose column. The charged column was then washed with benzene saturated with water until the eluate no longer fluoresced red. The eluting solvent was then changed to water-saturated ethyl acetate to which additional ethyl acetate was added in the amount of 33 per cent of the solution volume. Three zones were visible under ultra-violet light. The third zone fluoresced blue and contained scopolin. The eluate was then concentrated to dryness by evaporating an azeotropic mixture of water and isopropyl alcohol. The solid residue was recrystallized from methanol and proved to be scopolin (m.p. 214°–215° uncorrected; synthetic scopolin 215°–216°). The ultra-violet absorption spectra for the isolated and synthetic scopolin were identical (max. 286 and 337 mμ, min. at 264 and 304 mμ). Paper chromatographic R_F values of the isolated and synthetic scopolin were in good agreement in the four solvent systems, as shown in Table 1.

Isolated scopolin was hydrolysed with hydrochloric acid. The hydrolysate was extracted with ethyl acetate. The aglucone present was identical with reference scopoletin. The sugar in the water layer of the hydrolysate was found to be glucose.

Quantitative determination of scopolin and scopoletin. The procedure of Dieterman *et al.*[10] was used to determine the scopolin and scopoletin in tumour tissue. The results obtained are presented in Table 2. Each value represents an average of three determinations.

Table 2. SCOPOLIN AND SCOPOLETIN IN VARIOUS *Nicotiana* TUMOURS

Samples	μg Scopolin/g fresh wt.	μg Scopoletin/g fresh wt.
2*n* (*N. glauca* × *N. langsdorffii*)		
Old tumours from root and stem	125	1,143
New tumours from root and stem	142	8·8
4*n* (*N. glauca* × *N. langsdorffii*)		
Old floral tumours	1,433	285
New floral tumours	433	12·5
Old tumours from root and stem	533	20·5
New tumours from root and stem	1,150	87
4*n* (*N. suaveolens* × *N. langsdorffii*)		
Mixed tumours	70	0·9

It appears that there is no consistent relationship between the amount of scopoletin and the amount of scopolin. In tumours from (*N. glauca* × *N. langsdorffii*) a higher level of scopolin and scopoletin was found than in those of (*N. suaveolens* × *N. langsdorffii*).

Identification of chlorogenic acid. A portion of the original ethanol extract was fractionated on a Magnesol column to examine chlorogenic acid (3-caffeoylquinic acid) content. The eluate containing the first major yellow band was concentrated *in vacuo* at 35°. This aliquot was then streaked on 3 *MM* Whatman paper, and developed in the *n*-butyl alcohol–acetic acid–water system. The blue fluorescing zone, with R_F of 0·63, was eluted with methanol. The eluate was concentrated and proved to be chlorogenic acid by using several solvent systems with paper chromatography and also by hydrolysis. The R_F value of the isolated chlorogenic acid was identical with that of the authentic chlorogenic acid. The R_F value of the caffeic and quinic acids obtained from 1 N sodium hydroxide hydrolysate was identical with the authentic caffeic and quinic acids[11] (Table 1).

To examine the chemical and genetical background, the relative amount of scopolin, scopoletin and chlorogenic acid present was estimated in the parental tissue of these tumour-producing *Nicotiana* hybrids. This was done by visual comparison of samples with standards of various known quantity on paper chromatograms (ethyl acetate : pyridine : water, 2 : 1 : 2). As shown in Table 3, scopoletin is not detectable in all three parent species. Scopolin is almost absent in *N. suaveolens*, very low in *N. langsdorffii*, and present only in about one-third to one-fifth in *N. glauca* in comparison with its tumorous hybrid. Chlorogenic acid levels, however, are similar in the parents or the hybrid. Quantitative results, however, were not obtained for chlorogenic acid in tumorous tissue.

Table 3. RELATIVE AMOUNT OF SCOPOLIN, SCOPOLETIN AND CHLOROGENIC ACID IN *N. glauca*, *N. langsdorffii* AND *N. suaveolens*

Plant tissue	μg Scopolin/g fresh wt.	μg Scopoletin/g fresh wt.	μg Chlorogenic acid/g fresh wt.
2*n* (*N. glauca* × *N. langsdorffii*)	125	1,143	100
N. glauca			
Leaves and stems	44	0	113
Floral tissue	23	0	35
N. langsdorffii			
Leaves and stems	5	0	112
Floral tissue	0	0	175
N. suaveolens			
Leaves and stems	1	0	245
Floral tissue	0	0	70

In a separate investigation, using a variety (one sucker) from *Nicotiana tabacum*, scopoletin was also not detected in healthy plants, but a very minute quantity of scopolin was found in leaves and stems. In the roots, however, a considerable amount (118 μg/g) of scopolin was found.

This communication provides results on the sharp increase of scopolin and the new formation of scopoletin in the tumorous tissue of *Nicotiana* hybrids as compared with parental material. These compounds appear to be related to the formation of tumours in the F_1 hybrid.

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Utilization of Saccharose and Maltose by Yeasts

WORK on the utilization of saccharose by *Saccharomyces oleaginosus*[1], and other species, has been continued on other yeasts lacking invertase. The results with *S. rouxii* were: (*a*) negative assimilation in yeast nitrogen base Wickerham (YNB) with 0·5 per cent saccharose, and positive assimilation in the same medium with a 2 per cent sugar concentration; in the latter case, the assimilation is appreciably quicker in Erlenmeyer flasks; (*b*) slow fermentation in a 2 per cent concentration in yeast infusion (YI), and slightly more active fermentation in yeast autolysate (YA). With *S. italicus VB* 20 the assimilation of saccharose in YNB was also negative with 0·5 per cent saccharose, and positive with 2 per cent; the fermentation was extremely weak in YI and YA.

Further, a series of yeasts was chosen capable of assimilating saccharose, but not raffinose, and it was proved that they lacked beta-fructosidase; their behaviour in the presence of saccharose varied from extremely active fermentation (*C. tropicalis*) to very weak (*H. angusta Ac* 455, *C. lusitanae Ac* 475) to a negative fermentation (*C. pulcherrima, T. ernobii, H. californica*, and the majority of the *C. lusitanae* strains).

A similar behaviour with maltose was found only in the yeasts lacking invertase, *S. rouxii Me* 3, that fermented maltose (slowly in YI and more rapidly in YA) and did not assimilate it in YNB with 0·5 per cent sugar; in the same manner, two invertase-positive yeasts (*C. utilis Ac* 551 and *S. kluyveri Ac* 316) fermented maltose in YA, but not in YI.

Considering the general problem of utilizing saccharose and maltose, we note that the following points stand out, at least in the group of yeasts treated: (*a*) Whenever a yeast ferments maltose, it ferments saccharose, even though this fermentation be weak or may need special media. (*b*) A yeast may ferment saccharose in the absence of invertase, but can neither assimilate nor ferment maltose (*S. wickerhamii*), or can only assimilate it (*H. angusta Ac* 455). (*c*) There are yeasts that ferment glucose and assimilate maltose and/or saccharose, but are incapable of fermenting them. (*d*) Within one species, there may be appreciable variation between different strains with regard to the assimilation or fermentation of maltose and/or saccharose (*S. rouxii, S. kluyveri, S. italicus, C. lusitanae, C. utilis*).

From the quantitative point of view, for example the amount of sugar utilized, there are differences between cultures. For example, with *S. oleaginosus, S. hienipiensis, S. italicus, C. tropicalis*, some strains of *C. lusitanae*, and others, the maltose utilized in 10 days in YNB represents practically 100 per cent of the initial amount (2 per cent), while *S. rouxii, S. lactis, C. utilis* var. *major*, other strains of *C. lusitanae*, and others, the corresponding figure after 20 days is only 10 to 20 per cent. The culture medium used has a profound influence on the rate of sugar utilization. The same happens with saccharose: *C. tropicalis Ac* 433 utilizes 100 per cent of the initial amount (4 per cent) in 48 h, while *C. lusitanae Ac* 19 utilizes 17 per cent, *T. ernobii* 8 per cent, and *H. californica* 10 per cent. The addition of various peptones to the growth medium has no influence on the results. In the case of saccharose, YA may not be substituted by YNB plus vitamin-free acid hydrolysed casein, using *S. oleaginosus, S. italicus VB* 20, *S. hienipiensis, S. rouxii, H. angusta*. On the other hand, *S. rouxii Me* 3, which does not assimilate maltose in YNB, will do so if it is complemented by 'Bacto' vitamin-free casamino-acids. In small inoculations, adaptation to maltose or saccharose has no influence on utilization of the saccharose by *S. oleaginosus, S. hienipiensis, S. italicus VB* 20, *S. rouxii*, or by maltose by *S. rouxii Me* 3, unlike that which occurs with other inducible enzymes.

We see, therefore, that with regard to the utilization of saccharose and/or maltose by yeasts lacking invertase, it is possible to use them in simple media (YNB) or more complicated ones (YA) which may be fermentative or merely assimilative. We also observe that if there is invertase, the saccharose is fermented, and equally, if there is melibiase, the melibiose is fermented; on the other hand, maltose and saccharose, in the absence of invertase, may be assimilated and not fermented: a similar case occurs with beta-galactosidase in *S. marxianus*. This seems to coincide with the fact that beta-fructosidase (invertase) and alpha-galactosidase (melibiase) involve external hydrolysis.

From these results we may deduce that: (i) it is probable that utilization of saccharose by yeasts lacking invertase may be due, at least in some cases, to a different enzymatic system from that used in the utilization of maltose; (ii) in the utilization of saccharose and maltose diverse factors (such as medium, concentration of sugar, aerobiosis, strain of yeast) have such a decisive influence that the sugar utilization pattern is unstable; this must be taken into account from the taxonomic point of view; (iii) the utilization of saccharose in the absence of invertase without external hydrolysis having been confirmed, it seems logical to admit the existence of a similar system to that of maltozimase[2], but the fact that yeasts that ferment glucose are only capable of assimilating maltose[3] or saccharose remains unexplained.

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Action of Phospholipase C on Influenza Virus

INFLUENZA virus is known to contain large amounts of phospholipid. By treating the virus with phospholipase *C*, considerable amounts of acid-soluble phosphoric compounds are released, and the virus becomes sensitive to the action of proteinase from *Streptomyces griseus* (proteinase SG, ref. 1) with reduction of its infectivity.

The 'A2/Kumamoto Y/5/57' strain[2] of influenza virus (non-avid) was used for this experiment. Virus was labelled with ^{32}P by the method of Liu *et al.*[3], and was purified by three cycles of adsorption-elution from chicken red cells, followed by high- and low-speed centrifugation. The final product was suspended in veronal buffered saline, *p*H 7·8, and tested for contamination with non-viral ^{32}P by determining the Geiger count before and after adsorption with red cells. The preparations had an average hæmagglutinin titre of 10,240 and an average Geiger count of 5,480 c.p.m./ml. Of this radioactivity more than 98·5 per cent was carried by the virus particles, and was adsorbable on the red cells. Attempts were made to determine the relative distribution of the isotope in the virus phospholipid. Labelled virus was fractionated by a modification of the Schmidt–Thannhauser technique[4]. The cold trichloroacetic acid (TCA), hot alcohol–ether, hot TCA-soluble fractions, and the residue, were assayed for radioactivity. On average, 15, 50, 24 and 11 per cent of the label were found respectively in cold TCA, hot alcohol–ether, hot TCA fractions and residue. Phospholipase *C*, kindly supplied to us by Dr. H. Ikezawa of this Institute, had been purified from *Cl. perfringens* type *A* α-toxin by the ammonium sulphate and alcohol precipitation procedure[5]. *Streptomyces griseus* proteinase, which is known to have extremely broad substrate specificity, was obtained from the Institute of Physical and Chemical Research, Tokyo.

Aliquots of labelled virus were treated with (1) phospholipase *C*, varying the concentration of enzyme and

the incubation time; (2) phospholipase *C* followed by RNase; (3) phospholipase *C* followed by proteinase SG; (4) phospholipase *C* followed by proteinase SG and RNase; (5) proteinase SG; (6) proteinase SG followed by RNase; and (7) RNase. After treatment, they were chilled and added to cold TCA (final concentration 5 per cent), and centrifuged. The supernatants (acid-soluble fraction) and the precipitates were separated and counted, and the relative radioactivity of each acid-soluble fraction was calculated. The experimental conditions and typical observations, including virus infectivity, as titrated in the allantoic cavity of chick embryos after the enzymatic treatments, are shown in Table 1. A remarkable increase in relative radioactivity of the acid-soluble fraction was observed by the treatment of labelled virus with phospholipase *C*. When the virus was treated for 8 h with this enzyme, the increase reached to 27 per cent of the original label. As 50 per cent of the original label is considered to be due to the virus phospholipid, this value means that about 54 per cent of the virus phospholipid is released into the acid-soluble state. Infectivity after the treatment, as titrated in the allantoic cavity of chick embryos, was 6·5 ($\log_{10}$), whereas that of the control was 7·0. The additional treatment with RNase did not result in further increase of acid-soluble radioactivity. This suggests that virus ribonucleic acid is not yet exposed by the treatment with phospholipase *C* alone. Neither a significant increase of acid-soluble radioactivity nor a reduction of virus infectivity was observed by treatment with RNase alone, proteinase SG alone, and proteinase SG followed by RNase. On the other hand, treatment of the virus with phospholipase *C* followed by proteinase SG reduced its infectivity significantly, and subsequent additional treatment with RNase resulted in a further slight increase of acid-soluble radioactivity. A preliminary experiment using the picrylation method[1] showed that proteinase SG liberated significantly more amino-acid from the phospholipase *C*-treated virus than from this intact virus.

Table 1. Relative Radioactivity of Acid-soluble Fraction and Infectivity after the Successive Treatment of Influenza Virus with Phospholipase *C*; Proteinase SG; and RNase

Treatment			Relative radioactivity of acid-soluble fraction (%)	Infectivity (Log_{10})
Control (incubated without enzyme)			15	7·0
L	2 mg	1 h	37	—
L	2 mg	3 h	38	—
L	10 mg	8 h	42	6·5
LP		0 h	—	6·0
LP		3 h	42	4·5
LPR			49	4·3
LR			43	6·3
P			17	7·3
PR			17	7·3
R			15	7·0

Notes: L, phospholipase *C*; P, proteinase SG, 150 γ for 3 h; R, RNase, 50 γ for 2 h; LP, phospholipase *C* 10 mg for 8 h followed by proteinase SG 150 γ for 0 h or 3 h; LPR, phospholipase *C* 10 mg for 8 h followed by proteinase SG 150 γ for 3 h and RNase 50 γ for 2 h; LR, phospholipase C 10 mg for 8 h followed by RNase 50 γ for 2 h; PR, proteinase SG 150 γ for 3 h followed by RNase 50 γ for 2 h.
Diluent: 0·001 M $CaCl_2$ containing veronal buffered saline. After treatment, enzymes were inhibited by addition of anti-α toxin (for phospholipase *C*) and one-tenth volume of 0·1 M EDTA (for proteinase SG).

The simplest explanation of the observed results would be that virus phospholipid protects, enzymatically or constitutionally, a part of the virus protein which is essential for virus infection. The statement by Hoyle and Frisch–Niggemeyer[6] that, on entry into a host cell, influenza virus phospholipid is destroyed, seems to agree with this hypothesis. Anderson and Ada[7] reported that phospholipase *A* purified from the venom of the black snake failed to inactivate influenza virus, and they considered two possibilities: (1) that intact phospholipid was not necessary for infectivity; or (2) the virus phospholipid was not accessible to the action of the enzyme. The present work shows that the virus phospholipid was accessible to the action of phospholipase *C*.

We thank Dr. Thomas Francis, jun., for advice, Dr. H. Ikezawa for the sample of phospholipase *C*, and Dr. T. Komai for isotopic measurements.

This work was supported in part by a research grant from the United States Public Health Service (*RF*–39).

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Fatty Acids of Beef Heart Sphingomyelin

A ceramide lactoside, synthesized to approximate to the structure of the tumour hapten cytolipin *H*[1], was reported[2] to have a melting point of 86° C, which appeared low for a cerebroside analogue. A 25-mg sample was absorbed on 7 g silicic acid : 'Celite' (5 : 2) in chloroform, and eluted with increasing methanol concentrations in chloroform. At 5 per cent methanol, about 2 mg of ceramide (m.p. 84°–86° C) was obtained, while the bulk of the material, recovered at 30 per cent methanol, had an infra-red spectrum identical to that of the original material, and a melting point of 167°–170° C (measured on a Kohfler micro-hot stage). Under the same conditions, authentic cytolipin *H* melted at 174°–183° C. At this point, it appeared that the original sample had contained about 10 per cent ceramide (by weight), a contaminant which could not be detected in the infra-red spectrum at that concentration. But the differences in the new melting points could be accounted for by differences in the fatty acids residues of both compounds. A preliminary attempt at the identification of the fatty acid residues of the ceramide lactoside indicated that lignoceric acid (C_{24}, tetracosanoic acid) made up only one-third of the total fatty acids residues, while another one-third was made up on some odd-number saturated fatty acid. It was, therefore, of interest to determine the components of the fatty acid mixture in the sphingomyelin from which the ceramide was prepared for the synthesis of ceramide lactoside.

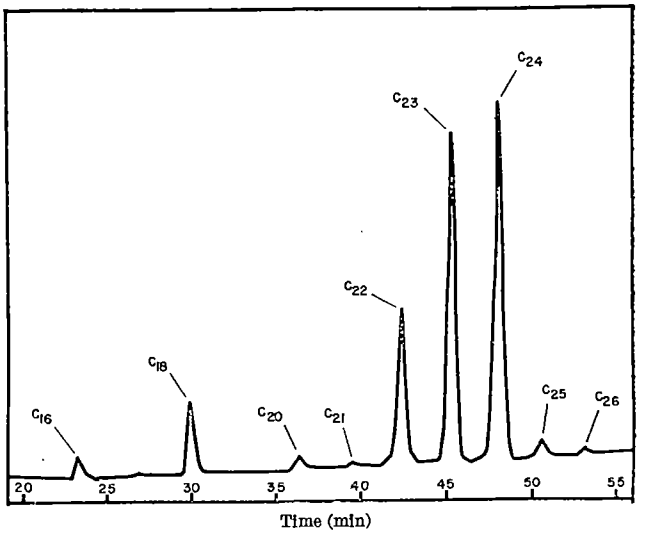


Fig. 1. Gas chromatogram of the methyl esters of sphingomyelin fatty acid residues. Detector: ^{90}Sr argon ionization. Column: 1 per cent *SE* on 'Chromosorb *W*' 80/100 mesh, 6 ft. × 1/8 in. internal diameter, glass. Column temperature; programmed from 125 to 300° C, at 3°/min. Cell temperature: 285° C. Flash heater: 295° C

Sphingomyelin was obtained by repeated recrystallizations in cyclohexane : acetone (1 : 1 v/v) of a beef heart lecithin-sphingomyelin sludge, until the infra-red spectrum showed the absence of absorption at 1,741 cm^{-1} (ester carbonyl from contaminating hydrolecithins). The melting point of sphingomyelins purified in this fashion was raised from 196° to 210°–211° C. A sample of purified sphingomyelin was refluxed for 3·5 h in 10 per cent sulphuric acid in methanol. The methanol was removed under reduced pressure, and water was added to the residue, which was then extracted with methylene chloride. The methylene chloride extracts were dried over anhydrous sodium sulphate, and evaporated to dryness. The waxy residue was recrystallized from methanol, and fractionated by gas chromatography, to give the pattern shown in Fig. 1. The individual peaks were identified by their retention time, and by cochromatography with pure methyl esters of fatty acids. Integration of the area under the peaks gave the relative proportions of the methyl esters in the sample, as recorded in Table 1.

Table 1. Composition of the Methyl Esters Mixture

Methyl ester of:	Per cent
Palmitic acid (C_{16})	1·7
Heptanoic acid (C_{17})	0·3
Stearic acid (C_{18})	7·7
Eicosanoic acid (C_{20})	1·5
Henneicosanoic acid (C_{21})	0·4
Docosanoic acid (C_{22})	16·8
Tricosanoic acid (C_{23})	33·6
Tetracosanoic acid (C_{24})	35·8
Pentacosanoic acid (C_{25})	1·7
Hexacosanoic acid (C_{26})	0·5

In the procedure outlined, the methyl esters of the lower fatty acids would be lost, should this sphingomyelin contain such residues. But there was no evidence of unsaturated, hydroxy, keto, or branched fatty acid methyl esters in the mixture. With the crude assumption that all the fatty acid residues of sphingomyelin were hydrolysed and esterified to the same extent, Table 1 represents an approximation of the ratio of the fatty acid residues in sphingomyelin. The presence of relatively large amounts of an odd-number fatty acid (tricosanoic acid, peak C_{23} in Fig. 1) was not unexpected, since normal even- and odd-number fatty acids from C_{18} to C_{28} occur in butter fat[3]. The chemical and physical properties of tricosanoic and tetracosanoic acids are so similar that it would be difficult to separate and identify them by classical methods. It is, therefore, not surprising that sphingomyelin has long been assumed to contain mostly docosanoic and tetracosanoic acids as fatty acid residues[4].

A similar sphingomyelin preparation had been used in the synthesis of the ceramide lactoside[1]. It appears now that the fatty acid residue of the ceramide lactoside is a mixture whose composition also approaches that shown in Table 1.

Pure fatty acids methyl esters were purchased from the Applied Science Laboratories, State College, Pennsylvania. The beef heart lecithin-sphingomyelin sludges were gifts of the Sylvana Chemical Co., Orange, New Jersey. The authentic sample of cytolipin *H* was donated by Dr. Rapport. This work was supported in part by a grant (*CA* 06991–01) from the U.S. Public Health Service.

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Determination of Dipeptidase Activities

Investigations of intestinal mucosal dipeptidases have been hampered in the past by the lack of suitable assay methods. The methods in use at present[1] require relatively large amounts of substrate and are inadequate for the examination of dipeptidase activities in crude tissue extracts or partially purified preparations. The new assay method described here has a wide application and is simple and rapid. It is convenient even when a large number of determinations is required.

The method utilizes the difference in absorption between peptides and amino-acids in the low ultra-violet region[2], in contrast to most current methods which are based on either the release of amino groups[3] or carboxyl groups[4]. Many substances, including proteins, carboxylic acids and buffer ions, also have a strong absorption in the low ultra-violet region and can interfere with the absorption to be measured. These interfering substances are present in relatively high concentrations in crude tissue extracts, and special measures must be adopted to remove them. If this is not done, a method based on the disappearance of the absorption due to the peptide bond will not offer any substantial advantage over those based on the release of amino or carboxyl groups[5].

By using ethanol, with a negligible absorption down to 210 mμ, it is possible to remove the interfering substances by precipitation. This permits the full utilization of the strong absorption of the peptide bond in the low ultra-violet region, and increases the sensitivity many times compared with earlier methods. The precipitation procedure also permits readings with a narrow slit width in the spectrophotometer and therefore eliminates the

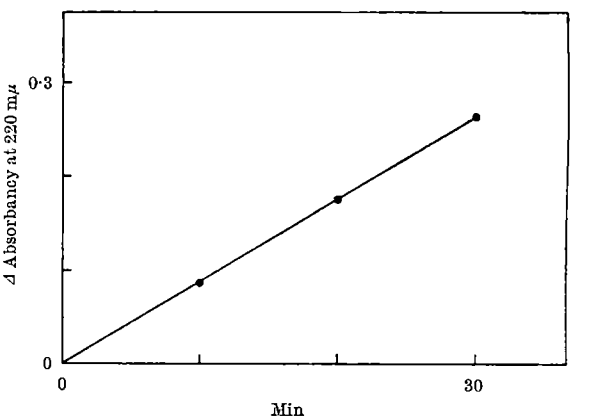


Fig. 1. Change of absorbancy at 220 mμ with hydrolysis of 3·75 μmoles of glycylglycine at 37° C and *p*H 8·0. The enzyme source was a mucosal homogenate from pig intestine, added in an amount containing 5·75 μg of nitrogen. No metal ions were added

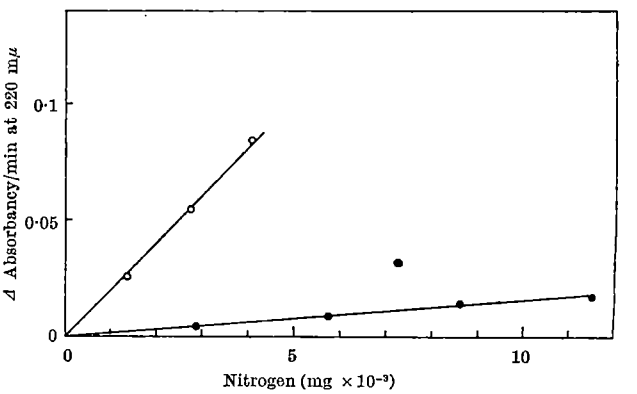


Fig. 2. Velocity of hydrolysis measured at 220 mμ, 37° C and *p*H 8·0 versus enzyme concentration. The enzyme source was a mucosal homogenate from pig intestine and was added in increasing amounts of nitrogen per sample. No metal ions were added. ○, 1·5 μmoles of glycyl-L-leucine. ●, 3·75 μmoles of glycylglycine

risk of interference from stray radiation occurring at these wave-lengths[6].

In general, the assay procedure is performed in the following manner. Fifty μl. of an aqueous dipeptide solution and 100 μl. of a 0·15 M sodium borate solution are mixed in centrifuge tubes. The tubes are kept in a water bath at 37° ± 0·05° C. Appropriate amounts of enzyme (for example, intestinal mucosal homogenate) are then added and thoroughly mixed with the substrate solution. After suitable time intervals, the reaction is interrupted when 1·3 ml. of ethanol is added and the resulting precipitate centrifuged off at 4,000 r.p.m. The clear supernatant is removed and read at 220 mμ in a Beckman spectrophotometer (Model DU) provided with 10 mm semi-micro cuvettes. Samples treated identically, but containing equivalent amounts of the corresponding amino-acids instead of dipeptide, serve as blanks. The concentrations of the substrate solutions were chosen to give an absorbancy for the supernatant from the zero time samples of about 0·9 at 220 mμ; for example, 0·03 M for glycyl-L-leucine and 0·075 M for glycylglycine. The *p*H of the buffer was adjusted to give an optimal *p*H-value for the enzymatic activity to be measured. Metal ions, when used for activation of the enzymes, were added to the digest mixture via the buffer solution.

The assay procedure gives straight-line relationship up to about 40 per cent hydrolysis. The results of a typical experiment are shown in Fig. 1. The velocity when measured by the outlined procedure is proportional to the enzyme concentration, as shown in Fig. 2. The assay method is accurate to within about ± 1 per cent, even when crude tissue extracts are used as the source of enzymes.

A full account of this work will appear elsewhere. This work was supported by grants from the Swedish Medical Research Council.

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PHYSIOLOGY

Formation of Isorhodopsin in the Frog's Eye during Continuous Illumination

Both in solution and in the intact rods the rhodopsin of the frog, *Rana temporaria*, absorbs light maximally at the wave-length 502 mμ (λ_{max} 502 mμ)[1]. Like the other vertebrate rhodopsins it is composed of a colourless protein, opsin, combined with the 11-*cis* isomer of retinal (formerly retinene)[2]. Light isomerizes the 11-*cis* retinal (the chromophore) to the all-*trans* configuration, thereby converting rhodopsin into all-*trans* lumi- and meta-rhodopsin. In the dark or in weak illumination the meta-rhodopsin (λ_{max} at about 480 mμ) completely hydrolyses to free opsin and pale yellow all-*trans* retinal, which reduces to colourless retinol (formerly vitamin A) (ref. 3). But it has been demonstrated that in solutions a bright light can isomerize the all-*trans* chromophores of lumi- or meta-rhodopsin into mixtures of stereoisomeric chromophores, all still attached to opsin. The 11-*cis* fraction of this mixture is rhodopsin, the 9-*cis* fraction isorhodopsin (a thermally stable but photosensitive rhodopsin analogue, λ_m486 mμ (refs. 3–5)). It has been demonstrated that this back-isomerization of coloured intermediates also takes place during flash-irradiation in suspensions of rods[5] and in the living rat eye[6], but the yield of isorhodopsin is less in these experiments *in situ* (about 20 per cent of the thermally stable pigment) than in the corresponding experiments with solutions (about 50 per cent)[4].

The coloured intermediates are, however, so short-lived that they occur in large amounts only after bright flashes of light. During continuous illumination the amounts of intermediates are so slight in the mammalian eye that their isomerization into isorhodopsin and back into rhodopsin cannot be detected. The formation of rhodopsin during permanent illumination is thus not faster than in the dark, when rhodopsin is regenerated only from free 11-*cis* retinal and opsin[6–8]. But in the frog's eye the situation is apparently different. The orange intermediate (λ_{max} 470–480 mμ) observed *in situ* in the frog's rods is more stable than the metarhodopsin in solution[5,9], and my experiments suggest that an isomerization of this intermediate into rhodopsin and isorhodopsin plays an important part in excised and opened frog eyes during strong and continuous illumination at 13°–14° C.

I have investigated the regeneration of rhodopsin in opened eyes. These eyes are excised from strongly light-adapted (60 min) frogs, then opened in white light and further illuminated for 25 min at 13°–14° C, and after that left to dark-adapt in a dark chamber at 13°–14° C for different lengths of time. The course of the regeneration is the same as with intact frogs[10]. The careful preparation of the opened eyes, the extraction and the spectrophotometric measurements have been described earlier[11]. In the actual experiments the digitonin solution and the ice-cold acetate buffer (*p*H 4·6), into which the eyes are placed immediately after regeneration, contained 0·1 M hydroxylamine in order to prevent a synthesis of rhodopsin or isorhodopsin *in vitro* or during the washing of the retinas.

The content (more exactly the extinction) of the red photosensitive pigments in the central parts of the light-adapted retinæ is 5·1 ± 0·1 per cent of the rhodopsin content (extinction) in the central parts of fully dark-adapted retinæ and during the first 10 min in the dark an average of 0·5 per cent is regenerated per min. Since the bleaching and synthesis of the photosensitive pigments are in equilibrium in the light-adapted eyes, we can also estimate the rate of synthesis in light, if we know the bleaching rate. Since the bleaching effect of the lamp on the rhodopsin in a dark-adapted retina is known, and the screening effect of the pigment epithelium in the central parts of the light-adapted retinæ can be estimated[9], it seems possible to calculate the rate of bleaching. In this way I have found that 1·5–2·5 per cent (of the extinction of the dark-adapted rhodopsin content) is synthesized per minute in the central parts of the light-adapted retinæ. This leads to the unexpected conclusion that rhodopsin synthesis is faster in light than during the first minutes in the dark. The difference spectrum of the pigment extracted from eyes strongly light-adapted for a considerable time, however, helps us to explain the situation. I have put together all the difference spectra I have obtained from these light-adapted retinæ and the resulting curve (Fig. 1) shows that λ is maximal at 490–495 mμ and is in fair agreement with the difference spectrum of a photosensitive pigment, which Bridges[4,5] has obtained by flash-illumination of frog rhodopsin (λ_{max} 502 mμ). In Bridges's pigment, λ_{max} = 494 mμ; by partial bleaching analysis it was proved to be a nearly 50 : 50 mixture of rhodopsin and isorhodopsin (λ_{max}, 486 mμ). This suggests that a photoisomerization of intermediates has occurred and that a considerable amount of isorhodopsin is formed in the opened eyes during continuous illumination. This effect of a long illumination is in agreement with Hubbard and Kropf's[3] observation that the relative quantum efficiency for converting cattle metarhodopsin (in solution) to rhodopsin is 5 times larger than the quantum efficiency for converting metarhodopsin to isorhodopsin, but that

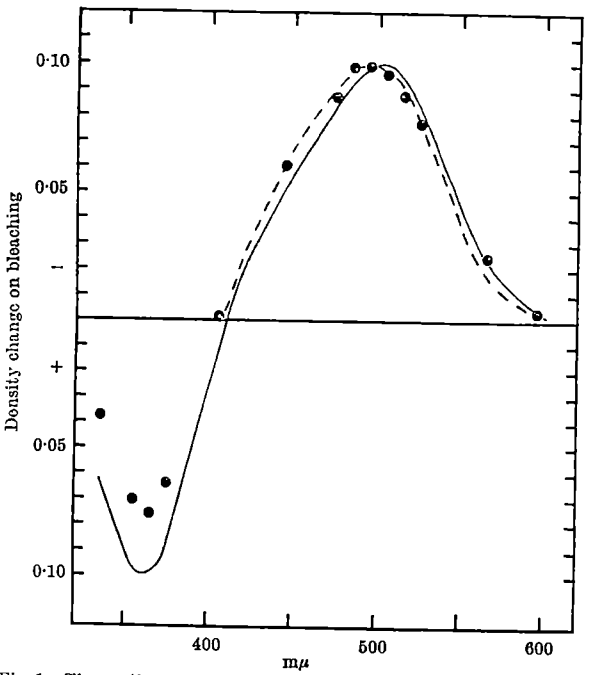


Fig. 1. The continuous line shows the difference spectrum of rhodopsin, extracted from fully dark-adapted frogs' retinæ. The broken line shows the difference spectrum of Bridges's (ref. 4) 50 : 50 mixture of rhodopsin and isorhodopsin, and the solid circles are measurements of the difference spectrum of a photosensitive pigment extracted from strongly light-adapted frogs' retinæ. All spectra refer to extracts which contain hydroxylamine

the rhodopsin bleaches about 3 times faster than isorhodopsin in a dim white light.

I have also investigated the photoisomerization *in vivo* by calibrating two lights, one blue (462 mμ) and one green (558 mμ), so that they had an equal bleaching effect on a rhodopsin solution, and by allowing the two dark-adapted eyes (opened in red light) from the same frog to bleach in these lights. Several such experiments were made, and the rhodopsin contents in both eyes were measured after different lengths of exposure to these coloured lights. After 1–20 min of bleaching the 'blue eyes' always contained significantly more rhodopsin than the 'green eyes'. After 10 min in the lights the blue and the green eye respectively contained 52 and 28 per cent of the rhodopsin content in an unbleached eye, but after that the differences became smaller. This difference between the blue and the green light suggests that the intermediate, which *in vivo* is isomerized back to rhodopsin, is Bridges's metarhodopsin-like orange intermediate[5] (λ_{max}, 470–480 mμ), and not a lumirhodopsin, because the absorption spectrum of the frog's lumirhodopsin is not displaced to shorter wavelengths from the spectrum of rhodopsin[12].

This investigation was supported by grants from the "Svenska Vetenskapliga Centrālradet", the "Leo och Regina Wainsteins Stiftelse" and the "Oskar Öflunds Stiftelse".

I thank Dr. K. O. Donner for many helpful suggestions.

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Effect of *d*-Gamma-tocopherol on the Incidence of Teratogeny in Vitamin E-deficient Rats

IT has been found that administration of an adequate dose of *d*,l-α-tocopheryl acetate (*d*,l-ATA, 1 mg or more) to vitamin E-deficient rats, not later than the 8th day of gestation, prevented fœtal resorption and insured birth of the usual number of full-term normal young[1]. When female rats were given vitamin E at too late a stage of pregnancy (after the 12th day of gestation) their fœtuses were partially or wholly resorbed or were dwarfed and dead. Congenital abnormalities occurred when a single dose of 1 mg or more of vitamin E was given at a critical time during gestation, between the 8th and 12th days. Such abnormalities included exencephalus, cleft palate, facial fissures, arrested development of the parietal and occipital bones, nasal fossæ and sternebræ, ectocardia and gastroschisis.

The development of the abnormalities has been traced[2]. Arrested development could be seen in 11-day embryos. By the 13th day of gestation, exencephalus was apparent. Preliminary observations on changes in the glycogen content of developing embryos have been made[3]. Œstrogen or progesterone has been found to abolish or reduce the incidence of these congenital abnormalities[4].

Variations in the composition of the rations affected the yield of the abnormal as well as the normal young, the incidence of resorption and the average number of implantation sites[5]. It was also found by chemical assay that maternal serum and liver tocopherol-levels were lowest in the vitamin E-sufficient group, highest in the vitamin E-deficient group and intermediate in the marginally E-deficient groups with abnormal young[6]. The tocopherol-level of the maternal skeletal muscle, however, showed the opposite trend. Furthermore, the tocopherol-levels of the abnormal fœtal sera, liver and carcasses were slightly, though not significantly, lower than those of the normal ones from the corresponding groups.

Since there are many forms of vitamin E, it was deemed of interest to determine the effect of these on the incidence of teratogeny. However, many forms were not readily available. In this study *d*-γ-tocopherol (*d*-GT) was used and its effects were compared with those of *d*,*l*-ATA.

Weanling female rats of the Holtzman strain, weighing approximately 40 g, were fed a vitamin E-deficient ration consisting of 49 per cent dextrin, 22 per cent lard, 18 per cent vitamin-free casein, 5 per cent brewer's yeast, 4 per cent salt mixture (U.S.N. XIV) and 2 per cent cod liver oil[5]. When they reached a weight of about 175 g they were mated with males which were fed 'Purina' pellets. After mating, as evidenced by the presence of sperm in the vaginal smear, females were assigned to different groups. In one series of experiments 1, 2, 4 or 8 mg of *d*,*l*-ATA in 1 ml. of corn oil ('Mazola') was given to each rat by gavage on the 10th day of gestation. Also, positive controls which received 2 mg of *d*,*l*-ATA during the first five days of gestation and negative controls which did not receive any vitamin E supplementation were used. In the second series of experiments, 1, 2, 4 or 8 mg of *d*-GT was given on the 10th day of gestation. Positive controls which received 2 mg of *d*-GT daily during the first five days of gestation were also used. All rats were laparotomized on the 21st day of gestation and the fœtuses examined for gross congenital malformations.

The reproductive performance of the vitamin E-deficient rats given *d*-GT and *d*,*l*-ATA is presented in Table 1. Rats with *d*-GT supplementation did not gain as much weight as those of corresponding groups on *d*,*l*-ATA. This was especially striking in the positive control group which gained only half as much weight as those given *d*,*l*-ATA. Rats given 1 mg *d*-GT gained as little weight as the negative control. The average gain in weight of rats given 2, 4 or 8 mg *d*-GT was only slightly higher than that of the negative controls, but lower than that of rats given as little as 1 mg of *d*,*l*-ATA. However, for some unknown reason,

Table 1. Reproductive Performance of Vitamin E-deficient Rats given *d*-Gamma-Tocopherol (*d*-GT) and *d,l*-Alpha-Tocopherol Acetate (*d,l*-ATA) during Gestation

Vitamin E given	Dose/day mg	Given on day(s) of gestation	No. rats bred	Avg. wt. gain/rat during gestation g	Live fœtuses: Normal % †	Live fœtuses: Abnormal % †	Live fœtuses: Abnormal % ‡	Dead fœtuses: Not resorbed % †	Dead fœtuses: Resorbed % †	Implantation sites* Avg. No. ± *S.D.*
d-GT	2	1–5	12	36	16§	0	0	0	84·0§	8·3 ± 2·3
	1	10	14	31	0·79§	0	0	0·79	98·4	9·1 ± 3·5
	2	10	17	43	2·03¶	0·68§	25§	0·68	96·6§	8·7 ± 2·2
	4	10	18	33	0	0	0	0	100§	8·4 ± 4·5
	8	10	17	39	0	0·79¶	100‖	0	99·2§	7·5 ± 3·7
d,l-ATA	2	1–5	19	74	100	0		0	0	8·6 ± 1·6
	1	10	20	46	7·3	9·7	36·4	1·0	82·0	10·3 ± 1·9
	2	10	20	47	8·5	15·0	63·8	2·0	74·5	10·0 ± 1·1
	4	10	20	60	17·7	12·8	42·0	0·4	69·1	11·3 ± 5·2
	8	10	18	36	26·6	2·8	9·5	2·1	68·5	7·9 ± 2·3
	0	—	24	28	0	0		0	100·0	10·5 ± 4·1

* Including those of live resorbed and non-resorbed dead fœtuses. † In terms of total number of implantation sites. ‡ In terms of total number of viable young. § $P < 0{\cdot}01$ relative to corresponding groups given *dl*-ATA. ¶ $P < 0{\cdot}05$ relative to corresponding groups given *dl*-ATA. ‖ $P < 0{\cdot}02$ relative to corresponding groups given *dl*-ATA.

the rats with 8 mg *d,l*-ATA did not gain more weight than those given 4 mg of the same vitamin although there was an increase in the incidence of normal young. At all four levels of *d*-GT supplementation there was a significant decrease in the percentage of normal and abnormal live young with a concomitant increase in the incidence of resorption when compared with rats that had received *d,l*-ATA. It is of interest to note that even in the positive control group the *d*-GT could only produce 16 per cent of live normal young whereas identical treatment with *d,l*-ATA yielded 100 per cent live normal offspring. However, the 10 mg of *d*-GT given to the positive control group in five separate doses did also prevent teratogeny altogether as in the case of *d,l*-ATA.

Recently, by the dialuric acid and hæmolysis assay test of vitamin E, Friedman *et al.* found that *d*-γ-tocopherol had about one-fifth the potency of the α form and that tocopherol esters were about as potent as free tocopherols[7]. In their work on the influence of various tocopherols on the incidence of encephalomalacia, Bunnell *et al.*[8] observed that *d*-α-tocopherol compared with *d*- or *d,l*-α-tocopheryl acetate gave higher incidence of encephalomalacia and lower blood tocopherol-levels. The intramuscular injection of 9 per cent 'Tween-80' emulsion of pure α-, δ- and γ-tocopherols showed γ-tocopherol to be nearly equivalent to α-tocopherol in preventing encephalomalacia, and δ-tocopherol to be ineffective. Scott *et al.*[9] noted that γ-tocopherol fed at a level of 20 mg/lb. of ration delayed onset of symptoms of exudative diathesis in some chickens, while *d*-α-tocopheryl acetate (5 mg/lb.) prevented all symptoms and promoted a good rate of growth. In examining the utilization of pure α-, γ- and δ-tocopherols by laying hens. Dju *et al.*[10] found that α-tocopherol induced the greatest deposition of the vitamin in the eggs while γ-tocopherol was one-fourth as effective as α-tocopherol. Selzer *et al.*[11] reported little or no protection against liver necrosis in rats when γ-tocopherol was fed on a molar basis equivalent to that at which α-tocopherol gave protection. In studying liver degeneration in rats on diets containing 30 per cent *Torula* yeast, Emerson and Dooley observed that *d*-γ-tocopherol afforded less than one-tenth the protection given by α-tocopherol[12]. Griffiths[3] showed a relative rate of 18·7 per cent for the liver storage of γ-tocopherol in the growing chick compared to 100 per cent for α-tocopherol. Our results indicated that *d*-γ-tocopherol was much less effective (84 per cent) than *d,l*-α-tocopheryl acetate in preventing fœtal death and resorption. This phenomenon was probably due to both the absence of the α form[14] and the absence of the ester which is 62 per cent more potent than the free form[15]. By virtue of its low biological potency[16], *d*-γ-tocopherol also eliminated or reduced the occurrence of congenital malformations. Furthermore, higher levels of supplementation of *d*-γ-tocopherol were no more beneficial than the lower ones since even at 8 mg it was still below the median fertility dose of 8·9 mg calculated out by Joffe and Harris[14]. This was contrary to the observed beneficial effect of corresponding higher levels of *d,l*-α-tocopheryl acetate as far as increase in the number of normal young and decrease in fœtal resorption were concerned.

It has been observed that an oral dose of γ-tocopherol is deposited in the ingested form in livers of rats[17]. Apparently no methylation to α-tocopherol occurs. This may, in part, explain the ineffectiveness of even higher levels of γ-tocopherol in improving the growth of the fœtuses and preventing fœtal death and resorption.

I thank Dr. P. L. Harris of Distillation Products Industries, Rochester, New York, for *d*-γ-tocopherol, and Dr. J. P. Kass of Pabst Laboratories, Milwaukee, Wisconsin, for the brewer's yeast.

This work was supported by grant *RG*-4993 from the U.S. Public Health Service.

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Site of Cardiac Glycoside Inhibition of Cation Transport

The cardiac glycosides have been shown to be specific inhibitors of active cation transport in a wide variety of tissues[1–4]. The specificity of this inhibition was first demonstrated by Schatzmann, who found that these glycosides blocked sodium extrusion from cold-stored red cells without affecting either lactate production or oxygen consumption[4].

The isolated toad bladder has been extensively used as an *in vitro* analogue for the investigation of a variety of transport processes. In the present investigation, the cardiac glycoside strophanthin *K* has been shown to

produce a marked inhibition of sodium transport in this tissue, an inhibition which was specifically blocked by increasing the serosal potassium concentration.

Hemibladders from freshly pithed *Bufo marinus* were dissected free and mounted between the halves of a modified Ussing short-circuit apparatus[6]. Boyle–Conway medium (Na, 104; K, 2·5; Ca, 1·6; Mg, 1·2; Cl, 78; HCO_3, 2·5; PO_4, 3·0; SO_4, 1·9; and glucose, 5·5 m.moles/l.) independently bathed the two surfaces of the membrane[7]. Aeration and continuous stirring were provided by a mixture of O_2 and CO_2, 95 : 5; the final *p*H was 7·6. Net transmembrane sodium transport from the mucosal to the serosal surface of the tissue was measured as its electrical analogue, the short-circuit current (SCC) (ref. 6).

After a stable baseline current had been recorded with conventional Boyle–Conway medium (Na, 104; K, 2·5 m.moles/l.) on both sides of the membrane, a high potassium Boyle–Conway medium (Na, 5; K, 102 m.moles/l.) was substituted at the serosal surface of the preparation. When the SCC was stable, strophanthin *K** was added to the serosal medium in a final concentration of 2×10^{-4} moles/l. Inhibition of sodium transport was assessed as the decrease in SCC measured as a percentage of the predicted value obtained by extrapolation of the control curve 10 min after glycoside was added.

The solutions were then removed, and replaced by the original Boyle–Conway medium (Na, 104; K, 2·5 m.moles/l.). After a suitable control period, strophanthin *K* was again added to the serosal medium in a final concentration of 2×10^{-4} moles/l. and the inhibition of sodium transport assessed as previously described.

The mean SCC in the five experiments was 57 (range 20–90) μamp in the presence of 2·5 m.moles/l. potassium at both surfaces of the membrane. When strophanthin *K* was added to the serosal chamber the mean decrease in SCC was 55 ± 10 per cent ($P < 0{\cdot}001$) after 10 min. In four of these experiments, the SCC fell to zero within 30 min, indicating total cessation of active sodium transport.

The substitution of high-potassium medium at the serosal membrane reduced the SCC to a mean of 30 (range 10–60) μamp. When strophanthin *K* was added to the serosal compartment in the presence of this high-potassium medium, however, there was no further reduction in SCC (4 ± 2 per cent). At no time did the SCC approach zero, in marked contrast to the results with normal potassium buffer.

Strophanthin *K* was totally ineffective in suppressing the SCC when added to the mucosal medium under any circumstances.

Sodium transport by the isolated surviving toad bladder has been extensively investigated in recent years[8,9].

Sodium ions penetrate the cell through the mucosal (urinary) membrane, which is selectively permeable to sodium, along a favourably oriented electrochemical gradient. The extrusion of sodium through the serosal membrane occurs against the electrochemical gradient for sodium and is by definition an 'active' process accomplished by a 'pump' mechanism, located in the serosal membrane, and is dependent on the metabolic integrity of the tissue[10]. The rate of sodium transport is determined by both the 'passive' permeability of the mucosal membrane to sodium ion and the activity of the serosal pump.

Bricker, Biber and Ussing[10] and Frazier and Leaf[11] recently showed that active sodium transport persists in the isolated frog skin and the toad bladder, respectively, in the presence of inside (serosal) potassium concentrations of 120 m.moles/l. This property was employed in the studies reported here for the evaluation of cardiac glycoside inhibition of the serosal sodium pump, when it was exposed to medium approximating extracellular cation composition (Na, 104; K, 2·5 m.moles/l.), and independently to high potassium serosal medium (Na, 5; K, 102 m.moles/l.). Since the composition of the mucosal medium was not altered (Na,104; K, 2·5 m.moles/l.), the concentration driving force for sodium penetration into the cell at the mucosal surface remained constant, and the effect of the potassium concentration on the serosal sodium pump could be independently evaluated.

It is apparent that the cardiac glycoside, strophanthin *K*, did not significantly modify sodium transport when the serosal sodium pump was exposed to a high-potassium environment. In contrast, a marked inhibition of sodium transport was noted when conventional 2·5 m.mole-potassium serosal medium was employed.

Caldwell and Keynes have demonstrated that ouabain inhibited sodium transport in nerve fibres when applied externally but not when injected intracellularly[12]. This lack of intracellular glycoside effect could result from either a marked spatial specificity of carrier–inhibitor interaction or a competitive inhibition of the glycoside effect by the normally high intracellular potassium concentration. The experiments described here, in which the potassium concentration of the medium to which the serosal sodium pump is exposed can be increased while the sodium concentration necessary for mucosal sodium penetration is held constant, favour the latter explanation.

The therapeutic effects of cardiac glycosides are widely held to be exerted through a modification of cation transport processes[13], although this has not been substantiated in all studies[14]. Since the normally high intracellular potassium concentration appears to inhibit the action of intracellular glycosides on cation transport processes, it is concluded that these effects are a function of their concentration in the extracellular medium *per se*.

In summary, the isolated surviving toad bladder was employed to evaluate the effect of cardiac glycosides on active sodium transport. Strophanthin *K*, 2×10^{-4} moles/l., markedly depressed the short-circuit current when the serosal membrane was in contact with medium containing 2·5 m.moles/l. potassium but had no inhibitory effect when medium containing 102 m.moles/l. potassium was substituted at the serosal surface. These observations suggest that the lack of inhibition by intracellular glycoside of sodium transport previously described in squid axon is attributable to the intracellular potassium concentration. As a corollary, the therapeutic effects of cardiac glycosides *in vivo* appear to be a function of their extracellular concentration alone.

I thank Dr. Isidore S. Edelman and Dr. Peter H. Forsham for help and advice.

This work was reported at the 36th Scientific Session of the American Heart Association, October 1963, Los Angeles, California. It was supported in part by Fellowship *A.S.P.* 15024 and Grant *A*–3870 from the National Institute of Arthritis and Metabolic Diseases, U.S. Public Health Service.

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* C grade, Calbiochemical Company. No further purification of this material or independent biological assay was attempted.

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PHARMACOLOGY

'Viractin': a Prophylactic Agent against Upper Respiratory Infections

'Viractin' is the name we are proposing for the active principle in the mycelium of *Streptomyces griseus* cultures which produce actidione[1,2], and which we have found to prevent selectively upper respiratory infections in man. The activity of viractin was first noted in 1946 while one of us (B. E. L.) was isolating, crystallizing, and characterizing actidione. It was observed then that the people working with the actidione process were not susceptible to upper respiratory infections.

The preparation that has been used for *in vitro* investigations, tissue culture, animal, and also clinical studies was developed from residues of actidione mother liquors (generously supplied by the Upjohn Co., Kalamazoo, Michigan). The active principle was concentrated by distillation from this residue which has a characteristic mouldy odour. The crude distillate is the fraction collected between 60° and 200° C at 200μ. The viractin concentrate used in all investigations reported here was obtained by redistilling the crude distillate and collecting the fraction boiling at 105°–135° C at 200μ. Neither the crude distillate nor the redistilled preparation contains actidione. Gas–liquid chromatography shows that the active concentrate contains approximately 30 peaks. Only two compounds, 2,4-dimethyl*cyclo*hexanone and maltol, have been identified in this mixture. The cyclic ketones are apparently the major components, and it is postulated that one or more of these ketones is the active principle. Concentrated sulphuric acid produces a highly coloured product with the major peak at 292 mμ. This peak has been used to assay the product and to serve as a guide in dispensing the material for clinical testing.

Tissue culture investigations have shown that viractin has no significant *in vitro* viricidal activity against seven human respiratory viruses, measles and polio virus at a concentration of 0·5 mg/ml. When tested by the intranasal route in mice infected with the influenza virus, it showed only marginal activity at 100, 200 and 400 mg/kg. The LD_{50} of the preparation is 200 mg/kg intranasally, 300 mg/kg intravenously and more than 300 mg/kg subcutaneously. Inhalation toxicity of the vapours of viractin has been determined in rats confined to a chamber of 80 l. capacity into which air was introduced at a rate of approximately 250 ml./min after being directed forcefully against the surface of the viractin distillate contained in a 25 ml. vial. Three of the ten male rats comprising the treated group were killed after exposure for five days to 2·5 gm of the volatilized concentrate, and the remainder after an additional four days of exposure to another 2·5 gm of the volatilized product. No gross detrimental effects were observed. In fact, the treated animals had gained slightly more weight than the controls. Gross and histopathological investigations were made of their respiratory tract and other organs. Microscopic examinations of the lungs, trachea, spleen, kidney, adrenals and heart showed no essential differences between exposed and control animals. In concentrations used clinically, viractin has shown no toxic symptoms in animals or man. The lack of an *in vivo* or *in vitro* assay method to demonstrate antiviral activity has seriously delayed this investigation.

Since there is no reliable assay method in animals (except for toxicity investigations) for antiviral preparations which are not viricidal, such as viractin, observations on the human being were undertaken cautiously. The concentrated distillate has a vapour pressure which makes it possible to administer viractin as an inhalant over prolonged periods at extremely low concentrations. It was first tested for its effectiveness in reducing the incidence of upper respiratory infection prophylactically, using the family as a unit. After collecting these preliminary results, it was realized that they would not be scientifically acceptable for a number of reasons, including the lack of adequate controls. Also, the criteria of what constitutes an upper respiratory infection are debatable among clinicians. It was decided to carry out a preliminary study in a hospital of the chronically ill (neurological and psychiatric patients) since the turnover of patients would be very low. The viractin was evaporated from cotton balls at room temperature in two wards of approximately 80 patients each for a period of five months. The cotton balls were replenished with viractin concentrate at bi-monthly intervals. The results were striking in that the patients in these two wards escaped the Asian influenza epidemic which went through the hospital while the test was under-way. The two wards used to control this investigation had 47 patients with acute attacks of influenza among the 112 patients. It was also effective in preventing the occurrence of the common upper respiratory infections, for only 8 patients out of the 160 in the test wards had upper respiratory infections during that 5-month period of observation. Viractin was again tested the following winter with equally good results.

Table 1. Effect of Treatment with Viractin on Upper Respiratory Infection in Man

No. of days the upper respiratory infection persisted	Experimental group. 310 patients for a period of 77 days. No. of patients with upper respiratory infection	Placebo group. 320 patients for a period of 77 days. No. of patients with upper respiratory infection
2	2	17
3	4	11
4	3	5
5 or more	0	11

From these preliminary clinical tests it was postulated that viractin acts prophylactically to prevent upper respiratory infections by combining competitively with the sites where the virus attaches itself to the host cells. This is not a novel idea without experimental evidence, because it has been shown by Davies *et al.*[3] that amantadine hydrochloride shows antiviral activity by interfering with the penetration of the virus into the host cell.

A double blind clinical study was then conducted in a hospital for the chronically ill during March 1–May 16, 1964, to determine the effectiveness of this agent. The product was dispensed on cotton gauze squares in four wards with an average population of 310 patients (neurological and psychiatric). We use the average population because the patient population is relatively constant at this hospital, never exceeding more than 10 patients admitted or discharged for any given week in the four experimental wards. The amount placed on each gauze square for evaporation at room temperature was 79,000 optical density units (optical density observed times dilution factor) calculated as the sulphuric acid chromogen at 292 mμ. This amount was allowed for 1,000 ft.^{3} of air space. These gauze squares were strategically placed only in the sleeping wards, and each square was replaced every 14 days. In this way, the active principle would be at a low and slowly decreasing concentration in the air for inhalation during each 14-day period. The placebo selected to control the investigation was a mixture of ethanol and amyl acetate in the same proportions in which they occurred in the test material, to which was added small amounts of kitchen bouquet for colouring and methyl salicylate for odour. This gave it a detectable but not easily recognizable odour. It was placed in four wards of the same hospital with an average population of 320 patients. The turnover of patients was the same as given for the experimental wards.

Since this was a double blind study, the physicians, nurses, and attendants were instructed to record all upper respiratory infections. Patients showing any symptoms of upper respiratory infections were recorded by the nurses and checked by the ward physician. Severity of symptoms was also recorded by a number code:

1, first mild symptom of upper respiratory infection; 2, moderate symptoms; 3, fever; 4, patients confined to the infirmary. Because the nurses and physicians had no way of positively diagnosing a true upper respiratory infection from an allergic response, psychosomatic upset, etc., they recorded all symptoms. We consider a true upper respiratory infection as one which persists for five or more days.

Table 1 gives the number of patients who had upper respiratory infection of 2, 3, 4 and 5 or more days duration for the entire investigation, control and experimental. The results indicate a lower incidence of upper respiratory infection in experimental wards for the 77-day observation period than in the wards with the placebo for the same time period. If we consider an upper respiratory infection of 5 or more days duration to be the ones representing a true upper respiratory infection in man, then viractin reduced the incidence to 0 in the experimental wards while the placebo wards had 11 patients with upper respiratory infection.

The cumulative evidence has shown that viractin holds promise as an effective prophylactic agent for upper respiratory infections. Additional evidence from several hundred other individuals and families over the past seventeen years is also worthy of note.

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Solubility Relations of Fluorine Compounds and Inert Gas Narcosis

Two main physicochemical theories of inert gas narcosis have been put forward. The Meyer–Overton[1] theory postulates that the intensity of narcotic action varies as the concentration of narcotic in the lipids. More recently, Pauling[2] and Miller[3] have suggested that the aqueous phases play a dominant part and relate narcotic activity to the tendency to form hydrates or more generally to induce structure ('icebergs') in water.

For most simple narcotics the predictions of both theories are in substantial agreement as the narcotic effects of simple molecules are broadly in keeping with many of their thermodynamic properties, boiling points, ideal solubilities or polarizabilities[4,5]. However, in the case of fluorinated compounds these general relations break down and provide a means of discrimination between the theories. Such substances deviate very markedly from ideality in solution. Certain fully fluorinated gaseous compounds have the lowest recorded solubilities in water although their solubilities in organic solvents are less exceptional.

Though the dissociation pressures of hydrates at 0° C have been used to estimate the degree of structure a solute induces in water another indication is provided by the entropies of solution[6]. The entropy of solution of SF_6 has been estimated as -22 e.u. at 25° C using measurements of its solubility in water over a range of temperatures[7]. The results are in good agreement with those of Friedman[8], though both sets differ by a factor of five from another determination[9]. Such a high entropy of solution would suggest a narcotic activity much higher than that observed (as would the dissociation pressure of its hydrate). Table 1 summarizes the data for a typical series of gases[10] and illustrates that solubility in the lipids (as represented by olive oil) is the best indication of narcotic activity, other factors showing little correlation when fluorinated compounds are considered. In the case of CF_4 for which no anæsthetic data are available the predictions of the two theories are very different. The hydrate theory would suggest an anæsthetic pressure of ~0·5 atm., a degree of narcotic activity unlikely to have gone unnoticed during the search for fluorocarbon anæsthetics, whereas the lipid solubility theory would indicate about 5 atm*.

Table 1

Gas	Anæsthetic pressure (atm.)	Diss. pressure hydrate 0° C (atm.)	$-\Delta S$ soln (25°C) (cal. deg^{-1} mole^{-1})	Bunsen coeff. olive oil (37° C)
He	~200	—	3	0·015
N_2	29	160	7	0·067
A	20	95·0	9	0·14
Kr	2·9	14·5	13	0·43
CH_4	4·6	26·0	11	0·28
SF_6	3·0	1·0	22	0·25
Xe	0·85	1·2	16	1·70

We thank Prof. W. D. M. Paton for his advice.

**Note added in proof.* Preliminary experiments have shown that CF_4, at a partial pressure of 3 atm., does not produce anæsthesia in mice.

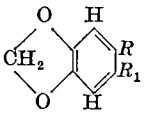
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Effects of Insecticide Synergists on Duration of Sleep induced in Mice by Barbiturates

A number of otherwise pharmacologically inactive compounds are known to prolong sleep induced by barbiturates in mammals[1]. The most widely investigated of these is '*SKF 525A*' (2-diethylaminoethyl 2,2-diphenylpentanoate), which prolongs the action of barbiturates and other drugs by inhibiting liver microsomal enzymes which degrade a wide variety of drugs[1]. When applied to insects, '*SKF 525A*' synergizes pyrethroid insecticides and antagonizes the toxicity of the organophosphorus poison, malathion[2]. In these respects, '*SKF 525A*' mimics the action of commonly used insecticide synergists such as piperonyl butoxide[2]. Although piperonyl butoxide and other chemically related synergists have been included in insecticide formulations for many years, their effects on drug activity in mammals have not been examined. In this communication the effects of two insecticide synergists on the hypnotic action of barbiturates are described.

The synergists (Fig. 1) examined were: (1) technical piperonyl butoxide containing 80 per cent 3,4-methylenedioxy-6-propylbenzyl butyldiethylene glycol ether and 20 per cent related compounds; (2) 96–98 per cent pure sesoxane (2-(2-ethoxy ethoxy)-ethyl 3,4-methylenedioxyphenyl acetal of acetaldehyde). The synergists were administered by intraperitoneal injection to 15–20 g female mice. Initially it was established that the synergists themselves (emulsified with 'Tween 20' in physiological saline) injected at dosages as high as 75 mg/kg did



	R	R_1
Piperonyl butoxide	$CH_2CH_2CH_3$	$CH_2(OCH_2CH_2)_2OC_4H_9$
Sesoxane	$O—CCH_3(OCH_2CH_2)_2C_2H_5$ (OH)	H

Fig. 1. Structure of piperonyl butoxide and sesoxane

not induce sleep or cause any visible harmful effects in mice observed for 24 h. Synergists were then given at 50 mg/kg to batches of 10 mice, and 30 min later the mice received 100 mg/kg hexobarbital sodium in saline. At the same time a control group of 10 mice were injected with 20 mg/kg of the emulsifying agent 'Tween 20' 30 min before receiving 100 mg/kg of the hexobarbital. A few minutes after being injected with hexobarbital, the mice in all three groups lost their 'righting reflex' and the time taken to regain this reflex was regarded as the sleeping time.

The results of this experiment are given in Table 1. They demonstrate that the synergists prolong sleep which is induced by the hexobarbital. Essentially the same results were obtained when either synergist was injected simultaneously with the barbiturate. Piperonyl butoxide was also found to extend the sleeping time of another barbiturate, pentobarbital sodium. Piperonyl butoxide (75 mg/kg), given simultaneously with 50 mg/kg pentobarbital ('Nembutal'), caused a batch of 17 mice to sleep an average of 220 ± 77 min compared with 33 ± 16 min for a control group of 18 mice (P = 0·00025–0·0005). (Two of the 19 mice originally injected with the pentobarbital and the synergist failed to recover from their sleep.) Piperonyl butoxide loses its ability to extend sleeping time within 24 h. Thus 19 mice treated with 75 mg/kg piperonyl butoxide 24 h before receiving 100 mg/kg hexobarbital slept an average of 17 ± 4 min compared with 24 ± 8 min for a control group of 18 mice (P = 0·1–0·05).

Table 1. Effect of Piperonyl Butoxide and Sesoxane on the Duration of Hexobarbital Sodium (100 mg/kg) Sleeping Time

Sleeping time ± S.D. (min)		
Control	Mice treated with 50 mg/kg piperonyl butoxide	Mice treated with 50 mg/kg sesoxane
25 ± 9	102* ± 29	143* ± 27

* Means significantly different from control; $P < 0·0005$ (Student's t test).

The means by which piperonyl butoxide and sesoxane prolong barbiturate action is not known. They do not appear to act by increasing the sensitivity of the site of action of the drugs, as mice receiving either synergist immediately following recovery from hexobarbital hypnosis did not return to sleep. Piperonyl butoxide and sesoxane and some related methylenedioxy phenyl synergists are known to block detoxication mechanisms in insects[3,4] and rats[5]. It would seem likely, therefore, that like '*SKF 525A*' the synergists also prolong the effects of barbiturates on mice by inhibiting drug detoxication.

Methylenedioxy phenyl synergists such as piperonyl butoxide are often included in pyrethrin insecticide formulations. In ordinary usage it is unlikely that mixtures of this kind would offer any risk to individuals taking barbiturates. Barbiturates are, however, recommended for supportive therapy in cases of accidental poisoning with synergized and non-synergized insecticides[6]. The findings obtained here suggest that this practice could bear re-examination, especially as some chlorinated and organophosphorus insecticides which are not usually combined with synergists are also known to lengthen the duration of action of barbiturate in mice[7].

The results presented in this communication raise three other interesting points. First, they indicate that insecticide synergists, of which a wide variety are known[3], could be useful tools in pharmacological research. Secondly, it is possible that the incorporation of moieties associated with synergistic activity (for example, methylenedioxy phenyl groups)[3] into suitable structured drug molecules could result in longer-lasting drug action. In this connexion, it is noteworthy that the methylenedioxy analogue of trimethoxyamphetamine is reported to be somewhat more potent to man than trimethoxyamphetamine or the closely alled compound, mescaline[8]. Finally, it has been shown that measures of pyrethrin-resistance are substantially reduced in the presence of methylenedioxy phenyl synergists[9]. These synergists might also have some practical application in overcoming drug-resistance in those bacteria and protozoa where resistance is determined by drug degradation mechanisms.

One of us (B. C. F.) is supported by a Department of Scientific and Industral Research research grant awarded to Prof. J. R. Busvine. Piperonyl butoxide was kindly supplied by the Cooper Technical Bureau, Berks, and sesoxane by Shulton Fine Chemicals, New York.

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TPNH Oxidation by the Prepuberal Rat Testis

TPNH generation in immature and adult rat testis *in vivo* is under hormonal control[1,2]. Greenberg and Glick, working with ACTH injections in the adrenal cortex, also found increased TPN reduction[3]. There is biochemical evidence that reduced TPN is required for steroid synthesis *in vitro*. Lynn and Brown[4] showed that TPNH is essential for testosterone microsomic synthesis. Hall and Eik-Nes[5] demonstrated in the rabbit testis *in vitro* that TPNH accelerates the conversion of ^{14}C cholesterol to ^{14}C testosterone. The experiment related in this communication was performed with the aim of elucidating whether or not reduced TPN is oxidized by the immature rat testis, and if this function is under hormonal control. Immature animals were selected because in them the dilution factors that interfere with enzymatic assays in adult animals[2] are not significant.

Immature A × C inbred rats of our colony were used, weighing 20 g at the onset of the experiment. The experimental animals were injected with 5 units per day of chorionic gonadotrophin for five days. At the end of the experiment the animals were killed by sudden decapitation. The biological effect of the hormone was measured by testis, seminal vesicle and prostate weight. The testicular tissue, free from its capsule, was homogenized in 10 per cent 0·25 M sucrose in a motor-driven Potter–Elvehjem homogenizer at 2,000 r.p.m. Then the homogenate was centrifuged at 1,800 and 10,000 r.p.m. under refrigeration. The two fractions obtained in this way were called fraction 1 and fraction 2.

The following assays were performed in fraction 1: G-6-P dehydrogenase by the method of Horecker and Kornberg[6], isocitric dehydrogenase by the method of Ochoa[7], and TPNH oxidase by the method of Kaplan[8].

In fraction 2, TPNH diaphorase was assayed by the method of Ernster[9].

The following results were obtained:

	Control	Experimental	Increase (%)
G-6-P dehydrogenase	35·6 ± 3·6	78 ± 2·7	116
Isocitric ,,	74·5 ± 9·2	120 ± 3·1	61
TPNH oxidase	0	0	0
TPNH diaphorase	43·2 ± 3·5	76·4 ± 7·2	76
Testis weight	60 ± 5	74·5 ± 2·9	25
Prostate weight	6·7 ± 2·7	12·2 ± 1·2	82
Seminal vesicle weight	3·2 ± 0·98	11·9 ± 1·2	270

G-6-P and isocitric dehydrogenase activities are expressed as millimicromoles of reduced TPNH per mg of protein in 5 min, TPNH diaphorase as millimicromoles of reduced dichlorophenolindophenol. Organ weights are expressed in relation to 10 g body-weight.

These results confirm our previous findings showing that the specific activities of the enzymes that generate TPNH in the prepuberal testis of the rat are under hormonal control; they also show that TPN diaphorase is under hormonal control, while there is no TPNH oxidation in either the normal rats or the stimulated ones. Another series of experiments[10] demonstrated by manometric techniques that TPNH generating systems cannot be coupled to respiration unless an exogenous mediator, vitamin K, is supplied.

There is a striking discrepancy between the increased activity of TPNH diaphorase elicited by the hormone and the absence of TPNH oxidation. Since TPNH diaphorase is considered a component of the respiratory chain and it is not possible to stimulate respiration in testis by TPNH generating systems, it seems plausible that in this system the electron acceptor for soluble TPNH diaphorase is other than cytochrome *c*.

Steroid synthesis is a microsomal function which entails hydroxylation. In view of the results obtained in this work we can assume that testis TPNH diaphorase may have hydroxylating activity analogous to that of the hydroxylases already described in the literature[11]. Philips and Langdon[12], in a study done on liver microsomal TPNH cytochrome *c* reductase, have suggested that this enzyme could have a function analogous to that which we propose for TPNH diaphorase.

One of us (N. A. S.) received financial support from the Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina.

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Alkaloid Content of Tobacco Seeds

There are many publications in various languages about the alkaloid content of tobacco leaves. The same cannot be said about tobacco seeds, and the few publications that exist do not all agree. G. Albo[1] accepted the probable presence of a solanin-like substance, but not nicotine. In his opinion this is a nutritive substance for the embryo, and therefore not to be expected in the developed plant. Scurti and Perciabosco[2] found neither solanin nor any alkaloid-like substance in tobacco seeds, but instead did find allantoin. According to G. Paris[3], the ripe seeds are free from nicotine. J. Chaze[4] investigated the existence and cytological formation of alkaloids in tobacco plants, and maintained that nicotine is not present in the seed. He showed that alkaloids do exist in the germinated seeds, in the vacuoles of cells of the growing seedling.

According to G. Klein[5], tobacco seeds contain trigonelline, but this was not confirmed by Abdoh and Taufel[6]. On the other hand, Abdoh and Taufel proved that trigonelline was not present in the tobacco seed, but found a compound with a pyridine ring, possibly nicotinamide or nicotinic acid. G. I. Vormann[7] examined the oil of tobacco seeds for alkaloids and found a very weak positive reaction with phosphotungstic acid. On the other hand, P. Radua[8] reported a lack of nicotine-positive reaction in the mature seeds of *N. tabacum*. The gradual degradation of nicotine in the presence of tobacco seeds was shown by Walter G. Frankenburg[9].

We have again re-examined the question of the occurrence of nicotine or other tobacco alkaloids in mature tobacco seeds.

Mature seeds, which were 1, 2, 3 or 4 years old from three species of Iranian tobacco plants—*Nicotiniana basmacerus*, *N. trabzant* and *N. tikolak*, acclimatized to the northern areas of Iran—were used in the present investigation.

20 g of seeds were ground in a porcelain pestle and mortar, then mixed with 150 ml. 30 per cent sodium hydroxide and finally extracted with 75 ml. of a mixture of benzene and chloroform in the ratio of 9 : 1 in a separating funnel. The procedure was repeated five times. The benzene and chloroform layers were separated and filtered. Ethanol and water (50 : 50) were added and the mixture acidulated with sulphuric acid (N/10). After shaking in a separating funnel the water layer, which contained alkaloids, was removed and concentrated to 5 ml. To this concentrate a solution of sodium hydroxide was added to bring the *p*H to 7·2. This concentrate was used in chromatography.

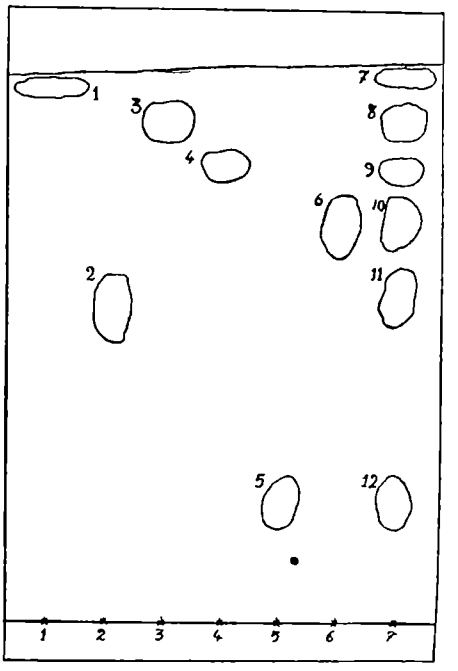


Fig. 1. Chromatogram of extract of fresh seeds. 1, Nicotyrine; 2, nornicotine; 3, nicotine; 4, nicotinamide; 5, nicotinic acid; 6, anabasine; 7, Nicotyrine; 8, Nicotine; 9, Nicotinamide; 10, anabasine; 11, nornicotine; 12, nicotinic acid. Solvent *n*-butanol/acetate buffer 0·1 M (*p*H 5·6), 50 : 50 v/v—upper layer is used

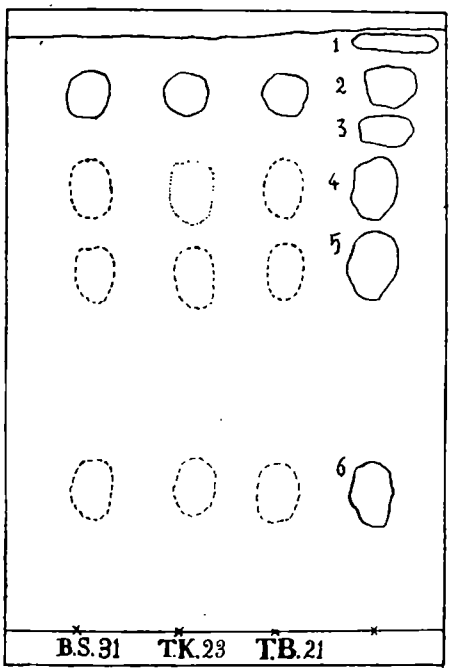


Fig. 2. Chromatogram of extract of 1-yr-old seeds. 1, Nicotyrine; 2, nicotine; 3, nicotinamide; 4, anabasine; 5, nornicotine; 6, nicotinic acid. Solvent *n*-butanol/acetate buffer 0·1 M (*p*H 5·6), 50 : 50 v/v—upper layer is used

Experiments with non-buffering and buffering papers revealed that the best system was achieved by spraying the paper with 0·1 M acetate-buffer (*p*H 5·6), and using the upper layer of normal butanol–acetate buffer 0·1 M (*p*H 5·6) in the ratio 50 : 50. We sprayed the Whatman No. 1 paper with acetate buffer and dried it in air. The samples were applied to this paper, and it was kept in a cylinder filled with the vapour of the lower layer of the above-mentioned mixture for 0·5 h to reach a state of equilibrium. It was then developed for 21 h at 23° C by the ascending method, in a closed glass cylinder, which was lined with paper saturated with the vapour of the lower phase of the mixture.

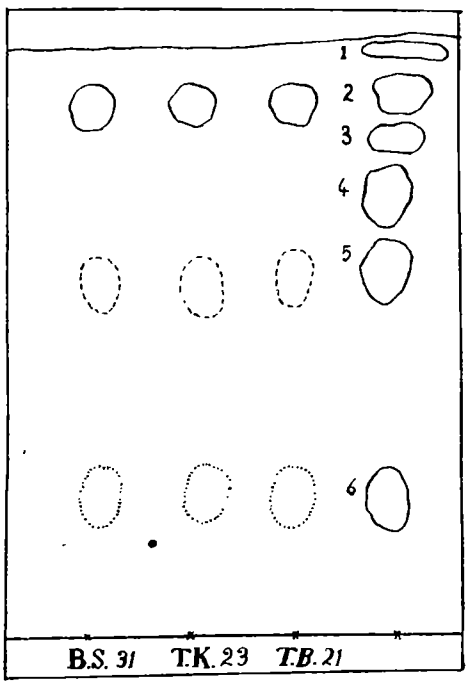


Fig. 3. Chromatogram of extract of old seeds. 1, Nicotyrine; 2, nicotine; 3, nicotinamide; 4, anabasine; 5, nornicotine; 6, nicotinic acid. Solvent *n*-butanol/acetate buffer 0·1 M (*p*H 5·6), 50 : 50 v/v—upper layer is used

The chromatograms were first examined with a 2 per cent alcoholic solution of 2,4-dinitrofluorobenzene and 5 per cent solution of sodium hydroxide. In the later experiments we used *p*-aminobenzoic acid and cyanogen-bromide fumes. Fig. 1 shows the chromatogram of six alkaloids and related compounds which were obtained from pure substances. In the experiments with one-year-old seeds, we first used the 2,4-dinitrofluorobenzene and sodium hydroxide solution. The results for all three species showed the appearance of nicotine spots.

To confirm the foregoing results we repeated the experiments with *p*-aminobenzoic acid and cyanogen-bromide fumes. The result showed the presence of four substances, nicotine, anabasine, nornicotine and nicotinic acid (Fig. 2). Of the two reagents, cyanogenbromide fumes were more effective.

Further experiments with the old seeds were carried out. The extract of old seeds (B.S.31, three years old; T.K.23, two years old; T.B.21, four years old) were examined with 2,4-dinitrofluorobenzene and sodium hydroxide, and nicotine spots were obtained from all three plant species. We repeated this experiment with the reagent P.A.B.A. and cyanogen-bromide fumes. We obtained nicotine, nicotinic acid and nornicotine spots in all three species, but found no anabasine (Fig. 3). Generally, the spots of anabasine, nornicotine and nicotinic acid were less pronounced than the nicotine spot; furthermore, the spots (especially of nicotinic acid) of the old seeds were clearer than those of the one-year-old seeds, and the spots of the T.K.23 species were weaker than those of the other two species.

We conclude, therefore, that the alkaloids nicotine, anabasine, nornicotine and nicotinic acid exist in mature seeds, and it appears that more alkaloids can be found in the fresh mature seeds than in the old seeds.

We thank Mr. Saphir Paulis for the supply of tobacco seeds and Messrs. E. Merk for the tobacco alkaloids.

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HÆMATOLOGY

Xg^a Investigations of the Family of a Child with a Ring *X* Chromosome

Elsewhere in this issue of *Nature* (p. 804) Pfeiffer and Büchner report their observations on the morphology and DNA synthesis of a ring *X* chromosome in a child with Turner's syndrome. The investigators kindly gave us the opportunity to test the blood of the patient and her relatives for *Xg*^{a}. (The anti-*Xg*^{a} serum was kindly provided by Dr. A. Cahan, Knickerbocker Biological Inc., New York.)

The first observations of the *Xg*^{a} testing in this family were: mother, brother and sister of the patient positive, father and patient very weakly positive or negative. Repeating the tests with the red cell suspensions of father and patient resulted again in doubtful reactions, even when a double volume of antiserum was used. Therefore,

absorption tests were performed: one volume of anti-Xg^a-serum was incubated with one volume of packed red cells for 30 min at 37° C. After incubation with the father's and the patient's red cells the absorbed sera reacted negatively or almost negatively with a suspension of the mother's erythrocytes. It may be concluded from these findings that some weak Xg^a activity is present on the father's and the patient's red cells.

A few possibilities are suggested as an explanation for these results: (1) the mother is homozygous Xg^aXg^a or heterozygous Xg^aXg^b; the patient received an Xg^a carrying chromosome which lacks its Xg^a locus by deletion. (2) The mother is heterozygous Xg^aXg^b and the child received the Xg^b carrying chromosome, which may have lost its Xg locus or not. In both cases the intact X chromosome in the patient must be the paternal one, carrying the very weak Xg^a factor. (3) The ring chromosome carries the mother's 'normal' Xg^a factor, the patient's very weak Xg^a reaction being due to the mixed occurrence of cells with the ring chromosome and XO cells. This seems improbable, however, since the patient's red cells reacted at least as weakly with anti-Xg^a as those of the father. In the presence of a normal X with weak Xg^a in a mosaic, with a normal Xg^a factor, a stronger reaction would be expected. (4) Another possibility is that both father and patient possess normal Xg^a factors, and that modifying genes are responsible for their weak manifestations, a hypothesis impossible to assess from available data.

As the mother has two normal Xg^a positive children, the probability of her genotype being Xg^aXg^a is about 80 per cent. The chance that she would transmit an Xg^b gene to a third child is therefore 10 per cent: the most probable interpretation of the observed facts is that the child received a maternal X chromosome from which the Xg locus had been deleted. This would indicate that the Xg locus is situated at the short arm, or far from the centromere at the long arm, of the X chromosome.

This work was supported by a grant from the U.S. National Institutes of Health, *NB* 04321-03.

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Separation of White Blood Cells

A convenient way of obtaining white cells from whole blood is simply to allow EDTA-blood to settle in siliconized glasses and then pipette off the leucocyte-rich supernatant after 1·5 h or more. A more rapid separation occurs when agents which aggregate erythrocytes are added to the blood[1]. The effect of these agents may be utilized even without mixing them with the blood. From physical chemistry it is known that a reaction between two compounds may be increased manifold if it occurs at an interface[2]. Similarly, the clumping of the erythrocytes may be accelerated when the process occurs at an interface.

Methylcellulose ('Methocel', Dow Chemical Co., Michigan), ethylhydroxyethylcellulose, methylhydroxypropylcellulose and hydroxyethylcellulose have been found to clump erythrocytes quite efficiently. Methylcellulose (MC) is mixed with 'Natrii' *N*-methyl-3,5-diacetamido-, 2,4,6,-trijodbenzoas ('Isopaque', Nyco Ltd., Oslo, Norway), which is an X-ray contrast for intravenous use. 'Isopaque' (Is) is suitable for many purposes in this field, because of its low viscosity at high concentrations, and its rather low osmotic activity.

A stock solution of Is with specific gravity of 1·20 (33⅓ per cent) has been mixed with solutions of MC of 0·8, 1·2, 1·6 and 2 per cent, to form final solutions with specific gravities of 1·08, 1·09, 1·10, 1·11 and 1·12. One ml. EDTA blood (EDTA 10 per cent, 1 : 50) is then applied on the top

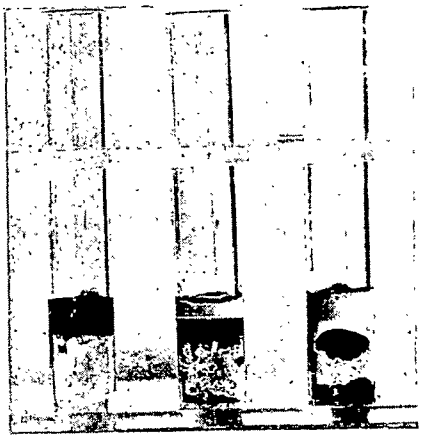


Fig. 1. Cell separation with a mixture of 'Isopaque' (33⅓ per cent) and methylcellulose (1·2 per cent). Specific gravity 1·09. The figure shows the situation after 1, 15 and 30 min

of these mixed solutions in siliconized centrifuge glasses with inner diameter of 13 mm. The blood column is about 7 mm high. The red cells are now clumped by MC at the interface, and fall down to the bottom of the glass (Fig. 1).

When the sedimentation of red cells is completed, the white cells follow them slowly downwards. Therefore, the supernatant should be pipetted off when there is a clear zone of 2–3 mm between the layers. The time for separation to occur will vary between 15–65 min in this system. The separation time is lengthened as the specific gravity or viscosity increases.

The supernatant contains platelets and 30–50 per cent of the white cells in the original blood volume. As to the contamination of red cells in the supernatant, the following points can be noted.

(1) When the specific gravity is kept constant, the number of red cells in the supernatant is reduced as the concentration of MC increases.

(2) If the concentration of MC is constant, the removal of red cells is more efficient as the specific gravity increases.

(3) The best separation is obtained with a rather high specific gravity and a low MC concentration.

Table 1 gives the result at five different levels. Each value represents the average of 10 samples, taken from different persons: 0·5 ml. (about five-sixths) of the supernatant was pipetted off from each glass. The mean white cell count in peripheral blood was 6,138/mm³.

Table 1. Separation of Blood Cells in the Methylcellulose 'Isopaque' System

Stock solutions (%)	Sp. gr.	MC conc. (%)	Leucocyte count in supernatant /mm³	Erythrocyte count in supernatant /mm³	Separation time (min)
Is. 33⅓ MC 2·0	1·08	1·25	4,451	3,309	18·0
Is. 33⅓ MC 2·0	1·09	1·11	4,430	2,512	24·5
Is. 33⅓ MC 1·6	1·10	0·80	4,303	1,999	35·0
Is. 33⅓ MC 1·2	1·11	0·54	4,313	1,636	44·5
Is. 33⅓ MC 1·2	1·12	0·48	3,700	925	58·0

The 'Isopaque'/methylcellulose system has served as a model system for other combinations of compounds, where the reaction principles are the same, for example, red cell removal. Some of these will be briefly described.

'Isopaque'/dextran. A 6 per cent solution of dextran ('Dextran 500', Pharmacia, Uppsala, Sweden) is mixed with Is (33⅓ per cent) to form solutions with specific gravities of 1·07–1·11. Separation time will be 10–60 min. The white cell yield is 30–50 per cent. The red cell number in the supernatant may be below 500/mm³. This system provides a very stable interface, since, when separation has occurred, plasma and white cells will remain at the top for hours.

'Isopaque'/'Ficoll'. Is (33⅓) is mixed with 'Ficoll' (Pharmacia, Uppsala, Sweden). Solutions with 10–15 per cent of 'Ficoll' and specific gravities of 1·09 are suitable.

Leucocyte yield is 40–60 per cent. Erythrocyte number in the supernatant is usually somewhat greater than in the two former systems.

'Ficoll'/EDTA. A 50 per cent solution of 'Ficoll' is mixed with 4–5 per cent EDTA to form a solution with specific gravity of 1·09. Separation time is about 40 min. White cell yield is 50–60 per cent. 1,000–2,000 red cells/mm³ are found in the supernatant.

EDTA/dextran. A 28 per cent solution of EDTA is mixed with 10–20 per cent solutions of 'Dextran 500' to form solutions with specific gravities of 1·11–1·14. Separation time will vary between 20 and 60 min. The white cell yield is 60–80 per cent. The red cell number in the supernatant will be somewhat higher than in the other systems. A 28 per cent EDTA solution is made in the following way: 35 ml. of distilled water is added to 28 g of EDTA and *p*H is then adjusted to 7·3 with a 30 per cent sodium hydroxide solution. Distilled water is then added to a final volume of 100 ml.

In some cases some small red clumps will be left exactly at the interfacial level. Therefore, to obtain a low red cell contamination, only the upper five-sixths of the supernatant should be pipetted off. The white cell yield is increased by using a greater volume of blood. With heparin as anticoagulant, 2·5 I.U. per ml. blood is suitable.

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IMMUNOLOGY

Cell-bound and Cell-binding Antibody in the Peripheral Blood of Immunized Rabbits

In a previous communication we reported on the production of antibody by peripheral blood leucocytes of rabbits[1]. The following work was undertaken to examine the relationship between cell-bound and cell-binding antibody in blood. The term 'cell-bound antibody' is used here to describe substances which at the time of testing were associated with blood leucocytes and enabled these cells to react specifically with the homologous antigen. The expressions 'cell-binding antibody' and 'cytophilic antibody' will connote globulin components of immune serum which become attached *in vitro* to cells in such a way that these cells are afterwards capable of specifically absorbing antigen[2,3].

A study of cell-bound and cytophilic antibodies appears to be of special interest in view of the continued need for a simple and sensitive direct *in vitro* test for reagins. The present model, although not dealing with reagins, was thought to give some indication as to the feasibility of such tests for cell-bound and cell-binding antibody.

Rabbits were immunized by eight intravenous injections of 1 ml. human serum. After 12 weeks of rest they were boosted with 1 ml. human serum intravenously. Five days later the animals were bled from the femoralis. 25 ml. of blood were immediately mixed with 5,000 units of heparin/0·5 ml. saline and 2·5 ml. Dextran solution ('Macrodex', Pharmacia, Uppsala, Sweden). After standing for 1 h at room temperature at an angle of 45°, the supernatant fluid with the leucocytes was withdrawn and centrifuged at 400 g for 7 min. Further lots of leucocytes were obtained by two more centrifugations of the remaining blood. The combined leucocytes were removed after washing with 10 ml. of a solution containing 50 ml. 5 per cent rabbit serum in Gey's solution and 2·5 ml. of 20,000 units heparin.

For evaluating the level of cell-bound antibody about $30–50 \times 10^6$ leucocytes, which still contained up to 50×10^6 microgram ^{131}I–human serum albumin (HSA), and (*b*) first pretreated with methanol for 5 min at room temperature, washed three times with Gey's solution and then incubated with labelled antigen as under (*a*). Blood leucocytes and red cells from normal rabbits served as controls. The results are summarized in Table 1.

Table 1. Cell-bound Antibody in Rabbit Blood

Cells	Animal No.	Antigen (^{131}I–HSA) adsorbed on to cells	No methanol (c.p.m.)	Methanol pre-treatment (c.p.m.)
Leucocytes from immunized rabbits	66	(*a*)	164	(*a*) 1,441
	78		677	903
	85	(*b*)	507	(*b*) 353
	89		37	1,492
	02	(*c*)	2,027	(*c*) 2,863
	06		5,263	3,688
Erythrocytes from immunized rabbits	66	(*a*)	60	(*a*) 691
	78		15	87
	85	(*b*)	31	(*b*) 12
	89		8	16
	02	(*c*)	0	(*c*) 7
	06		0	10
Normal leucocytes		(*a-c*)	33	(*a-c*) 42
Normal erythrocytes		(*a-c*)	1	(*a-c*) 3

Applied antigen in experiment (*a*) 0·5 μg ^{131}I–HSA = 2,751 c.p.m.
Applied antigen in experiment (*b*) 0·5 μg ^{131}I–HSA = 4,643 c.p.m.
Applied antigen in experiment (*c*) 0·5 μg ^{131}I–HSA = 11,103 c.p.m.

The blood leucocytes from immunized rabbits carried large amounts of cell-bound antibody. It is evident from the controls that this fixation of antigen is not due to red cells present in the leucocyte preparations, since in nearly all the rabbits even 5×10^8 red cells had no appreciable antigen-binding capacity. The effect of methanol was somewhat variable. In four out of six blood leucocyte preparations methanol increased the fixation of antigen. This observation would tend to suggest that some of the antibody responsible for antigen-binding was present intracellularly and made available by methanol pretreatment of cells, an interpretation compatible with our previous findings that blood leucocytes can produce antibodies[1].

Our findings[3] of the existence of cytophilic antibodies in antisera to various antigens suggested that part of the antigen-fixing capacity of blood leucocytes from immune rabbits may have been caused by fixation of humoral cytophilic antibody on to the leucocytes.

The sera of the immunized rabbits were therefore tested in our 'standard technique' for the titration of cytophilic antibody using blood leucocytes and spleen cells from normal rabbits[4,5]. Table 2 gives the results. Cytophilic antibody levels in the immunized animals, when tested with $1·5 \times 10^8$ normal nucleated spleen cells, showed the expected levels. In contrast, only low cytophilic titres could be measured with the same sera when 4×10^7 normal blood leucocytes were used. As is shown in Table 1, a similar cell number from immunized rabbits had a much higher affinity for the antigen. These results suggest that the antigen-binding capacity of blood leucocytes from immunized rabbits was probably due only to a small extent to passive adsorption of

Table 2. Cytophilic Antibody Levels in Immunized Rabbits

Type of rabbit cells used for antibody fixation	Sera Animal No.	Antigen (^{131}I–HSA) adsorbed on to cells (c.p.m.)
Normal leucocytes	66	69
	78	17
	85	7
	89	19
	02	27
	06	225
	Controls	7–12
Normal erythrocytes	All immune and normal sera	7–22
Normal spleen cells	66	637
	78	106
	85	21
	89	55
	02	305
	06	1,705
	Controls	12–25

circulating antibody on such cells. The main contribution to antigen fixation must therefore have been made by antibody-producing cells in blood, as demonstrated earlier.

In summary, the results suggest the possibility of measuring the levels of cell-bound and cell-binding antibody in blood. Whether this method can be applied to the measurement of circulating and blood cell-bound reagins, or factors associated with the delayed-type hypersensitivity, remains to be investigated.

This work was supported by the Swiss National Foundation for Scientific Research, grant 2635.

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7 S Globulin, Immunologically Identical to 19 S Gamma-1 (beta-2)-M-globulin, a New Protein of Horse Serum

KLAUSNER[1] described, at the beginning of the century, a precipitation produced by distilled water in syphilitic sera. This reaction was afterwards found in kala-azar (especially by Brahmahari and later by Sia; also called the Sia test), in malaria, in Waldenstroem's macroglobulinæmia, in the syndrome called African splenomegalia and in sleeping sickness. Chorine[2] showed that it consists in fact in an extension of the normal euglobulin precipitation to the alkaline area. The same extension was found by Felton[3] in the serum of horse immunized by pneumococci. Later it was demonstrated that this is the case for all horses immunized intravenously with bacteria. We show here that in equine serum the fraction responsible for this alkaline precipitation is composed mainly of a 7 *S* globulin, immunologically identical to 19 *S* γ-1 (β-2)-M-globulin.

Our work was performed with the sera of horses immunized intravenously with pneumococci, with meningococci, or with plague bacilli. The serum, diluted to 1/15 with 0·01 M phosphate buffer, was brought to *p*H 8. Indeed at this *p*H all the γ-group globulins are already anionic and therefore the heteropolar interactions which cause euglobulin precipitation[4] cease. The precipitate was washed 3–4 times with diluted phosphate buffer at *p*H 8 and lyophilized. Here we will call this precipitate 'Sia factor'. The quantity of Sia factor, lower than 0·1 per cent in normal horse serum, is 1–2 per cent in antibacterial horse sera[5]. Free electrophoresis shows the diffuse band of γ-globulins. The results of analytical ultracentrifugation depend largely on the value of the ionic strength. In the presence of concentrations lower than 2 per cent anhydrous sodium sulphate and lower than 5·5 per cent sodium chloride three components are present: a 7 *S* globulin, an atypical macroglobulin (9–12 *S*), and a typical macroglobulin (18–19 *S*). With higher salt concentrations, two components only are present: a 7 *S* and a 19 *S* globulin. The proportion of the latter does not change notably with the ionic strength; therefore it is the atypical macroglobulin that yields 7 *S* globulin when the ionic strength increases (Table 1). The 18–19 *S* component predominates in the case of normal equine serum (about 70 per cent); the atypical macroglobulin-yielding 7 *S* component predominates in the Sia factor of immune horse sera (75–95 per cent). The Sia factor is immunologically homogeneous in the case of immune sera. It has, on the immuno-electrophoretogram, the mobility of γ-1-globulin, and its long precipitation line presents the double curvature usually noticed with γ-1 (β-2)-*M*-globulin (Fig. 1). Two antigenic components are present in the Sia factor of normal horse serum; one has the immuno-electrophoretic appearance of 7 *S* γ-globulin and the other, that of the γ-1 (β-2)-*M*-globulin. A relation of partial immunological identity exists between the 7 *S* γ-globulin of normal horse serum and the Sia factor of immune horse sera (Fig. 2).

Table 1. RESULTS OF ANALYTICAL ULTRACENTRIFUGATION
Sia factor of horse anti-plague serum

Na_2SO_4 (%)	$(s_1)^w_{20}$ Proportion (%)	S	$(s_2)^w_{20}$ Proportion (%)	S	$(s_3)^w_{20}$ Proportion (%)	S
0·4	11·3	7·05	83·0	11·7	5·8	19·6
0·81	25·4	6·78	65·6	11·8	9·0	18·5
	s_1+s_2 Proportion (%)		S			
1·2	95·6		8·41		4·4	18·3
2·02	96·6	6·94			3·4	18·5
0·26 per cent protein						

Sia factor was fractionated on DEAE-cellulose column. Three bands are obtained under comparable conditions either with the preparation from normal or from immune sera. The first band is eluted by 0·02 M phosphate buffer of *p*H 8; the second by 0·04 M phosphate buffer of *p*H 8; and the third by 0·2 M sodium monophosphate. In the case of normal horse serum, the first band is practically pure 7 *S* γ-globulin; the second and third bands give an

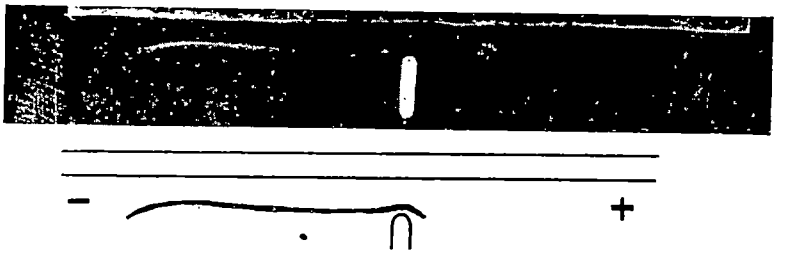


Fig. 1. Immuno-electrophoretogram of crude Sia factor obtained from anti-meningococcal horse serum. Rabbit anti-horse serum in the antibody trough

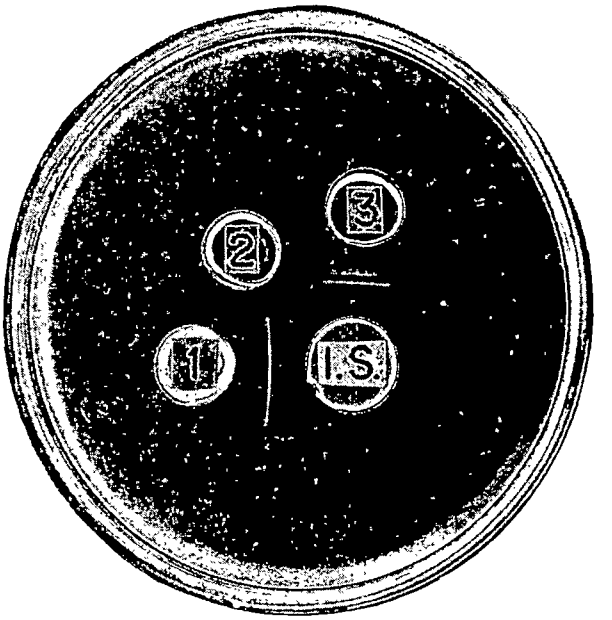


Fig. 2. Ouchterlony test comparing Sia factor to 7 *S* γ-globulin. Well 1, crude Sia factor of anti-meningococcal horse serum; well 2, 7 *S* γ-globulin of normal horse serum; well 3, normal horse serum; *I.S.*, crude anti-Sia factor serum of rabbit

immunological identity reaction with the Sia factor of immune horse sera, but the second band consists almost exclusively of the atypical macroglobulin yielding 7 *S* globulin in salt solutions of high ionic strength, where the third is composed for about 90 per cent of 18–19 *S* globulin. The first and second chromatographic bands seem to be identical in the case of immune sera. They are practically pure atypical macroglobulin yielding 7 *S* globulin in salt solutions of high ionic strength. All the 18–19 *S* globulin is found in the third band in a proportion varying between 20 and 70 per cent. The remainder is the atypical macroglobulin (Table 2). The molecular weights evaluated by light scattering in Brice's apparatus, in the presence of variable ionic strength, are presented in Fig. 3. The constant value of 170·000 in salt solutions of high ionic strength seems to become infinite when the ionic strength tends towards zero, suggesting a variably

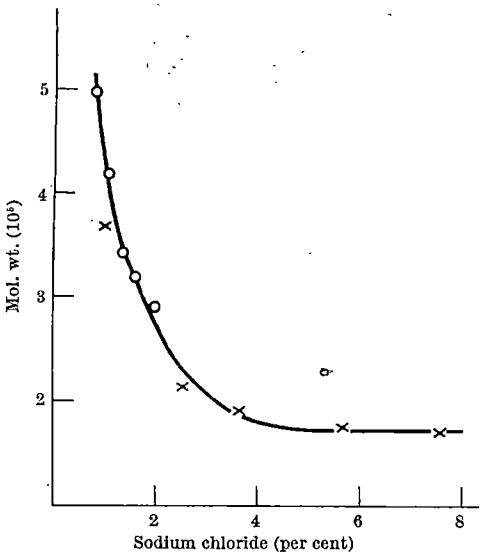


Fig. 3. Variation of the molecular weight of Sia factor first chromatographic band with the ionic strength. Sia factor was obtained from horse anti-plague serum. Molecular weights evaluated by light scattering. Circles and crosses refer to independent experiments. 0·12 per cent protein

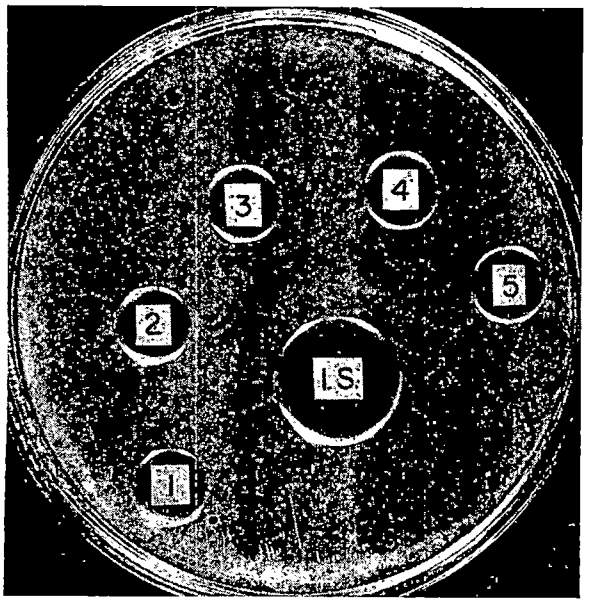


Fig. 4. Ouchterlony test comparing chromatographic fractions of the Sia factor of anti-plague horse serum with the practically pure macroglobulins of normal horse serum. Well 1, 7 *S* γ-globulin of normal horse serum; well 2, macroglobulins of normal horse serum; well 3, chromatographic band I; well 4, chromatographic band III; well 5, 7 *S* γ-globulin of normal horse serum; *I.S.*, rabbit anti-Sia factor serum saturated with 7 *S* γ-globulin of normal horse serum

Table 2. Sia Factor of Anti-plague Horse Serum

Analytical ultracentrifugation of the chromatographic bands

	Na_2SO_4 (%)	$(s_1)^w_{20}$ Proportion (%)	S	$(s_2)^w_{20}$ Proportion (%)	S	$(s_3)^w_{20}$ Proportion (%)	S
Band I							
0·19%	0·6			100·0	9·3		
Protein	1·2			100·0	8·2		
	2·5	91·0	6·9	9·0	?		
Band III							
0·16%	0·6			89·2	9·1	10·8	19·2
Protein	1·2			86·4	7·8+11·4	13·9	18·4
	2·5	76·5	6·6	4·4	10·0	19·2	18·4

aggregated precipitating system in weak ionic strength salt solutions. The three chromatographic bands are immunologically identical and they yield an identity reaction with a fraction of normal horse serum containing about 95 per cent 18–19 *S* globulins when examined by Ouchterlony test in the presence of anti-Sia factor rabbit serum (Fig. 4). The macroglobulin fraction of normal horse serum was obtained by Filitti-Wurmser and Hartmann's[6] modification of the technique of Koenig *et al.*[7].

The first and second chromatographic band of the Sia factor of immune horse sera, representing in a pure state the native monomeric γ-1 (β-2)-M-globulin, contains 16·4 ± 0·1 per cent nitrogen and a little less than 1 per cent of hexoses. It therefore differs from the polymeric γ-1 (β-2)-*M*-globulin also by having practically lost its carbohydrate part. It is constituted largely, perhaps entirely, of antibodies. Its minimum solubility is at *p*H 8. Normal horse serum contains about 0·01 per cent of this entity and immune horse sera 0·8–2 per cent.

Human serum differs in many respects from horse serum. Nothing precipitates in the normal human serum at *p*H 8 in solutions of low ionic strength. The precipitate produced in pathological sera is constituted exclusively of 7 *S* γ-globulin and of typical 19 *S* γ-1 (β-2)-*M*-globulin; the latter predominates (about 90–95 per cent).

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RADIOBIOLOGY

Tritium Labelling of Antibodies

A large number of alkylating agents react with proteins while forming relatively stable compounds. Ethyl methane sulphonate (EMS) reacts in proteins mainly with non-ionized amino groups and ionized sulphydryl- and carboxyl-groups[1]. Below *p*H 8, the carboxyl groups constitute the majority of the alkylated groups, and above this *p*H-level the amino groups are also alkylated. By varying the *p*H it is possible to vary the pattern of the groups which are alkylated[2], and this can be utilized to attach tritium-labelled groups to proteins. In the work recorded here we used tritium-labelled EMS, which was synthesized from commercially available tritiated ethanol (ethanol-2-*T*, Radiochemical Centre, Amersham) and methanesulphonic anhydride.

210 mg of EMS (0·518 c. per mole) in a 21-ml. solution of 1 per cent human globulin in 0·5 M $NaHCO_3$ were incubated at 37° C in a water bath. Samples of 3 ml. were withdrawn at certain intervals and dialysed at a temperature of 5° C against 0·9 per cent NaCl, the latter being changed frequently until a constant value of outer liquid

radioactivity was reached (about 24 h). One-ml. portions of the protein samples were evaporated in aluminium trays, weighed, and measured in a windowless gas flow proportional counter. The amount of protein was sufficient to reach infinite thickness for tritium β-particles. To convert counts per minute to ethyl groups per gram of protein, the labelled protein was compared to known amounts of the original EMS solution + protein in a liquid scintillation counter. Fig. 1 shows c.p.m. and the corresponding number of ethyl groups, in gram equivalents attached to 10^5 g of protein as a function of time.

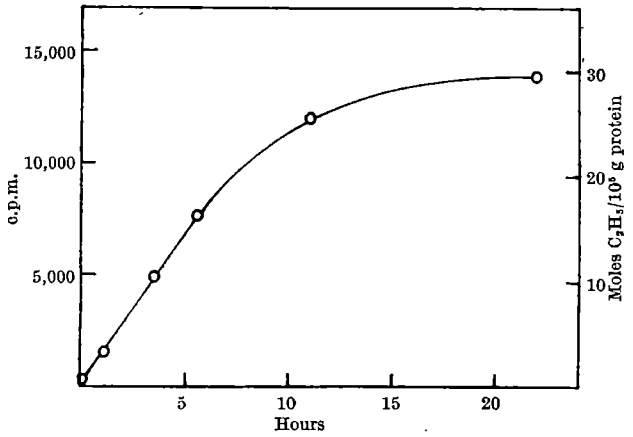


Fig. 1. Time study of the alkylation of the protein

Stability against hydrolysis. The stability of the alkylated protein against hydrolysis was investigated at five different *p*H levels. Samples of labelled globulin of known tritium content were dialysed at 37° C against phosphate buffer at *p*H 5·0, 6·3, 7·3, 7·7 and 8·9, and the release of tritiated ethanol was measured as a function of time. In Fig. 2, the remaining percentage of ethyl groups was plotted against time.

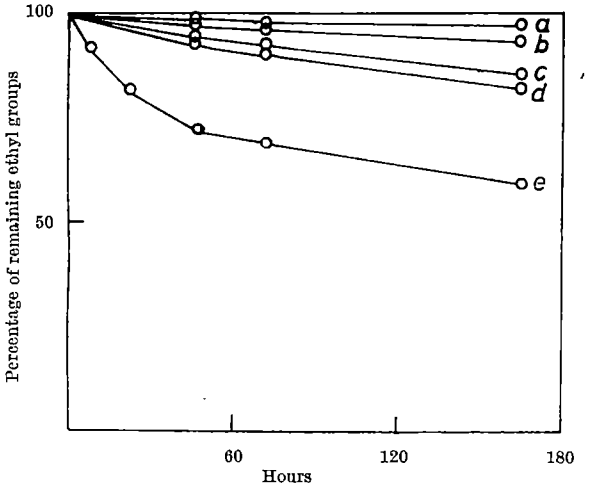


Fig. 2. Hydrolysis of ethyl protein as a function of time at *p*H: *a*, 5·0; *b*, 6·3; *c*, 7·3; *d*, 7·7; *e*, 8·9

Influence on immunological activity. To test its influence on the immunological activity of the globulin, the labelled protein was titrated against human erythrocytes. No serious damage in this respect was observed (Fig. 3).

Change in electrophoretic mobility. The mobility of the labelled globulin on paper electrophoresis in veronal buffer *p*H 8·6 was reduced to about 95 per cent of that of the untreated protein.

In this investigation we used EMS with a specific activity of only 0·518 c./mole. It is technically easy to increase this activity by a factor of 1,000; however, 10 mg of protein labelled with EMS of specific activity of

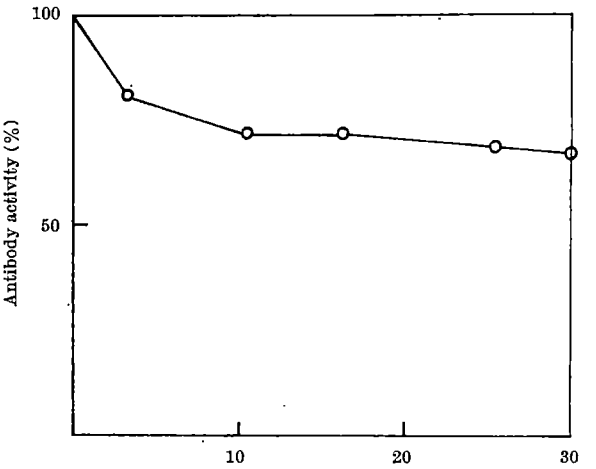


Fig. 3. Influence of the alkylation on the antibody titre

500 c./mole, gives approximately 35,000 ergs/h from tritium decay. This corresponds to 3·5 rads/h in a 100-ml. solution, which must be taken into account in *in vivo* experiments, but which is of minor importance with regard to protein destruction when working with *in vitro* systems.

Preliminary experiments with erythrocytes have shown that it is also possible to label whole cells in the manner described here. In this system, we found it convenient to measure the radioactivity by autoradiography or in a liquid scintillation counter after hydrolysis of ethyl ester bonds in alcoholic KOH and distillation of the alcohol.

The labelling process is strongly dependent on such factors as *p*H, temperature, and the concentrations of EMS and protein. The method described here might, therefore, be improved with respect to the utilization of the radioactive material and the stability of the labelled material.

This work was supported by grants from the Swedish Natural Science Research Council, the Swedish Medical Research Council and the Swedish Atomic Research Council.

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BIOLOGY

Duration of Embryonic Hypersensitivity and its Relation to the Level of Differentiation of the Chick

The chick embryo is capable of developing hypersensitivity of the immediate type (anaphylaxis) to foreign proteins during most of its developmental life[1,2]. It has also been demonstrated that, from the 12th day of incubation, sensitization of the delayed type to transplantation antigens can be elicited by injection of whole blood[3]. Due to the fact that chick embryos also are susceptible to immunological suppression and are not capable of humoral antibody production[4,5], a new interpretation was needed to account for such distinctive immunological properties of the chick embryo. In proposing such a theory it was postulated that, during development of the embryo, tissue-bound antibodies can be elaborated by cells derived from inactive mesenchymal precursor cells. Such tissue-bound antibodies, in common with classical antibodies, have anaphylactogenic properties[1]. It was assumed that chicks, sensitized as embryos, would be capable of manifesting hypersensitive reactions for only a short time after

hatching. This was based on a common observation that chick embryos and young animals injected with antigen do not manifest anaphylaxis when tested one month or more after the initial injection[4,6]. The term hapto-antibody was suggested for these tissue-bound embryonic antibodies which manifest this anaphylactic property.

In order to determine if the tissue-bound hapto-antibodies possessed this anaphylactogenic property transiently or permanently, the following experiments were carried out.

Embryonic chicks were injected intravenously with 4 mg of human γ-globulin. One group was injected with antigen on the 12th day of incubation, at a time when the chick embryo is not susceptible to immunological suppression. The other group of chick embryos was injected on the 15th day of incubation when immunological suppression had been established[4]. From such experiments it could be ascertained if hypersensitivity, established in the embryo, might be of different duration in animals which are likely to manifest immunological suppression at 45 days post-hatching than chicks which do not exhibit immunological suppression. About 60 per cent of both groups of chicks hatched. These hatched chicks were given a second (shocking) injection of 40 mg of human γ-globulin with 4 mg of dried whole guinea-pig serum (Difco, Detroit, Michigan), 1, 2 or 3 weeks after hatching. The control groups of chick embryos were not given the sensitizing injection, however; they were given the shocking injection of human γ-globulin and guinea-pig serum.

Table 1. Effect of age on the anaphylactic response of chickens which as embryos had been given a sensitizing intravenous injection of 4 mg human γ-globulin (HGG) on either the twelfth or fifteenth day of incubation

Group	Sensitizing antigen	Second (shocking) injection of antigen into the heart at various times post-hatching: Age (Weeks)	Antigen	Amount (mg)	No. of chicks exhibiting an anaphylactic response: Sensitized at 12 days: None	Mild	Severe	Sensitized at 15 days: None	Mild	Severe
A	None	1	HGG	40	9	1	0	8	0	0
B	HGG	1	HGG	40	2	6	1	1	8	1
C	None	2	HGG	40	8	1	0	8	0	0
D	HGG	2	HGG	40	2	8	0	3	7	0
E	None	3	HGG	40	17	0	0	9	0	0
F	HGG	3	HGG	40	18	0	0	9	0	0

The anaphylactic responses following the shocking injection were either mild, lasting about 5 min, or severe, lasting about 15 min. Such gradations of responses have been described previously[1]. In these experiments no fatal anaphylactic response was observed. This is in agreement with the observation that the 3-week-old pullet showed a less severe anaphylactic reaction with histamine than the adult cock[7]. The results summarized in Table 1, group *B*, clearly demonstrate that both mild and severe responses were elicited when sensitized chicks were given a shocking injection 1 week post-hatching. Somewhat similar results were obtained in the group *D* chicks which were challenged 2 weeks after hatching. The two positive responses which were observed in control groups can be explained as previously stated[1]. In contrast, however, by the third week following hatching no anaphylaxis could be elicited when the shocking injection was given (group *F*). It was also observed that the anaphylactic response exhibited in chicks 1 week of age is not as severe as when the challenge was given on the third day after hatching[1].

These results support the thesis that hapto-antibodies are characterized by a transient state of involvement in anaphylactic responses. This is in keeping with the idea that, during differentiation of the immunological system, antigens are able to induce various types of immunological responses depending on the level of differentiation of the cellular population at the time antigen is administered. Thus, one will be able to observe embryonic hypersensitivity, immunological suppression or humoral antibody depending on the time when the antigen is injected[1].

These experiments verify one of the assumptions of Van Alten and Schechtman[1] that the embryonic anaphylactic state is relatively short-lived in contrast to the durable anaphylactic states of mature animals.

This investigation was supported by U.S. National Science Foundation grant *GB*-1685.

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Effect of Time of Year on the Roughage Intake of Housed Sheep

The nutritional requirements of animals are said to vary with the amount of activity performed and with the environmental temperature. It is also said that darkness reduces activity and the maintenance of an air temperature above critical level leads to better food utilization.

As changes in nutritional requirements may be reflected by differences in the level of voluntary food intake, voluntary hay intake results were routinely obtained from sheep kept permanently indoors in individual pens measuring approximately 4 ft. 6 in. × 3 ft. 0 in. They were daily offered a small amount of mixed concentrates, always of the same composition and quality and always entirely consumed. In addition each received up to a maximum of 1·0 kg chopped hay a day. It was noticed that this voluntary hay intake varied with the season of the year and for this reason some continuous data which were available were examined. The results relate to the intakes of two young Shetland wethers and two mature Scottish Blackface ewes over a period of 23 months and are summarized in Table 1.

These animals were healthy and thriving throughout and had a good appetite. The two Shetland wethers (7 and 9) were completely normal. Each Blackface ewe (4 and 441) was fitted with a permanent ebonite rumen cannula and 441 had also had the right cervical vagus nerve severed and the left cervical vagus nerve exteriorized in a skin loop. There was no reason for assuming that these animals were adversely affected in any way.

There are small gaps in the data due to animals having been used for other purposes for short periods. Food intake was measured daily during the week and over two days at the week-end, as a routine laboratory procedure. The monthly mean air temperature was calculated

Table 1. Summary of Data obtained
Sheep (mean daily hay intake, g)

	9	7	441	4	Mean	Mean air temp. (° F)
May	405	530	766	—	567	54
June	600	667	700	—	656	58
July	688	676	800	890	764	62
August	753	683	940	1,000	844	62
September	696	684	985	1,000	841	62
October	689	654	869	929	785	54
November	596	662	738	893	722	44
December	428	594	682	747	613	40
January	349	626	691	788	614	36
February	352	605	779	732	617	44
March	468	586	799	829	671	53
April	552	507	756	834	662	48
May	669	550	736	863	705	54
June	851	736	957	988	883	61
July	997	895	1,000	1,000	973	64
August	999	—	985	—	992	64
September	997	—	923	—	960	60
October	991	914	1,000	963	967	56
November	974	833	946	932	921	46
December	886	883	950	926	911	41
January	435	831	969	829	761	40
February	631	815	939	855	810	43
March	854	837	847	773	828	43

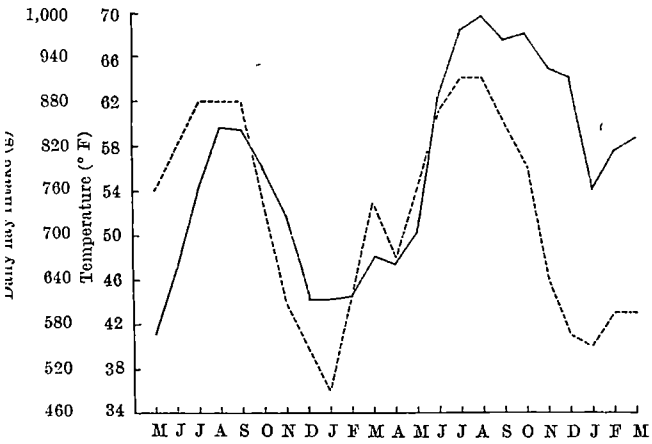


Fig. 1. Relationship between environmental temperature and hay intake. ——, intake; - - - -, temperature

from the maximum and minimum temperatures for each day, also routinely recorded. The results are further summarized in Fig. 1.

It is evident that all four sheep ate more hay a day during summer than in winter. The same hay was not used throughout. Housed sheep show preferences for different qualities and parts of hay and for no apparent reason one lot of hay may be less acceptable to some or all sheep than is another similar lot. However, it is unlikely that this is the reason for the variation in intake noted here. It is also most unlikely that the quality of hay offered was in any way responsible.

It is known that appetite is depressed by high environmental temperatures in the case of cattle[1], goats[2] and sheep[3] and in non-ruminants. In the outdoors sheep shearing has been found to lead to an increase in food intake[3]. In the rat, Brobeck[4] showed that food intake is a mechanism of temperature regulation and is not necessarily determined by total energy expenditure.

It might be argued that the foregoing result was due to seasonal changes in the activity of the animals. There is evidence that darkness reduces activity, for example, grazing sheep spend more time resting and less time grazing by night than by day[5,6] and do not travel between 22.00 and 03.00 h in summer[6]. Pastured dairy cattle also graze little at night and in addition they ruminate mainly at night[7], which is also true of housed sheep[8], and this tendency is not necessarily related to feeding times[9] but is related to the times of light and darkness[10]. With both sheep[11] and goats[2] the lowering of environmental temperature may lead to an increase in activity which would therefore operate against any decrease in activity due to darkness. It is therefore unlikely that activity is the all-important factor.

It is possible that the increase in intake of food is a direct relationship with length of daylight, but this appears unlikely since the rise in intake during June–August follows the rise in temperature and is in opposition to the decreasing hours of daylight, whereas if hours of light are concerned the relationship argued would require to be a positive one. However, the curves for intake and temperature do not always follow one another exactly since there is sometimes a lag of one or two months and this may also be true where the hours of daylight are concerned.

Douglas[12] has stated that, other things being equal, ventilation is more important for milk yield in dairy cattle than is temperature. The development of the winter coat and the reduction of activity in winter darkness may offset the effects of a lowered environmental temperature so that there may be no change in metabolic needs. For example, Doney[13] has found that the fleece of Blackface sheep apparently has some effect in reducing weight loss under adverse climatic conditions. The Eskimo is said to carry his environment around with him in his loose fur clothing and is thus virtually independent of low climatic temperature[14], and recently Cresswell[15] stated that "for all intents and purposes the sheep carries its own environment around with it in the form of its fleece". On the other hand, Hunter[16] found that hill ewes, presumably in full fleece, are sensitive to exposure, particularly in spring. With these housed sheep it is unlikely that length of coat was the only factor, since during June, when the animals were shorn, food intake did not reach its maximum, and since the period January–May is one of full coat one might expect a steady intake or even a fall in response to rising air temperature, instead of which there was a steep rise following the steep air temperature rise. Thus length of coat does not appear to offer an adequate explanation.

Mehrotra and Mullick[17] noted that sheep in Egypt showed a summer preference for leaves, but less concentrates were eaten so that the total dry-matter intake fell. This may be the answer in the present case, that is, appetite for hay increased with temperature, but since the intake of concentrates was always well below potential voluntary intake it was not possible to have a rise in winter concentrates intake and a fall in summer. However, this in itself is not an entirely satisfactory explanation since sheep at range do not normally receive concentrates. Food is scarce and of poorer quality during winter grazing under 'natural' conditions; it is therefore possible that some mechanism for improving the efficiency of food utilization may have been developed in the sheep.

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Destruction of Homologous Cells in Tissue Culture by Nuclei of Immune Lymphocytes

THE mechanism of action of immune lymphocytes in transplantation immunity and other delayed-type immune responses is still obscure. It is almost generally assumed that these cells carry on the surface of their cytoplasm protein anti-determinant groups, that is to say, they behave as cells with fixed antibodies. We have suggested[1] that the effect of immune lymphocytes is a particular case of their morphogenetic action and is not connected with the antibody-like structures of their cytoplasm.

This suggestion has been experimentally checked in the work recorded here.

The experiments were carried out in the system described by Rosenau and Moon[2], namely, a culture of Earl's *L* cells and lymphoid cells of *BALB/c* mice immunized with *L* cells. Immune lymphoid cells (designated here as lymphocytes) and their nuclei were obtained from *BALB/c* mice on the fourth day after the last (intrasplenic) immunization with *L* cells. For this purpose, the spleens were aseptically isolated and minced by scissors. One-third of the mass was used for isolation of intact lymphocytes and two-thirds for isolation of their nuclei.

Lymphocytes were obtained by means of 2–3 gentle strikes in a Potter glass homogenizer in Medium 199 and filtration of the cell suspension through two layers of gauze; the cell suspension (about 95 per cent nucleated cells were lymphocytes) was brought by Medium 199 to a final concentration of 2,000,000 lymphocytes per ml. The nuclei were isolated by means of the combined Schneider and Petermann[3] and Allfrey[4] methods. Fragments of spleens were homogenized in a Potter homogenizer in a 30 per cent sucrose solution with 0·02 per cent calcium chloride; 6 per cent citric acid was used to bring the final sucrose concentration in the homogenate to about 1 per cent (not more), according to Dounce's[5] recommendations. Homogenization was carried out until disappearance of visible particles in transparent light.

The homogenate was diluted three times with a 30 per cent sucrose solution with 0·02 per cent calcium chloride and filtered afterwards through two and four layers of gauze and then through two and four layers of cambric. The nuclei were sedimented in the centrifuge at 2,000 r.p.m. and then twice washed in 8·5 per cent sucrose solution with 0·2 per cent potassium chloride for 10 min at 1,200, 1,000 and 800 r.p.m. respectively. To eliminate any traces of citric acid, nuclei were washed twice with large volumes of a lactalbumin-hydrolysate solution, and afterwards re-suspended in Medium 199 to a final concentration of 2,000,000/ml. and 4,000,000/ml. Stained smears were carefully examined under the microscope to check the morphology of nuclei and absence of any cytoplasmic contaminations. Control lymphocytes and nuclei from non-immune *BALB/c* mice were treated in the same way.

Trypsinized *L* cells were cultivated 24 h in test tubes in 90 per cent Medium 199 plus 10 per cent of bovine serum. Thereupon the medium was eliminated, the tubes with the culture rinsed with pure Medium 199, and 1 ml. of a suspension of normal or immune lymphocytes or nuclei in Medium 199 with no serum added to each tube.

Five groups of tubes with *L* cells were used in every experiment with the following additions: first, with normal lymphocytes; secondly, with normal nuclei; thirdly, with immune lymphocytes; fourthly, with immune nuclei; fifthly, without any addition.

Four to seven tubes were used in each group. After addition of lymphocytes or of their nuclei the tubes were incubated for 48–60 h at 37°.

The destructive action of lymphocytes or of their nuclei was determined by counting *L* cells in every tube.

Our experiments conclusively confirm the data of Rosenau and Moon[2] as to the destructive action of immune lymphocytes on homologous cells.

Our evidence shows that nuclei of immune lymphocytes behave in a similar way. These immune nuclei surround *L* cells and stick to their surface. This picture enhanced up to a maximum after incubation for 18–48 h. *L* cells lost their processes, rounded up, and a considerable part of them degenerated.

In each field of vision one could see such pictures and cell-free spaces. The nuclei of normal non-immune lymphocytes showed less tendency to attach to *L* cells; they did not cause any destruction, nor did the density of the cell layer diminish. The effect of nuclei of immune lymphocytes was quite similar to that of intact immune lymphocytes. Some tendency of both normal and immune nuclei to agglutination was noted.

In all tubes incubated with nuclei of immune lymphocytes a statistically significant decrease in the number of *L* cells was noted. Immune nuclei caused quite distinct destruction of *L* cells whereas normal nuclei exhibited no such action (Tables 1 and 2).

Similar results have been obtained in three more experiments. The number of *L* cells in tubes with no lymphocytes or nuclei was similar to that in tubes with normal lymphocytes and their nuclei, so that tubes containing only *L* cells are not presented in Tables 1 and

Table 1. Destruction of *L* Cells by Immune Lymphocytes and their Nuclei

(Exp. No. 11)

No. of tubes in every group	Quantity of *L* cell per 1 ml. after 48-h incubation with: Immune lymphocytes	Normal lymphocytes	Immune nuclei	Normal nuclei
1	50,000	96,000	12,000	58,000
2	40,000	122,000	18,000	58,000
3	36,000	152,000	16,000	88,000
4	38,000	110,000	14,000	68,000
5	50,000	96,000	20,000	58,000
6	30,000	134,000	10,000	74,000
7	26,000	100,000	6,000	48,000
8	—	60,000	—	46,000
	$M = 35{,}500$	$M = 107{,}500$	$M = 13{,}700$	$M = 62{,}250$
	$\sigma \mp 7{,}700$	$\sigma \mp 24{,}500$	$\sigma \pm 4{,}400$	$\sigma \pm 13{,}020$
	$m \mp 3{,}000$	$m \mp 8{,}700$	$m \mp 1{,}700$	$m \mp 4{,}600$

Table 2. Destruction of *L* Cells by Immune Lymphocytes and their Nuclei

(Exp. No. 16/2)

No. of tubes in every group	Quantity of *L* cell per 1 ml. after 48-h incubation with: Immune lymphocytes	Normal lymphocytes	Immune nuclei	Normal nuclei
1	110,000	318,000	60,000	140,000
2	157,000	332,000	40,000	118,000
3	90,000	300,000	64,000	190,000
4	92,000	276,000	90,000	180,000
	$M = 112{,}000$	306,500	63,500	157,000
	$\sigma \pm 26{,}600$	$\sigma \pm 20{,}940$	$\sigma \pm 17{,}800$	$\sigma \mp 29{,}300$
	$m \pm 13{,}300$	$m \pm 10{,}470$	$m \pm 8{,}900$	$m \pm 14{,}600$

2. Complete elimination of cytoplasm is directly confirmed by constant examination under the microscope and indirectly by experiments in which lymphocytes and nuclei were suspended in Medium 199 supplemented with 10 per cent bovine serum inactivated at 56°.

Under these conditions, the effect of immune lymphocytes was retained, whereas that of immune nuclei was completely eliminated, probably by serum nucleases.

The very fact of the destructive action of immune nuclei refutes the role of hypothetic lymphocyte-bound antibodies or antibody-like rearrangements of their cytoplasm.

The question arises: How do immune lymphocytes destroy homologous cell-antigens?

The following possibilities may be considered. (1) Lymphocyte-bound antibodies are contained in both cytoplasm and nuclei or some nuclear structures have antibody-like properties. (2) The nucleus of the immune lymphocyte does not act through the agency of protein anti-determinants, but transmits to homologous cells some information which determines the synthesis of such anti-determinants as we considered in (1); this can be realized through: (*a*) transmission of DNA or of its individual informative moieties, or (*b*) the complex immune lymphocyte nucleus-homologous cell functions as an integrated system in which transmission of common messenger-RNA occurs from the immune nucleus, or (*c*) while active ribosome-like nuclear structures are transmitted from the immune nucleus to the homologous cell.

The first alternative, namely, the action of nuclear protein anti-determinants, seems highly improbable for the mere reason, at least, that the pattern of cell disintegration in transplantation immunity differs from that caused by antibodies in the presence of the complement.

The second alternative seems more convincing. The mechanism of lymphocyte-induced destruction of homologous cells is similar to the primary cytopathic effect in virus diseases.

If the role of the immune lymphocyte is not linked with the anti-determinants of its cytoplasm doubt is necessarily cast on the widespread concept as to the role of these hypothetical cytoplasmic antibody-like structures in the 'recognition' by immune lymphocytes of homologous

cell-antigens. In fact, there does not apparently exist any such 'recognition' at all; instead the effect of lymphocytes in the delayed type responses, especially in transplantation immunity is a particular case of their normal morphogenetic function[1].

The major function of lymphocytes consists in maintaining a constant level of differentiation of cells in various tissue systems by means of continued transmission of morphogenetic information.

In a similar way they transmit such information to a homograft. But in homotransplantation immunity there occur immune lymphocytes although the question is still open whether or not these cells result from instructive transformation or from clone selection.

Immune lymphocytes carry 'anti-information' to antigens of the grafts, that is, a system controlling complete or partial synthesis of protein anti-determinants to the graft antigens. Realization of this synthesis is unnecessary. Immune lymphocytes transmit this anti-information in the ordinary way both to normal tissues of the organism and to cells of the homograft. For normal body cells it is immaterial whether or not individual parts of the template received from lymphocytes can secure synthesis of a protein with anti-determinants to cell antigens of another strain of mice. As to the graft which consists of cells of this other strain of mice, incorporation of such anti-information is fatal.

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Purification of Algal Cultures with Caffeine

Ducker and Willoughby[1] have suggested a method for eliminating bacteria from algal cultures. If a technique could be devised for selectively killing fungi and non-photosynthetic protozoa, the selection of pure strains of algae would be considerably simplified.

I have observed that when algae are raised on salt media they are remarkably resistant to caffeine. Concentrations of 0·01–0·03 M kill the fungi and non-photosynthetic protozoa and, if anything, stimulate the algae. In reconstitution experiments and wild isolates I have succeeded in eliminating from cultures of *Euglena* and *Chlamydomonas* contaminants including species of *Aspergillus*, *Penicillium*, *Saccharomyces* and *Paramecium*. This worked in various media including pond water, pea infusion, modified Cramer-Myers salt medium[2] and Beijerinck's mineral medium with acetate[3]. The fungi were not killed in '*Euglena* medium'[4] or the enriched medium of Gibor and Granick[5], possibly because of the adenine content.

This method is not invariably successful; *Scenedesmus*, for example, is considerably more sensitive than *Euglena*. It should, however, be included among techniques to be tried in case of necessity.

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Oxygen Diffusion from the Roots of Some British Bog Plants

SEVERAL workers have shown that the roots of various plants are able to oxidize an anaerobic medium. With rice, van Raalte[1] was able to demonstrate that one oxidizing agent responsible was probably oxygen itself. Coult and Vallance[2] also showed that oxygen diffused from the sub-aerial parts of *Menyanthes trifoliata* L. into an anaerobic solution.

More recently, and concurrently with the work given in this communication, van Raalte *et al.*[3], using a 'polarographic' technique, have shown that oxygen diffuses from the roots of barley grown in anaerobic solutions.

The results presented here were also produced 'polarographically' using platinum versus saturated calomel electrode, the oxygen diffusion being measured in μamp. The platinum electrode is in the form of a small cylinder coated on the outside with 'Araldite', the reactive surface being on the inside.

The roots of the experimental plant are inserted with the electrodes into a deoxygenated solution, which is then 'sealed' with paraffin oil. At this stage a root can be inserted into the cylinder electrode, and a manual 'polarograph' taken, a 60-sec equilibration period being allowed for each reading. (Note that the plateau current is directly proportional to the oxygen diffusion.)

If the aerial parts are placed in an atmosphere of nitrogen, or cut and sealed with 'Vaseline', the oxygen diffusion from the root drops immediately. The minimum diffusion eventually recorded corresponds to the residual current[4] (Fig. 1). Further, if after a prolonged period the nitrogen is replaced by air or oxygen, or the aerial parts re-cut below the 'Vaseline' (zero time, Fig. 2), within 2 min oxygen again diffuses from the root. This latter observation is in agreement with the work of Barber *et al.*[5], who found that $^{15}O_2$ travelled down from the aerial parts of rice to the root tip in a similar time.

It has been found with *Eriophorum angustifolium* Honck. that, if air or nitrogen is replaced by oxygen, the diffusion rate from the root increases to five times that produced with the aerial parts in air (Fig. 1).

Moving the electrode farther up the root has shown which regions of the root are concerned in the release of

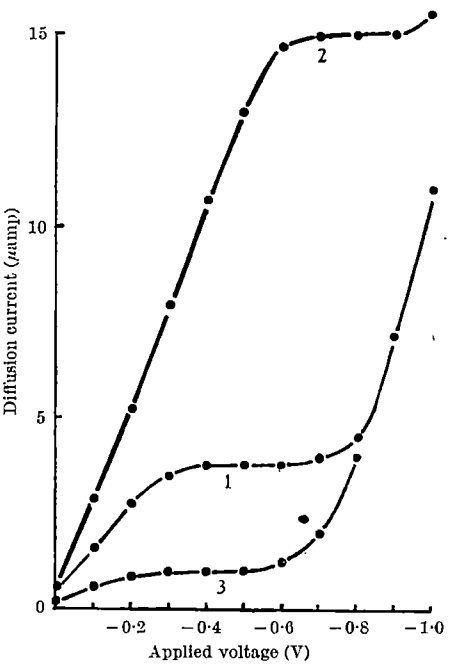


Fig. 1. *Eriophorum angustifolium*. (1) Oxygen diffusion round the root tip; aerial parts in air; (2) oxygen diffusion round the root tip: aerial parts in 100 per cent oxygen; (3) oxygen diffusion round the root tip ≡ residual current: aerial parts in 100 per cent nitrogen

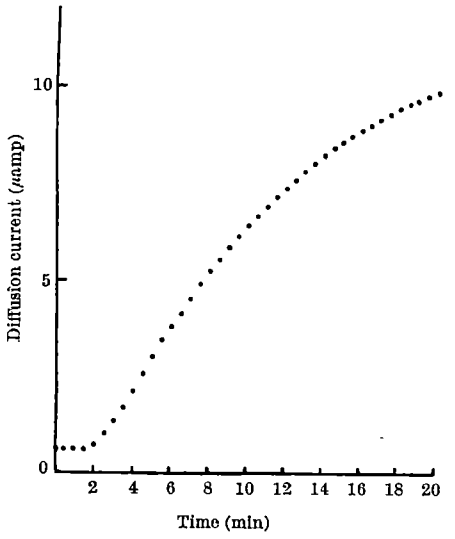


Fig. 2. *Menyanthes trifoliata.* Oxygen diffusion from the root plotted at −0·6 V. The aerial parts were in an atmosphere of nitrogen for 1·5 h before re-exposure to air at zero time

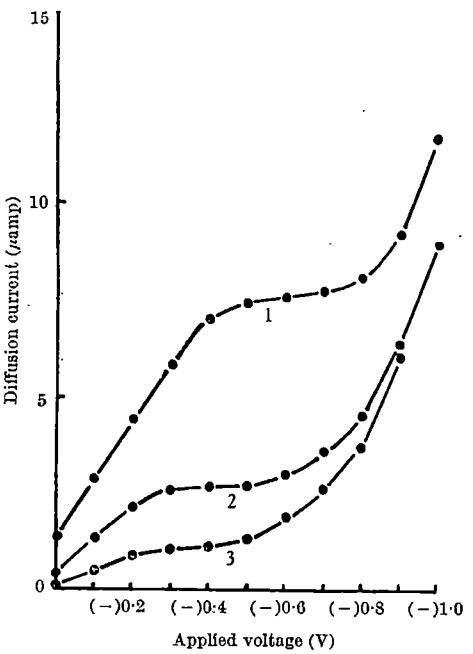


Fig. 3. *Eriophorum angustifolium.* (1) Oxygen diffusion round the root tip 0–1 cm; (2) oxygen diffusion round the root tip 0·5–1·5 cm; (3) oxygen diffusion round the root 1–2 cm

oxygen to the surrounding solution (Fig. 3). With *Eriophorum angustifolium* and *Molinea coerulea* (L) Moench. usually only the first centimetre of root tip is involved. With *Menyanthes trifoliata*, although the first 4 cm may be involved, the greatest oxygen diffusion value is found from the first centimetre of root tip.

Rates of diffusion which have been worked out for these species are as follows: *Menyanthes trifoliata*, 16 × 10^{-8} g O_2 cm^{-2} root surface min^{-1}; *E. angustifolium*, 13 × 10^{-8} g O_2 cm^{-2} root surface min^{-1}; *Molinea coerulea*, 1 × 10^{-8} g O_2 root surface min^{-1}.

These results suggest that there may be a similarly graded tolerance to reducing conditions by these species. Thus, as the rate of oxygen diffusion from *Menyanthes* roots is greater than the rate from the roots of *Eriophorum*, it might be expected that *Menyanthes* will withstand stronger reducing conditions than *Eriophorum*, and, similarly, *Eriophorum* than *Molinea*.

This is in accordance with measurements of redox potentials made at certain sites occupied by these species.

I thank Dr. D. J. Boatman for his advice.

This work is supported by a grant from the Department of Scientific and Industrial Research.

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MICROBIOLOGY

Fatty Acid Composition of Lipids extracted from Three Spherical Bacteria

Abel[1] suggested that taxonomic importance be attached to fatty acid components of bacterial lipid and proposed a simple and rapid scheme for the isolation and characterization of the lipid. Previous workers have shown slight similarities or differences in fatty acid contents of *Lactobacilli*[2], group '*N*' *Streptococci*[3], mesophilic and psychrophilic yeasts[4] and *Sarcina* species[5]. In several micro-organisms Bloch[6] showed that vaccinic acid is the principal acid in anaerobes whereas oleic acid predominates in aerobes. We have compared the fatty acid spectra of three spherical bacteria which are strikingly different in cellular arrangement, Gram staining, and habitat.

The micro-organisms were: (1) a marine isolate tolerant to 15 per cent sodium chloride tentatively assigned to genus *Gaffkya*; (2) *Micrococcus cryophilus*, A.T.C.C. 12226, a psychrophile; (3) *Veillonella alcalescens* A.T.C.C. 12641, an obligate anaerobe. Cells of micro-organisms listed were grown under appropriate conditions at optimum temperature in 20-l. lots of lipid-free trypticase soy broth (BBL) and harvested at the maximum stationary phase by centrifugation, and were washed twice with distilled water. Aqueous suspensions of cells were treated for 1 h in a 10 *KC* Raytheon magnetostrictor sonic oscillator (Raytheon Corp., Waltham, Mass.). Sonicated cells were dried *in vacuo* at 45° C over P_2O_5. Lipids were extracted from the dehydrated flaky material with chloroform–methanol 2 : 1 (v/v), according to the procedure of Folch[7]. Extracted lipids were weighed and recorded as percentages of cellular dry weights, and 20–40-mg aliquots were transesterified with 8 ml. of dry methanol and six drops of concentrated sulphuric acid. The resulting methyl esters, extracted and purified by the method of Luddy[8], were subjected to gas liquid chromatographic analysis on stainless steel columns employing the aerograph *A90C* (Wilkens Instrument and Research, Inc., Walnut Creek, California) 'Apiezon *L*' and diethylene glycol succinate polyester (DEGS) as the liquid phases. The methyl esters were identified by comparing 'carbon numbers'[9] with those of authentic esters. The lipid content for *Gaffkya* sp. was 7·1 per cent; *M. cryophilus* was 6·1 per cent and *V. alcalescens* was 10·1 per cent.

The data are presented in Table 1, where all fatty acids present in the concentration of 5 per cent or more are considered as major components, and dashes represent those present in less than 1 per cent. *Gaffkya* sp. has six major fatty acids; *M. cryophilus* three; and *V. alcalescens* six. Abel[1] detected essentially the same number of major fatty acids in *Gaffkya tetragena* as in our halo-tolerant *Gaffkya*, but differences are seen: (1) in the relatively high proportion of C_{20} acid present in the former; (2) the principal unsaturated C_{15} acid which was absent in the halo-tolerant *Gaffkya*.

Table 1. Fatty composition as g/100 g of methyl esters of unfractionated lipid extracted from *Gaffkya* sp., *Micrococcus cryophilus* and *Veillonella alcalescens*. Averages of duplicate runs

Fatty acid	*Gaffkya* sp.	*M. cryophilus*	*V. alcalescens*
9 : 0*	1·8	—	—
12 : 1	9·2	—	—
13 : 0	—	—	5·6
13 : 1	3·1	—	—
14 : 0	8·8	—	1·7
15 : 0	28·5	—	10·2
15 : 1 or 16 : 0 *br*	—	—	1·7
16 : 0	15·9	7·6	5·3
16 : 1	1·2	29·1	7·4
17 : 0	—	1·2	4·5
17 : 1 or 18 : 0 *br*	—	—	45·4
18 : 0	16·3	2·9	—
18 : 1	2·0	—	10·7
18 : 8	—	55·5	2·0
19·0 *cy*	—	1·3	—
20 : 0	11·1	—	—
Unidentified or in trace amounts	2·1	1·4	5·5

* First digit denotes number of carbon atoms; second, number of double bonds; *cy* indicates the cyclopropane ring of lactobacillic acid and *br*, a branched-chain acid.

M. cryophilus shows a relatively high proportion of unsaturated fatty acid and the absence of fatty acids with fewer than 16 carbons. The spectrum is markedly different from that of the mesophilic *Micrococcus ureae*, although similar results have been reported in psychrophilic yeasts. Our findings seem to corroborate the lipid distribution of plants and animals[10] living in a temperate or cold region, producing highly unsaturated lipids at low environmental temperatures. Prill[11], using *Aspergillus fischeri*, and Singh[12], employing *A. nidulans*, however, found the opposite correlation. Factors other than temperature may be involved in the regulation of degree of unsaturation.

V. alcalescens showed as the major component a 17 : 1 or 18 : 0 acid, so that additional investigations will establish whether its fatty acid spectrum exhibits a high proportion of unsaturated fatty acids and hence differs from that of the anaerobe *Clostridium*[13].

If the cultures are representative of the genera *Gaffkya* and *Veillonella*, these can be distinguished by the predominant fatty acid present, and in psychrophiles, *M. cryophilus* may represent fatty acid components characteristic of the low-temperature group. Investigations of additional members of these genera may corroborate the taxonomic or phylogenetic significance in lipid composition.

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Use of a Fluorescent Brightener for tracing the Passage of *Salmonella* in Eggs

During investigations concerning the contamination of eggs by *Salmonella*, cultural methods for detecting the passage of the organisms through the shell into and beyond the membranes provided little evidence concerning possible sites of entry and subsequent translocation. The work of Darken[1,2] on the uses and properties of fluorescent brighteners suggested a suitable means of tracing these bacteria.

The most suitable material available for this purpose was 'Calcofluor White *M2R*, New', a water-soluble diamino-stilbene-disulphonic acid brightener, which differed from the compound used by Darken[2] in that it contained two diethylamino substituents on the triazine rings. A concentration of 0·05 per cent (w/v) of this compound in nutrient agar (Difco) plates supported the growth of various species of *Salmonella* without any apparent inhibition or mutagenesis[3]. The brightener was readily absorbed by the cells, which were collected after an incubation of 24 h at 37° C, washed and suspended in isotonic saline.

The bacteria were brightly fluorescent and the suspending fluid non-fluorescent when observed under ultra-violet radiation. Fluorescence microscopy confirmed that the individual cells were fluorescent. This effect did not alter in the saline suspension after an incubation of 21 days at 37° C.

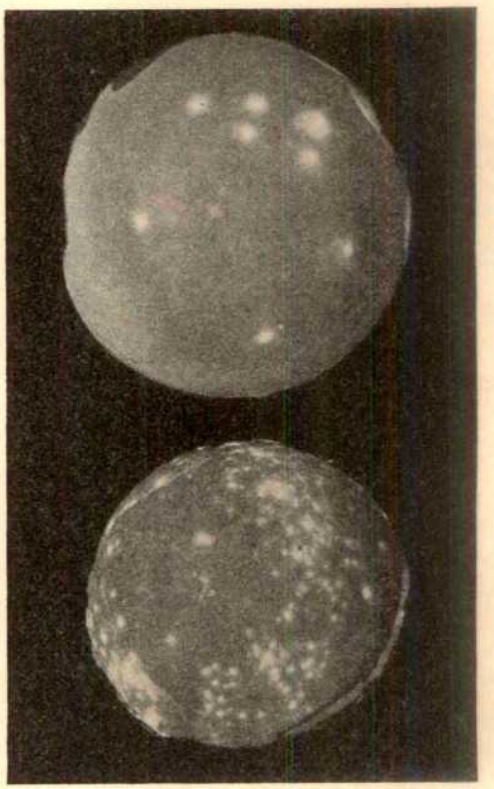


Fig. 1. Colonies of *S. anatum* treated with a fluorescent brightener, developing on the interior surfaces of moderately and heavily contaminated eggs. Photographed under ultra-violet light. (× 1)

Fresh hens' eggs held at 37° C for 3 h were immersed for 30 min in an iced saline suspension of treated *Salmonella anatum* (approximately 10^4 organisms per ml.). Thereafter the eggs were incubated for 5–7 days at 37° C. On breaking and examining the interiors of the shells under ultra-violet light, fluorescent spots were readily observed (Fig. 1). These spots were composed of micro-organisms identical by cultural and serological criteria with the *Salmonella* of the original suspension. Despite considerable multiplication the individual cells comprising the spots were still fluorescent. Untreated *Salmonella* suspensions provided no such fluorescent spots and their presence inside the egg shells could only be ascertained by cultural methods.

The visualization of the sites of entry and the degree of contamination by *Salmonella* in eggs could be used to determine the efficiency of control measures.

This work was supported in part by funds provided by the Western Utilization Research and Development Division of the U.S. Department of Agriculture. The 'Calcofluor White *M2R*, New' was provided by the American Cyanamid Co., Chicago.

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Carbon Dioxide-dependent Morphogenesis in *Arthrobotrys conoides*

Carbon dioxide has been shown to influence morphogenesis in micro-organisms, higher plants and animals. Among the processes affected by carbon dioxide (or bicarbonate) concentration are sporangial differentiation in *Blastocladiella emersonii*[1], mould-yeast dimorphism[2,3], spherulation in *Coccidioides immitis*[4], sporulation by bacteria[5] and fungi[6,7], root development by pea[8], and sexual differentiation in *Hydra*[9]. This list can now be extended to include morphogenesis in *Arthrobotrys conoides*. *A. conoides* is a nematode-trapping fungus which captures prey in adhesive networks of hyphal loops. Usually, these organelles of capture are not formed spontaneously by the fungus but develop in response to the presence of prey. Morphogenesis is induced by a metabolic product of nematodes which has been partially purified[10–12] and designated nemin[13]. The tests described here provide evidence that nemin-induced morphogenesis in *A. conoides* is dependent on carbon dioxide.

Petri plates containing maize-meal extract agar were inoculated with *A. conoides* and stored in air at 28° C until the fungus developed as a single colony which covered approximately one-third of the agar surface in each plate. Some of these colonies were treated with nemin (added as an aqueous extract of ascarids) to induce morphological differentiation and then incubated, with untreated controls, for three days at 28° C in desiccators containing the following concentrations of carbon dioxide in air: (*a*) 0·00 per cent (1·0 N KOH placed in bottom of desiccator); (*b*) 0·03 per cent (air); (*c*) 10 per cent (air adjusted by introducing into the desiccator manometrically measured amount of carbon dioxide from a tank). A quantity of 1·0 N H_2SO_4 was placed in desiccators (*b*) and (*c*) to approximate the dehydrating effect of the KOH in desiccator (*a*). The influence of concentration of carbon dioxide on growth was measured as the change in diameter of colonies during storage in the desiccators, and cultures were examined microscopically for morphological change. The results of independent tests performed in our separate laboratories using two strains of *A. conoides* are summarized in Table 1.

A. conoides grew but formed no traps in the absence of carbon dioxide. Growth in air (0·03 per cent carbon dioxide) exceeded that in an atmosphere devoid of carbon dioxide, and, in air, the fungus formed traps in response to treatment with nemin. In no case did traps develop in controls that were not treated with nemin. Although nemin-induced morphogenesis was carbon dioxide-dependent, the influence of concentration of the gas on growth and trap formation varied with the strain of *A. conoides* tested. Growth and the response of strain *NB* to nemin were both greatest in air, and reduced by an absence or an abundance of carbon dioxide. Strain *C* developed best in air, but the morphogenic response of the fungus to nemin was maximum in an atmosphere containing 10 per cent carbon dioxide. Optimum levels have not been established; but growth and nemin-induced morphogenesis in *A. conoides* appear to be independent functions of carbon dioxide concentration.

Table 1. Influence of Concentration of Carbon Dioxide on Growth and Trap Formation by Two Strains of *A. conoides* treated with Nemin

Reaction	*NB* strain CO_2 (%)			*C* strain CO_2 (%)		
	0	0·03	10	0	0·03	10
Growth*	15	20	10	12	20	8
Morphogenesis†	−	+ +	+	−	+ +	+ + +

* Radial expansion of colony (mm).
† Abundance of traps: −, none; +, few; + +, many; and + + +, very many.

This work was supported in part by U.S. National Science Foundation grants *G*–19211, *GB*-548 and *GB*–1508.

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CYTOLOGY

Absence of Late Replication of a Human X-Ring Chromosome

As autoradiographic examination of human and other mammalian chromosomes after incorporation of tritiated thymidine has revealed a characteristic pattern of replication during the late *S* phase for the heteropycnotic *X* chromosome and some autosomes, the lymphocyte culture technique associated with a simplified autoradiographic method may be used for diagnosis and investigations of the sex chromosomes even in smaller laboratories.

In lymphocytes of an intelligent girl, twelve years of age, with typical features of Turner's syndrome and negative sex chromatin in leucocytes and mucosa nuclei—the patient being one of a series of 40 females with chromatin negative Ullrich–Turner syndrome—we found two cell lines with 45 and 46 chromosomes respectively in a ratio of about 40/60. Cells with 46 chromosomes were characterized by a little odd-shaped chromosome which in 41 out of 113 complete mitoses had a ring configuration. Its diameter did not exceed the length of a chromosome in group 21–22. Bipartite (Fig. 1) and quadripartite configurations were like those shown by Lucas[1] and Gropp[2] on ring chromosomes of No. 18.

Autoradiographic examination using Ilford *K*-2 nuclear emulsion instead of stripping film for the exposure of the fixed, spread and orcein stained chromosomes following incubation for 6 h of tritiated thymidine failed to show late labelling of the ring chromosome which has been considered as a deleted *X*. Grains in 113 mitoses have been counted and *X*/45 ratio[3] was determined for several degrees of labelling. In mitoses which contained a few grains, thus marking late *S* phase, we saw clearly labelled

autosomes of group *B*, *D* and *E* (16), whereas over the ring there were usually no, or only very few, grains. The *R*/45 ratio for mitoses with fewer than 50 grains was 0·027; for all 113 mitoses, 0·015. The regression line is given in Fig. 2.

Although our results cannot be compared with those given by Rowley *et al.*[4], who examined an *X*-ring chromosome by modified techniques (shorter time of incubation, stripping film exposure), we may conclude that our ring chromosome does not exhibit late replication as found by these authors. This finding does not fulfil the hypothesis that the abnormal *X* chromosome is always the late replicating one. Otherwise the result of an examination of the normal *X* chromosome in human females and of the isochromosome of the long arm of *X* of which the regression lines are shown in Fig. 2 fits well with the data given by Muldal *et al.*[3]. This abnormal behaviour of a deleted *X* chromosome may be explained by the observation that pericentric and terminal regions complete DNA-synthesis at an earlier state than interstitial parts of the late replicating *X* chromosome[5,6]. We have seen this phenomenon in three different normal *X* chromosomes and are led to the conclusion that these interstitial parts have been deficient in our ring chromosome. The remainder of the chromosome behaves, therefore, apparently as an earlier replicating isopycnotic chromosome.

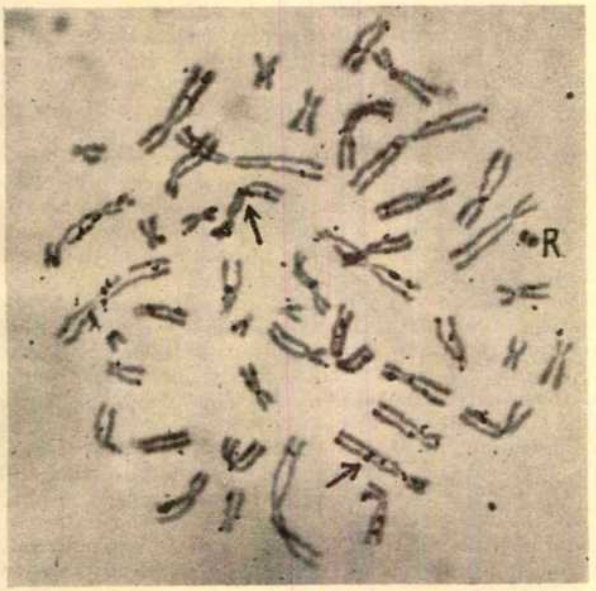


Fig. 1. Bipartite configurated ring chromosome which is not labelled in a mitosis with few grains thus marking late stage of the *S* phase. Two *B* chromosomes are labelled at identical regions (→)

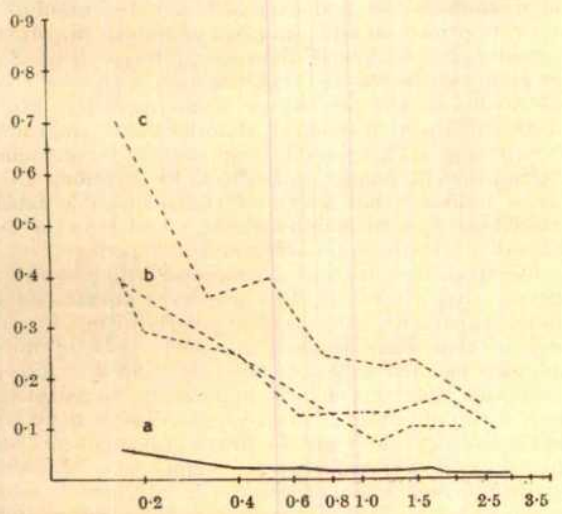


Fig. 2. *X*/45 (*R*/45) ratio plotted against mean counts of several degrees of labelling, mean labelling of total count being 1·0. *a*, Our ring chromosome; *b*, two normal female *X* chromosomes; *c*, isochromosome for the long arm of *X*

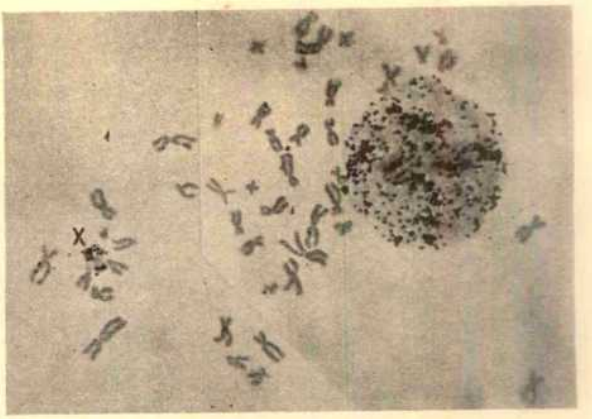


Fig. 3. Selective late labelling of interstitial areas on the long and short arm of a normal human *X* chromosome. DNA synthesis in the pericentric zone must have been completed before tritiated thymidine added

The appearance of our patient excludes the possibility that the ring chromosome is derived from an autosome, but we are unable to disprove that it represents a *Y* chromosome of which the *Y*/45 ratio equals that of *R*/45.

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Percentage Incorporation of Leucine labelled with Carbon-14 into Isolated Mitotic Apparatus during Early Development of Sea Urchin Eggs

THERE is evidence that during division in sea urchin eggs a certain amount of protein specifically necessary for division is synthesized. Hultin[1] has shown that the division of these eggs is blocked at 'streak stage' by puromycin. Gross and Cousineau[2] have found, in autoradiographs of *Arbacia punctulata* eggs, that most of the labelled amino-acid incorporated in earliest cleavage is found in the region of the mitotic apparatus; the localization of label is particularly pronounced when supernumerary asters (cytasters) are induced in developing eggs by treatment with heavy water. On this basis, these authors suggest that the protein synthesized in earliest cleavage plays a structural or catalytic part in the operation of the mitotic apparatus. The suggestion is substantiated by the finding of Stafford and Iverson[3] that labelled amino-acid incorporated by fertilized eggs may be found in the isolated mitotic apparatus.

The experiments recorded here give the degree of localization in the isolated mitotic apparatus of amino-acid incorporated in earliest cleavage. The material used was *Paracentrotus lividus*.

Eggs and sperm were obtained by injection of sea urchins with 0·5 M potassium chloride. After washing

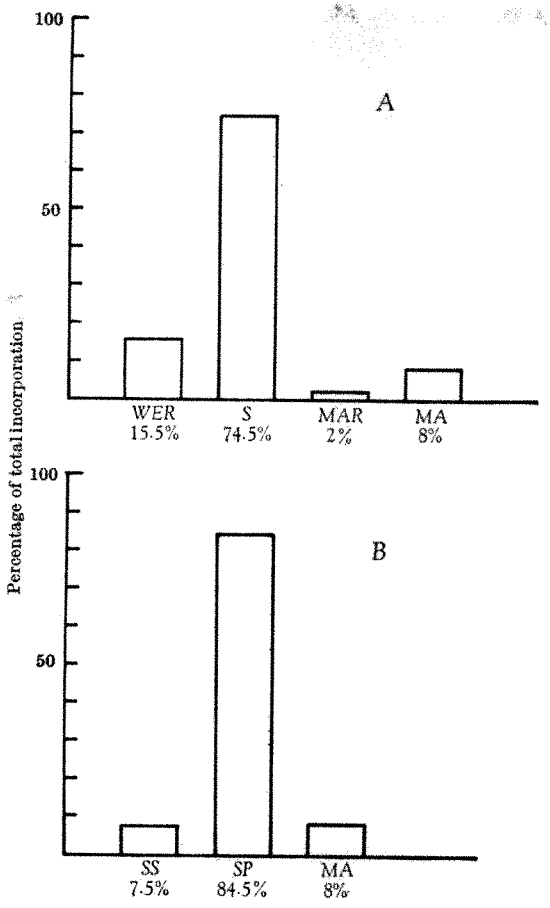


Fig. 1. *WER*, whole egg rinse; *S*, supernatant; *MAR*, mitotic apparatus rinse; *MA*, mitotic apparatus; *SS*, soluble supernatant; *SP*, pellet

several times with sea-water, the eggs were fertilized, and the fertilization membranes and gelatinous coats were removed by passing the eggs repeatedly through bolting silk, followed by several washes in sea-water. The embryos were then incubated until the time of mitotic apparatus isolation with 0·61 mμmole/ml. of leucine-^{14}C (160 mc./mmole), at 22° C. The mitotic apparatus was isolated using the procedure of Kane[4], either unmodified or with substitution of 0·01 M citrate buffer at *p*H 6·0 for the corresponding phthalate buffer. Results were substantially the same in the two cases. High yields of mitotic apparatus were obtained. A direct count, made during a typical isolation in a trial run (citrate buffer), showed a yield of 91 per cent.

During isolation, fractions were obtained and sampled for radioactivity according to the following schedule. After washes in isotonic sodium chloride–potassium chloride, 19 : 1, the eggs were suspended in isolation medium and centrifuged in a hand centrifuge to give a supernatant free of intact eggs—the whole egg rinse—and a pellet of swollen but undispersed eggs. The latter was resuspended in isolation medium, and the eggs were dispersed by agitation until the homogenate, which contained the mitotic apparatus, was free of intact eggs. The homogenate containing mitotic apparatus was then centrifuged sufficiently to give a supernatant free of mitotic apparatus and a pellet of unwashed mitotic apparatus. The latter was again resuspended in isolation medium, and recentrifuged to give a supernatant, the mitotic apparatus rinse, which was also free of mitotic apparatus and a pellet which constituted the mitotic apparatus fraction. A sample of non-mitotic apparatus fraction was also made up by pooling equal fractional parts of the whole egg rinse, supernatant and mitotic apparatus rinse fractions, and this was centrifuged for 90 min at 36,000 r.p.m. in a Spinco ultracentrifuge with a No. 40 rotor, to obtain a supernatant containing the fraction which was 'soluble' in the isolation medium used (molar hexanediol, *p*H 6·0), and a pellet of non-mitotic apparatus particulate material, presumably including ribosomes, mitochondria, and other particles sedimentable in more conventional homogenization media.

For determination of incorporated radioactivity, samples were fixed with an equal amount of 10 per cent trichloroacetic acid containing a 1,000-fold excess of leucine-^{12}C, the TCA-insoluble material was collected on 'Millipore' filters, and repeatedly washed with leucine-^{12}C in 5 per cent TCA, and the 'Millipore' filters were mounted in planchettes and counted in a thin end-window Geiger counter. From previous experience, we consider this radioactivity to be in protein.

Fig. 1 shows the percentage of total radioactivity that is found in the various fractions for an isolation of mitotic apparatus at first metaphase (88 per cent of the isolated mitotic apparatus at metaphase). In agreement with the results of Stafford and Iverson[3], some radioactivity is recovered in the mitotic apparatus fraction, but incorporation into mitotic apparatus was never in excess of 10 per cent, although the experiment was repeated several times.

In view of the autoradiographic findings of Gross and Cousineau[2], we had expected a much larger percentage of incorporation into the isolated mitotic apparatus. However (unless we are dealing with a species difference), we find that a considerable portion of earliest incorporation of amino-acid, which is localized in the mitotic apparatus region of eggs fixed *in vivo*, is not retained in that region during isolation of mitotic apparatus.

We have to some extent further characterized this fraction of labelled material which is detachable from the mitotic apparatus region during isolation of mitotic apparatus. In the first place, most of it is particulate in the isolation medium used. This can be seen from Fig. 1*B*, which shows that by far the larger part of the non-mitotic apparatus label is in the particulate fraction. The material is therefore either particulate to start with, or it is precipitated by the isolation medium.

In the second place, it appears that the detachable material is most strongly localized in the mitotic apparatus region at metaphase, in fact moving cyclically into and out of the mitotic apparatus region as the latter grows and recedes. This is in fact the behaviour found by Gross and Cousineau[2] for the labelled material in autoradiographs, but for mitotic apparatus, isolated at prophase and metaphase, we find that although the absolute incorporation into an equal number of mitotic apparatuses is greater at metaphase than at prophase (Fig. 2), in fact giving an increase of incorporation with time that is close to linear, the percentage of incorporation into the mitotic apparatus fraction is almost exactly the same in the two cases. Therefore, it is apparently the detachable material that fluctuates cyclically in localization.

It is unlikely that much of the detachable labelled material consists of spindle fibres, astral rays, or other classically recognizable structural components of the mitotic apparatus, because microscopic evidence strongly suggests that these are well preserved in the isolated mitotic apparatus; this is particularly true of spindle fibres in that Kane's[5] electron micrographs of mitotic apparatus isolated by his method show an abundance of the 20-mμ filaments which apparently constitute the classical spindle fibres. Moreover, as discussed by Gross and Cousineau[2], there are a number of components other than the classical structural components of mitotic apparatus which are concentrated in the *in vivo* mitotic apparatus region (see, for example, the electron micrographs of Harris[6]). Some of these components may be concentrated passively by the exclusion from the growing mitotic apparatus region of the yolk and other large

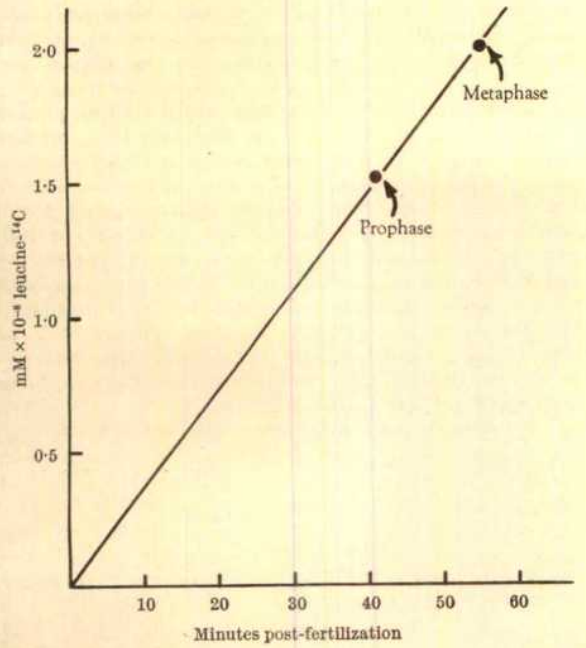


Fig. 2

particles which constitute a significant fraction of the cytoplasm of sea urchin eggs. These components include ribosomes, vesicular elements which Harris calls the 'matrix' of the mitotic apparatus[6], and possibly also soluble proteins. Furthermore, the amounts of both vesicular elements and ribosomes appear reduced in the electron micrographs of Kane[5], when fixation is in the isolation medium.

Our results do not bear directly on the broader question of whether the detachable material plays a part in the functioning of the mitotic apparatus. We have not, in fact, ruled out the possibility that the detachable material is bound *in vivo* to structural components of the mitotic apparatus and that the binding is in some way disrupted by the isolation medium. Whether or not it is so bound, the *in vivo* localization of the detachable material in the mitotic apparatus region is consistent with a functional role, for example, an enzymatic role as suggested by Gross and Cousineau[2].

If we cannot dismiss the possibility that the detachable material is bound under physiological conditions, neither can we dismiss the possibility that the bound material is, properly, detachable. Some label remains with the isolated mitotic apparatus and might in fact represent incorporation into the structural components mentioned above. However, in view of the small percentage of label which remains with the mitotic apparatus, and in view of the fact that the isolation of mitotic apparatus as a cohesive unit requires special stabilizing conditions, it also seems possible that the label which is isolated with mitotic apparatus represents a part of the detachable material which has 'coprecipitated' with mitotic apparatus in the course of its stabilization, perhaps irreversibly. The possibility of such a redistribution of label is illustrated by an experiment, done in collaboration with Dr. G. Giudice, which shows that when metaphase eggs, after exposure as above to leucine-^{14}C, are homogenized in a more conventional medium (0·44 M sucrose containing 0·005 M citrate and 0·01 M *tris*(hydroxymethyl)-aminomethane, at *p*H 7·8) considerably more of the incorporated radioactivity is found in the 'soluble' fraction, obtained by the same centrifugation procedure, than the 10 per cent reported above for conditions under which mitotic apparatus can be isolated; if the 'soluble' fraction is then made molar in hexanediol and titrated to *p*H 6·0, then an amount of label greater than that which is recoverable with isolated mitotic apparatus is precipitated from the soluble fraction, as judged from the fact that it appears in the pellet on recentrifugation.

Autoradiographic investigations of leucine incorporation in early development of *Paracentrotus* eggs are being carried out.

This investigation was supported by U.S. Public Health Service postdoctoral fellowships 1–*F2–CA*–6933–01 and 1–*F2–GM*15–45101. We are indebted to Dr. Paul R. Gross for helpful discussion of the manuscript.

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VIROLOGY

Growth of Bovine Parainfluenza-3 Virus in Chick Tissues growing on Polythene

A BOVINE strain of myxovirus parainfluenza-3 was first isolated by Reisinger *et al.*[1] in cultures of embryonic bovine kidney cells. Recently, isolates have been made by intra-amniotic inoculation of 14-day-old chick embryos[2]; but evidence of a successful isolation was only obtained by titration of the amniotic fluids on embryonic bovine kidney cells.

Using the method of tissue culture on polythene described by Fulton[3], a bovine strain of parainfluenza-3 virus was found to multiply in tissues from 10- to 11-day-old chick embryos. The virus in freeze-dried monkey kidney culture fluid was passed twice in rhesus monkey kidney cultures growing in bottles and was collected by freezing and thawing. The supernatant had a hæmagglutinin titre of 1/128 when tested with 0·5 per cent guinea-pig erythrocytes at 4° C.

The virus was inoculated on chick lung, chick muscle, chick skin or chick intestine growing on disks of polythene. The disks were floated on Medium 199 and were

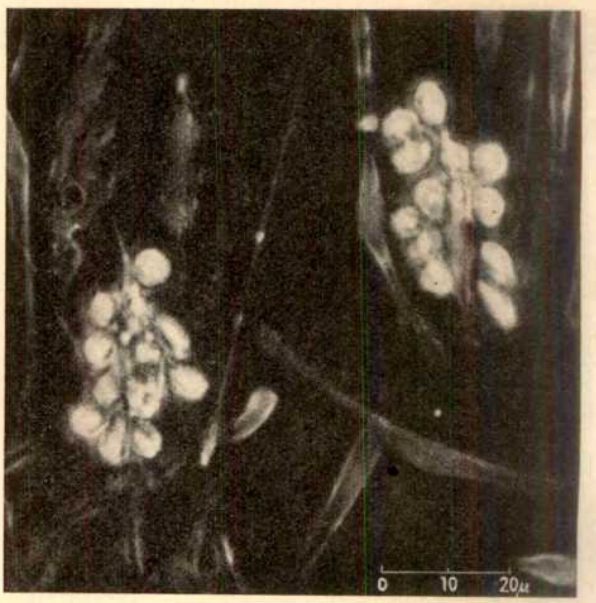


Fig. 1. Foci of hæmadsorption of guinea-pig erythrocytes after infection of chick lung with a bovine strain of myxovirus parainfluenza-3. Anoptral negative phase-contrast illumination. (*c.* × 875)

examined with phase-contrast illumination after 48 h. There was no apparent difference between the infected and the control disks; but if a 2 per cent suspension of guinea-pig erythrocytes was allowed to sediment on to the disks at 4° C, infected cells in all the tissues were labelled by the adsorption of erythrocytes. The foci of hæmadsorption were observed only on spindle-shaped cells (Fig. 1). The guinea-pig erythrocytes were hæmolysed within 15–20 min if the disks were stored at 4° C. When the disks were kept at room temperature, the erythrocytes were eluted from the cells very rapidly.

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SOIL SCIENCE

Studies of Sensitivity and Electro-kinetic Potential in Leda Clay

In a previous communication[1], an anomalous difference was pointed out between two supposedly similar geological deposits from widely separated regions of the world. Whereas a relationship between sensitivity (the ratio of undisturbed shear strength to remoulded shear strength) and pore-water salt concentration has been reported for some Norwegian marine soils, this was not true for Leda clay from the Ottawa–Hull area in the St. Lawrence Lowland region of eastern Canada.

This has now been substantially confirmed at several new locations in the Ottawa–Hull area. Except for one location, the pore-water salt content is low and the sensitivity is not uniquely dependent on the amount of salt present. Fig. 1 shows that at specific conductivities of less than 4×10^{-3} ohm^{-1} cm^{-1} the sensitivity varied from 11 to 1,000+; as expected, at high specific conductivities the sensitivity remained relatively low.

What has been particularly puzzling, and for which no reason has been advanced, is the wide variation in sensitivity that is observed in this area for soils of low pore-water salt concentrations, which sometimes appear almost identical in other respects such as strength, water content, grain-size distribution, and clay mineral content.

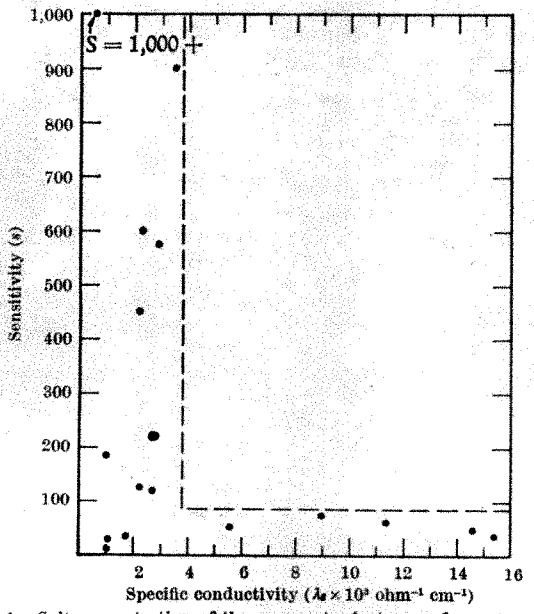


Fig. 1. Salt concentration of the pore water in terms of specific conductivity, as a function of sensitivity

Measurements of the electro-kinetic potential in undisturbed samples of this soil have shown that potential increases directly with increasing sensitivity. The electro-osmotic technique was selected because it has an important advantage for this type of investigation as it can be adapted to measure the electro-kinetic potential of undisturbed soil in its natural pore-water electrolyte environment. Because of the difficulties that are involved in electro-osmotic measurements and the uncertainties in using the appropriate values for dielectric constant and viscosity in calculating the potential, the numerical values recorded in this communication have a formal significance only, although they may be more confidently used for comparative purposes.

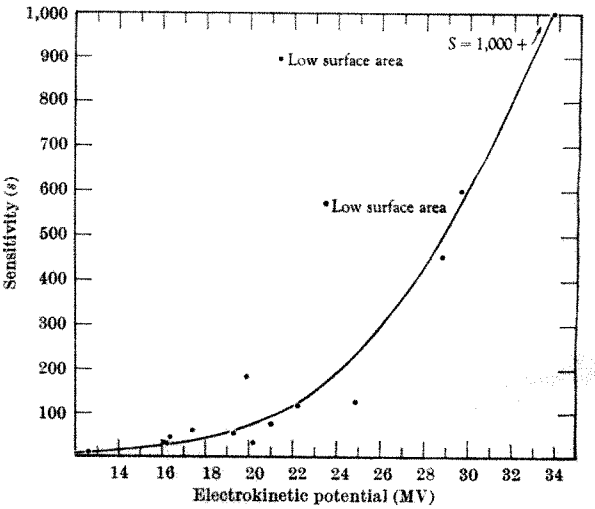


Fig. 2. Electrokinetic potentials of undisturbed Leda clay as a function of sensitivity

The results shown in Fig. 2 are for the same soils as in Fig. 1. Although there is scattering, the trend is thought to be sufficiently definite to be significant. The greatest deviations are for those soils that have a low surface area as compared with the average, although the clay-size content is essentially the same. One must assume from this that there are also purely physical factors which influence sensitivity.

The trend of the measured relationship between sensitivity and electro-kinetic potential—a first step in our investigations—simplifies the understanding of the wide variations in sensitivity, and such variations in potential need no longer be postulated to explain the phenomena. It follows that the higher the electro-kinetic potential, hence also the interparticle repulsion, the greater will be the loss in strength expressed in terms of sensitivity. The basic causes for the large variations in electro-kinetic potential at low pore-water salt content are not known in most instances, but in at least one case a change in the Na^+/Ca^{++} ratio in the pore water has been thought to be responsible.

In other investigations, measurements of the electro-kinetic potential at various stages of leaching (field pore-water salt concentration 10–15 gm/l.) gave an increase in potential as leaching proceeded. This supports Rosenqvist's theory of leaching to explain the formation of Norwegian quick clays. There is some doubt, however, based on recent geological evidence[2], about the marine origin of Leda clay; it is now thought that a large proportion of the Leda clay deposits in the Ottawa–Hull area consists of re-worked marine sediments that have been re-suspended and subsequently sedimented in an almost fresh-water environment as the salinity of the

Champlain Sea was decreasing. In one location near Ottawa a salty marine clay remnant appears to be flanked by such deposits. A similarity which is characteristic of all the deposits is the flocculated structure, but it is known that rather small concentrations of electrolyte during sedimentation are required to bring this about. If, in fact, these are essentially fresh-water deposits, the existing theory for sensitivity based on the salt-leaching is not satisfactory. Some progress is being made in exploring sensitivity on a new basis.

The main intent of the present communication is to show the trend in the measured relationship between electro-kinetic potential and sensitivity. Details of the apparatus and technique and more complete results are to be published shortly.

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GENETICS

'Selfers' and High Mutation Rate during Meiosis in *Ascobolus immersus*

DEMEREC[1–3] applied the term 'selfers' to "auxotrophic mutants of bacteria in which transduction with homologous phage gives rise to significantly larger numbers of wild-type variants than occur by spontaneous reversion in uninfected control bacteria". Lissouba *et al.*[4] found that some mutants affecting ascospore pigmentation in *Ascobolus immersus* produce wild-type 'recombinants' in homo-allelic crosses, whereas numerous other whitespore mutants when selfed never give wild-type revertants. However, this phenomenon has not been investigated in detail. We have examined one such mutant, '186', more precisely, because it produced the 'recombinants' in relatively high frequency. Homo-allelic crosses of this mutant were carried out and tetrad analysis applied. It was characteristic that wild-type spores were found mainly in asci with 6 : 2 segregation, namely, 6 spores '186' and 2 wild. In the case of normal back mutation before meiosis, asci with 4 mutant and 4 wild spores should be obtained. Among 127,964 tetrads examined, 38 of the 6 : 2 and 3 of the 4 : 4 asci were found (frequencies 0·000297 and 0·000023, respectively). Asci with segregation 4 : 4 in homologous cross may represent the normal rate of spontaneous back-mutations that correspond to Demerec's control experiments, because only one set of genes is present during mitosis. In turn, the 6 : 2 tetrads can originate only during meiosis. In meiosis two sets of genes are present in the nucleus, so that the situation is analogous to the transduction experiments with 'homologous phage'. Assuming that the 4 : 4 asci result from back-mutations in mitosis and the 6 : 2 asci from back mutations in meiosis, it is obvious that the frequency of this phenomenon is about 10-fold higher in meiosis than in mitosis. Presumably this difference is even greater if we take into account the clonal effect which works only on back-mutations in mitosis, increasing the number of wild-type nuclei that could enter meiosis.

There are at least three possible explanations of the 'selfers' phenomenon: (*a*) unequal crossing-over; (*b*) copy-choice (non-reciprocal exchange) connected with illegitimate conjugation of similar components of structures involved in recombination; and (*c*) stimulation of mutability of the locus by its homologue. Demerec excluded the first and second possibility in his experiments. The third, however, gives actually no information of the mechanisms involved. We crossed all six white spores from one 6 : 2 ascus obtained in the cross '186' × '186' with the mutant '186' to see if there would be any difference in the frequency of the 6 : 2 asci in these crosses, because one could expect that two spores—the second product of unequal crossing-over—would behave differently from the other mutant spores. No difference was found between these crosses and the initial one as to the frequency of the 6 : 2 and 4 : 4 asci, which makes the hypothesis of unequal crossing-over rather improbable in this case. It is impossible to check the second hypothesis because we have no deletion mutant covering the site examined (as in Demerec's case).

In this situation we cannot distinguish between the second and the third hypotheses, which assume an increase of the mutation rate in meiosis. Whatever the mechanism of origin of the asci described, it is evident that in the site examined the mutation rate (if it is really mutation) in meiosis is higher than in mitosis. This is in agreement with the results obtained by Magni and von Borstel[5] with a number of genes in *Saccharomyces*. Magni[6] found later that there is no preferential mutation rate in either of the two chromosomes involved. We crossed double mutant '231' × '186' ('231' is located about 1·3 crossing-over units away from '186' and has the same phenotypic expression as '186') with the mutant '186'. In this situation we expected the frequency of the 6 : 2 asci to equal a half of that found in the cross '186' × '186', if the reversion occurs at random in all of the four strands. Among 95,972 asci examined, 15 of the 6 : 2 and 1 of the 4 : 4 tetrads were found (frequencies 0·00015 and 0·00001, respectively), which was in agreement with our assumption and also with Magni's results. On the other hand, Magni found that reversion was highly correlated with the exchange of the outside markers, suggesting that the phenomenon in question was due to the unequal crossing-over.

The analogy between our results and those obtained by Demerec and by Magni is striking. Possibly it may be a more general phenomenon that intimate contact of the two homologous fragments of genetic material can stimulate reversions. The molecular basis of this phenomenon is still unknown.

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PSYCHOLOGY

Androgen-induced Reversal of Parental Behaviour in the Female Rabbit

DURING the course of recent experiments designed to investigate endocrine influence on the developing nervous system androgens have been given to pregnant guinea-pigs[1,2] and rats[3] without apparently disturbing parental behaviour at, or after, parturition. The effect of such treatment on the pregnant rabbit does not seem to have been previously reported. This has now been examined by injecting six rabbits during pregnancy with testosterone propionate in arachis oil. Three ('Chinchillas') received five injections of 5 mg beginning on the 10th day of pregnancy and on each 4th day afterwards; the other three animals ('New Zealand Whites') were all injected daily with 5 mg from the 17th until 21st day, 2·5 mg from the 22nd until 25th day and 1·0 mg from the 27th until

29th day. In other respects, such as caging, cleaning, lighting, temperature and diet, the animals were treated exactly as were non-injected pregnant rabbits. Litters were born within the gestation period found to be normal in this Department (31 ± 2 days).

None of the mothers exhibited normal parental behaviour. When given straw they made no attempt to build a nest and they did not pluck their fur before parturition. After the young were born the mothers showed no retrieval behaviour, but left the offspring lying about the cage for some hours, discouraging attempts at suckling, before chewing them. All members of each litter were eaten or fatally mutilated within 24 h from birth. Although such cannibalism sometimes occurs with normal rabbits[4] it has been rare in this laboratory. Since the behaviour of the male rabbit is characterized by a comparable initial indifference to offspring followed by cannibalism this phenomenon may perhaps be regarded as a reversal of parental behaviour.

It would be of interest to know why androgens during gestation cause behavioural reversal in some species but not others, and whether such effects are correlated with the presence (in the rat and guinea-pig) or absence (in the rabbit) of a mechanism for the cyclic release of gonadotrophins. The male rat does not exhibit spontaneous cyclic release of gonadotrophins and it has been shown[5] that intrahypothalamic injections of testosterone in this animal initiate female parental behaviour. However, another mechanism, unconnected with presence or absence of reproductive cycles, may be involved. Spayed rabbits, given daily injections of progesterone, build maternal nests of fur when the administration of the hormone is discontinued[6], and nest-building in this species has been attributed to the cessation at parturition of progesterone secretion by the corpora lutea[7]. It does not seem likely that this phenomenon is prevented by giving androgen during pregnancy. More probably, the action of testosterone in the pregnant rabbit is to render the hypothalamic regions involved in parental behaviour refractive to reduced blood levels of progesterone. It may tentatively be postulated that disturbances of maternal behaviour including the wish not to suckle in animals and, perhaps, humans may be associated with an abnormal release of androgens from the adrenal cortex occasioned by such factors as emotional stress.

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STATISTICS

Estimating the Malthusian Parameter from Census Data

In reporting[1] the results of experiments designed to examine consequences of the theory surrounding the concept of the innate capacity for increase, or Malthusian parameter[2,3], attention was directed to an unexplained discrepancy. From a linear regression of log total numbers on time, the experimental populations increased by an average of 6·082 times per three weeks, with 1 per cent confidence limits of 6·026 and 6·138, whereas age-specific life and fertility data independently obtained suggested that the value should have been 4·478. This discrepancy alone would have meant no more than that the experimental methods used in obtaining the life and fertility data depressed some or all of the rates of development, etc., but, by coincidence as it now seems, another method of evaluating the census observations suggested a value of 4·664, which is in accord with that calculated from the life and fertility table data. This value was obtained as follows: it was assumed that the population growth could be described as a first order process:

$$Mn_t = n_{t+1}$$

where M is a matrix the elements of which are numerical values of the dependence of stage i at time $t + 1$ on stage j at time t ($i, j = 1, 2, \ldots, s$), where there are s morphologically distinguishable stages in the population, and n_t and n_{t+1} are column vectors specifying the number of individuals in each stage at times t and $t + 1$. From census observations made at intervals of 3 weeks, M was estimated for this period by:

$$M = (V^{-1} W)'$$

where V is the variance/covariance matrix between the numbers of individuals at time t and W is the covariance matrix between the numbers of individuals at times t and $t + 1$. The first estimate, that forming the basis of the earlier discussion[1], will be referred to as M_1 and was:

$$M_1 = \begin{bmatrix} 0{\cdot}250 & 1{\cdot}998 & 4{\cdot}734 & -0{\cdot}009 \\ 1{\cdot}596 & 0{\cdot}173 & 25{\cdot}023 & 12{\cdot}021 \\ 0{\cdot}152 & 0{\cdot}042 & -0{\cdot}110 & 1{\cdot}032 \\ -0{\cdot}133 & 0{\cdot}854 & 1{\cdot}022 & -0{\cdot}231 \end{bmatrix}$$

which has a dominant latent root of 4·664 with an associated stable (column) vector of [0·455 0·875 0·055 0·152]'.

Dr. R. W. Howe, of this Laboratory, pointed out to me that this estimate of M used variances and covariances which had been corrected for the mean; if the experimental methods had achieved the results for which they were designed, namely, the removal of harmful 'density' effects, then regressions should pass through the origin, that is, corrections for the means should not be made. A new estimate of M was then made; this will be referred to as M_2. This matrix:

$$M_2 = \begin{bmatrix} 0{\cdot}341 & 1{\cdot}803 & 4{\cdot}119 & -0{\cdot}785 \\ 1{\cdot}203 & 0{\cdot}998 & 27{\cdot}776 & 15{\cdot}309 \\ 0{\cdot}125 & 0{\cdot}101 & 0{\cdot}085 & 1{\cdot}264 \\ -0{\cdot}160 & 0{\cdot}875 & 0{\cdot}919 & -0{\cdot}279 \end{bmatrix}$$

has a dominant latent root of 5·521 with a stable (column) vector of [0·347 0·925 0·058 0·141]'. This latent root is obviously in much closer agreement with the increase implied by the slope of the linear regression estimate of the totals on time.

Since there does not seem to be an algebraic method for the estimation of the standard errors of the latent roots of a matrix, the elements of which have known or estimated variances, it is not clear whether the 1 per cent confidence limits for this latent root include the linear regression estimate. At present, therefore, whether a discrepancy remains is uncertain. Further investigation of the underlying distributions[1,4] and their implications will be necessary before this problem can be solved.

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, November 23

INSTITUTE OF ACTUARIES (at Staple Inn Hall, High Holborn, London, W.C.1), at 5 p.m.—Mr. J. R. Ford and Mr. C. M. Stewart: "Population Trends in Great Britain".

BRITISH SOCIETY FOR THE HISTORY OF SCIENCE (in the Council Room of the Science Museum, Exhibition Road, South Kensington, London, S.W.7), at 5.30 p.m.—Dr. Marie Boas Hall: "Henry Oldenburg and the Art of Scientific Communication".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. R. F. Hoskins: "Loop Gain and Return Difference of Transistor Amplifiers".

ROYAL INSTITUTION OF NAVAL ARCHITECTS (at the North East Coast Institution of Engineers and Shipbuilders, Bolbec Hall, Newcastle upon Tyne), at 6 p.m.—Mr. W. D. Horsley: "The High Speed Generator—Eighty Years of Progress" (Sir Charles Parsons Memorial Lecture).

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Lady Williams, C.B.E.: "Vocational Training". (Second of three Cantor Lectures on "A Comparative Study of World Trends in Education".)

INSTITUTION OF MECHANICAL ENGINEERS, MANIPULATIVE AND MECHANICAL HANDLING MACHINERY GROUP (in the Renold Building of the College of Science and Technology, Manchester), at 6.45 p.m.—Discussion on "The Mechanics of High-Speed Weaving".

Tuesday, November 24

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p.m.—Mr. D. J. Robinson: "A Visit to Venezuela's El Dorado".*

UNIVERSITY OF LONDON (at the Institute of Education, Malet Street, London, W.C.1), at 5.15 p.m.—Dr. Joyce Long: "Universities and Those Outside Them".*

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, London, S.W.1), at 5.30 p.m.—Mr. J. L. Beckett and Mr. H. R. Oakley: "A Combined Scheme of Sewage Disposal and Refuse Composting in Leicester".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 5.30 p.m.—Sir Lawrence Bragg, F.R.S.: "Waves and Vibrations". (Afternoon lecture for Sixth Form Boys and Girls from Schools in London and the Home Counties. Further lectures on November 24, and December 1 and 2.)

UNIVERSITY OF LONDON (at Imperial College of Science and Technology, London, S.W.7), at 5.30 p.m.—Prof. E. P. Wohlfarth: "Magnetism in Theory and Practice" (Inaugural Lecture).*

UNIVERSITY OF LONDON (at Queen Elizabeth College, 61–67 Campden Hill Road, London, W.8), at 5.30 p.m.—Dr. P. T. Haskell: "The International Fight Against the Locust".*

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. M. G. P. Stoker: "Tumour Viruses". (Ninth of fourteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)*

ROYAL AERONAUTICAL SOCIETY (joint meeting with the Institution of Electrical Engineers, at Savoy Place, London, W.C.2), at 6 p.m.—Discussion meeting on "Unconventional Methods of Generating Electrical Power in the Aircraft of the Future".

INSTITUTION OF MECHANICAL ENGINEERS, GRADUATES' AND STUDENTS' SECTION (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6.30 p.m.—Mr. A. J. Barter: "Locomotive Maintenance".

SOCIETY OF CHEMICAL INDUSTRY, PLASTICS AND POLYMER GROUP (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. J. I. Cunneen: "Cis-trans Isomerism in Polyisoprenes".

SOCIETY FOR ANALYTICAL CHEMISTRY, SPECIAL TECHNIQUES GROUP (at the Chemical Society, Burlington House, Piccadilly, London, W.1), at 7 p.m.—Twentieth Annual General Meeting, followed by Scientific Films.

Wednesday, November 25

INSTITUTION OF CHEMICAL ENGINEERS (in the Connaught Rooms, Great Queen Street, London, W.C.2), at 9.45 a.m.—Discussion Meeting on "The Economist's View of the Process Industries".

INSTITUTE OF PETROLEUM (in association with the Institution of Heating and Ventilating Engineers, at the Royal Aeronautical Society, 4 Hamilton Place, London, W.1), at 4 p.m.—Symposium on "District Heating".

ROYAL STATISTICAL SOCIETY (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.15 p.m.—Mr. S. Paul Chambers, C.B., C.I.E.: "Statistics and Intellectual Integrity" (Inaugural Address of the President).

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. D. St. J. Seigne: "Marine Electric Propulsion".

CHALLENGER SOCIETY (at the Linnean Society, Burlington House, Piccadilly, London, W.1), at 5.45 p.m.—Dr. T. F. Gaskell: "The Prospects of Oil or Gas from Under the North Sea".

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, JOINT I.E.R.E.–I.E.E. COMPUTER GROUPS (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Discussion on "Logic Circuit Symbols" opened by Mr. J. A. Lawrence and Mr. J. H. Smith.

INSTITUTION OF MECHANICAL ENGINEERS (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Sir Robert Cockburn: "Materials and the Engineer" (Thomas Hawksley Lecture).

ROYAL METEOROLOGICAL SOCIETY (in the Conference Hall, County Hall, Westminster Bridge, London, S.E.1), at 6 p.m.—Dr. J. Hallett: "The Origins and Growth of Ice in Our Atmosphere".

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP—FOOD ENGINEERING PANEL (joint meeting with the Industrial Water and Effluents Group, at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Mr. A. Henchley and Mr. R. Cunningham: "Effluent Disposal in an Edible Oil Refinery and Margarine Factory".

INSTITUTION OF ENGINEERING INSPECTION (at the South-West Essex Technical College, Forest Road, Walthamstow, London, E.17), at 7.30 p.m.—Mr. J. C. McCullagh: "Inspection in the Radio and Electronics Industry".

Thursday, November 26

FERTILISER SOCIETY (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 2.30 p.m.—Prof. J. F. Sutcliffe: "The Absorption of Inorganic Nutrients by Plants" followed by a discussion.

INSTITUTION OF ELECTRICAL ENGINEERS, SCIENCE AND GENERAL DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Colloquium on "Plasma Jets".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. W. Lane-Petter: "Sophisticated Laboratory Animals". (Tenth of fourteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation).*

INSTITUTION OF MECHANICAL ENGINEERS, LUBRICATION AND WEAR GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Standards in Britain for Lubrication and Lubricants".

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (joint meeting with the Heavy Organic Chemicals and Chemical Engineering Groups, at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Dr. Ing. B. Orsoni: "The Italian Heavy Organic Chemical Industry".

Friday, November 27

UNIVERSITY OF LONDON (at the Institute of Neurology, National Hospital, Queen Square, London, W.C.1), at 5.30 p.m.—Prof. W. Feindel (Montreal): "Radio-Isotopic Studies on Cerebral Circulation in Man".*

INSTITUTION OF MECHANICAL ENGINEERS, APPLIED MECHANICS GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion Meeting on "New Pathways with Kinematic Linkages".

BRITISH SOCIETY FOR THE HISTORY OF SCIENCE (in Room 45, Leicester College of Technology, The Newarke, Leicester), at 7 p.m.—Dr. John Butt: "James Young, Scientist, Industrialist, Philanthropist".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Dr. P. H. Greenwood: "Explosive Speciation in African Lakes".

Saturday, November 28

BOTANICAL SOCIETY OF THE BRITISH ISLES (in the Botany Department, British Museum (Natural History), Cromwell Road, London, S.W.7), from 2 p.m. to 5.30 p.m.—Annual Exhibition Meeting.

LONDON COUNTY COUNCIL (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Zoe, Lady Hart Dyke: "The Silk Makers".

GLACIOLOGICAL SOCIETY (joint meeting with the Scott Polar Research Institute, at the Scott Polar Research Institute, Cambridge), at 8.30 p.m.—Mr. John T. Andrews: "Baffin Island".

Monday, November 30

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (joint meeting with the I.E.R.E. Medical and Biological Group, at Savoy Place, London, W.C.2), at 2.30 p.m.—Discussion on "Lasers".

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 2.30 p.m.—Anniversary Meeting.

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.15 p.m.—Dr. T. F. Gaskell and Captain G. S. Ritchie, D.S.C., R.N.: "Beneath the North Atlantic".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT LECTURER (graduate, preferably with an interest in microbiology) IN BOTANY—The Principal, Wigan and District Mining and Technical College, Wigan (November 27).

LECTURER (with an M.Sc. or Ph.D. in microbiology or bacteriology, and experience in parasitology) IN MICROBIOLOGY at the University of Nigeria, Nsukka, Eastern Nigeria—The London Representative, University of Nigeria, 33 Craven Terrace, London, W.2 (November 30).

SENIOR LECTURER IN BIOCHEMISTRY; LECTURERS IN CIVIL ENGINEERING (2); a SENIOR LECTURER IN RESEARCH (Physics); and a SENIOR LECTURER IN RESEARCH (Electrical Engineering)—The Principal, Nottingham Regional College of Technology, Burton Street, Nottingham (November 30).

CHAIR OF SOCIOLOGY—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (December 1).

LECTURER or ASSISTANT LECTURER IN PHARMACOLOGY—The Secretary, Chelsea College of Science and Technology, Manresa Road, London, S.W.3 (December 1).

RESEARCH ASSISTANT (with a good honours degree in physics or chemical engineering and a special interest in particle mechanics or fluid dynamics or rheology) IN THE MINING ENGINEERING DEPARTMENT, to work on particle fluid flow in model systems, with special reference to blood—The Registrar, The University, Nottingham (December 1).

EXPERIMENTAL OFFICER/SENIOR EXPERIMENTAL OFFICER (with experience in the application of modern analytical techniques and in the organization of routine analytical work, and preferably experience in the application of auto-analytical procedures) to take charge of a chemical and biochemical analytical laboratory—The Secretary, Hill Farming Research Organization, 29 Lauder Road, Edinburgh, 9 (December 2).

LECTURERS (2) (with experience in numerical analysis and/or systems programming) IN THE DEPARTMENT OF COMPUTER SCIENCE—The Registrar, The University, Manchester, 13 (December 5).

RESEARCH ASSISTANT (graduate interested in genetics, biochemistry or microbiology) IN THE DEPARTMENT OF BOTANY, to work on problems concerned with the genetics and physiology of yeast—The Registrar, Queen Mary College, Mile End Road, London, E.1 (December 10).

LECTURER IN PHYSIOLOGY—The Secretary, The Queen's University, Belfast, Northern Ireland (December 17).

LECTURER/SENIOR LECTURER (graduate in veterinary science, agricultural science or rural science, with extensive postgraduate experience and preferably a higher degree) IN ANIMAL HUSBANDRY at the University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, December 18).

READER IN COMPUTING at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Brisbane and London, December 18).

SENIOR LECTURERS, LECTURERS OR ASSISTANT LECTURERS IN MATHEMATICS—The Registrar, University of East Anglia, Earlham Hall, Norwich, NOR. 05G (December 18).

SENIOR LECTURERS OR ASSISTANT LECTURERS IN PHYSICS—The Registrar, University of East Anglia, Earlham Hall, Norwich, NOR. 05G (December 18).

CHAIR OF PHYSICAL METALLURGY—The Registrar, University College of Swansea, Singleton Park, Swansea (December 31).

CHAIR OF PHYSICS—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (December 31).

SENIOR LECTURERS OR LECTURERS IN PURE MATHEMATICS AND APPLIED MATHEMATICS at Monash University, Melbourne, Australia—The Registrar, Monash University, P.O. Box 92, Clayton, Victoria, Australia, or The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, December 31).

TEACHING FELLOW (with an appropriate honours degree or equivalent qualifications, and preferably a background of organic and/or experience in organo-analytical chemistry) IN THE SCHOOL OF CHEMISTRY, University of New South Wales—The Agent General for New South Wales, 56–57 Strand, London, W.C.2; and The Appointments Section, The University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia (December 31).

CHAIR OF SOCIOLOGY—The Registrar, The University of Essex, Colchester, Essex (January 5).

I.C.I. RESEARCH FELLOW IN CHEMISTRY, BIOLOGICAL CHEMISTRY, ENGINEERING, PHARMACOLOGY, PHYSICS, or in a related subject—The Registrar, The University, Senate House, Bristol, 2 (February 1).

SENIOR LECTURER IN THE DEPARTMENT OF PATHOLOGY, University of Melbourne, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, February 1).

ENTOMOLOGIST (with a degree in zoology, postgraduate experience in the ecology of mosquitoes and other blood-sucking flies, and administrative experience) in the Cayman Islands, to undertake research and control of nuisance mosquitoes, with responsibility for the laboratory, field and laboratory staff—The Appointments Officer, Ministry of Overseas Development, Room 404, Eland House, Stag Place, London, S.W.1, quoting Ref. RC/205/36/01.

GRADUATE RESEARCH ASSISTANT IN MEDICAL MYCOLOGY—The House Governor, Brompton Hospital, London, S.W.3, quoting Ref. R.A.I.

JUNIOR LECTURER OR LECTURER (medical graduate) IN THE BACTERIOLOGY DEPARTMENT—The Dean, Guy's Hospital Medical School, London Bridge, S.E.1.

LECTURER (graduate) IN ZOOLOGY—The Bursar, Harper Adams Agricultural College, Newport, Shropshire.

LECTURERS OR SENIOR LECTURERS (well qualified and preferably significant research and/or industrial experience) IN (a) INORGANIC CHEMISTRY; (b) PHYSICS; and (c) METALLURGY OR MATERIALS—The Academic Registrar, Loughborough College of Technology, Loughborough, Leicestershire, quoting Ref. 25/G.

MASTER TO TEACH MATHEMATICS throughout the School including Sixth Form; and a MASTER TO TEACH BIOLOGY to ordinary level and BOTANY AND ZOOLOGY to advanced level—The Headmaster, King's College School, Wimbledon, London, S.W.19.

POST-DOCTORAL RESEARCH FELLOW IN THE DEPARTMENT OF CHEMISTRY for the study of the gas phase reactions of methylene and ethylidene—The Secretary and Registrar (Room 9), The University, Highfield, Southampton.

REGIONAL TRIALS OFFICER (graduate in horticulture or with equivalent qualifications) at Stockbridge House, Yorkshire, for variety trials of vegetable crops—The Secretary, National Institute of Agricultural Botany, Huntingdon Road, Cambridge.

SENIOR LECTURERS (with good academic qualifications in chemical engineering, or an allied discipline) IN CHEMICAL ENGINEERING—The Academic Registrar, Loughborough College of Technology, Loughborough, Leicestershire, quoting Ref. 24/G.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

The British Steel Castings Research Association. Recommended Procedure for the Ultrasonic Examination of Steel Castings. Pp. vi + 49. (Sheffield: The British Steel Castings Research Association, 1964.) [610

The Institute of Navigation. Annual Report 1963. Pp. 18. (London: The Institute of Navigation, 1964.) [610

Glasshouse Crops Research Institute. Annual Report 1963–64. Pp. 18. (Rustington, Littlehampton: Glasshouse Crops Research Institute, 1964.) [610

Maxwell's International Subject Bibliographies. No. 2: Oceanography including Marine Biology. Pp. 25. No. 4: Design and Construction of Scientific Laboratories. Pp. 5. No. 5: Dermatology, Venereology and Physiology of the Skin. Pp. 14. No. 6: Orthopaedics including Rehabilitation of the Physically Handicapped. Pp. 16. No. 7: Spectroscopy and Spectrometry. Pp. 15. No. 15: Graphic Arts and Industries. Pp. 41. No. 25: Cartography. Pp. 12. No. 33: Industrial Toxicology and Occupational Health. Pp. 19. (Oxford: Robert Maxwell and Co., Ltd., 1964.) [610

National Soil Survey of Ireland. Soil Survey Bulletin No. 1: Soils of Co. Wexford. By M. J. Gardiner and Pierce Ryan. Pp. xiii + 171 (31 plates). (Dublin: An Foras Taluntais (The Agricultural Research Institute), 1964.) 30*s*. [610

University of London. University College Calendar, 1964–65. Pp. lxxxvii + 470. (London: University College, 1964.) [610

University of Aberdeen: School of Agriculture. Miscellaneous Publications No. 2: Index of Scientific and Economic Studies of Particular Significance to the Pig Industry of the United Kingdom, Part 3. By J. B. Dent. Pp. xv + 65. (Aberdeen: School of Agriculture, The University, 1964.) 6*s*. [610

The Association of the British Pharmaceutical Industry. First Report of the Expert Committee on Drug Toxicity submitted to the Association on 24th July, 1963. Pp. 24. (London: The Association of the British Pharmaceutical Industry, 1964.) [610

University Grants Committee. Returns from Universities and University Colleges in receipt of Exchequer Grant, Academic Year 1962–63. (Cmnd. 2456.) Pp. v + 46. (London: H.M. Stationery Office, 1964.) 5*s*. 6*d*. net. [610

Other Countries

United States Department of the Interior: Geological Survey. Water-Supply Paper 1736: Compilation of Records of Surface Waters of the United States, October 1950 to September 1960. Part 12: Pacific Slope Basins in Washington and Upper Columbia River Basin. Prepared under the direction of E. L. Hendricks. Pp. xii + 415 + plate 1. Water-Supply Paper 1739: Compilation of Records of Surface Waters of Hawaii, July 1950 to June 1960. Prepared under the direction of E. L. Hendricks. Pp. vii + 136 + plates 1–5. Water-Supply Paper 1747: Geology and Hydrology of the West Milton Area, Saratoga County, New York. By F. K. Mack, F. H. Pauszek and J. R. Crippen. Pp. viii + 110 + plates 1–6. Water-Supply Paper 1750–B: Summary of Floods in the United States during 1959. Prepared under the direction of E. L. Hendricks. Pp. vi + 101. Water-Supply Paper 1754: Preliminary Results of Hydrogeologic Investigations in the Valley of the Humboldt River near Winnemucca, Nevada. By Philip Cohen. Pp. v + 59 + plates 1–6. (Washington, D.C.: Government Printing Office, 1964.) [510

United States Department of the Interior: Geological Survey. Water-Supply Paper 1758: Surface-Water Hydrology of Coastal Basins of Northern California. By S. E. Rantz. Pp. v + 77 + plates 1–6. Water-Supply Paper 1773: Geology and Ground-Water Resources of the Anchorage Area, Alaska. By D. J. Cederstrom, Frank W. Trainer and Roger M. Waller. Pp. v + 108 + plates 1–4. Water-Supply Paper 179–AA: Stream Hydrology Related to the Optimum Discharge for King Salmon Spawning in the Northern California Coast Ranges. By S. E. Rantz. Pp. iii + 16. Water-Supply Paper 1779–D: Hydrology and the Effects of Increased Ground-Water Pumping in the Anchorage Area, Alaska. By Roger M. Waller. Pp. iv + 36 + plates 1–5. Water-Supply Paper 1779–F: Hydrologic Studies of Small Watersheds, Honey Creek Basin, Collin and Grayson Counties, Texas, 1953–59. By C. R. Gilbert, G. G. Commons, G. E. Koberg and F. W. Kennon. Pp. v + 98 + plates 1–3. Water-Supply Paper 1779–I: Ground Water in the Sandpoint Region, Bonner County, Idaho. By Eugene H. Walker. Pp. iv + 29 + plate 1. Water-Supply Paper 1779–J: Geology and Water Resources of the Bluewater Springs Area, Carbon County, Montana. By Everett Z. Zimmerman. Pp. iv + 24 + plate 1. Water-Supply Paper 1779–P: Geology and Ground Water of the Luke Area, Maricopa County, Arizona. By R. S. Stulik and F. R. Twenter. Pp. iv + 30. Water-Supply Paper 1779–Z: Selected Bibliography on Laboratory and Field Methods in Ground-Water Hydrology. By A. I. Johnson. Pp. iii + 21. (Washington, D.C.: Government Printing Office, 1964.) [510

United States Department of the Interior: Geological Survey. Professional Paper 352–E: Geomorphology of Segmented Alluvial Fans in Western Fresno County, California. By William B. Bull. Pp. iv + 89–129 + plates 6 and 7. Professional Paper 403–D: Petrology of the Limestones of Guam. By Seymour O. Schlanger. With a section of Petrography of the Insoluble Residues by J. C. Hathaway and Dorothy Carroll. Pp. iv + 52 + plates 1–21. Professional Paper 415: Geology and Mineral Deposits of the Thomas and Dugway Ranges, Juab and Tooele Counties, Utah. By Mortimer H. Staatz and Wilfred J. Carr. Pp. vi + 188 + plates 1–9. Professional Paper 442–D: Channel Patterns and Terraces of the Loup Rivers in Nebraska. By James C. Brice. Pp. iv + 41. (Washington, D.C.: Government Printing Office, 1964.) [510

United States Department of the Interior: Geological Survey. Professional Paper 429–B: Ecology of Benthonic Species. By Patsy B. Smith. Pp. iv + 55 + plates 1–7. Professional Paper 440–L: Data of Geochemistry. Technical Editor: Michael Fleischer. Chapter L: Phase Equilibrium Relations of the Common Rock-Forming Oxides Except Water. By George W. Morley. Pp. ix + 158. Professional Paper 448–B: General Geology of the Mississippi Embayment. By E. M. Cushing, E. H. Boswell and R. L. Hosman. Pp. iv + 28 + plates 1 and 2. Professional Paper 452–A: Principles of Underfit Streams. By G. H. Dury. Pp. iv + 67 + plates 1 and 2. Professional Paper 452–B: Subsurface Exploration and Chronology of Underfit Streams. By G. H. Dury. Pp. iv + 56. Professional Paper 474–F: Water Content of Micas and Chlorites. By Margaret D. Foster. Pp. iii + 15 + plate 1. Professional Paper 497–B: Removal of Water and Rearrangement of Particles During the Compaction of Clayey Sediments—Review. By Robert H. Meade. Pp. iv + 23. (Washington, D.C.: Government Printing Office, 1964.) [510

New Zealand. Report of the Director-General of Forests for the year ended 31 March 1964. Pp. 59. (Wellington: Government Printer, 1964.) [610

Population Reference Bureau, Inc. Population Bulletin, Vol. 20, No. 5 (September, 1964): New Patterns in U.S. Fertility. Pp. 113–140. (Washington, D.C.: Population Reference Bureau, Inc., 1964.) 50 cents. [610

Year-Book of the Institute of Nutrition, 1963. Pp. xiv + 86. (Budapest: Institute of Nutrition, 1964.) [610

Annals of the New York Academy of Sciences. Vol. 115, Article 2: Computers in Medicine and Biology. By Walter E. Tolles and 96 other authors. Pp. 543–1140. (New York: New York Academy of Sciences, 1964. 9 dollars. [610

Editorial and Publishing Offices of "NATURE"
MACMILLAN (JOURNALS) LIMITED
ST. MARTIN'S STREET, LONDON, W.C.2
Telephone Number: Whitehall 0081. Telegrams: Phusis London W.C.2

Annual subscription: Inland £13, Overseas £13 15s., payable in advance, postage paid to any part of the world.

Advertisements only should be addressed to
T. G. Scott & Son, Ltd., 1 Clement's Inn, London, W.C.2.
Telephone Number: Holborn 4743

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SCIENCE AND TECHNOLOGY AND THE NEW LABOUR GOVERNMENT

AFTER his recent election as Prime Minister, the Right Hon. Harold Wilson lost no time in re-organizing the Government and appointing Ministers and other personnel to carry out the policies already announced or to be developed in due course.

This includes science and technology: the various science, technology and education Ministers have already been selected and their names announced, and even scientific advisers who are not members of either the House of Commons or the House of Lords have been, or are about to be, appointed. Consultation with non-partisan scientists by the Government of the day is not a new idea, either in Britain or elsewhere; but it is nevertheless very worth while, indeed essential, and it seems that the Prime Minister is determined to take full advantage of it.

These are early days, of course, so any form of criticism is best deferred; nevertheless, we must recognize that it is quite clear that the Prime Minister and his Government are determined to do the best they can, in their own way (after consultation with distinguished scientists both within and outside Parliament), for the advancement of science and technology and their application in industry and medicine and for other benefits to the community.

Whether the Government takes the right, and most advantageous, course remains to be seen, though even at this initial stage it is encouraging to know that men who have already proved themselves in the various branches of science, technology and education have been appointed to the highest political and advisory posts. (Incidentally, it is to be hoped that as a result of the Government's aims and objectives, at any rate in science and technology, set out for public approval, and the appointment of appropriate men to carry them out, we shall no longer read (mainly in the lay Press) such ugly terms as 'boffins' and 'backroom boys'.)

It is perhaps significant and indicative of the importance attached in the new Government to science that so early as during the debate on the Queen's Speech at the opening of the new Parliament on November 3, Mr. Robert Maxwell (Member for Buckingham), who is well known throughout the world of science and science journal and book publication, should have, in his maiden speech, made the organization of the Government for science and technology his main theme.

During his speech, Mr. Maxwell directed the attention of the House of Commons to the great benefits which industry and the country can obtain by making use of the results of research in science, engineering and medicine. After discussing various problems which need solving in his own constituency, graciously commenting on the good work done there by his Parliamentary predecessor, Sir Frank Markham, Mr. Maxwell said:

"I very much welcome the Government's proposals to help our industries to gain the full benefit of advances in scientific research and technology. At this point, I wish to refer to the strictures which the Leader of the Opposition seemed to think it right to cast on the creation of the Ministry of Technology. . . . The House may wonder what authority I have to deal with these matters. I was chairman of one of the working parties appointed by my right hon. Friend the Member for Coventry East (Mr. R. H. S. Crossman) in his previous capacity as Opposition Front Bench spokesman on science and education, and chaired the committee on science, Government and industry. Also, I am a publisher of scientific magazines and books and I am in the closest touch with scientists from all over the world. . . .

"I hope to be able to prove to the House that the new Ministry of Technology is certainly one of the answers that the Government needed in order to get British industry to apply the results of research faster and better than it has done in the past. It is now well understood that the growth of our economy, the welfare of our citizens, our national security and the aid that we can afford to give to under-developed countries all depend to a growing extent on the effective use that our industries make of new technology.

"In recent years the rate of increase in our gross national product per worker and *per capita* has slowed down and has been substantially less than the increase of almost all highly industrialized nations. It is apparently not fully realized that scientific discovery followed by technological research and development produces nothing, other than knowledge, for society. They must be followed by their applications through the combined use of capital, and equipment and human resources—labour and management—to produce an economic good.

"It is commonly accepted that management in British industry, both private and nationalized, by and large has failed badly to make use of science and technology as an aid to increased productivity and profitability. For example, of the first fifty ethical drugs prescribed by doctors under the National Health Service, in order of sales value used in this country, only three were discovered and developed in this country—ordinary penicillin and the new Beecham penicillins, 'Broxil' and 'Penbritin'.

"It is generally agreed that one of the major obstacles preventing the wider application and use of science and technology in British industry is that there does not seem to be, at present, an effective organization or method to convey to individual companies, their managements and foremen, the new technology in a form which points the way to its practical applications.

"The other major problem is the great gulf, and lack of communication, which exist between the pure scientists and the applied scientists, the universities, the technical colleges, the trade research associations, industry and Government, and, finally, the gap which exists between management and scientists in individual firms.

"The whole issue may, therefore, be summed up as being a problem in communication of information and the need to change attitudes of mind. I submit that the creation of the Ministry of Technology is a massive and positive step in bringing about the necessary alteration and to obtain the needed change of attitude.

"The Government should provide something which has been lacking in our country for a long time—a sharp and independent means for recognizing when the mission of a Government research and development establishment has lost its validity, and the practical means for re-directing the establishment into more productive channels either within or outside the Government Department that originally sponsored it. When the independent nuclear deterrent is abolished, the problem of what to do with the

Aldermaston Weapons Research Establishment is a good example of the kind of problem which I have in mind.

"The present system of awarding development contracts tempts private companies to talk their way into a development programme with promises of results which wise technical judgment would deem unattainable. . . . The present arrangement does not provide for adequate penalties for failure to achieve promised results, nor does it give sufficient incentives for a high level of technical performance. It offers incentives to contractors to make systems complex and expensive or to prolong the development work. All this is most wasteful of our vital scientific and engineering manpower as well as of the taxpayer's money.

"Finally, the present defence research contract arrangement with its built-in competitive incentives and inadequate penalties for poor technical performance leads to the proliferation of many research and development groups in private industry of sub-critical size or quality. This is another important example of where the new Government's changes in the organization of science and engineering may prove to be most helpful and valuable both in saving taxpayers' money and in making better use of our scarce national resources in science and engineering.

"The Government and our scientific and engineering community should make it one of their major joint tasks to employ our new-found ability to combine the great diversity of scientific and engineering skills and disciplines to make a massive assault on very large-scale national problems. The effectiveness of employing this new Government tool has been demonstrated during the Second World War and in the massive American and Russian space programmes. The social innovation of use in peace-time of this new Government tool is of even greater consequence in the long run than the scientific and technical innovations on which most of our attention is presently focused. This is the spill-over from defence of the greatest national and social consequence, and we as a country have so far failed to use this instrument in peace-time. There can be no doubt that the development of this new capability has endowed us as a nation with great new powers. I am sure that the new Government will use this social invention for peaceful purposes and not just confine it to the defence sector.

"The Government should show the way how to use research and development in the modern inter-disciplinary way through industry to improve and raise the quality and excellence of the environment in which we work and live. Familiar examples of the material waste and erosion of the æsthetic environment which are very complex and which can only be solved on a multidisciplinary basis are traffic congestion and air and water pollution.

"The strength of British science depends on the initiative, imagination and intelligence of individual working scientists and engineers. The best possible programme formulated at the top can be made entirely ineffective by the people who are carrying it out. The purposes of organization for science and engineering in the Government must be to ensure quicker identification and support of new ideas and maintain support for basic research and development to guarantee that the most important national technological jobs are tackled by the most able people. I am convinced that the Government's arrangements for the organization of science and technology will do that."

It is hoped to publish later in *Nature* Mr. Maxwell's description and views of the new Labour Government's organization for science and technology in Great Britain. It is hoped that he will also show how the new Government's organization for science and technology compares and differs from those now in existence in the other scientifically advanced major countries.

The opinions and ideas of Mr. Maxwell are what we have been awaiting for a considerable time. However, one cannot help but feel that much that is talked about development of scientific progress into industrial practice is so much political ammunition, and only the next few months will show if the present Government is successfully on the way to taking full advantage of scientific research.

THE SCIENTIFIC SPIRIT

The Scientific Intellectual

The Psychological and Sociological Origins of Modern Science. By Lewis S. Feuer. Pp. xii+441. (New York and London: Basic Books, Inc., Publishers, 1963.) 10.00 dollars.

HARDY, in *A Mathematician's Apology* (pp. 18–19, Cambridge University Press, 1948), once referred to the motives which, in his opinion, lead men to prosecute research of practically any kind, be it psychology, classics or mathematics; they were "intellectual curiosity", "professional pride", and finally, "ambition, desire for reputation . . . even the power or the money, which it brings". Prof. Feuer, in this weighty volume, attempts to dig more deeply, to peer beneath the surface of such conscious and mundane considerations, using some of the vernacular of psychoanalytical jargon in the process. His question is, what inspired—at least at the psychological level—the rise of the scientific attitude and of the revolution in science, particularly since the seventeenth century? His major conclusion is that the scientific movement and the scientific intellectual are born from the "hedonist-libertarian spirit". Some of the traits of the hedonist ethic are freedom from a sense of "primal guilt", "asocial" in character, if not downright "subversive". Since the seventeenth century beginnings, scientists find "joy and pleasure in scientific activity itself"; they are utilitarian and individualistic, and above all they seek a conception of the universe which is "less fear-ridden, less anxiety-surrounded" than its predecessors. They also seek to rid themselves of "dependence-relations", an expression of a strong authoritarian super-ego: priests, elders and confessors often seem to have retarded scientific progress, and the individualism of the Jewish and Protestant spirit may be expected to yield useful results for scientific progress.

Feuer takes to task particularly earlier explanations of the rise of science, such as R. K. Merton's view that Puritanism and ascetic Protestantism were strongly linked with the growing curiosity concerning the phenomena of Nature, a view itself traced back both to Marxist and Weberian notions. In particular, he rejects (though without argument) a contrary theory of a brother psychoanalyst, Norman O. Brown, that rather than hedonism, "the essence of the scientific spirit [is] to be mercilessly ascetic, to eliminate human enjoyment from our relation to nature, to eliminate the human senses" (compare p. 316, *Life against Death*, Vintage ed., 1961, p. 316), a passage which in turn reflects the views of the distinguished French writer on the psychoanalytical background of science, Gaston Bachelard, whom Feuer does not mention at all.

Feuer indeed does not spend much time on confuting alternative hypotheses, nor consider any individual counter examples. Instead, we get a vast array of a scholarly collected (or rather 'selected') multitude of facts and features which, though they may not 'prove' the author's thesis, are at least intended to illustrate it. This, as every good scientist knows, is an exceedingly dangerous under-

taking. Feuer's wholesale paraphrase of the complex social phenomenon called 'modern science' under one single aspect, and his illustrative method of backing his doctrine, to one reader at least smacks all too much of the ancient alchemists' apocryphal search for the universal solvent. There is no serious argument against alternative interpretations, no sustained breaking down of the complex idea of science; nor is the psychoanalytical approach employed with much more than popularizing terminology. Sometimes, simplification is taken to absurd extremes. To cite just one instance: Chapter 3 discusses the "nominalist recovery of the sense of reality". Here, the medieval doctrine of the "reality of universals" is contrasted with incipient nominalism, as exemplified in the philosophy of Occam. There is no mention here of further important alternatives, such as Aristotelian (rather than Platonist) realism, nor of conceptualism. We are told (p. 87) that the realm of universals "was the projective universe of the celibate scholastics", Duns Scotus being cited as an example. But Occam, though eventually expelled from the Franciscan order, was surely a celibate also! Besides, it is misleading to imply that realism died in the fourteenth century. Surely it has had important defenders to the present day; a glance at any text-book on the foundations of mathematics will show this. In any event, when the psychoanalytic writer tries to 'explain' philosophical and social phenomena by means of such questionable concepts as "the reality-principle" and "reality-thinking"—notions which, though they may have their origin in Freud, are dismissed by other psychoanalysts as naïve (compare Brown, *op. cit.*, p. 235), we may say that it will be well to sit back and wait until the psychoanalysts have put their own house in order before we can be sure that their putatively illuminating commentaries are more than *obiter dicta*, and constitute a serious approach towards the sociology of science.

This is not to say that a patient reader may not extract much that is of interest from this volume. It covers a very large field, and in the process it has dug up or collected from primary sources much that will be useful to the careful scholar, ranging as it does from Asia via the Jews to the scientific intellectual in the United States (Chapter 11). Moreover, it is useful to be confronted by a more sophisticated approach towards the phenomenon of 'the scientific spirit' than the more simple-minded and more usual notion of the 'patient collection of facts'.

GERD BUCHDAHL

POPULAR ASTRONOMY ILLUSTRATED

The Flammarion Book of Astronomy

Translated into English by Annabel and Bernard Pagel. Pp. 670. (London: George Allen and Unwin, Ltd., 1964.) 100*s*. net.

ASTRONOMIE Populaire, by Camille Flammarion, was first published in 1880. It was an immediate success, and did much to establish Flammarion's reputation as a writer on popular science. Many subsequent editions have appeared in French, suitably brought up to date, and in 1894 an English translation was produced by J. Ellard Gore.

The last French edition appeared in 1954, by which time there was very little of the original text left. The whole book had been overhauled and expanded, with new sections written by various eminent French astronomers. Only the general plan, as laid down by Flammarion, remained unaltered.

A new English translation has long been overdue, and its appearance is therefore particularly welcome. It has been based on the 1954 text, translated by Annabel and Bernard Pagel, and again revised; there are sections by various French astronomers, including A. Danjon, A. Dollfus and C. Fehrenbach, while some material has also been added by one of the translators, B. Pagel, of the Royal Greenwich Observatory. The presentation leaves nothing to be desired; in view of the lengthy text coupled with the lavish illustrations (some of which are in full colour), the price of £5 is by no means excessive.

The style is non-technical, and the whole text may therefore be followed by the beginner, but a great deal of information useful to the serious student is also given. The general impression is one of excellence, and the book will certainly find its way into most scientific libraries.

The first section deals with the Earth as an astronomical body, with special emphasis on its motion in space. This is followed by almost 70 pages devoted to the Moon, illustrated with many fine photographs taken at the Pic du Midi and other major observatories. Considerable space is given to problems of the lunar surface itself, to which French astronomers have always paid great attention; the Moon's movements are also described in detail, and there is a chapter on lunar eclipses.

The next hundred pages deal with the Sun in all its aspects. This means giving an outline of elementary spectroscopy, and the result is very satisfactory; the chapter headed "Inside the Sun" is, for example, a model of clarity. Following this, the planets of the Solar System are described one by one. Some observers, including myself, will consider that the colours on the planetary drawings are rather too definite, but this is a matter of opinion, and it is not easy to give any precise standards of reference.

Minor planets are included with the large bodies of the Solar System, but comets and meteors are treated in a separate section. There is then a long description of the stellar universe, which will be particularly useful to the serious student even though there is relatively little about cosmology. Book 7, "The Instruments of Astronomy", is relatively brief, since it covers less than twenty pages and could well have been expanded, though it is clear and concise. Artificial satellites and space vehicles are treated separately, and at the end of the book there are useful tables of astronomical data.

It is inevitable that there should be a few points of criticism, particularly as the book is so long and so comprehensive. First, it must be said that the translation, though satisfactory on the whole, is not flawless; lunar observers will look in vain for the "Mare Pluvium", while the Sinus Iridum is translated as "Gulf of Irises". There are also some cases of curious grammar, and a few sentences which should have been tidied up. There are, moreover, various minor misprints which will presumably be weeded out in the next edition.

The other defect in the book is one which can scarcely be avoided in a project of this magnitude. Progress in astronomy is now so rapid that it is difficult to keep up to date even in the case of a book which can be produced quickly, and in an elaborate publication there is no hope of avoiding a considerable time-lag between the final preparation of the text and the subsequent appearance of the book. This means that recent developments cannot be included, which is unfortunate but inevitable. It is a pity that some particularly important discoveries have had to be ignored; for example, there is nothing about quasi-stellar objects or about the close-up lunar photographs obtained by the U.S. vehicle *Ranger VII*.

On the other hand, it seems that too little attention has been directed to making the book as up-to-date as was possible in the circumstances. Some of the sections give the impression of having been written years ago and left unaltered, though in a few cases footnotes have been added. A typical example occurs on p. 202, where there is a footnote stating that "at the time of writing news was received that on December 12, 1952, the solar spectrum was photographed from a height of 80 kilometres". Even the brief note about the pictures of the Moon's far side, obtained in 1959 by the Russian probe *Lunik III*, seems to have been tacked in as an afterthought. Further-

more, on p. 572 we read that "collisions between galaxies are very noisy in the radio wave band", though elsewhere (p. 547) it is correctly stated that the whole theory of colliding galaxies has now fallen out of favour. The whole of the section dealing with artificial satellites and interplanetary probes is out of date, and it would have been wiser to make it complete up to the latest possible date (presumably 1962 or 1963) and explain why subsequent developments could not be included.

However, these shortcomings are very minor when the book is considered as a whole, and it would be most unfair to place undue emphasis on them. On balance, this is certainly the best book of its type to have been produced in English during the past quarter of a century, and it will undoubtedly run to many editions. Revisions can be made from time to time, but the bulk of the text will need no drastic amendment for some years, except in the unlikely event of some spectacular discovery which overturns many theories which have become thoroughly established. All those who have been concerned in the production deserve the highest congratulations, and it is to be hoped that the book will have a wide circulation.

PATRICK MOORE

CRYOGENIC PROCESSES IN GEOLOGY

An Introduction to the Theory of the Formation of Frozen Rocks

By I. A. Tyutyunov. Translated from the Russian by J. O. H. Muhlhaus. (International Series of Monographs on Earth Sciences, Vol. 6.) Pp. xiv + 94. (Oxford, London, New York and Paris: Pergamon Press, 1964.) 50*s*.

THIS most welcome and fascinating book is the first to describe the physical and chemical changes that occur in regions of permafrost. Although active permafrost research is a restricted investigation, the contents of this book should arouse widespread interest in the more general fields of rock weathering, erosion, soil formation and clay mineralogy. The translation from the original Russian text has been ably carried out, although some passages appear to be rather unwieldy.

The main theme throughout the book is concerned with the energy that can be liberated by water during sequences of freezing and thawing and how, as a result, minerals covered by films of water are vigorously attacked. Permafrost investigations are covered by the field of cryogeny which is concerned with the complex physical, chemical and mineralogical changes in the upper strata of the lithosphere and hydrosphere at a negative geothermal level of heat exchange. The liberation of energy is described in terms of crystal fragmentation by water resulting in a tremendous increase in potential energy at the water surface layer. The rate and efficiency of conversion of energy in terms of mineral alteration in areas of permafrost are related to the degree of condensation of the potential surface energy and to the concentration of substances (cations, anions) in reactions at such locations. The site where energy changes occur at the water/mineral surface interface is referred to as the 'double surface layer'. The changes involved are dealt with in detail and the formation of cryogenic minerals is described, such as those formed by interaction of Na, K, Mg with Cl, SO_4. Through the process of freezing, water provides a significant agency for influencing the concentration and nature of solute present in terrestrial water.

The power of pellicular water in terms of potential energy and penetration is considered to parallel the forces of tectonic processes and is ranked as an outstanding geological factor that must be seriously considered in such environments. Changes concomitant with freezing are described as differentiation processes, and essentially result from dehydration and coagulation of colloids during freezing. The stronger the coagulation of suspended material in the soils the greater the result in the progressive increase in the segregation of ice, to such an extent that ice schlieren are formed—a cryogenic texture.

The book introduces various physical and chemical theories in simple terms and develops them to an advanced state. Although the salient points of permafrost investigations are outlined, geologists would perhaps welcome a more detailed account, particularly as this is the only book on the subject. Also a further chapter describing the practical effect of physical and chemical processes that occur in frozen rocks would be useful for mining and construction engineers.

Permafrost investigations are a rather specialized field of research; however, the approaches and conclusions discussed in this book will undoubtedly be welcomed by those interested in earth sciences.

E. I. HAMILTON

HANDBOOK OF ELECTROPLATING

Handbook of Industrial Electroplating

By E. A. Ollard and E. B. Smith. Third edition. Pp. x + 400. (London: Iliffe Books, Ltd.; New York: American Elsevier Publishing Company, Inc., 1964.) 65*s*.

IN their foreword Messrs. Ollard and Smith disclaim any attempt to produce a text-book, and the title happily defines their work as a *Handbook of Industrial Electroplating* and this it indisputably and most excellently is. No one, accustomed to the problems that arise daily in a modern plating shop and who has studied this book, can fail to believe that a copy of it is an essential item in the personal equipment of every plating shop manager.

Excellence in commercial electrodeposition, to a greater degree perhaps than in most crafts, depends on infinite capacity for taking care, and emphasis on this fact runs like a thread of gold through the whole book. It is certain that the reader who specializes in any section of the vast field covered by the authors will be stimulated to seek out and understand the theories which have contributed so much to the present rapid development of knowledge in this field. Such stimulation alone is rich reward for the possession and study of this book.

This constant need for care in the management of a modern electroplating shop is underlined by the fact that the authors have, with great insight, devoted more than one quarter of the actual reading matter to testing both depositing solutions and the deposits themselves. For some, these pages will themselves be of absorbing interest and for others an invaluable source of reference.

The growing body of regulation covering industrial effluent disposal fully justifies the attention the authors have devoted to this subject. None whose duty it may be to "design, erect or run" plating plants can afford to ignore the information on this aspect that is so readably set out in this book.

The references to, and information about, automatic and semi-automatic plants whet the appetite for more, and it is to be hoped that in later editions the authors will devote a full chapter to this aspect of plating-shop equipment. Much of the success or failure of operating platers depends on the human factor, and the advent of the completely automated plant must be the dream of many a plating-shop manager.

For many, the most valuable section is that devoted to costing and organization, especially the paragraph on estimating and the very valuable statistics on productivity. It is to be hoped that future editions will continuously bring these statistics up to date and will amplify them with some indication of the relationship between productivity and the percentage of installed vat time actually used in the period the statistics cover. Those interested in national productivity, and curious as to

where the shop, for which they may be responsible, stands in regard to the plants listed, will have food for thought in the fact that the largest shop, based on people employed, achieves barely half the turnover per employee achieved by the smallest shop, and little more than one-third of that achieved by the plant which is less than one-third its size. Of course, the comparative nature of the work, which the authors do not give, could, in part, explain these wide variations. Unfortunately, they accord too closely with Britain's general pattern, and it is to be hoped that in bringing these figures up to date in later editions the authors will give similar figures culled from the United States, to the benefit of their British and American readers alike.

The rapid advances in techniques for anodizing aluminium for exterior architectural work, that have been made over recent years, suggest that both revision and amplification of the authors' references to this subject would merit attention in future editions. Some comment, in the paragraphs on trichloroethylene degreasing, on the troubles experienced by some shops, which have used this process for cleaning aluminium intended for exterior architectural work, would also be welcomed in later editions. The short paragraph devoted to dyes for anodized coatings is too short to do the authors justice.

The attention to *BS.* 3382 in the chapter on plating threaded parts is surely inspired. It is becoming increasingly clear that the requirements of this specification are no more stringent than the minimum required to sustain and accelerate the growth of this market, which seems to have expanded more strongly since the publication of *BS.* 3382 than ever before.

The formulæ, so generously set out, cover much valuable information that, in the not distant past, would have been shrouded in commercial secrecy. Permission to publish such data displays confidence in the belief that what benefits the industry must benefit its members, and evokes the hope that later editions will include a glossary of troubles that platers must expect to meet and suggested methods to avoid or overcome them.

In conclusion, it must be said again that the authors have achieved in full their aim to produce "a handbook for those whose duty it is to design, erect, maintain, or run an electrodeposition plant" and, without doubt, this book should be on the shelves of all whose work is covered by the authors' description. J. Fox-Williams

BIOLOGICAL CLOCKS

The Physiology of Diurnal Rhythms

By Janet E. Harker. (Cambridge Monographs in Experimental Biology, No. 13.) Pp. vii + 114. (Cambridge: At the University Press, 1964.) 20*s.* net; 3.95 dollars.

PLANTS and animals show diurnal (or circadian) rhythms in their physiological processes and behaviour. In a natural environment, the timing of the phases of any rhythm is, within a pattern imposed by the genetical constitution of the organism (to use Dr. Harker's own words), primarily affected by light conditions, including both intensity and photofraction. But superimposed on the effects of light, variations may be induced by fluctuating temperature and humidity, amount of food present, age and sex of the organism, and the number of other animals of the same species present. However, once the phases of a circadian rhythm have been set, the rhythm will persist under constant conditions for many months or even throughout the life-time of an animal.

The phenomenon can be explained on alternative hypotheses. Either there is an endogenous mechanism, or there are external time signals which are perceptible to the organism, but not to the research worker. In a search for time-cues which would permit the latter hypothesis, F. A. Brown and his colleagues have invoked and investigated the roles of lunar and solar cycles and of changes in terrestrial magnetism and barometric pressure. According to Brown, for example, the diurnal pattern of oxygen consumption of organisms in a pressure-controlled chamber can be affected by the barometric pressure one to seven days ahead.

Dr. Harker's stated view is that neither theory can at present be proved. No evidence has been recorded to disprove the effects observed by Brown, even though his contention is not accepted by the majority of workers. Moreover, the adaptive significance of a short endogenous rhythm is not obvious; an immediate reaction to the prevailing environment would have many advantages. Nevertheless, the tenor of Dr. Harker's book supports the view that the ultimate controlling system of circadian rhythms is to be found within the organism, even though phasing factors are supplied by the environment. There seems to be little doubt, she says, that some clock-like cellular mechanism exists which is responsible for the observed physiological and biological rhythms. On another level she cites her own beautiful demonstration that a cockroach made arrhythmic in locomotor activity by keeping it for a long period in continuous light develops a diurnal rhythm when joined parabiotically with a rhythmically active cockroach which has been kept in alternating 12-hour periods of light and darkness. The source of the activity rhythm has been traced to a secretion of a group of neurosecretory cells lying on either side of the sub-œsophageal ganglion.

This attempt to summarize the major argument of Dr. Harker's monograph does not do full justice to its comprehensiveness or its logical presentation. The field of descriptive and experimental work on diurnal rhythms is explored and documented; their significance and the possible nature of biological clocks is fully discussed. The book should initiate a sphere of readers into an important branch of biology which has only developed within the past two or three decades. F. J. Ebling

RESEARCH ON WATER POLLUTION

Advances in Water Pollution Research

(Proceedings of the International Conference held in London, September 1962.) Edited by B. A. Southgate, Prof. W. W. Eckenfelder and Prof. E. A. Pearson. Vol. 1: Pp. xliii + 437. Vol. 2: Pp. vi + 578. Vol. 3: Pp. vi + 437. (London and New York: Pergamon Press, 1964.) 300*s.* net per set.

THE first International Conference on Water Pollution was convened with a view to disseminating information on recent advances in knowledge resulting from research in relevant fields, and was a logical outcome of the steady growth in the effort being devoted in an increasing number of countries to pollution prevention and control. The Conference was organized into three sections, the proceedings of each of which—comprising the sixteen papers presented and the accompanying discussion—form the main subject-matter of a separate volume. The first of these is concerned with the problem of pollution in streams and rivers, the second with the development of methods for treatment of effluents and of the solids derived from them, and the third with the disposal of effluents in estuaries and in the sea.

It is abundantly clear from these proceedings that water pollution research presents those concerned in it with a fascinating variety of problems covering a wide range of complex chemical, physical and biological phenomena. In the first volume the subjects covered include such diverse topics as the correlation of mass-transfer coefficients for the absorption of atmospheric oxygen in rivers with their hydraulic characteristics, the effects of liquid shear on invertebrates, the response of fish to temperature gradients, the occurrence of patho-

genic bacteria in rivers and the identification of trace organic chemicals. The second volume includes accounts of work on oxygen transfer, on the kinetics of aerobic and anaerobic treatment processes, on de-watering of sludges, on sedimentation and on methods of removal of nitrogen, phosphorus and biologically non-degradable organic compounds. The third volume deals largely with the development of rational procedures for the siting of outfalls for the dispersal of sewage, industrial, and radioactive effluents in the sea and with the prediction of the effects of polluting discharges and of natural phenomena on the distribution of dissolved oxygen and nitrogenous compounds in estuaries; the problem of pollution of the sea by oil is also examined.

In the case of each paper presented, at least one formal discussion contribution was prepared by a selected participant, and most of these rank as short papers in their own right; in addition, a good deal of pertinent discussion from the floor has been reported accurately in substance. In general these discussions form a most useful part of the *Proceedings* and in some cases even outweigh in value the paper under discussion or, by putting its contents into proper perspective, greatly enhance its value.

On the whole, these *Proceedings* are a chronicle of steady progress rather than revolutionary advance, interspersed, however, with the occasional exceptionally good paper such as one on the theory of the kinetics of gas absorption and one on the prediction of effects of pollution on the condition of water in an estuary.

There is no doubt that anyone concerned with the technical aspects of pollution problems would find a great deal that would be of interest in these volumes, and the research worker in the field should certainly have access to them.

A. L. Downing

PHYSIOLOGY OF MOLLUSCS

Physiology of Mollusca

Vol. 1. Edited by Karl M. Wilbur and C. M. Yonge. Pp. xiii+473. (New York and London: Academic Press, 1964.) 114*s*. 6*d*.

PHYSIOLOGISTS and zoologists alike look forward with interest to new volumes in the Academic Press series on physiology. The present volume, the first of two on the *Physiology of Mollusca*, is a welcome addition to the line and will be a useful work of reference for a long time. However, this volume illustrates the weakness of using a phylum or class as a frame of reference for a physiologist. Too often the reader feels that the most meaningful comparisons would be with members of other phyla rather than with other molluscs. Earlier volumes in the series, such as those on the fishes and the insects, had a unity, possibly derived in part from the adaptation of their particular group to one predominant environment. This unity is lacking in the present volume, and only occasionally, for example in the chapters on shell and muscle, are there signs of a peculiarly molluscan physiology emerging. This feeling of diffuseness is aggravated by our limited knowledge of molluscan physiology. Two volumes were devoted to each of the groups previously covered in this series and molluscs are also to receive two volumes. To fill them the subject matter has been widened to include ecology, embryology, comparative anatomy and systematics. Each of these chapters is admirable in its way and the authors have placed the emphasis on the physiological aspects of their topics, but when the two volumes together are so expensive as to be outside the pockets of many workers on the subject, a single more restricted volume might have been more valuable. The distribution of topics between the two volumes is rather arbitrary and makes it almost essential to buy them together. Muscles are dealt with in Volume 1, nervous physiology in Volume 2, ionic regulation in Volume 1, excretion in Volume 2, chromatophores in Volume 1, and pigment in Volume 2.

The book begins with a survey of molluscan structure and systematics. This is a fine essay in selection and compression by J. E. Morton and C. M. Yonge which readers not too familiar with the molluscs will find useful, yet few people interested in the molluscs will be without Morton's excellent little book which covers the same ground in a more expansive form. In the following chapters G. Newell surveys the ecology of intertidal molluscs and W. R. Hunter the ecology of nonmarine forms. Reproduction is a topic in which a peculiarly molluscan physiology may emerge, but V. Fretter and A. Graham's up-to-date survey shows that the subject is still largely in a descriptive phase. The wide variety of forms of hermaphroditism which occurs in the molluscs may surprise some mammalian physiologists. Embryonic development is described by C. P. Raven and this is followed by a largely technical account of the large-scale culture of marine bivalve larvæ by P. R. Walne.

The elegant geometry and convenient rigidity of the shell make the molluscs attractive material for the quantitative study of growth and ageing. Measurement and interpretation of growth are described by K. M. Wilbur and G. Owen, who also discuss the influence of metabolism, temperature and nutrition on growth rates. This is followed by an account of the specifically molluscan problem of shell formation by Wilbur, including the metabolism of shell formation, the organic matrix and the various forms in which calcium carbonate can crystallize. There is interesting evidence of a relationship between the nature of the organic matrix and the crystal type. This is a topic which may well throw light on the whole subject of skeleton formation, but, as Wilbur points out, it is curious that detailed information on the composition of the extrapallial fluid from which the shell is deposited is often lacking.

Osmotic and ionic regulation in the molluscs is dealt with by J. D. Robertson. One of the determining features of molluscan physiology here is the high permeability of the body wall which affects both the marine and freshwater forms. Robertson also discusses ionic and osmotic regulation in the tissues, a subject neglected until recently.

The wide variety of muscle conveniently available in the molluscs has made the group of unique importance to muscle physiologists. G. Hoyle provides an excellent critical survey of a complicated and disputed field and his article will be of value to all physiologists. Fine structure and biochemical studies are beginning to elucidate the structure of even the slowest molluscan muscles but, in contrast, knowledge of the details of the nervous control of the muscles is still sadly deficient. Two chapters by Morton describe a variety of minor topics such as bioluminescence and pigment cells, and locomotion. The mobility of the molluscan body has led to a remarkable range of methods of locomotion including both jet propulsion and a less-well-known screw locomotion in some pelagic gastropods. Pelagic molluscs have also developed two methods for obtaining neutral buoyancy. One depends on their powers of ionic regulation to produce a solution less dense but isosmotic with sea water, the other on the use of osmotic pressure to abstract water from a rigid shell to produce what is essentially a vacuum. These two phenomena are described by E. J. Denton, who was largely responsible for their discovery.

The volume is well produced and indexed and few errors were observed, although G. S. Carter did not state that hæmocyanin increased the oxygen carrying capacity of the blood by 3 per cent (p. 69) but to 3 volumes per cent. The description of the innervation of the anterior byssus retractor muscle (p. 331) would be clearer if the visceral connective, mentioned in the text, were labelled in the diagram and if the cerebropedal connective, also referred to in the text, were labelled as such and not as the cerebral connective.

W. T. W. Potts

ORIGIN OF THE CANYON DIABLO NO. 2 AND NO. 3 METEORITES

By Dr. DIETER HEYMANN
Enrico Fermi Institute for Nuclear Studies, University of Chicago

OF the thousands of irons which have been recovered from the Arizona Meteor Crater the vast majority are coarse octahedrites. However, careful searches by Nininger have turned up a small number of anomalous individuals designated as Canyon Diablo No. 2 and No. 3 (refs. 1 and 2). Both are medium octahedrites; they have a higher nickel content than Canyon Diablo No. 1 and often show a distorted and granulated structure.

There is no doubt that Canyon Diablo 2 and 3 are truly distinct from Canyon Diablo 1. The kamacite bands of Canyon Diablo 1 vary in width, ranging up to as much as 4–5 mm; whereas the kamacite bands in Canyon Diablo 2 average 1·4 mm in width. The nickel content of Canyon Diablo 1 is 7·11 per cent as against 8·09 per cent in Canyon Diablo 2, and the palladium concentrations are 3·98 p.p.m. and 5·30 p.p.m. respectively[3]. Nininger explained these chemical and structural differences in two ways. He suggested that Canyon Diablo 2 was a swarm of small iron meteorites, captured by the main mass while the latter was in orbit round the Sun. He also offered the alternative explanation that Canyon Diablo 2 and 3 were distinct falls but thought this possibility less likely. The capture hypothesis was not widely accepted because of the obvious unlikelihood of such an event. Goldberg *et al.* pointed out that the terrain round Meteor Crater is possibly the most extensively searched area for meteoritic material. This area is about 65 km² or $1{\cdot}3 \times 10^{-7}$ times the Earth's surface. A fall frequency on the Earth of about 35 iron meteorites a year[4] means that this area of 65 km² was hit on the average once in 230,000 years and twice in 460,000 years. (I have assumed that 6·7 per cent of all fallen meteorites are irons.) From other investigations[5] it appears possible that iron meteorites can survive on the surface of the earth for a million years or longer. The hypothesis of a distinct fall is, therefore, not altogether unlikely.

The evidence for distinct origins of Canyon Diablo 1, 2 and 3 is not very convincing, however, despite the chemical and structural differences. Canyon Diablo is one of the largest iron meteorites known. Pieces of the parent mass may have been spalled off during passage through the atmosphere and fallen near Meteor Crater. There is considerable disagreement as to the size of the projectile which produced the crater. Öpik[6] estimates a mass of 2·6 million tons, Shoemaker[7] gives 63,000 tons and Bjork[8] places the mass between 30,000 and 194,000 tons, depending on impact velocity. The lowest estimate corresponds to a diameter of 19·5 m. Thus it appears almost certain that the diameter of the projectile was at least 10 m. The three Canyon Diablo varieties might have been broken out from diametrically opposed locations, and, since nothing is really known about chemical and structural variations of iron meteorites on such a scale, a third hypothesis deserves consideration in my opinion, namely the one originally rejected: that Canyon Diablo 1, 2 and 3 did all come from the same object.

An essential difference among these hypotheses is that the first two assume the anomalous Canyon Diablo to have existed in space as small individual entities, whereas the third one assumes them to have resided in a single, large parent mass. These two possibilities can be distinguished experimentally inasmuch as they should result in markedly different concentrations of cosmogenic rare gases. Signer and Nier[9] have calculated the distribution of the cosmogenic nuclides helium-3, neon-21 and argon-38 in spherical meteoroids of different diameters, and their results are shown in Fig. 1.

This plot is based on measurements on the Grant meteorite which has a cosmic-ray exposure age of 640 ± 100 million years[10]. Between the break-up of its parent body, 640 m.y. ago, and its capture by the Earth, the

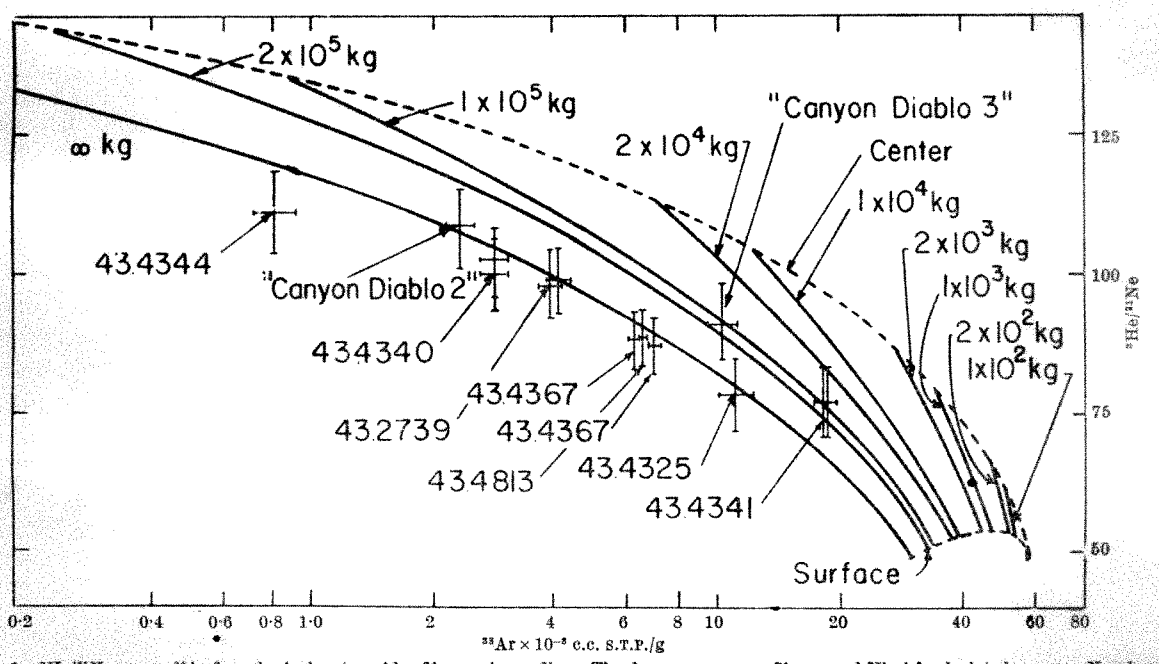


Fig. 1. $^3He/^{21}Ne$ versus ^{38}Ar for spherical meteoroids of increasing radius. The drawn curves are Signer and Nier's[9] calculated curves. Numbered points are measurements on Canyon Diablo 1

meteorite accumulated cosmogenic nuclides which were produced by high-energy nuclear reactions between cosmic-ray particles and the atomic nuclei of the meteorite. The concentration of any stable nuclide in a sample depends, of course, on the duration of the irradiation and the location of the sample below the surface of the meteorite before the latter entered the Earth's atmosphere. While this location cannot be derived from the observed location in the recovered object, due to considerable ablation losses in the atmosphere, the $^3He/^{21}Ne$ ratio in the Signer–Nier plot is a sensitive indicator of the depth of a sample; the higher this ratio the greater was the depth.

A measurement of helium-3, neon-21 and argon-38 in any meteorite which has the same exposure age as Grant should give a point on this plot corresponding to the pre-atmospheric mass of the meteorite and the location of the sample inside the object. A difference in cosmic-ray exposure age alone would merely result in a proportionate difference in argon-38 concentration and the point would be located on a curve which can be obtained from Fig. 1 by a simple horizontal displacement of the Signer–Nier curves. Unfortunately, the pre-atmospheric mass of a meteorite and hence the location of a sample in the object are usually unknown, but in special cases like Canyon Diablo it is clear that points must lie close to a Signer–Nier curve of infinite mass. Note that each sphere of given mass (masses range from 100 kg to infinity, that is, $\geqslant 2 \times 10^5$ kg) has a maximum $^3He/^{21}Ne$ ratio which occurs at the centre of the sphere.

In Fig. 1, I have also plotted my experimental results on seven different Canyon Diablo 1 specimens. A detailed discussion of the experimental procedure will appear elsewhere[11]. Evidently the results agree quite well with a Signer–Nier curve for a meteoroid of very large mass. The only specimen which is definitely outside the correlation is 43.4341. From the mean displacement of the Canyon Diablo 1 points to the left of the Signer–Nier curve for infinite mass, I conclude that the normal Canyon Diablo has a lower exposure age than Grant, namely 540 ± 100 million years, which is not in disagreement with a recently measured exposure age of 665 ± 65 million years by the ^{40}K–^{41}K method[12]. Shown also in Fig. 1 are results obtained on samples of Canyon Diablo 2 and 3. The results of individual measurements are given in Table 1.

Table 1. Cosmogenic Rare Gases in Canyon Diablo No. 2 and No. 3 Meteorites

Weight of sample (g)	Concentration, 10^{-8}			c.c. S.T.P./g		Ratios		
	^3He	^4He	^{21}Ne	^{36}Ar	^{38}Ar	$^3He/^{21}Ne$	$^3He/^4He$	$^3He/^{36}Ar$
1. Canyon Diablo No. 2, specimen No. 371.2								
0·3921	37·9	157	0·35	1·5	2·3	108	0·241	16·5
0·2834	34·7	151	0·33	1·4	2·3	105	0·219	15·1
0·2832	37·7	151	0·34	1·1	Lost	110	0·249	
Average	36·8	151	0·34	1·3	2·3	108	0·240	16·0
2. Canyon Diablo No. 3, specimen No. 586.1								
0·9183	180	664	1·96	6·57	10·3	92	0·271	17·5
0·6559	170	664	1·87	6·48	10·2	91	0·256	16·7
Average	175	664	1·92	6·53	10·3	92	0·264	17·0

The recovered mass of Canyon Diablo 2 is a few kilograms. I take this to mean that the meteorite was small, less than 500 kg if it was a 'satellite' or a distinct fall, unless most of its mass disappeared through weathering. The helium-3 content of Canyon Diablo 2, specimen 371.2, is about eight times lower than the highest helium-3 concentration measured in a Canyon Diablo iron: 301×10^{-8} c.c. S.T.P./g (43.4341). This result, taken alone, does not necessarily place Canyon Diablo 2 inside the main mass, since it is conceivable that this meteorite is a distinct fall and has a cosmic-ray exposure age of only 64 ± 12 million years. But this is unlikely. For one thing, all exposure ages of medium octahedrites measured so far are uniformly higher than 300 million years[13]. Moreover, the $^3He/^{21}Ne$ ratio of this specimen, 108 ± 7, is much higher than what one would expect to find inside a meteorite of a few hundred kilograms. The highest ratio observed in Grant (recovered mass 480 kg) is 82 ± 4 (ref. 10). Thus the $^3He/^{21}Ne$ ratio makes it extremely likely that specimen 371.2 was once inside a large mass: 10^4 kg or larger. Finally, the Canyon Diablo 2 point lies remarkably close to the curve defined by Canyon Diablo 1. In view of these facts, I conclude that the simplest explanation for the observed cosmogenic rare gas abundances is that Canyon Diablo 2 was once part of Canyon Diablo 1; was located approximately 50 cm below its pre-atmospheric surface; and was exposed to cosmic-ray irradiation for the same length of time as the main mass.

In the case of Canyon Diablo 3, the conclusion cannot be as definite. This iron has the third highest cosmogenic rare gas abundance of 47 Canyon Diablo individuals which I have examined. Moreover, its $^3He/^{21}Ne$ ratio does not fit well with the curve defined by Canyon Diablo 1, as can be seen from Fig. 1. The $^3He/^{21}Ne$ ratio of 92 is higher than what one would expect for this specimen: 82. Yet one detail in Fig. 1 leads me to the tentative conclusion that Canyon Diablo 3 also belonged to the main mass. Specimen 43.4341 seems to be displaced to the right of the Canyon Diablo 1 curve, yet it appears to belong to the normal variety. This could be explained in two ways. Either Canyon Diablo was broken out from its parent body 1 æon ago and suffered a second collision 540 million years ago * or specimen 43.4341 was located in a protrusion on the surface of the main mass. It can be easily shown that in the latter case the production ratios of cosmogenic nuclides are determined by the size of the protrusion, not so much by the radius of the main mass. In the present case the protrusion apparently had a mass of about 10^5 kg and a radius of 1·4 m.

Is it a coincidence that the cosmogenic rare gases in Canyon Diablo 3, specimen 586.1, can be explained with the same set of assumptions? It, too, could have come from a location in the main mass which was exposed for one æon and survived the collision 540 million years ago. Or it could likewise have come from a protrusion of about 10^5 kg which was exposed during 540 million years.

If all the varieties of the Canyon Diablo meteorite indeed come from a single parent mass, then it follows that substantial structural and compositional variations existed in the nickel-iron phase of the parent body of Canyon Diablo over distances of 10–100 m. This adds a new element of flexibility to all theories on the origin of iron meteorites.

I thank Prof. E. Anders for suggesting this experiment and for his advice. I also thank Prof. Carleton Moore, who made samples of Canyon Diablo Nos. 2 and 3 available. This work was supported in part by the U.S. Atomic Energy Commission contract *AT*(11–1) 382 and the National Aeronautics and Space Administration grant *NsG*–366 research.

* There is good evidence that iron meteorites suffer repeated collisions in space which produce new surfaces. Fragments from under such new surfaces have shorter exposure ages than the main mass (see: Vilcsek, E., and Wänke, H., *Proc. Symp. Radioactive Dating, Athens*, 1962, 381; IAEA, Vienna, 1963). I myself have measured four Canyon Diablo 1 specimens not shown in Fig. 1, which indicate another, still shorter exposure age for this meteorite of 150–200 million years.

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[See also p. 867 of this issue—Editor.]

COSMIC-RAY-INDUCED PARTICLE TRACKS IN A METEORITE

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THE existence of ancient or 'fossil' charged particle tracks in natural minerals was first established by Price and Walker in the examination of samples of terrestrial mica samples[1,2]. It was shown by these authors that particle tracks in terrestrial mica samples must be due predominantly to the spontaneous fission of uranium impurities. A new dating method based on the known spontaneous fission rate of uranium-238 was proposed[2] and has now been applied to a number of terrestrial systems[3–8].

Based both on calculation and direct experiment with high-energy proton beams[9,10], it has been realized for some time that samples such as meteorites which have been exposed to the primary cosmic radiation for long times should contain cosmic-ray-induced tracks which can be used to obtain information both about the cosmic radiation and the meteorite history. Indeed, the idea of finding fossil cosmic-ray-induced tracks was the original motivation for the search by Price and Walker for fossil-particle tracks in natural minerals. We wish to report here what we believe are the first observations of such cosmic-ray-induced tracks in samples of meteoritic material.

The observations to be described are preliminary in the sense that we cannot at this time definitely determine which of several possible cosmic-ray particles are responsible for the observed tracks. After presenting the experimental data we discuss the problem of theoretical interpretation and conclude that the majority of the observed tracks come either from spallation recoils or heavy primary ions. Additional experiments which should serve to solve completely the problem of track origin are indicated briefly.

Particle tracks in minerals can be revealed by a proper etching treatment which selectively attacks the trail of radiation-damaged material produced by the passage of a heavy charged particle; this initial damaged region is enlarged to a size where a hollow tube or track can be seen in an optical microscope. The etching treatment in olivine for good track revelation is somewhat more complicated than that which has been reported for mica[11] and other minerals[6]. It consists of a sequence of alternate etches of 4 min in a concentrated solution of hot potassium hydroxide (29 g KOH; 9 g H_2O at 160° C) and 30 sec in a 5 per cent hydrofluoric acid solution at room temperature. Different samples etch at somewhat different rates and the number of sequences for optimum revelation varies somewhat; typically, two sequences are required. Both the acid and base treatment are necessary since neither one alone will give narrow tracks. This treatment works well on polished 100 faces of olivine but not on other principal planes where somewhat different procedures must be used to obtain cylindrical tracks. Irrespective of the crystal orientation, however, a potassium hydroxide etching treatment will generally give some sort of etch pit if a track is present. Thus a base etching treatment can be used as a preliminary way of distinguishing interesting samples which contain a high density of tracks. The rather complicated etching behaviour of olivine is interesting in itself and will be reported in more detail elsewhere. Fig. 1 shows an example of etched particle tracks on a polished 100 surface of terrestrial olivine which has been irradiated with fission fragments from a californium spontaneous fission source.

Olivine is a common constituent of meteorites and in the pallasites it is possible to obtain large single crystals suitable for orientation and subsequent polishing. Experiments by Fleischer *et al.*[12] have shown that particle tracks are produced in olivine at the low temperatures expected in space and that, once formed, they should be retained for $T >> 10^{10}$ years even at high pressures. In a preliminary search for cosmic-ray-induced events in meteorites we obtained olivine crystals from the following pallasites: Pavlodar (Siberia), Brenham (Kansas), Molong (New South Wales), Brahin (U.S.S.R.), Krasnojarsk (U.S.S.R.), Admire (Kansas), Springwater (Canada) and Magnesia (Smyrna, Turkey). Parts of these samples were broken into small fragments which were etched for 15 min in the hot solution of potassium hydroxide and then examined for etch pits. In all the samples except those from

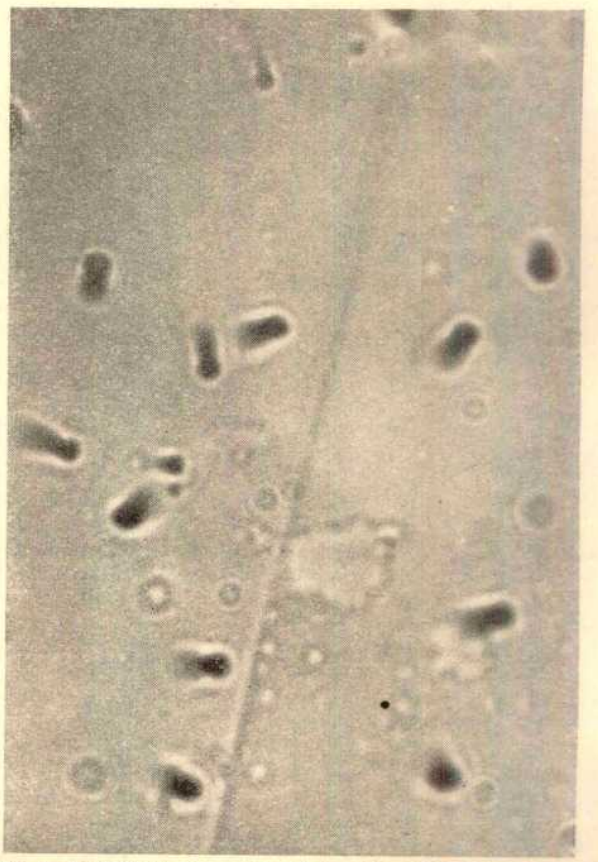


Fig. 1. Etched fission fragment tracks in olivine. This picture shows a 100 surface of a terrestrial olivine crystal which has been bombarded with fission fragments from a californium-252 spontaneous fission source and then etched as described in the text

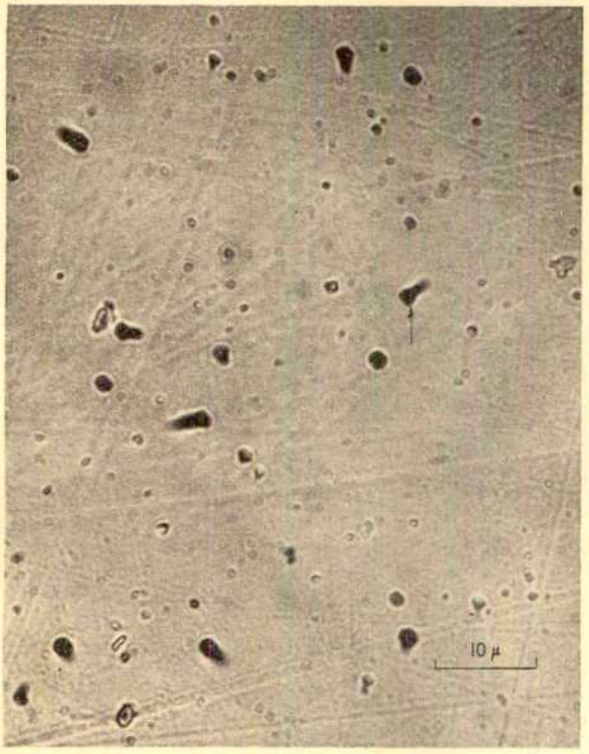


Fig. 2. Cosmic-ray-induced tracks in meteoritic olivine. This shows a polished 100 surface of an olivine crystal from the pallasite Pavlodar which has been etched as described in the text. The black lines similar to that indicated by the arrow are believed to be particle tracks produced by cosmic-ray interactions in the meteorite

Pavlodar[13] only an occasional etch pit was observed. In Pavlodar, however, we found an anomalously large density of etch pits indicating the possible presence of particle tracks. Crystals from this meteorite were then oriented by X-rays and cut and polished to give 100 faces which were afterwards etched as described above. The orienting and polishing of the remaining samples are now being carried out.

Before etching, the olivine crystals from Pavlodar show a very occasional short track-like inclusion; after etching, there is uniform concentration of short, cylindrical etch figures which we believe are due to fossil tracks produced by cosmic-ray interactions in the meteorite. Fig. 2 shows a representative portion of an etched 100 surface with a number of tracks visible. The tracks range in length from the minimum observable ($< 1\mu$) to approximately 10μ. The average length of definitely identifiable tracks is $\sim 3\mu$; but it is clear that there are many small etch pits which are present, probably of particle origin, which would lower this average value if they could be properly measured. The density of tracks which have a definite 'tail' and are readily identifiable is $\sim 5 \times 10^5/\text{cm}^2$. If the small etch figures are included the value is about three times greater. Although the tracks are distributed uniformly in density throughout the crystal, there is a marked angular anisotropy in the 100 plane. If an arbitrary line is drawn in the plane to distinguish 'up' and 'down' it is found that about 3–4 times as many tracks have their tails pointed up as down.

Dislocations can also give rise to cylindrical etch figures and could conceivably be responsible for the etch figures which we are ascribing to particle tracks. For the reasons discussed here we believe this explanation to be very improbable. First, the appearance of the meteoritic etch figures is quite different from the typical appearance of dislocation etch figures in olivine. These latter etch figures are usually very long ($> 100\mu$) and frequently show pronounced curvature. They almost always occur in localized areas and are usually accurately aligned one with the other in these zones. By contrast, the meteoritic etch figures are quite short and have a uniform density. Although they exhibit a general anisotropy they are by no means accurately aligned and all possible directions are represented.

Another strong argument for the particle-track origin is provided by annealing experiments performed on Pavlodar. When a sample was heated at 600° C for 20 min and afterwards re-etched, the density of short etch figures had dropped to zero. Occasional long dislocation etch figures, however, were still observed. Since it is known from the independent work of Fleischer *et al.*[12] that this heat treatment is sufficient to erase charged-particle tracks in olivine, the results lend support to the charged-particle origin of the etch figures in the unheated sample.

Finally, there are several sources of charged particles in the cosmic radiation which can easily account for the observed tracks. Fleischer *et al.*[14] have shown that the rate of loss of energy of a charged particle must exceed a certain critical value $(\text{d}E/\text{d}x)_c$, in order to form an etchable track. Although the value of $(\text{d}E/\text{d}x)_c$ has not yet been accurately determined for olivine, comparison of the length of fission fragment tracks in olivine with those in mica (where a value of $1{\cdot}5 \times 10^{10}$ eV/(g/cm²) has been previously measured) indicates that $(\text{d}E/\text{d}x)_c$ in olivine lies between the limits 2 and 3×10^{10} eV/(g/cm²). (All values of energy loss are referred to aluminium as a standard.) Theoretical calculation shows that there are at least six possible sources of particles whose rates of loss of energy could exceed the critical value necessary to produce tracks in meteoritic olivine: (1) spontaneous fission of uranium impurities; (2) cosmic-ray-induced fission of heavy elements; (3) cosmic-ray-induced spallation recoils of medium and heavy elements; (4) meson jets from very-high-energy cosmic-ray interactions[15]; (5) heavy ions in the primary cosmic-ray flux[16]; (6) magnetic monopoles[17] —particles first postulated by Dirac and not yet observed in Nature.

Tracks from these different sources would be expected to vary considerably in their appearance, their density, and their distribution within the meteorite. For example, cosmic-ray-induced fissions would give rise to '*v*-tracks' because the fission of the excited nucleus takes place in flight. Spallation recoils, on the other hand, would give very short, straight tracks. Slowed-down heavy ions would give different track-lengths depending on their masses, but would be quickly absorbed in the meteorite and thus would show a strong spatial variation of their density: very-high-energy protons producing meson jets would, on the contrary, show very little spatial variation. Spontaneous fission tracks should be distributed isotropically regardless of the position of the sample in the original meteorite; but all the other track sources could give rise to strong anisotropies if the test sample had originated from one edge of the meteorite.

The average length of the observed tracks and their angular distribution are clearly incompatible with a spontaneous fission origin and this source can definitely be ruled out as a contributor of the bulk of the observed tracks. The absence of any '*v*-tracks' similarly precludes the induced fission possibility. Further, the published figures on shower multiplicities and angular distributions for meson jets[15] indicate that the density of meteoritic tracks is much too high to be accounted for by this mechanism.

Calculated values of rates of energy loss for Dirac monopoles[18,19] show that doubly charged monopoles would probably register tracks though they would be close to the threshold for detection. The flux of such particles would have to be $\gtrsim 3 \times 10^{-11}/\text{cm}^2$ sec to account for the observed density. This value is slightly less than the upper limit measured by Malkus[20] in a terrestrial experiment, and on the basis of existing information we cannot completely rule out the monopole source though we regard this as an extremely unlikely explanation.

It can be shown that heavy cosmic-ray primaries which are known to be present in the cosmic radiation are capable of producing the observed density of tracks. Taking only iron nuclei into account and calculating the number of particles whose rate of loss of energy exceeds the critical value as a function of depth from the outside of the meteorite, we find that a typical space exposure of $\sim 10^8$ years[21] will give an unobservably high value of $> 10^7/cm^2$ near the surface. The calculated density drops off rapidly with depth because of nuclear absorption and reaches an unobservably low value at ~ 20 cm below the surface. Typical ablation distances which have inferred from other experiments range from 2 to 15 cm (ref. 22) and, in the absence of knowledge concerning the location of our sample with regard to the original surface of the meteorite, the heavy ion source remains a strong possibility for explaining the observed results. In this connexion it should be noted that Pavlodar was the smallest meteorite of the set which was examined. The total mass of Pavlodar was only 4·5 kg, while the others, with the exception of Magnesia, all had a total mass exceeding 50 kg. The observed track-lengths are somewhat shorter than we would expect for iron nuclei but could be accommodated within the present uncertainty in $(dE/dx)_c$ for olivine.

In our opinion, the most likely explanation for the bulk of the observed tracks is the production of spallation recoils by cosmic-ray interactions with medium atomic weight elements present in the olivine. The density, ρ, of visible recoils in a sample which has been exposed to the cosmic radiation for a period of time, T, is given by: $\rho = n\sigma d\varepsilon N_0 T$, where n is the flux of cosmic rays, σ is the average interaction cross-section, d is the average track-length, N_0 is the number of atoms/cm³, and ε is probability per interaction for the production of a visible track. Taking $n = 3$ p/cm² sec, $\sigma = 1$ barn, and $T = 10^8$ years, we find that $\rho = 1{\cdot}4 \times 10^{11}\varepsilon$. Thus the observed track density of $\sim 10^6$/cm² can be explained if only $\sim 10^{-5}$ of the total number of interactions give rise to visible tracks. Put another way, medium atomic weight impurities, which will almost always give heavy enough recoils to produce tracks, need only be present at a concentration of 10^{-5} to explain the observed results. Previous proton acceleration experiments in mica[9,10] have shown that visible spallation recoils are produced with a probability of $\sim 0{\cdot}05$. One sample of terrestrial olivine[10] gave $\varepsilon < 5 \times 10^{-5}$, but this is not particularly meaningful since it is clear that each sample must be calibrated independently with a proton beam because of the possible role of medium weight impurities.

It is expected that the following work should serve to settle completely the question of the track origin in Pavlodar: (*a*) determination of the response of individual crystals of Pavlodar to irradiation with high-energy protons and also thermal neutrons; (*b*) measurement of $(dE/dx)_c$ for olivine; (*c*) comparison of results obtained with other meteorites—particularly those the cosmic-ray exposure age of which has been measured and where it will be possible to measure the spatial variation of track densities within the meteorite. This work is now in progress.

Whatever the final explanation of the tracks in Pavlodar, it seems likely that fossil track investigations will be a fruitful source of information both about the meteorites and the cosmic radiation.

We thank Prof. J. Orcel, director of the Laboratory for Mineralogy and Crystallography of the Museum of Natural History in Paris, for his generous co-operation in providing the necessary samples. One of us (R. M. W.) held a U.S. National Science Foundation fellowship during the time this work was started, and he would like to thank both the National Science Foundation and the director of the Laboratory for Physical Chemistry at Orsay, Prof. Y. Cauchois, for their help. Finally, we would thank R. L. Fleischer and P. B. Price of the General Electric Research Laboratory for their advice.

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CHANGES IN THE EARTH'S MOMENT OF INERTIA

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VARIOUS theories of the Earth's evolution have suggested that its moment of inertia has gradually changed. Urey[1] inferred a cold origin of the Earth from chemical arguments. He supposed that initially the Earth was a uniform mixture of iron and silicate bodies, and that the iron has only slowly collected to form the core. I developed this idea as part of a theory of continental drift[2] and was able to obtain[3] an empirical law of the growth of the Earth's core, from which can be calculated the change with time of the Earth's moment of inertia[4,5]. Fig. 1 shows my result and the change in the length of the day which would have occurred in the absence of any external torque on the Earth. Lyttleton[6], in a theory of mountain building, has revived Ramsey's theory of the core–mantle boundary as a phase change. However, he argues that, in an initially cold Earth, there would have been no core but that as the temperature rose an increasing volume of silicate would go over into a dense phase. The resulting contraction of the Earth is held to account for mountains. Lyttleton finds that the Earth's moment of inertia has decreased by 30 per cent during the past 3×10^9 years. Thus both these theories predict that the Earth's moment of inertia was greater in the past than at present.

Other theories predict that the moment of inertia has increased with time. Egyed[7] has advocated a theory of an expanding Earth. He considers that an annual increase of the radius of between 0·5 mm and 1·0 mm is likely. Creer[8], on the basis of palæomagnetic reconstructions of the continents, suggests a similar rate of expansion.

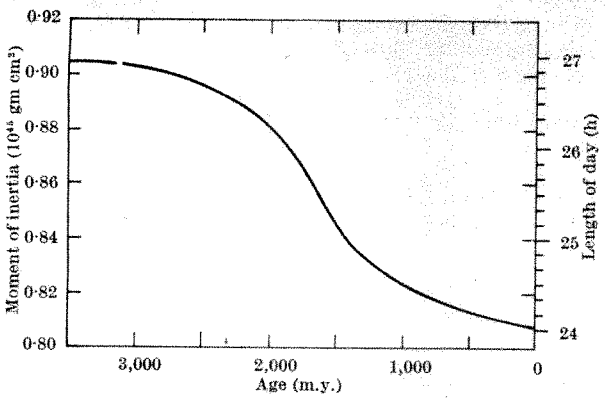


Fig. 1. Moment of inertia and length of day against time for growing-core hypothesis

Carey[9], from his interpretation of oroclines, has suggested a doubling of the Earth's surface area (and thus of its moment of inertia) since the Palæozoic. Heezen[10] has also postulated an expansion of the Earth of comparable magnitude to explain tensional features on the ocean floor.

My purpose is to point out that it is now likely that all these theories can be put to experimental test. A method of determining the rate of the Earth's rotation in the geological past has been suggested by Wells[11]. He shows that daily growth rings on the epitheca of corals can probably be recognized, and hence finds that the number of (presumably solar) days in the year in Devonian times was about 400. He states that counts on 12 corals lie between 385 and 410 and we may estimate the standard deviation as about ± 7 days. Wells[11] and Munk and Davies[5] regarded the result as reasonable in view of the known deceleration of the Earth due to lunar tidal friction.

However, Scrutton[12] has recently shown that it is also possible on certain Middle Devonian corals to recognize what appear to be monthly bands. He finds 30·59 days in the Devonian month. Counts on 10 corals give the standard deviation as ±0·13 day. If this is correct, the tides must be influencing the growth perhaps due to the change in sunlight with the height of the water. Thus the synodical month is determined by this method. To convert this to the number of days (s) in the sidereal month we divide by $1 + 30{\cdot}59/(w + 1)$, giving 28·4 days, where w is the number of sidereal days in the year (399). This is very important geophysically because it opens up the possibility of separating changes in the Earth's rotation due to changes in its moment of inertia from those due to lunar tidal friction, because the latter changes the length of the month as well as the day. Let t, Y and T be the lengths of the sidereal day, the year and sidereal month respectively for any geological period, and t_0, Y_0, and T_0 the present values. Then by Kepler's third law:

$$r^3/T^2 = G(M + m)/(2\pi)^2$$

where M and m are the masses of the Earth and Moon, and r is the mean Earth–Moon distance and G the gravitational constant. If L is the orbital angular momentum of the Moon at any geological period and L_0 the present value, then by Kepler's second law, neglecting orbit eccentricity:

$$L/2m = \pi r^2/T$$

Thus:

$$[G^2(M + m)^2 m^3].T = 2\pi L^3$$

Similarly, if $M_\odot$ is the mass of the Sun and N and N_0 the Earth's past and present orbital angular momenta:

$$G^2 M_\odot^2 M^3 . Y = 2\pi N^3 \qquad (1)$$

Thus:

$$\frac{m^3}{M_\odot^2 M}\left(1 + \frac{m}{M}\right)^2 \frac{T}{Y} = \frac{L^3}{N^3}$$

at any time in the past.

$$\beta(L_0 - L) = (N_0 - N)$$

where β is the ratio of the solar tidal retarding couple on the Earth to the lunar one. Theory gives the present value[13] as 1/5·1 if the equations of motion are linear and as 1/3·4 if the friction is proportional to the square of velocity. Because $N_0/L_0 \simeq 10^6$, N/N_0 is closely equal to 1:

$$\therefore \frac{T_0}{Y_0} \cdot \frac{w}{s} = \left(\frac{L_0}{L}\right)^3$$

thus:

$$L_0/L = 1{\cdot}016 \pm 0{\cdot}003$$

and is independent of G.

Lunar tidal friction results in angular momentum being transferred from the Earth's rotation about its axis to the Moon's orbit. Hence from the above value of L_0/L we may find that the average lunar tidal decelerating torque on the Earth over 370 m.y. is $3{\cdot}9 \times 10^{23}$ dyne cm (since the Devonian). This agrees with that given from measurements[14] of the longitudes of the Sun and Moon over the past 2–3 centuries ($3{\cdot}9 \times 10^{23}$ dyne cm). It has long been known that the value obtained from ancient eclipse data is twice as great[14,15]. This strongly suggests that the latter reductions are unreliable, as Munk and MacDonald[16] thought.

Now:

$$(1 + \beta)(L_0 - L) = 2\pi I/t - 2\pi I_0/t_0$$

where I and I_0 are the Earth's past and present moments of inertia respectively. Using the ratio (4·83) of the Moon's orbital angular momentum to the Earth's spin angular momentum:

$$1 - L/L_0 = [-1 + (I/I_0)(w/365{\cdot}25)(Y_0/Y)]/4{\cdot}83(1 + \beta) \qquad (2)$$

If:

$$Y = Y_0$$

then:

$$I = I_0(0{\cdot}999 \pm 0{\cdot}003) \text{ for mean } \beta = 1/3{\cdot}7$$

and:

$$I = I_0(0{\cdot}994 \pm 0{\cdot}003) \text{ for mean } \beta = 1/5{\cdot}5 \qquad (3)$$

The values of the errors may not be very realistic in view of the uncertainties of this new field and the restricted nature of the sampling of corals so far made.

These results dispose convincingly of Carey's and Heezen's extreme hypotheses. This is in agreement with the palæomagnetic evidence, which has been used by Ward[17] to determine the radius of the Earth in the Permian by relating within one continent the angles of magnetic inclination and the distances between the sites at which they were determined.

However, Dirac's[18] suggestion that G varies inversely as the time since the origin of the universe must be taken into account as some proponents[19] of the expansion theory have taken it to be the cause.

From equation (1):

$$Y/Y_0 = G_0^2/G^2$$

Then from equation (2):

$$I > I_0(0{\cdot}85 \pm 0{\cdot}003) \text{ for } \beta = 1/3{\cdot}7$$
$$I > I_0(0{\cdot}84 \pm 0{\cdot}003) \text{ for } \beta = 1/5{\cdot}5$$

for the age of the universe cannot be less than that of the solar system $4{\cdot}5 \times 10^9$ years, $Y/Y_0 > 0{\cdot}84$. However, as Fowler and Hoyle[20] have shown that the galaxy is 1×10^{10}–$1{\cdot}5 \times 10^{10}$ years old, Y/Y_0 lies between 0·93 and 0·98, thus the results of equation (3) become:

$$I_0(0{\cdot}98 \pm 0{\cdot}003) > I > I_0(0{\cdot}93 \pm 0{\cdot}003) \text{ for } \beta = 1/3{\cdot}7$$
$$I_0(0{\cdot}97 \pm 0{\cdot}003) > I > I_0(0{\cdot}92 \pm 0{\cdot}003) \text{ for } \beta = 1/5{\cdot}5$$

The results should be compared with the values in Table 1.

Table 1

Theory	Change in radius	I/I_0
Lyttleton	−0·01 mm/yr	1·030
Urey-Runcorn		1·005
Carey-Heezen		0·5
Egyed	0·5–1 mm/yr	0·89–0·94
Dicke	0·05 mm/yr	0·994
Creer	0·5–1·2 mm/yr	0·87–0·94

The observations need to be greatly extended, but they do not favour the expansion theories of Egyed and Creer unless G varies. There is evidence[21] from radioactivity against the latter. Lyttleton's figure would only be too high if it were assumed that the rate of change of the Earth's moment of inertia was uniform with time, which is not necessary. The discussion would be on much firmer ground, though, if it could be demonstrated, by determining w and s for other geological periods, that I/I_0 varies smoothly with time, and L/L_0 in accord with theory.

A preliminary account of this work was given at a conference on the Earth–Moon system at the Goddard Institute for Space Studies, Columbia University, New York. I thank Mr. C. T. Scrutton and Dr. G. Backus for their advice.

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NEW U.S. RESEARCH STATION IN THE ANTARCTIC PENINSULA

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THROUGH the combined efforts of the twelve nations signatory to the Antarctic Treaty and with advice from the Scientific Committee on Antarctic Research, scientists from these countries are delving deeply into the mysteries of the Antarctic Continent. These efforts comprise a major investigation of an environment which should enable us to understand better its relation to the rest of the world. The United States programme, to explore the Continent adequately and to take full advantage of the natural laboratory offered, is being constantly evaluated, and suggested projects of research are supported wherever possible. It is therefore reasonable, as research in certain regions passes the point of greatest scientific return, to establish sites in new areas which offer more fertile research possibilities. In this way the facilities of "Laboratory Antarctica" and the efforts of scientists can be used most effectively.

With the onset of the International Geophysical Year and the development of plans for that remarkable co-ordinated international research programme, the United States opened up a total of seven stations: Byrd, Ellsworth, Hallett, Little America, McMurdo, Pole and Wilkes. At the end of the International Geophysical Year, Little America was closed, the custody of Ellsworth Station was transferred to Argentina, and the custody of Wilkes Station was turned over to Australia. For some years a few American scientists continued to work at these stations in close co-operation with the scientists of these two countries. Ellsworth Station has now been closed, and its custody returned again to the United States, and Wilkes Station is now entirely manned by Australians.

For four years the American programme was carried on at the four remaining stations. In 1963, the United States installed a new station called Eights Station (after James Eights, the first American scientist to work in Antarctica) at the base of the Antarctic Peninsula at 78° W. and 78° 18′ S. for the purpose of investigating conjugate point and other upper atmospheric phenomena, and meteorology.

It became apparent some years ago that the vast ocean areas surrounding Antarctica and extending for some 1,000–1,500 miles north offered great potentials in modern research. For this reason, the American programme added to its efforts a sea-going research station, the U.S.N.S. *Eltanin*, which began her programme of research in and around the Scotia Arch, the Drake Passage, and the South Pacific.

University biologists in the United States who have been involved in programmes on the Continent and on the *Eltanin* have repeatedly expressed their interest in obtaining facilities for a sophisticated biological research programme in the peninsula area to complement and supplement the work being done on the *Eltanin*. For this reason, the establishment of a new American scientific station in the peninsula area has been under consideration for some time. The U.S. National Science Foundation, which bears the responsibility for planning, managing, and financing American scientific programmes, and the U.S. Navy, which is responsible for the logistics for the American programme, prepared a prospectus of potential locations for a station in the area. A wide reconnaissance of these sites was conducted during January–February 1963 aboard the Navy ice-breaker U.S.S. *Staten Island*. Even on this trip the biologists who accompanied the ship collected nearly 30,000 specimens. The group which made the reconnaissance of the sites recommended the Arthur Harbor vicinity on Anvers Island as the most promising location for the station, scientifically and logistically. Following this recommendation, early in 1964, a group of U.S. Navy construction experts surveyed the chosen location for a suitable site to construct the station.

The Falkland Islands Dependency Survey had chosen the Arthur Harbor area for a base which was built in 1955 and occupied until 1958. At that time, work was carried on by the British group in geology and topographic surveying, and observations were made in natural history. Many specimens were collected, and recently Dr. M. W. Holdgate[1] published observations of birds and seals made during the 1955–57 period. Dr. P. R. Hooper[2] has also written about the geological investigations made during that period. The availability of information from the countries which have carried out research in the area makes it possible to avoid duplication of efforts and confine the investigations to more specialized problems.

The British authorities have very graciously made the present hut at Arthur Harbor available for the initial phase of the American programme in the area. Along with the hut, which will be used to accommodate the laboratories, will be an additional building for living purposes. The new station will be called Palmer Station.

It is to be assembled by a small detachment of U.S. Navy Seabees who, along with the group which will stay over the winter, will be living aboard the ice-breaker U.S.S. *Edisto*. Once the station is complete, the five scientific and four Navy support personnel will move in for the winter, and the ship will proceed to other tasks.

In order to enlarge the effective working area of the station, a 125-f. wooden vessel designed for special service in and around the ice is being planned. Such a vessel would make possible biological, geological, and other investigations in the coastal areas of the peninsula and the off-lying islands.

The potentials for biological field investigations in the Antarctic Peninsula will, it is believed, attract research workers interested in problems of cold adaptation and physiology and, generally, in terrestrial and marine environmental phenomena. The biological laboratory planned for the Palmer Station will be equipped with standard apparatus and general supplies to meet the requirements for investigations of this type.

The peninsula area has been of interest to American geologists for many years, partly because of the strong likelihood that it may be the southern end of a land tie between South America and Antarctica. Although geological investigations both north and south of the Drake Passage have been carried out, much additional work on the history and structure of the peninsula remains to be done.

Palmer Station will be a most useful addition to the programme of the upper atmospheric scientist. It will provide a site for conjugate point studies which make possible the determination of some of the magnetospheric properties many earth radii out in space. The type of investigations which will be conducted may be the study of very-low-frequency whistler phenomena, the study of the absorption of particles which have been evaporated off the Sun, the study of the electro-jet which is a transitory current in the upper atmosphere, or a combination of these.

During the coming year, the Bernice P. Bishop Museum, Honolulu, Hawaii, will carry out ecological studies of Antarctic soil arthropods throughout the year to gather data on cold tolerances, temperature, humidity, food and hibernation requirements. In addition, continuous air plankton samplings will be maintained for recording the dispersal of land arthropods or other organisms by air currents. Complete soil and water analyses are planned for the ecological and climatological studies. Carefully controlled experiments will be initiated to analyse seasonal stresses on land arthropods. Collections will also be made of all arthropods, and particularly bird and seal parasites for faunal and systematic studies.

Glaciology and meteorology research will be carried out by scientists from Ohio State University's Institute of Polar Studies. Here they will investigate the balance of the ice cover and search for evidence of recent glacier fluctuations. Raised beaches will be sought also for evidence of sea level changes and datable material. A meteorological programme will include surface observations of temperature, wind, pressure, and cloudiness.

In keeping with the spirit of co-operation in Antarctica scientists from other countries will be welcome to join in the work at this station.

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NEWS and VIEWS

The Royal Society of London: Award of Royal Medals

Her Majesty The Queen has been graciously pleased to approve recommendations made by the Council of the Royal Society for the award of the two Royal Medals for the present year as follows: to Prof. M. J. Lighthill, Royal Society research professor at the Imperial College of Science and Technology in the University of London, for his distinguished contributions to knowledge of the flow of compressible gases, and the mathematical theory of distributions; to Prof. F. W. R. Brambell, Lloyd Roberts professor of zoology in the University of Wales at the University College of North Wales and honorary director of the Agricultural Research Council's Unit of Embryology, for his important contribution to our understanding of the passage of protein from maternal to fœtal circulations.

Marine Biological Laboratory at Plymouth: Dr. F. S. Russell, C.B.E., F.R.S.

As a centre for the prosecution of research into every aspect of marine biology, the Plymouth Laboratory is unexcelled in the world. This stature it has attained by virtue of the outstanding qualities of three directors, E. J. Allen, Stanley W. Kemp and F. S. Russell, who successively have guided its growth over a continuous period of just on seventy years. For more than half this long period, F. S. Russell, who retires on September 30, 1965, has been associated with the Laboratory. After notable service with the Royal Naval Air Service and the Royal Air Force in the First World War and a year as an assistant director of Fisheries Research to the Government of Egypt, he joined the Marine Biological Association as an assistant naturalist in 1924. Later he was a member of the Great Barrier Reef Expedition in 1928–29, and spent the years of the Second World War in London as wing commander on Air Staff Intelligence. He succeeded Stanley Kemp as director in 1945. A pioneer worker on zooplankton, he was among the first to investigate diurnal migrations and relate these to variations in light intensity, while his name will always be associated with the discovery of plankton indicators through his investigations into the distribution of species of *Sagitta*. Early interest in medusæ, stimulated by the late E. T. Browne, culminated in the publication in 1953 of *The Medusae of the British Seas*, a notable addition to the great series of monographs in which, over the past two centuries, naturalists have described the varied members of the British marine fauna. During Dr. Russell's years as director, the facilities both in the Laboratory and for collecting and observing at sea have greatly increased. While war damage necessitated complete reconstruction of what was the Director's House, major additions for research and teaching have been completed, while the Laboratory is now served by two vessels, R.V. *Sarsia* and R.V. *Sula*, 130 ft. and 61 ft. long, respectively, and by a motor launch. The present condition of the Laboratory is described in *The Journal of the Marine Biological Association* (43, 281; 1963). With the building up of these facilities has gone a steady increase in the scientific staff, which now numbers twenty, including three Follows of the Royal Society. Visitors from all parts of the world come to work in the Laboratory and the magnificent library or to study the facilities. All has been achieved by careful planning and an unusual capacity for lucid exposition. Few major laboratories can have been conducted with less fuss and bother, fewer still with such a disarming sense of quiet humour. Retirement will represent a welcome return to research for Dr. Russell but with the satisfaction, shared, one knows, by Mrs. Russell, who has helped so much to bring it about, of realizing that, probably as no one else could have done, he has carried the Plymouth Laboratory into this post-war scientific age with universally acclaimed success.

Prof. J. E. Smith, F.R.S.

THE Council of the Marine Biological Association has appointed Prof. J. E. Smith to be director of the Marine Biological Laboratory at Plymouth in succession to Dr. F. S. Russell. Prof. Smith holds the chair of zoology at Queen Mary College, London, and is vice-principal of the College. He is chairman of the National Committee for Biology, chairman of the Grants Committee (Biology) of the Department of Scientific and Industrial Research, and chairman of the Advisory Committee on the Dove Marine Laboratory to the Development Commission. He is a member of the British National Antarctic Survey Committee of the Royal Society, and the Biological Unit of this Committee is attached to his Department. He is a Trustee of the British Museum (Natural History). He has made outstanding contributions to our knowledge of the nervous system of echinoderms and other marine animals, and to the embryology of marine animals and the ecology of the marine fauna.

Industrial Metallurgy at the College of Advanced Technology, Birmingham: Prof. J. C. Wright

DR. JOHN C. WRIGHT has been appointed professor of industrial metallurgy at the College of Advanced Technology, Birmingham, as from October 19, 1964. Prof. Wright, who is 34 years of age, was educated at the Grammar School, Dudley. Joining the Development and Research Department of the Mond Nickel Co., Ltd., in Birmingham in 1948, he studied part-time at the College of Technology for the external B.Sc. degree of London University, and was awarded this with first-class honours in 1954. After graduating he joined Imperial Chemical Industries Metals Division in Birmingham as a technical officer to assist in the development of titanium-melting techniques and, in 1956, he was awarded a senior research fellowship at Wolverhampton and Staffordshire Technical College to work on problems associated with pulverized fuel-fired gas turbines and was awarded the Ph.D. degree of the University of London in 1958. In the same year he was appointed to the staff of the College of Advanced Technology, Birmingham, as a senior lecturer in metallurgy and, in 1959, he took up his present position at the college as reader in industrial metallurgy. In 1961, Dr. Wright was elected a member of the Council of the Institution of Metallurgists. His present research interests are associated with the applied physical metallurgy of the deformation of metals—particularly of the newer metals.

Enrico Fermi Professorship of Physics, Columbia University: Prof. Tsung-Dao Lee

DR. TSUNG-DAO LEE, Nobel laureate and professor of physics in Columbia University, has been appointed the first incumbent of the newly established post of Enrico Fermi professor of physics in the University. Dr. Lee shared the 1957 Nobel Prize in Physics with Dr. Chen Ning Yang, member of the Institute for Advanced Study, Princeton University, for their research disproving the conservation of parity principle. At that time, Dr. Lee was thirty years old, the youngest full professor in modern times at Columbia. Born in Shanghai on November 25, 1926, Tsung-Dao Lee graduated in 1943 from the Kiangsi Middle School in Kanchow. He then attended the National Chekiang University in Kweichow but, in 1945, fled from advancing Japanese invaders to Kunming, where he attended the National Southwest Associated University. For his work in physics he was awarded a Chinese Government fellowship in 1946, which enabled him to study at the University of Chicago, where he received his Ph.D. in 1950. Dr. Lee went to Columbia as an assistant professor from the Institute for Advanced Study in 1953, and became a full professor in 1956. During 1960–62 he served as professor of physics in the Institute for Advanced Study, and at the same time served as adjunct professor of physics at Columbia. He returned to Columbia as a full-time member of the teaching staff in 1962. During the past summer Dr. Lee taught in Varenna, Italy, and spent two and a half months carrying out research in Geneva, Switzerland.

Enrico Fermi, in honour of whom the new professorship was named, was also a Nobel laureate in physics and served as professor of physics in Columbia during 1939–45. He helped to build the first atomic pile at Columbia and went to the University of Chicago on leave from Columbia in 1942 when the Manhattan Project, which developed the atom bomb, was transferred to the Chicago institution. Dr. Fermi died in 1954.

Gold Medal of the Royal Astronomical Society of Great Britain: Dr. Maurice Ewing

DR. MAURICE EWING, director of Lamont Geological Observatory, Columbia University, has been awarded the Gold Medal of the Royal Astronomical Society of Great Britain for his contributions to marine geophysics. It is the first Gold Medal the Royal Astronomical Society has given specifically for geophysical work. From now on the society will award two Gold Medals, one for astronomical and one for geophysical work. The citation recalls that in 1949 Dr. Ewing was made director of the Lamont Geological Observatory, Palisades, N.Y., which is now "perhaps the most comprehensive geological and geophysical research unit in the world. Over the years the two aspects of Ewing's work, geophysical work at sea and the study of surface waves as a method of gaining more information concerning the crustal and mantle rocks of the earth, have gone from strength to strength, with new methods constantly being developed. The work at sea now requires two deep-sea research vessels, and there are no oceans where Lamont scientists have not been working. In marine research, Ewing's name will always be coupled with turbidity currents, abyssal plains, mid-ocean ridges, and crustal thicknesses, and in seismology with the dispersion of Love and Rayleigh waves and the application of this study to the understanding of the Earth's deep structure".

H. B. Bigelow Medal in Marine Science: Dr. B. C. Heezen

THE Bigelow Medal, established in 1960, is awarded by the Woods Hole Oceanographic Institution for "significant inquiries into the phenomena of the sea". Dr. Heezen, the third recipient, is assistant professor of geology at Columbia University, and his contribution to marine science lies in the field of marine geology, particularly the physiography of the Atlantic and Indian Oceans. His interpretation of the sedimentary and tectonic processes governing the physiography of the ocean floor has been developed in the light of energetic work both in the laboratory and in the field.

Science in Parliament: National Parks

THE debate in the House of Lords on November 10 and 11 on the Address to the Throne ranged widely. Following Lord Mitchison's statement that National Parks would fall within the responsibilities of the Minister of Land and Natural Resources, Lord Molson specifically urged the need for legislation to increase the powers of the National Parks Commission. The Standing Committee on National Parks of the Council for the Preservation of Rural England had written some months ago to the Prime Minister on the whole subject of the preservation of amenities, both within and without the National Parks. The Prime Minister had invited them to prepare a full memorandum and had stated that this would be taken into account when the Government formulated its land policy. Lord Molson said that the memorandum had just reached its final form and would shortly be sent to the new Government, but in replying for the Government

neither Lord Stonham nor the Lord Chancellor gave the assurance that it would receive favourable consideration for which Lord Molson hoped.

Desalination of Sea-water

Lord Molson also supported some observations made by Lord Weir on the desalination of sea-water and expressed the hope that the new Department, to which responsibility for water supply and conservation had been specifically transferred, would direct special attention to the need for increased economy in the use of water and give expert consideration to the Morecambe Bay barrage project. Lord Weir dealt with desalination in some detail, directing attention to the report published this year by the United Nations. Moreover, he stressed the significance of the multi-flush distillant developed independently and almost simultaneously in 1956 by two British engineers, Dr. R. S. Silver and Dr. A. Frankel, giving 11 lb. of distillate from 1 lb. of steam instead of the previous 4 lb., and halving the capital costs. Between 1959 and 1964 the two British firms involved had secured orders for forty installations, or some 70 per cent of the available market. While welcoming the grant made by the Department of Scientific and Industrial Research to the Water Research Association to investigate the true incremental costs of additions to conventional water supply, he hoped that besides attention being directed to the invisible factors, the possibilities of distillation of sea-water, utilizing nuclear reactors as a source of heat, would not be overlooked. He also welcomed the establishment by the Department of Scientific and Industrial Research of an advisory committee and its financing of research into desalination economics. Lord Bessborough, speaking on the following day, supported Lord Weir, and added that the Americans greatly valued Britain's co-operation in this field and wished to see it extended.

Inland Waterways

On November 11, Viscount St. Davids pressed the claims of the waterways to be considered in a co-ordinated transport system. Their working loss had already been reduced from more than £1 million to just more than £500,000. Including capital and interest the working loss had been reduced by 26 per cent. With a further measure now being prepared by the British Waterways Board, he thought that the waterways would pay their way, but he also stressed the importance of the use of the waterways for purposes other than transport. Britain was in great need of major waterways which could carry cargoes of substantial size where speed was not of the first importance. Lord Lindgren, Parliamentary Secretary to the Ministry of Transport, assured Lord St. Davids of his interest in the waterways and that he had taken careful note of what he had said. He hoped that the Board's further review would enable the Government to decide on its policy and to start preparing the necessary legislation. On transport generally, the Government's policy was towards an integrated system covering road, rail, inland waterways, air and coastal shipping, using the most suitable vehicle for the journey to be made and the traffic to be carried, and geared to meet the economic and social needs of Britain.

Iron and Steel Industry

During his speech in the debate on the iron and steel industry in the House of Commons on November 9, the Minister of Power, Mr. F. Lee, said that since the industry's research effort had been criticized in a special report from the Iron and Steel Board in 1963 (see *Nature*, 201, 853; 1964) research expenditure seemed to have increased appreciably in the industry as a whole. Four major companies were planning substantial additional facilities for laboratory research, and the British Iron and Steel Research Association was to construct a new iron-making laboratory. The Government, however, knew of no plans for increasing the resources devoted to long-range research or for providing adequate facilities for large-scale plant work within the industry. On this point Mr. J. H. Osborn said, later in the debate, that the expenditure of £9·3 million on research in 1963 almost reached the figure recommended by the Board, and that the manpower devoted to research increased by 25 per cent last year.

Industrial and Technological Manpower

In his Budget statement in the House of Commons on November 11, the Chancellor of the Exchequer, Mr. J. Callaghan, said that large and important parts of Britain's industrial and technological manpower and capacity were locked up in production and research for the Government. It would be an urgent task to see how far this pattern could be modified in the national interest to release resources for more productive purposes and for expanding exports. Of the £2,000 millions spent on defence, more than £350 million was spent overseas, and it was the Government's intention to relate commitments to resources and ensure that the Armed Forces could discharge their tasks with greater effectiveness and economy. Government expenditure in the civil field would also be examined and it was intended to ask a task group of senior officials, including scientific and economic advisers, to sort out their projects and appraise their economic priority. The Chief Secretary to the Treasury would review the remaining fields of civil expenditure, and would consider whether the established priorities were right or whether men and plant could be released for other work. The basic objective would be to reveal any areas where expenditure was not yielding full value in social and economic terms. However, it was also recognized that there were fields in which increased Government expenditure could assist modernization and accelerate technical progress.

British House Journals Competition

House journals (alias staff magazines, works newspapers, etc.) are valuable vehicles of two-way communication between people in the board room and the shop floor or counter. They range from the office-duplicated bulletin to the glossy magazine that vies with the best on the bookstalls. One of the principal objects of the British Association of Industrial Editors, the professional body for house journal editors, is improvement in the standard of industry's private press. To this end, an annual national open competition is held, which is judged by a panel of experts nominated by the Institute of Journalists, the National Union of Journalists, the British Federation of Master Printers and the Society of Industrial Artists. Among the awards are four major trophies: (1) the best journal distributed to employees, presented by the Monotype Corporation, Ltd.; (2) the best journal for external readership, presented by the Kings Town Engraving Co.; (3) the best-designed house journal, presented by the De La Rue Co., Ltd.; (4) the best newspaper, presented by Lord Crook. In addition, the Shell-Mex and B.P. Trophy is awarded for the best house story of the year. The next competition closes on January 15, 1965. Entry forms can be obtained from the competition secretary, Mr. John Eve, c/o Standard Telephones and Cables, Ltd., Therese House, Glasshouse Yard, Aldersgate Street, London, E.C.1.

The Grassland Research Institute

Much of the work of the Grassland Research Institute, Hurley, is concerned with the interactions of soil, pastures and grazing animals in relation to other parts of the environment (*Experiments in Progress*, No. 16: Annual Report for 1962–63. Pp. 100+4 plates. Hurley, near Maidenhead: The Grassland Research Institute, 1964. 10*s*.). The research programme is planned to maintain

close contact between the eight departments of the Institute. An important aspect of the work is testing grass and clover strains from other latitudes both as pure stands and mixed with indigenous species. A new approach to reseeding is provided by destroying swards chemically. There are indications that different species react differently to particular chemicals and selective killing of species may be possible in the future. The growth characters of species and varieties are being studied in field and pot experiments as well as in a controlled environment. It has been shown that light breaks given to extend day-length in spring hastens ear formation in Timothy with increase in seed yield. It is now well established that increase in soil nitrogen under ley has a dominant influence on crop production. The physical condition of the soil is also improved by leys, and experiments are in progress to see how far this affects the availability of potash and phosphate. Extra-mural experiments in progress on private farms give information about grass management under a range of soil and environmental conditions. Among research projects in animal nutrition are *in vitro* digestibility studies on a wide range of herbages gathered at different stages of growth and the use of enzymes for digesting specific tissues such as fibre. The interaction of animal and pasture within intensive systems of husbandry is being investigated in a series of experiments. Animal agronomy is concerned, in broad aspect, with animal production in response to type of sward, and management and methods of determining the quality and amount of herbage eaten by the grazing animal are being investigated.

The Wildfowl Trust, Slimbridge

S. T. JOHNSTONE comments in the fifteenth annual report of the Wildfowl Trust that the long spell of severe weather in 1963 brought about the deaths of some 9 per cent of the total collection at Slimbridge (Pp. 150+45 photographs. Edited by Hugh Boyd and illustrated by Peter Scott. Slimbridge: The Wildfowl Trust, 1964. 17*s*. 6*d*.). The limited heating accommodation available soon became crowded with the more delicate waterfowl. Those that were taken in at the start of the cold spell survived well, but those that were caught up as the Arctic conditions continued suffered heavy losses. Cuban, fulvous and red-billed whistling duck were casualties. On the other hand, Javan, spotted and white-faced whistling duck, which were given priority, came through without loss. A species which had heavy losses was the North American ruddy duck, and while there had been twelve pairs in the Rushy Pen and some odd males in the autumn, when the spring arrived these had been reduced to five females and eight males. The extreme cold delayed nesting, and the first eggs were three weeks late. Once the weather broke the breeding season reached its peak very quickly. There was a record number of birds raised —more than 1,300 at Slimbridge and 300 at Peakirk. 104 kinds nested and 96 kinds were reared. Andean geese were reared for the first time, bringing the number of forms of waterfowl reared at Slimbridge to 123. The collection now contains some 2,250 birds of 160 kinds and races. Unfortunately, the blackheaded duck (*Heteronetta atricapilla*) is no longer represented. The male, the only one in Europe, that had been at Slimbridge for five years, died during the cold weather. Interesting arrivals have been additions to the greater, Cuban and Chilean flamingos, and a male bronze-winged duck for a tame female—it is hoped that having laid eggs for the past four years, she will do so again this year and that the eggs will be fertile. One of the two female buffleheads has been exchanged for a male.

World Directory of Crystallographers

A THIRD edition of the *World Directory of Crystallographers* is being prepared. The editor of this edition is Dr. D. W. Smits, of Groningen, Netherlands, and biographical information is being collected for each country by a sub-editor. It is intended to include in the *Directory* all practising crystallographers, including graduate students. Readers in the United Kingdom who consider their names should be included in the *Directory*, but who have not received a questionnaire by November 30, are requested to write to Dr. P. T. Davies, Shell Research, Ltd., Thornton Research Centre, P.O. Box 1, Chester.

Soils in North-west Ghana

TECHNICAL Report No. 62 of the Agricultural Research Institute (Ghana Academy of Sciences) is on "Soils of the Seilo-Tuni Land Planning Area, Wala District, Near Wa, Upper Region" and is by H. B. Obeng. (Pp. 196+4 maps. Agricultural Research Institute, Kumasi, 1963.) The area in question comprises 420 square miles in the north-west of Ghana. It has a long dry season (October–April) when the water problem is acute; the average annual rainfall is 44 in., the humidity varies between wide limits, the mean annual maximum and minimum temperatures are 98° F and 69° F. Most of the area lies on Cape Coast and Dixcove granites, but Lower and Upper Birrimian rocks underlie a considerable part and there are smaller areas of mixed parent material and of Black Volta alluvium derived from the above-mentioned rocks. It is probable that much of the area has been raised and degraded several times because there are ironpan-capped remnants of peneplains and river terraces at various heights above the present flood plains. The topography is gently undulating with broad valleys, and the vegetation is mainly short grass savannah with scattered shrubs and trees. There is a large variety of soils; sheet erosion is very common and between the upland concretionary soils and the valley bottom soils there are wide areas of colluvial drifts, falling within the group of Savannah Ochrosols, providing the best soils for crops such as yams, guinea-corn, maize, beans and groundnuts. About 25 per cent of the area is considered suitable for mechanized cultivation and 40 per cent for grazing; the remainder should be reserved for woodland and water-shed protection. The bulk of the report comprises a description of the soil associations and detailed recommendations for the improvement of agricultural practices. The four maps of geology, soils and Land Capability are on a scale of approximately 1 in. to 1 mile.

Priorities in the Physiology of Reproduction

THE fifth International Congress on Animal Reproduction and Artificial Insemination was held in Trento, Italy, during September 6–13. It was attended by about 1,200 scientists from more than 60 countries, and at the closing session the Congress approved a memorandum setting out the problems in this field which, in the light of present knowledge, appear to call for most urgent attention, or to offer most promising investigation. Research in mammalian reproduction has two major objectives, the increase of food production on one hand and the control of population expansion on the other. Divergent as these interests are at the applied level, both are served by basic research, much of which is also related to other aspects of physiology. Furthermore, although the control of human reproduction commands so much attention, it must not be forgotten that human infertility and fœtal mortality, menstrual disorders and other gynæcological problems still call for investigation. Indeed, recent progress in these important fields has been far from spectacular, and the 'conclusions' of the congress were obviously right in displaying no complacency in this regard. Among the more general themes suggested for future work, immunological aspects of reproduction, especially the antigenicity of gametes and fœtal–maternal incompatibility have prior place. They constitute a rewarding field of study. Environmental influences on reproduction are still felt to be in need of elucidation, particularly in relation to the interaction of environmental and hereditary factors. Another field of funda-

mental research of immense importance, both economically and in medical terms, is that of neuro-endocrinology. At a more 'practical' level, the congress agreed that "It is important that research be continued on the factors affecting sex determination because of its economic importance", and went on to define means of expressing farm animal fertility in uniform terms so as to facilitate comparison of records, and to make recommendations concerning hygiene and legislation in animal health.

Research in Immunology

Research in Immunology (WHO Technical Report Series No. 286) should interest everyone who is concerned with this field of investigation, which is nowadays advancing so rapidly and extending its influence in many directions. The Report deals with immunological problems under the following headings: I, immunoprophylaxis and immunotherapy; II, immunopathology; III, tissue antigens and transplantation; IV, immunochemistry; V, research programme in immunology. Each section comprises the report of a scientific group convened by the Director-General of the World Health Organization. Two annexes summarize the recommendations made for research and give an outline of current problems in immunology. The Report is obtainable in the United Kingdom from H.M. Stationery Office, the price being 8*s.* 6*d.*, and it is also published in French and Spanish.

CIBA Fellowships

Several fellowships will be awarded for tenure during the academic year 1965–66 at continental universities or institutions for full-time research in chemistry, physics or some other allied scientific subject. They will be awarded to graduates of universities situated in the United Kingdom of Great Britain or Northern Ireland or in the Republic of Ireland, or to members of those universities graduating in 1964. It is anticipated that some fellowships will be awarded to recent graduates and other fellowships to candidates who have already taken their Ph.D. degree, or who have already spent some time in industry. The basic award for Fellows who have already undergone training in research and wish to continue post-doctorate studies will be £1,000 per annum. For candidates who have obtained a first degree in science and wish to undergo training in research, the basic award will be £700 per annum. Awards may be increased by appropriate additional allowances depending on place of study and marital status, etc. The total value of each fellowship will be determined by the Advisory Panel. The amount awarded will be sufficient to cover the Fellow's full living and other expenses (including all fees and cost of travel) during the tenure of his fellowship, which will be for one year, renewable for a further one or two years. Twelve CIBA Fellows are at present working at Aarhus, Brussels, Copenhagen, Geneva, Genoa, Heidelberg, Munich, Stockholm, Strasbourg and Zurich.

University News:

Birmingham

The following appointments have been announced: *Lecturer*, Dr. N. Baggett (chemistry); Mrs. Christine A. McCauley (computing in the Department of Mathematical Physics). *Research Fellows*, C. Ashley (Imperial Chemical Industries Research Fellow in the Department of Mechanical Engineering); Dr. I. L. Dillamore (Imperial Chemical Industries Research Fellow in the Department of Physical Metallurgy); Dr. Teh-Hsing Wei (statistics in the Department of Pure Mathematics).

Bristol

The following have been elected to professorships: Dr. A. D. Buckingham (theoretical chemistry); G. F. C. Rogers (engineering thermodynamics); Prof. A. R. Battersby (organic chemistry). Announced also are the following appointments to lectureships: Dr. K. H. G. Ashbee (metallurgy and ceramics, within the Department of Physics); M. Chisholm (geography); Dr. K. B. Linton (medical bacteriology); Dr. S. Moran (pure mathematics); N. F. Richards (civil engineering); Dr. R. A. Sawyer (aeronautical engineering).

Edinburgh

The following appointments have been announced: *Senior Lecturers*, Dr. D. W. Moss (clinical chemistry); Dr. D. C. Simpson (medical physics); Dr. J. M. Last (public health and social medicine). *Lecturers*, Dr. E. M. Dixon (astronomy); Dr. J. S. McKinley McKee (biochemistry); Dr. Winifred L. Stafford (biochemistry); Dr. J. F. Collins (biophysics); P. Edmond (clinical surgery); H. Whitfield (computer unit); E. W. Powell (dermatology); A. M. Nicolson (electrical engineering); Dr. D. C. S. Hutchison (medicine); Dr. N. M. B. Doan (public health and social medicine); G. K. Crompton (respiratory diseases and tuberculosis); W. L. Ford (therapeutics).

Southampton

The following appointments have been made: Dr. R. L. Bell, reader in physical metallurgy in the University of London, to the chair of engineering materials in the Department of Mechanical Engineering from a date to be arranged. Dr. L. Brent of the National Institute for Medical Research, to the chair of zoology from April 20. Dr. W. A. Gambling, reader in electronics in the University, to the second chair of electronics. Dr. G. B. Trasler, lecturer in the Department of Sociology and Social Studies in the University, to the chair of psychology.

Synthesis of Organosilicon Monomers

It has been brought to the Editor's attention that a British edition of this book (reviewed in *Nature*, 204, 512, 1964) is published by Heywood Books (Temple Press Books, Ltd., London, W.C.1) to whom orders should be addressed by purchasers in the United Kingdom, the Commonwealth (excluding Canada) and Europe. The price is 130*s.*

Announcements

Mr. D. B. Collett, a director of Dunlop Rubber Co., Ltd., has accepted the invitation to deliver the twentieth Foundation Lecture of the Institution of the Rubber Industry. This will take place at the Midland Institute, Birmingham, on May 19, 1965.

A meeting on "Plant Waxes, Cutin and Associated Compounds", organized by the Phytochemical Group, will be held in London on January 7. Further information can be obtained from A. H. Williams, Research Station, Long Ashton, Bristol.

A symposium on "Energy Transfer in Radiation Processes", arranged by the Association for Radiation Research, will be held in University College, Cardiff, during January 7–9. Further information can be obtained from Dr. G. O. Phillips, Department of Chemistry, University College, Cardiff.

The 1965 Gordon Research Conferences on "Electrochemistry: Electrode Processes", "Polymers" and "Basic Chemistry of Aging" will be held in Santa Barbara, California, during January 25–February 5. Further information can be obtained from the director of the conferences, Dr. W. George Parks, University of Rhode Island, Kingston, R.I.

A joint meeting of the North of England Section and the Special Techniques Group of the Society for Analytical Chemistry will be held in Leeds on December 11. A paper on "Some Aspects of Polarography" will be presented by M. L. Richardson. Further information can be obtained from the Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1.

MATERIALS SCIENCE AND TECHNOLOGY

A CALL FOR ACADEMIC AND INDUSTRIAL COLLABORATION

A CONFERENCE on "Materials Science" was held at the Whateley Hall Hotel, Banbury, during October 2–4; this was the second of its kind to be held in Britain. The first[1], held in May 1963, was arranged by the Institution of Chemical Engineers. Since then, the Materials Science Club has been formed, and this year's conference was held under the auspices of this new organization, with Prof. E. W. J. Mitchell (University of Reading) co-ordinating the arrangements.

The Club itself is independent of the existing professional institutions, and its purpose is to hold meetings at which research and education in materials science and technology can be discussed in an informal atmosphere. While being independent and informal, it does, however, hope to exert an influence on developments in education and on Government research.

The conference's tone of informality was set by a stimulating lecture, "Toughness", delivered after the opening dinner by Dr. F. J. P. Clarke (Atomic Energy Research Establishment, Harwell). A further lecture which, like Dr. Clarke's, was not intended to form part of the main business of the conference was given next day by Dr. A. C. Rose-Innes on the subject "New Superconducting Materials and their Uses".

The conference consisted of three half-day sessions. The first of these was entitled "Collaboration in Teaching and Research between Universities and Industrial Laboratories" and consisted of six papers. Dr. K. Hoselitz (Mullard Research Laboratories) established what was later seen to be the general feeling of the meeting, that much more collaboration between universities and industry is necessary. He pointed out that, although some university staff already acted as consultants for industry and industrial firms place research contracts with universities, an extension of this practice is desirable. Universities could reciprocate by inviting consultants from industry to advise on both research and teaching, personnel might be exchanged for short periods and industrial scientists should act as part-time lecturers.

Dr. J. Hill (Atomic Energy Research Establishment, Harwell) described the great variety and extent of the collaboration which is practised between Harwell and teaching institutions. He outlined the system of research contracts, the interchange of staff, the work carried out at Harwell by university research students and the short-term research appointments. On the teaching side, courses for university lecturers are provided, demonstration equipment is lent to universities and many staff act as examiners for higher degrees. Dr. Hill's experiences appeared to contradict those of Dr. Hoselitz, and this marked difference between the collaboration which a university may expect from an industrial firm and a Government laboratory was emphasized by the next speaker, Prof. E. W. J. Mitchell, who enquired whether commercial security was the reason for industry's greater reluctance to invite university staff into their laboratories.

Prof. Mitchell showed how the universities were, at least to some degree, already collaborating with industry in most of the ways which the previous two speakers had mentioned, and pointed out that the structure of university government did not in itself prevent an extension of this kind of activity. He warned, however, of the dangers and futility of superficial collaboration. For effective collaboration, an industrial scientist lecturing to postgraduate students must be brought into the stream of university student life in some way, and a university lecturer acting as an industrial consultant should be invited to join research projects at the earliest planning stage and not used merely as an experimental supervisor.

The next speaker, Dr. H. M. Finniston (International Research and Development Co., Ltd., Newcastle), stressed the role which the universities could assume in educating industrial scientists, and asked them to consider whether they were using the most effective aids to this purpose. Lectures could be filmed and taped, for example, and made available to a wide public. Market research could be carried out by universities in order to discover which subjects were of most direct interest to industrial scientists at the present moment, and courses should be provided in these subjects. Dr. Finniston spoke also of the tendency of young scientists nowadays to choose a university career in preference to industry, and suggested that industry might use co-operation as one means, although an indirect one, of obtaining the services of these men.

Dr. L. Holliday (Shell Chemical Co., Ltd.) emphasized that the driving force in establishing this collaboration must come from the universities; industry itself is usually very willing to co-operate. A function which industry is well placed to perform is the carrying out of surveys of useful research programmes through trade associations. He spoke of the importance of proximity in fostering collaboration, and hoped that research associations would take this into consideration in choosing a site for a new laboratory, and that a university would perhaps consider seriously the possibility of having research associations on its campus.

The last speaker of this session, Dr. E. G. Stanford (Aluminium Laboratories, Ltd.), disagreed with earlier speakers in one respect, that he did not wish to see universities becoming too concerned with seeking industrial collaboration, but they should follow their own research interests. On the other hand, he wanted to see a considerable increase in liaison between industry and all institutions concerned with higher technological education. Too little liaison exists at present. He stressed a point that Dr. Holliday had made, that lecturers must go out into industry to find what is needed in research before deciding what subject to work on; the success of collaboration depends largely on the readiness of lecturers to go out and learn what problems industry is dealing with.

During the discussion which followed, it became apparent that, while everyone present agreed that a great increase in collaboration would be a healthy development, both for universities and industry, there were marked differences of opinion regarding the mechanism of collaboration which would be most effective and also regarding the influence which it could be expected to have on students.

Unanimity was, however, to be found among speakers with a common background. For example, Prof. J. G. Ball (Imperial College of Science and Technology), Prof. K. M. Entwhistle (Manchester College of Science and Technology) and Dr. N. Thompson (University of Bristol) spoke of the responsibility of a university to provide an integrated scientific education, both lecturing and research being directed to this purpose. With this in mind, industrial experience for undergraduates could be regarded as irrelevant, and industrial lecturers on undergraduate

courses are unnecessary. Postgraduate studies are another matter, and students at this stage should learn something of industry, but it is still important that by far the greater part of research for higher degrees, which must be regarded as an extension of the undergraduate work, should be carried out in the university itself.

A different point of view was expressed by Dr. C. P. Sandbank (Standard Telecommunication Laboratories, Ltd., Harlow), who is a consultant to the Electrical Engineering Department of the University of Edinburgh. In a subject in which the technology is changing rapidly and fundamentally, in which assemblies of electronic and electro-mechanical devices are being replaced by minute crystals, it is not sufficient to rely on information being fed to universities by staff with recent industrial experience. It is obviously better if some people working in industry have the formal responsibility of introducing up-to-date material to the students. There are difficulties in the way, such as commercial security and the lack of rapport between visiting lecturers and students, but the climate in industry is becoming more sympathetic, and the machinery which has enabled university staff to contribute to the success of industrial research can no doubt be extended to encourage the reciprocal process.

Dr. Holliday brought out again a point which Dr. Finniston mentioned in his address, the importance to industry of encouraging the best graduates from universities to take up industrial work and the difficulties encountered at present in doing this. To overcome these, he urged universities to allow research students to carry out a substantial part of their work in industrial laboratories. The value of doing this is already appreciated in applied sciences, but not in the pure sciences.

It was surprising to find that, in a discussion of the relevance of industrial experience to undergraduate studies, sandwich courses should come in for so little comment. It was left to Mr. K. M. Ginsburg (Battersea College of Advanced Technology) to raise the subject, and Dr. W. A. Holmes-Walker (Metal Box Co., Ltd.), who has experience of the industrial side of the scheme, spoke of the effectiveness of this type of training for undergraduates who intend to enter industry. Very few other speakers took up the subject.

The establishment of part-time chairs in universities for industrial scientists was discussed, and the system employed in Holland and other European countries was described by Prof. R. W. Cahn (University College of North Wales, Bangor). Several speakers described how a similar scheme, on a much smaller scale, operates in Britain. There is no difficulty in using funds from the University Grants Committee for this purpose, and many firms would encourage their senior men to take these positions. There was general agreement that, as an experiment at least, more part-time posts with senior rank in the universities should be created.

It is difficult to sum up briefly the results of the discussion, beyond that a general desire for collaboration exists, but it would be fair to comment that the desire appears to be more on the part of industry, rather than of the universities. All the university scientists who spoke were well aware of the debt which they already owe to industry for its help, particularly in the form of research grants, and several of them pointed out that university research is almost wholly financed from sources other than the University Grants Committee, but a deliberate break with tradition would be required to bring industry closer to the students than it is at present. The impression left by the discussion was one of industry trying to encourage universities to come out rather than of universities begging industry to come in.

The second session of the conference had the title "Materials Science in Universities", and the chairman was Dr. Holliday. The first speaker was Prof. K. M. Entwhistle (Manchester College of Science and Technology). He directed attention to the fact that, in thinking about a particular subject such as materials science, it is necessary not to lose sight of the general purpose of a university education. Undergraduates should be presented with a demanding and challenging course of study which could stimulate critical, logical and independent thinking; and, at the same time, the university, as part of society in general, should create in the student an attitude of mind which makes him eager to contribute to it. Materials science he defined simply as an integrated and scientific study of the relation between the structure and properties of matter. The *ad hoc* methods used in the past in the development of materials are still necessary in the solution of practical problems, but theoretical understanding of the behaviour of solids is rapidly improving, and materials science now is a study in which theory and practice interact. For this reason, there is much room for experiment in the way the subject is treated as an academic discipline. But there is a ribbon of continuity which runs through the theory of the behaviour of solids which makes the theory at present accepted sufficient to present a challenging and satisfying course. Prof. Entwhistle went on to consider whether materials science could most profitably be introduced at the undergraduate or the postgraduate stage. His suggestion was that a limited number of institutions should experiment with undergraduate courses, with existing courses in subjects such as metallurgy remaining in their present form, but at postgraduate level it is vital that an enormous extension of the general study of materials should take place.

The second speaker in this session was Prof. R. Edgeworth-Johnstone (University of Nottingham), who, as a chemical engineer, described himself as a consumer of materials science, in the respect that it is a subject provided for his students by other departments. The kind of problems he faces are: What kind of course should chemical engineers ask for? What proportion of time should be devoted to different materials? How is such a course to be related to industrial work? The answer to some of these would be provided if a survey could be undertaken of the relative importance in economic terms of the various classes of materials. Prof. Edgeworth-Johnstone considered that materials science within a university organization is unlikely to appear as a separate discipline, and the possibility is that existing departments may group themselves together. In this case, care should be exercised to ensure that a balanced view of materials as a whole can be taken by the unified group. The purpose of the group, however it is formed, should be to provide a course which will teach engineers to make a critical assessment of the materials they use. Finally, he described the organization of design study groups and emphasized the economic importance of including in these groups a materials specialist.

It was almost inevitable that the discussion which ensued should be concerned with existing or projected university courses in materials science. The undergraduate course at the University of Reading is already in its second year, a more technological course at Bradford Institute of Technology has just begun, and a similar course, but with a heavier bias towards metallurgy, is to begin next year at Battersea College of Advanced Technology. A postgraduate course being offered at Woolwich Polytechnic was described by Dr. D. J. Morantz, and the course which is now running for the second time at the Imperial College of Science and Technology was outlined by Dr. J. C. Anderson. This latter course was the subject of much discussion. Enrolment of suitable students had been difficult at first, but this is now improving. The greatest difficulty, however, and one to which no immediate solution is available, is that of obtaining maintenance grants for the postgraduate students. But for the generosity of one or two firms, such as International Research and Development Co., Ltd., grants would be almost unobtainable, and there seems to be no hope of an

extension of postgraduate teaching until this situation is altered.

The title of the final session of the conference was "The Appreciation of Materials Science and Technology in Schools", and the chairman was Prof. Mitchell. At first sight this would appear to be an unusual subject for a conference of this kind, but it was soon apparent, both in the talks and in the discussion, that the attitudes of schoolchildren and their teachers are of great concern to university teachers, particularly in the applied sciences.

Prof. J. G. Ball, the first speaker, spoke of the almost total lack of appreciation of the nature of applied sciences, including materials science, which, in his experience, exists among schoolboys. There is a barrier in their minds between pure sciences on one hand, and what they regard as second-rate technological subjects on the other. Physics and chemistry, the sciences with which they are familiar, seem to be unnecessarily remote from real materials and real life. He described his attempts to provoke interest in metallurgy in sixth-formers, and particularly his discovery that their ideas of the subject were only of the craft aspect of it, of forges and lathes, and they had no idea of the intellectual side. This intellectual challenge was, in his opinion, the approach which should be made in order to correct the erroneous ideas which boys have picked up. An interesting note of uncertainty came into Prof. Ball's address, as he confessed that he was not yet completely convinced that a course in pure science was not the best preparation for a career in applied science, for some students at least. He concluded his address with suggestions for ways in which an interest in materials science might be aroused in schools, all of which have been used in existing subjects to a small extent. Careers pamphlets issued by professional organizations, conferences with schoolmasters, displays and exhibitions provided by universities were some of the means suggested.

Mr. M. Deere (Reactor School, Atomic Energy Research Establishment) outlined the work being carried out by the Reactor School at Harwell to give schoolmasters and their pupils an opportunity to meet technologists and to learn from them the nature of their work. Teachers attend courses on which the lectures are given by senior scientists. Facilities are provided for science teachers to undertake research work at Harwell, and scientists visit schools to give lectures and to give advice on scientific projects carried out by pupils. Courses for mathematics teachers have also been held, and courses for careers masters are planned. Boys in the first year of sixth-form work will be invited to courses which will be designed to bring them into direct contact with the technical work carried out at Harwell by discussing with the scientists real problems which are being tackled there. The purpose of this is not recruitment, but is rather what Prof. Ball spoke of earlier, an attempt to give boys a realistic image of the work of an applied scientist and to reveal the intellectual challenge which the work provides.

Early in the discussion which followed, Mr. R. S. Barron (Dauntsey's School) described a scheme which is being used in his school to give sixth-form boys an opportunity to become acquainted with engineering. Each boy carries out, over a period of 12 months, a project of his own choosing, and a report of the work is assessed for the Advanced Level of the General Certificate of Education by the Cambridge Examination Board. Advice on the work and assistance with the provision of expensive equipment are sought from industry and from Government research establishments. It came as a surprise to many in the audience to find that the Advanced Level examination scheme is flexible enough to permit enterprise of this kind.

Mr. M. H. Devenport (Eton College) attributed the lack of interest in applied science among schoolboys partly to the subject's lack of glamour when compared with, say, physics and its association with the Nobel Prizes. He was of the opinion that a move towards a less academic type of science in schools could be brought about through the existing examination system, without radical changes, provided that engineers give a clear lead in suggesting lines of work which are interpretable at sixth-form level. Several technologists present were dismayed by Mr. Devenport's remarks about the way their subject failed to inspire boys, but had to admit the truth of them, and suggestions for making applied science more appealing carried little conviction. There was no doubt in anyone's mind that a great deal of work of the kind described by the various speakers would have to be done before a noticeable change in schoolboy attitudes could be expected.

It was left to Prof. Entwhistle to point out that there appeared to be no reason why schoolgirls should not be encouraged to study materials science. It was the first time in the conference that they had been mentioned.

R. STEADMAN

[1] *Nature*, **199**, 233 (1963).

THE EUROPEAN ATOMIC ENERGY COMMUNITY

THE seventh annual report of the European Atomic Energy Community (E.A.E.C.)*, commonly known as Euratom, covers the period March 1963–February 1964 and consists of two distinct volumes, both in French. Volume 1 discusses in general terms the need for, and the economics of, the development of nuclear energy facilities in Europe; the co-operative programme of research undertaken by the six member states of Euratom; the relationship between Euratom and industry; the establishment of nuclear research centres and the dissemination of information about nuclear energy; health and safety precautions; and the links between Euratom and other nuclear energy organizations. The evidence, including the relevant technical facts and figures, for the statements and conclusions described in Volume 1 is given in the 37 articles which form the contents of Volume 2.

The illustrated report (*Eur* 1850*e*), though perhaps not so comprehensive as the annual report, is a much more readable and useful picture of the progress achieved by Euratom. It is in English and confines discussion in its 19 sections to a limited number of aspects of the technical and research work of the organization. Each section is written by one or more members of the group actively engaged in the particular aspect discussed. In the brief introduction to the report reference is made to the treaty signed in Rome on March 25, 1957, by the Governments of the member states (Germany, Belgium, The Netherlands, Italy, France and Luxembourg) establishing Euratom, and to the tasks and objectives of the Community. The aim is to foster progress in the peaceful uses of nuclear energy. Its main activities are carried out at, or directed from, the Joint Nuclear Research Centre, comprising the four establishments at Ispra (Italy), Petten (Netherlands), Geel (Belgium) and Karlsruhe (Germany). Ispra is the main establishment and has at its disposal large-scale digital and analogue computers essential for reactor studies, and there is a separate research programme on automatic translation and documentation. The European library of nuclear codes recently established by O.E.C.D. is to be housed at Ispra. The

* Communauté Européenne de l'Énergie Atomique, Euratom. Septième Rapport Général sur l'Activité de la Communauté, Mars 1963–Février 1964. Pp. 118. (Bruxelles: Euratom, 1964.) Documentation jointe au Septième Rapport Général sur l'Activité de la Communauté, Mars 1963–Février 1964. Pp. 213. (Bruxelles: Euratom 1964).

reactor physics, materials and engineering departments of the establishment are almost entirely concerned with the *Orgel* reactor project. The *Orgel* reactor is fuelled by natural uranium (in the form of ceramic compounds or alloys), heavy water moderated and cooled by high-boiling organic compounds. The structural materials consist of special preparations of light metals. Section 3 of the report by J. C. Leny and S. Orlowski describes in detail the *Orgel* project and the associated *Orgel* critical experiment and the *Essor* test reactor, which is now being constructed and which is expected to go critical at the end of 1966.

The Central Bureau of Nuclear Measurements, the establishment at Geel, is envisaged to become, in the nuclear field, a bureau of standards similar to the well-known national standards laboratories. The laboratories of the Central Bureau as planned in 1960 are now nearing completion; and by the end of 1964 the Bureau will have received all its heavy equipment. The pulsed 3-MeV Van de Graaff accelerator with a Mobley bunching system is in operation and the pulsed electron linear accelerator, of the travelling-wave type (*S* band, approx. 10-cm wave-length), is now being installed. The duties of the Bureau include not only the preparation and conservation of primary standards and their intercomparison with those of other laboratories, but also the improvement of standards, instruments and methods of measurement in addition to the accurate measurement of nuclear data. A full description of the Bureau together with its equipment for fissile and stable isotopes is given in Section 2 of the report.

Petten is a general-purpose establishment, the main function of which at present is to operate the test reactor (*HFR*) provided by the Dutch Government through the Reactor Centrum Nederland. The establishment is responsible also for the management of certain other important associations—*BR*2, Dragon, *THTR* and *Kema*—and a general programme of study related to them. The reactor *HFR* is virtually identical with the reactor *ORR* at Oak Ridge, and, consequently, close relations exist between the Petten and Oak Ridge establishments with regard to high-temperature reactors.

The specialized establishment, the Transuranium Institute at Karlsruhe, now in the course of construction on a site assigned to Euratom by the Gesellschaft für Kernforschung, is to be devoted to the non-military uses of plutonium in the nuclear energy industry. The laboratory will be brought gradually into use from about mid-1964 and is expected to be complete by 1966.

In addition to the sections dealing with the research establishments, there are sections dealing with thermonuclear research, radiobiology, radiation protection, industrial development of power reactors, marine reactors, the Euratom–United States joint programme, and the activities of the Eurisotop Bureau. The total personnel employed by Euratom on January 1, 1963, was 1,910, of which some 1,500 were members of the research establishments. The total is expected to rise to 3,200 by the end of the five-year programme in 1967.

NUFFIELD PROVINCIAL HOSPITALS TRUST

THE Nuffield Provincial Hospitals Trust has published its sixth report, recording the progress of schemes described in the previous report and announcing new projects during the past three years. A wide range of activities has been supported, and 150 projects are listed under 15 general headings*.

In welcoming the Ministry of Health's entry into the field of providing finance for service and operational research, the Trustees note that "this policy is to be applauded and the competition it introduces will be stimulating. Of course, it does not follow that there will henceforth be an abundance of money available for the kind of study which indicates how health services can be made more effective, or whereby resources can be deployed in a better way. Yet the implication is that Britain is entering a period of more intensive scientific enquiry into its health services—surely a wise investment from which excellent dividends may be expected in the future". The Trust's own contribution to this investment during the period under review amounted to £1,305,000. During the period covered by the report 13 books have been published on sponsored research, and several other projects have resulted in reports published under other imprints.

The most important single new subject appearing in the report is postgraduate medical education. The Trustees' decision to allocate a large sum of money (£308,750) to help promote schemes in the provinces has stimulated a great deal of activity. The aim of the Trustees, to stimulate the development of a comprehensive national policy for the continuing education of doctors, and so lead to the improvement of medical-care services, seems in a fair way to being realized.

The following selection gives an indication of the range of the Trust's interests: A grant of £20,000 over five years was allocated to the Oxford Regional Hospital Board for operational research studies of a number of aspects of hospital problems. £5,000 went to the United Birmingham Hospitals for a survey of health services for children in the City of Birmingham, and £1,000 to the University of Nottingham for a study on recruitment to, employment of, and demand for, certain of the professions supplementary to medicine; physiotherapy, occupational therapy, dietetics, orthoptics, and radiography. £2,400 over two years was made available for the secondment of a nurse to work in the Casualty Department of the General Infirmary at Leeds to investigate the cause and prevention of accidents in the home.

The North-Eastern Regional Hospital Board, Scotland, received a grant of £50,000 for the building of an in-patient and day hospital unit for child psychiatry in Aberdeen, and to cover the first year's running costs. The Aberdeen area, the report states, is particularly suitable for a project of this kind "in view of the basic demographic knowledge already available about the area, the close integration between the hospital service and the university department in mental health, the presence of an active research organization and the contacts already established with the local health and education authorities". £12,000 has been allocated for the provision of a parents' hostel at Smith's Hospital, Henley-on-Thames, which has facilities for some fifty psychotic or severely disturbed children. It has been found that little success attends efforts to treat these children unless the staff work very closely with the children's parents, particularly the mothers, since the corner-stone of treatment is the restoration or encouragement of this relationship. To an increasing extent the child is treated through the mother, because only rarely can anyone else give the necessary time and individual attention to bring about the return of the child to his or her normal position in the family.

A grant of £4,000 was allocated in order to make possible a study of community mental health services in Devonshire, the main purpose of which is to discover the problems and difficulties experienced by patients who have been

* The Nuffield Provincial Hospitals Trust. Sixth Report: A Record of the Progress of Schemes and Descriptions of New Projects 1961–1964. Pp. 152. (London: The Nuffield Provincial Hospitals Trust, 1964.)

mentally ill and received hospital treatment but who have now returned to live in the community. The Department of Psychological Medicine, University of Edinburgh, has been granted £15,725 over three years to develop a psychiatric treatment service for alcoholics, while £100,000 has been reserved for the development of a health centre at Witney, near Oxford: "All the general practitioners of the town will practise from surgeries in the centre, which will also provide accommodation for local health authority and dental clinics and consultant outpatient clinics, as well as X-ray and pathological facilities run by the Oxford Regional Hospital Board."

A further allocation of £92,250 has been made to the Hospital Engineering Unit at the University of Glasgow to undertake a programme of research work concerning problems met in heating, ventilating and air-conditioning of hospitals, including the means of attaining proper standards of performance. Much of the practical work will be carried out in an experimental ward unit which the Scottish Home and Health Department has built at Hairmyres Hospital, East Kilbride, Glasgow, but research will also be carried out at other hospitals. Another project concerning the physical problems of hospital design involves the allocation of £15,000 to the United Oxford Hospitals and the Oxford Regional Hospital Board jointly to enable a special unit to be built at the Churchill Hospital, Oxford, in which experiments can be conducted into the lay-out of wards and departments and where equipment and procedures can be tested.

£35,000 has been ear-marked for an experimental intensive-care unit which has now been opened at Broadgreen Hospital, Liverpool. The report comments that the successes claimed for progressive patient care in the United States have won adherents in Britain for the persuasive principles involved. However, there has been little practical experience of the system, or of any of its stages in Britain, although experiments and investigations in the care of acutely ill patients, and management of post-operative cases, have always been a feature of Britain's hospitals.

SMOKELESS AIR

THE autumn issue of the quarterly *Journal of the National Society for Clean Air* (35, No. 131; 1964, London) opens with some appropriate lines: "Ships, towers, domes, theatres and temples lie Open unto the fields, and to the sky; All bright and glittering in the smokeless air". This obviously epitomizes the aims of this organization, which have to-day become a matter of international concern.

Only a few decades ago the United Kingdom and the United States were the only countries actively organized to combat air pollution in big industrial cities, where the problem of contamination of the air breathed, in its progressively toxic aspects, had become a matter of both general and medical concern. "Today we find organization, investigation, research and legislation in many countries and in every continent . . . the natural result of the tide of industrialization flowing from the North Atlantic countries to almost every corner of the globe."

Progress of the movement since the early pioneer days is clearly revealed in the current number of the *Journal*, which contains a full first-hand report of the Council of Europe's Clean Air Conference at Strasbourg (June–July 1964). This has been described as the most comprehensive and longest event ever held on the problems of air pollution. Just previously, on the initiative of the Air Pollution Control Association of America, with the help of the U.S. Public Health Service, a meeting of six national air pollution organizations, including the United Kingdom, was convened and held at Washington, June 1964. The Strasbourg conference revealed not only what 14 member countries (including observers from the United States) had in common in air pollution problems, how different national situations determined procedure but, equally, despite the impracticable measure of uniformity in law and administration, the strong desire towards what is modestly described as 'harmonization' of mutual activities.

Fundamentally, at least in some parts of the United Kingdom, probably just as pertinent overseas, much depends on the availability and price of smokeless fuels, especially for domestic consumption; the position is not satisfactory at the present time, taking an overall survey, on either count. But air pollution is not only a question of utilization of smokeless solid fuel. As is well known, the Ministry of Transport in Britain has for some time been conducting a drive against smoke emitted by incorrectly maintained Diesel-engined vehicles using our public highways. The much-publicized national checks on some of the worst offenders have already had a disciplinary value, but, as another article in the *Journal* points out, the policy advocated by the Society is: ". . . that offenders should be required to put their vehicles in good order and that they should be prohibited from operating until this was done. This . . . is a better and more certain incentive to good practice than purely punitive measures".

Smokeless air, even better, pure air, as an environment in which both to live and to work, may be an ideal, perhaps unattainable in heavily built-up and industrialized areas, but at least the problem is a live one in most civilized countries to-day, and the organization responsible for this publication is doing great service in co-ordinating the efforts of everyone concerned in reaching the ultimate goal in Britain.

TRANSPLANTING MATURE DATE PALMS IN IRAQ

THERE is nothing very unusual nowadays in the transplanting of large trees for particular purposes. It is done in towns where the immediate effect of a large tree is required for amenity reasons and to save the wastage and delay in time brought about by the vandalism to which young trees are sometimes subjected in urban areas. So long as the special equipment is available, and the expense can be met, the operation has justified itself in the success it has had in temperate climates where advantage is taken of the dormant season of the trees, but it is a different matter in a hot climate where the rate of transpiration is one of the factors increasing the difficulty of the operation.

Mr. R. H. Nocton, an agronomist specializing in tropical agriculture, has recently prepared a paper describing the transplanting of 87 mature date palms in Iraq. The site was the New University of Baghdad and the trees ranged from 30 to 60 ft. in height. The operation was completely successful, and the following points are of particular interest.

The soil was alluvial to a depth of about 30 ft., fine textured and grading into sub-soil sand. This provided a good transplanting medium because water-holding capacity is better with fine-textured soils. From the nutrient point of view there were various difficulties such as the alkaline nature of the soil, *p*H 8–8·5, which tends to restrict the availability of certain nutrients to the trees and also the deficiency of important elements and the absence of nitrogen. Salinization of the lower root zone occurred, but this is known not to represent a serious factor where irrigation is practised. The climate made transplanting difficult because Iraq is tropical, with very hot summers, and always dry.

It is necessary to ensure the same orientation of the tree in the new site—especially the older the tree—and so a metal disk was nailed to the north side of the bole. Transpiration was arrested by pruning off the fronds to leave 6–8 per tree which were sprayed with water-dispersible plastic. Likewise, the root ends which were exposed on digging were sealed with plastic and the large bowl of earth, some 6 ft. in radius at the surface and about 7½ ft. deep, was covered with hessian during transportation. The tree with its bowl, a total of from 14 to 38 tons, was lifted by crane and moved to the new site in an upright position on a low loader. Planting took place 3–6 ft. deeper than normal to ensure stability and to promote the growth of roots. The hole was filled to within 2 ft. of the top and the space kept full of water for a month. Surface irrigation was undertaken and, although the Tigris is slightly saline, this did not matter because the major part of the irrigation occurred when the river was at its highest and the salt content was at its lowest level. In order to create a microclimate under the trees, a sprinkler irrigation system was used which not only increased the humidity from 18 to 80 per cent but also promoted root growth. New roots were produced on some trees two months after transplanting and a growth regulator was applied to any tree not showing new roots after three months.

The success of the experiment shows that physiological problems can be overcome. The economics largely depend on the availability of equipment, such as a suitable crane, low loader and excavator. The method is not only applicable to amenity planting but it is suggested by the author that it could be used to introduce 20–30-ft.-high date palms as shade trees in citrus plantations, thus saving some 15–20 years to the farmer. In this case the trees could be planted 3 ft. deeper than normal and the resulting formation of new roots would prolong their lives.

Copies of the paper can be obtained from Mr. R. H. Nocton, 591 Alwiya, Arasa, Iraq. C. J. Taylor

ISOLATION AND IDENTIFICATION OF THE INITIAL PEAK OF THE EARLY RECEPTOR POTENTIAL

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RECENTLY, Brown and Murakami[1] observed a new receptor potential in the eye of the cynomolgus monkey using intra-retinal microelectrodes. They found that the new response is produced only with high-intensity stimulus flashes and is characterized by an extremely short latency (< 0·1 msec) and a strikingly high resistance to anoxia. The amplitude maximum of the response was found to occur at the ciliary connexion between the outer and inner segments of the receptors. Brown and Murakami have labelled this response the early *RP* (receptor potential). Afterwards Cone[2] has shown that a similar response occurs in the electroretinogram (ERG) of a variety of vertebrate eyes. He found that in the albino rat the early *RP* almost certainly depends on the rhodopsin in outer segments of rods because the early *RP* has the same spectral sensitivity as rhodopsin and the amplitude of the early *RP* is linearly proportional to the pigment bleached by a flash. His evidence, therefore, strongly supports Brown and Murakami's conclusion that the response originates in the receptors.

These authors further noted that in some vertebrate eyes the early *RP* has a biphasic wave-form[2,3], a small corneal positive phase (the positive peak) preceding the dominant, corneal negative phase (the negative peak). We have now observed a similar wave-form in the excised eye of the albino rat by lowering the temperature of the eye somewhat below the physiological temperature (Fig. 1 *B*). Apart from opposite polarity the most notable feature that distinguishes these two peaks appears to be their strikingly different temperature dependence. By lowering the temperature sufficiently it was possible to abolish the negative peak and isolate the positive peak (Fig. 1 *C*). Significantly, the positive peak persists well into sub-zero temperatures. This behaviour suggested to us that the two peaks are produced by separate processes. Although the corneal negative component of the early *RP* has been shown to be a receptor potential, the positive component has never been investigated as a separate process. We, therefore, sought to determine whether or not the positive peak also originates in the receptors. If the positive peak is a receptor potential, because of its shorter latency it is probably even more closely linked to the 'primary events' of visual excitation than the negative peak.

Using the apparatus previously described[2] we undertook to observe the early *RP* in the ERG of albino rats (Sprague-Dawley) at various temperatures. The stimulus flashes were produced by a 65-joule photographic strobe lamp (Honeywell Strobonar 65*C*) having less than a 0·7-msec flash duration. To minimize artefacts the strobe lamp was housed in a soft-steel box, and the electrodes were shielded from the flash as much as possible. With these precautions the artefacts were found to be negligible. The effects of temperature could be studied on excised eyes, because the early *RP* has such a high resistance to anoxia[1,2]. However, since visual pigment does not regenerate in the excised eye of the rat, each set of observations was performed on an eye freshly excised from a fully dark-adapted animal. The electrodes were placed against the cornea and the back of the excised eye. The eye and the electrodes were then mounted in a chamber partly submerged in a thermal bath. Wratten neutral density filters were used to vary the stimulus intensity. The wavelengths of the stimulus flash were varied by using a set of five Baird-Atomic interference filters transmitting at 420, 450, 500, 560 and 620 mμ. The band-widths of the filters at 10 per cent transmission were found to be about ± 6 mμ.

The signals obtained in the rat at 35°, 25° and 0° C are displayed in Fig. 1. At the physiological temperature the negative peak completely dominates the positive peak (Fig. 1 *A*). As the temperature is lowered, however, the amplitude of the negative peak decreases rapidly from

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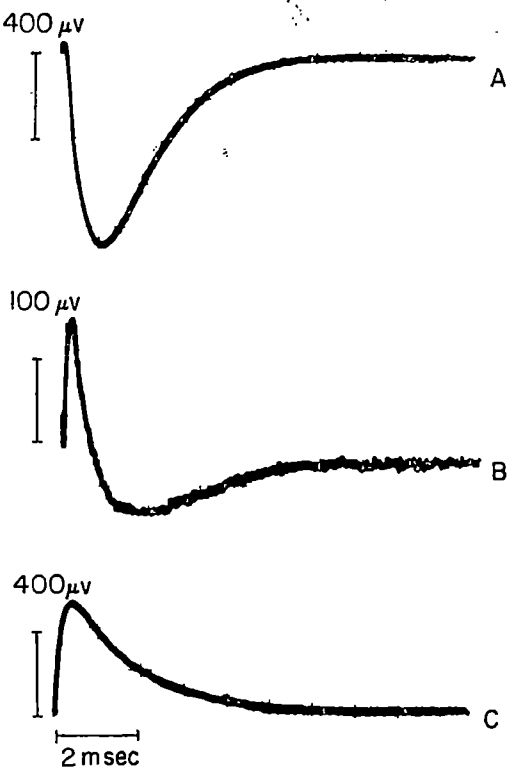


Fig. 1. Temperature dependence of the early *RP*. The positive and negative peaks of the early *RP* were elicited from the dark-adapted, excised eye of the albino rat by presenting a maximum energy, white flash at various temperatures: (*A*) 35° C, (*B*) 25° C, (*C*) 0° C

about 1 mV to about 100 μV in going from 35° to 25° C (Fig. 1 *B*). The negative peak is almost completely abolished at about 5° C. On the other hand, the positive peak becomes more and more prominent until it is essentially isolated below about 5° C (Fig. 1 *C*). Once the peak is isolated, its amplitude remains relatively stable at about 300–500 μV. The signal is still present at about −10° C. Qualitatively similar results have also been obtained with excised goldfish eyes. In this case, however, the biphasic response persists to much lower temperatures than in the case of the rat. In addition to these amplitude variations it may be seen in Fig. 1 that the responses, particularly the negative peak, slow down considerably as the temperature is lowered. Such changes in pulse speed with temperature should be valuable in excluding certain mechanisms which might be thought to generate these electrical responses.

As may be seen in Fig. 1, the positive peak always precedes the negative peak whenever both peaks are observed in the same record. This is consistent with the notion that the positive peak always has a shorter latency. Although the accuracy of the latency measurement is ultimately limited by the finite rise time of the stimulus flash, with the present equipment we can conservatively set an upper limit of 50 μsec for the latency of the positive peak at 0° C. At the physiological temperature the latency is probably much shorter.

The positive peak of the early *RP* has been observed in the cat, goldfish, frog[2] and cynomolgus monkey[3] at ordinary temperatures. Now, with the inclusion of the albino rat in this group, the positive peak has been obtained in every animal so far investigated. In the rat a suggestion of its presence can sometimes be detected even near the physiological temperature, provided the response is observed at sufficiently high sensitivity and sweep speeds. The effect of cooling seems to be mainly to suppress the negative peak without affecting the positive peak substantially. In both animals so far investigated the positive peak has been isolated by lowering the temperature.

The strikingly different temperature dependence exhibited by the two peaks strongly suggests that they are dependent on different processes. The positive peak could represent either: (1) an earlier, separate step in visual excitation, or (2) an event unrelated to visual processes. We sought to test the second possibility by identifying the pigment responsible for the positive peak. Since the positive peak has such a short latency, it cannot possibly be produced far from the absorbing pigment. Therefore, if the positive peak could be shown to be dependent on the visual pigment in the rod outer segments, it must follow that the positive peak, in all likelihood, is a receptor potential. Such an observation would exclude the second possibility. A series of experiments designed to identify the pigment were performed near 0° C on the excised eye of the albino rat. At this temperature the positive peak is almost completely isolated, and its amplitude is near its optimum value. The albino rat was used, because in the ERG of this animal only one visual pigment, rhodopsin, need be considered, and the eyes of the animal contain very few other pigments.

Since the negative peak has already been shown to be a receptor potential, it is pertinent to mention that, apart from the polarity and the temperature dependence, the properties displayed by the two peaks of the early *RP* are very similar. They both appear in the same high-intensity stimulus flashes; they both exhibit extremely short latencies; and they are both highly resistant to anoxia. In the series of experiments recorded here further similarities came to light. First, the amplitude of the positive peak was found to be reduced by successive flashes of high intensity in a manner consistent with the bleaching or excitation mechanism of visual pigments involving photoregeneration[4,5]. Furthermore, the amplitude of the positive peak, when plotted against the flash energy, produced a curve closely resembling that obtained by Cone[2] for the negative peak. Thus the amplitude of the positive peak appears to be linearly proportional to the amount of pigment bleached by the stimulus flash as in the case of the negative peak.

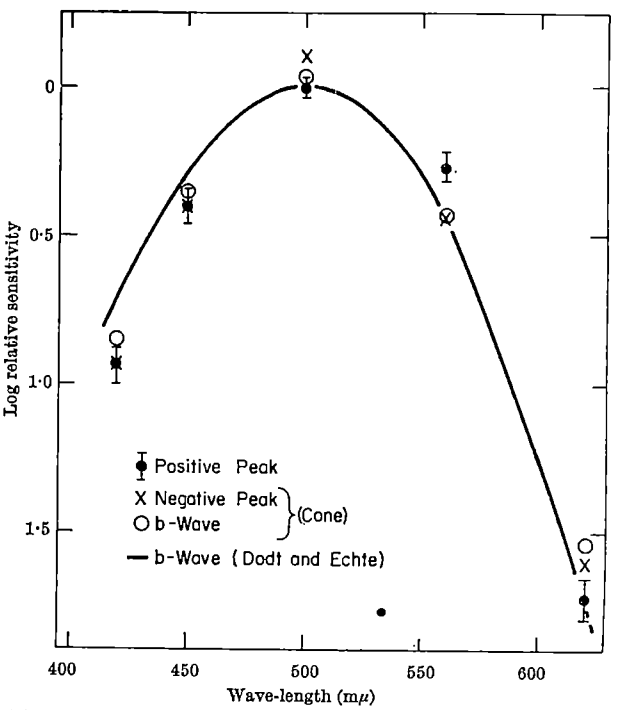


Fig. 2. Comparison of the spectral sensitivities of receptor potentials in the albino rat. The spectral sensitivity of the positive peak (•) has been superposed on the spectral sensitivities of the negative peak (×) and the *b*-wave (○) published in Fig. 2 of ref. 2. The solid line is the spectral sensitivity of the *b*-wave redrawn from Fig. 4 of ref. 6

Perhaps the most direct and convincing way of identifying a pigment is to measure a spectral sensitivity of the given response. The spectral sensitivity of the positive peak was obtained by finding the stimulus intensities needed to elicit equal responses at five different wavelengths. The results are presented in Fig. 2 along with the spectral sensitivities of the negative peak and the dark-adapted *b*-wave measured by Cone[3] and the spectral sensitivity of the dark-adapted *b*-wave obtained by Dodt and Echte[6]. The errors indicated on the positive peak spectrum do not include systematic errors arising from the optical arrangements, the calibration of the flash spectrum, etc. They represent statistical errors only. Since essentially the same experimental arrangement was used for the present measurement and Cone's earlier measurements, the systematic errors are expected to play only a minor part in the comparison of these results. We have remeasured the action spectra of the *b*-wave and the negative peak with our present arrangement to ascertain that no significant changes have taken place in experimental conditions since Cone's earlier measurements. As may be seen in Fig. 2, our positive peak spectrum is in good agreement with Cone's negative peak and *b*-wave spectra. There appears little doubt that all three responses are produced by the same pigment.

These results, particularly the action spectrum, almost conclusively point to rhodopsin as the pigment responsible for the positive peak. As possible sources of the rhodopsin, both the rod outer segments and the pigment epithelium need to be considered. Dowling[7] has shown, however, that no rhodopsin exists in the pigment epithelium of the albino rat. It therefore appears almost certain that the rhodopsin responsible for the positive peak resides in the rod outer segments. Since the latency is so short, the positive peak is probably generated very close to the rod outer segments. We therefore conclude that the positive peak is a receptor potential which manifests itself through a process earlier and distinct from that producing the negative peak.

Brown and Murakami[1] and Cone[2] have suggested that the early *RP* is probably closely connected with the initial photochemical events in visual excitation because of its short latency and its linearity with respect to the amount of rhodopsin bleached. Of the two peaks of the early *RP* the positive peak with its shorter latency is probably associated with an earlier step in the process of visual excitation. To the best of our knowledge it is the only electrical response recorded in the mammalian eye at subzero temperatures. Since a great deal of information regarding the initial events in the bleaching of rhodopsin has been obtained by investigating photolysis of the pigment at low temperatures[8], the positive peak of the early *RP* may provide a valuable means of linking the early physico-chemical events with the electrical responses of the eye.

This work was carried out under contract with the U.S. Atomic Energy Commission, and was supported by U.S. Public Health Service post-doctoral traineeships. We thank Prof. John R. Platt for his advice.

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CRANIAL EPIPHYSES IN MAMMALS

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EPIPHYSES are anatomical mechanisms necessary to the post-natal development of most of the bones of the mammal skeleton, being found (principally as the pressure- and traction-epiphyses of Parsons'[1] classification) in association with the vertebræ and ribs, the limb girdles, and the long bones at least of the limbs. Despite their considerable functional importance in skeletal maturation such structures are apt to be ignored by the standard treatises on vertebrate morphology or else to be accorded but incidental mention in connexion with the ossification pattern of a long bone. It would thus appear that, as objects of study, epiphyses have been relatively neglected except by human morphologists. Knowledge concerning epiphysis development, structure and variation is more abundant for man than for any other mammal and it may be that the human pattern of epiphysis topography has become implicitly accepted as canonical for mammals generally. If this be so, it may explain the absence so far of any record of the occurrence of an epiphysis in connexion with any mammalian cranial bone. Certainly cranial epiphyses are unknown in man and his primate kin; but that such structures may—indeed do—occur in some mammals, at least, this article purports to show.

An undoubted epiphysis (ossiculum mastoideum) is present in relation to the temporal bone of the otariid pinnipedes and another epiphysis occurs in relation to the lacrimal bone of tapirs, elephants and rhinoceroses.

The ossiculum mastoideum is observable in the genera *Arctocephalus*, *Eumetopias*, *Otaria* and *Neophoca* (and is probably present also in *Callorhinus* and *Phocartos*). Its development and fate have been studied by Cave and King[2] in the extensive series of *Arctocephalus* specimens in the British Museum (Natural History), which include examples of all growth stages from birth to old age.

In the young *Arctocephalus* pup the squamo-mastoid suture occupies a sulcus bounded anteriorly and posteriorly by bony lips, to the posterior of which are attached the sternomastoid and other powerful neck muscles. Progressive growth of these lips converts the sulcus into an oval vallecula, the floor of which, like the posterior lip itself, manifests in older pups a typically epiphyseal configuration. Meanwhile, in the cartilage filling the vallecula, the mastoid ossiculum—a traction epiphysis on the mastoid process—makes its appearance as a thin, curved, osseous flake. It enlarges progressively to an average maximum size of some 16 mm × 10 mm in the adult male and some 8 mm × 5 mm in the adult female. As a discrete ossicle, synchondrosed to the mastoid process and separable therefrom by maceration, it persists until late adult life, when it effects complete synostosis with that process and leaves no trace whatever of its former independence.

The antorbital process of the lacrimal bone of tapirs manifests a half-moon-shaped, anteroposteriorly compressed epiphysis, which detaches readily prior to the commencement of its synostosis with the lacrimal. Its presence is indicated by such specimens as B.M.1912.4.22.1 (*Tapirus indicus*), B.M.1948.5.21.3 (*Tapirus terrestris*), B.M.1872.1.24.2 (*Tapirus roulini*) and C.58.3 (*Tapirus* sp.).

A hemispherical epiphysis is associated with the antorbital process of the lacrimal bone in the Asian and the African elephant. Obtrusive examples of its presence in *Elephas maximus* are provided by B.M. specimen 1879.11.21.693 and by the mounted skeleton in the Ghar Dalam Museum, Malta. In *Loxodonta africana* material it is present in B.M. specimens 10410, 1921.1.1.36 and 49.697; it is incompletely demarcated by an epiphyseal line in B.M. specimens 49.695 and 1937.5.20.2; in B.M. specimen 1928.11.13.1 a portion of one epiphysis remains *in situ* and is passively movable on the lacrimal bone.

A lacrimal epiphysis occurs in all rhinoceros genera. In *Rhinoceros unicornis* B.M. specimens 1951.11.30.2 and 3.2.13.1 this epiphysis remains *in situ*; from B.M. specimens 1952.4.1.1 and 70.3.10.18 it has been lost after death.



Fig. 1. *Arctocephalus tropicalis gazella.* Right mastoid epiphysis (*E*) *in situ*



Fig. 2. *Ceratotherium simum.* Left lacrimal bone showing lacrimal epiphysis (*E*) *in situ*



Fig. 3. *Ceratotherium simum.* Right lacrimal bridge showing bed (*EB*) of detached lacrimal epiphysis, between the superior and inferior lacrimal foramina

B.M. specimen 1902.12.18.1 displays the corresponding epiphysis of *Rhinoceros sondaicus*.

In *Didermocerus sumatrensis* (which agrees with the African form *Ceratotherium simum* in having the lacrimal fossa invariably bridged posteriorly by bone) a lacrimal antorbital epiphysis is present; it appears bilaterally in B.M. specimens 1948.1.14.1 and 1461*b*, and unilaterally in B.M. specimens 1949.2.1.1, 79.3.11.1, 86.12.20.18 and 1952.4.1.2.

The *Diceros bicornis* lacrimal epiphysis is observable *in situ* in specimens B.M.99.6.29.11 and C.58.11 (private collection).

In *Ceratotherium simum* material the epiphysis is present bilaterally in specimens C.58.7, 58.8 and 58.9; its former independence and extent are indicated by epiphyseal lines or scars in specimens C.58.5, C.58.18, B.M.1948.1.28.1 and B.M.1948.1.28.2.

The lacrimal epiphysis in *Rhinoceros*, *Didermocerus* and *Diceros* is a flattened hemispherical cap on the antorbital process. Exceptionally (for example, *Rhinoceros*, *Diceros*) it may develop from two centres of ossification or (for example, *Didermocerus*) may extend medialwards into the lacrimal fossa parietes. In *Ceratotherium* it is responsible for the ossification of the posterior arm, the apex and part of the anterior arm of the osseous bridge over the lacrimal fossa and for some portion of the lateral wall of that fossa.

All the forms manifesting a lacrimal epiphysis possess thick tarsal plates and a well-developed and active nictitating cartilage; the epiphysis is a traction epiphysis developed in response to the tension exerted on the antorbital process by the attached and powerful tendo oculi. The lacrimal antorbital process is itself essentially a 'muscular' process for the attachment of the medial canthic structures, the development and activity of which may (as in the elephants) render this process the dominant portion of the lacrimal bone.

It is not suggested that mastoid and lacrimal epiphyses are restricted to the genera mentioned above. The present observations are submitted to draw attention to the hitherto unnoticed association of traction epiphyses with certain bones of the mammalian cranium. A fuller account of these structures will be published elsewhere.

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ARE PEPTIDE ANTIBIOTICS SMALL PROTEINS?

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IN the 35 years since Fleming's report[1] of the anti-Gram-positive bacterial effects of the peptide antibiotic, penicillin, nearly 65 other peptide antibiotics have been mentioned in the literature. These substances produced by a wide variety of micro-organisms contain one or more amino-acids or moieties derived from amino-acids. Some are effective in inhibiting the growth of various bacteria, Gram-positive and/or Gram-negative, others the growth of yeasts and fungi, and some have anti-neoplastic properties.

The recent interest in the genetic code and the biogenesis of proteins[2] has stimulated studies on the origins of the peptide antibiotics[3–6]. The findings that the 'small' physiologically active peptides, bradykinin[7] and angiotensin[8], arise from the specific enzymatic hydrolysis of larger proteins might be looked on as justification for the hypothesis that antibiotic peptides are also derived by similar mechanism, their biosynthesis being directed by 'special' genetic codes. The literature does not support the view that antibiotic peptides are formed from larger proteins, and we conclude that a different type of mechanism must be involved in the biosynthesis of antibiotic peptides from that concerned with formation of cellular proteins.

A summary of some of the biological and chemical properties of 42 of the better-characterized peptide antibiotics is presented in Table 1. Structures have been proposed for 21, and nine of these have been confirmed by chemical synthesis: benzylpenicillin, cephalosporin *N*, gramicidin *S*, enniatin *B*, actinomycin *C*, valinomycin, amidomycin, and polymyxin. In many cases, the micro-organisms producing these antibiotics synthesize a group of closely related substances rather than a single entity, and these have been grouped in this tabulation. Also the closely related antibiotics produced by different micro-organisms are mentioned as a group, for example, vernamycins *B*, ostreogrycins *B*, mikamycins *B* and staphylomycins *S*; streptothricin, streptolin, geomycin, racemomycin, myrothricin, and roseothricin. The members of these groups differ from each other usually only by a single amino-acid, the rest of the molecule being the same. Some peptide antibiotics are composed entirely of amino-acids, while others contain amino-sugars, fatty acids, pyrimidine derivatives and other moieties in addition to the peptide portion of the molecule. A list of the amino-acids found in hydrolysates of peptide antibiotics is presented in Table 2.

The peptide antibiotics appear to have several common features: (1) a wide variety of unusual constituents, including D-amino-acids, not found in proteins are found in antibiotic peptides; (2) the peptide antibiotics are cyclic peptides; (3) all are resistant to hydrolysis by peptidases and proteases of animal or plant origin.

A brief inspection of Table 2 is already convincing evidence against the concept that peptide antibiotics would be produced by the general mechanism which is operative in protein synthesis. The four bases of ribonucleic acids, if arranged in coding triplets, can provide information for 64 components[2]. Since several triplets correspond to (or 'code for') the same amino-acid[2], the number of components for which this coding system can provide sufficient information is considerably less than 64. This so-called 'degeneracy' of the code does not interfere with protein synthesis, because only about 20 amino-acids occur in proteins. The number of amino-acid components found in peptide antibiotics is already approaching 64, and, therefore, a degenerate three-letter code is certainly insufficient for the information which is necessary to put the components in a predetermined order, that is, in a well-defined sequence. If, in order to reconcile the number of building stones and the scope of coding, the degeneracy is disregarded, then the well-established universality of the genetic code has to be sacrificed too. But, even with this unattractive assumption that the code for peptide antibiotics is not degenerate and the genetic code is not universal, the number of possible components soon will be exhausted. Furthermore, the occurrence of 'antibiotic families' also indicates that the information provided for the synthesis of peptide antibiotics is less definitive than the coding system of protein synthesis. Therefore, the conclusion that peptide antibiotics are not produced by the same mechanism as proteins is not too hazardous.

Experimental evidence for differences in the biosynthetic procedures of peptide antibiotics and of proteins was found by Mach, Reich and Tatum[3], who examined the biosynthesis of tyrocidin by *B. brevis*. We cannot agree, however, with their conclusion that such a difference should exist also for other polypeptides of similar size. Since peptide hormones, some of which have molecular weights of the same order as peptide antibiotics, do not have unusual amino-acid components, the above considerations on coding are not valid for them. Moreover, since bradykinin and angiotensin are formed by enzymatic cleavage of proteins, this might well be the case with other peptide hormones. On the other hand, the studies of Zadrazil, Kroupa, Sormova and Sorm[6] on the biosynthesis of polymyxin and of Eikhom, Jonsen, Laland and Refsvik[5] on the biosynthesis of gramicidin *S* contribute to our contention that, from the point of view of biosynthesis, peptide antibiotics are not small proteins.

If the information needed for the sequential arrangement of building stones of antibiotics is different from the coding device of protein biosynthesis, there are still two possible mechanisms which can be postulated for such a purpose: (1) a yet undiscovered coding system involving nucleotides; (2) the amino-acid building stones are assembled on the surface of one or more enzymes. The assumption of an independent coding system for peptide antibiotics is rather unpalatable: it is easy to accept the existence of the genetic code because the products of protein synthesis, for example, the enzymes, are indispensable for life. Hence, evolution could, and probably had to, lead to such an intricate mechanism. Peptide antibiotics, however, with a few exceptions, are probably not absolutely essential for the survival of the micro-organisms which produce them. Some variants of antibiotic-producing cultures do not produce the antibiotic and apparently carry out all other metabolic functions found in the antibiotic producers. Thus it is possible that antibiotic peptides represent only products of metabolic pathways which were abandoned during evolution or that they are disposal mechanisms of undesirable metabolic by-products, for example, D-amino-acids. Therefore, the second possibility-that peptide antibiotics are assembled from specific pre, cursors without an independent coding system, but on a specific (enzyme ?) surface, seems to be the more reasonable

Table 1. Some Chemical and Biological Properties of Peptide Antibiotics

Name	Antibiotic-producing organisms	Antibiotic spectrum (organisms inhibited)	Est. mol. wt.	Amino-acids reported		Other components
				D-Amino-acids	L-Amino-acids	
Actinomycins (ref. 9)	*Streptomyces antibioticus*	Gram + bacteria antineoplastic	1,300	Alloisoleucine, valine	Sarcosine, *N*-methyl-valine, proline, threonine, *N*-methyl-isoleucine, *N*-methyl-alanine, hydroxyproline	Actinocinin
Alazopeptin (ref. 10)	*Streptomyces griesoplanus*	Antineoplastic	400	—	Alanine	6-diazo-5-oxo-hexanoic acid
Albomycin (ref. 11)	*Streptomyces subtropicus*	Gram + bacteria	1,140	—	Serine, *N*-hydroxy-ornithine	Iron
Amidomycin (ref. 12)	*Streptomyces* species	Yeast	1,000	Valine	—	D-α-hydroxyiso-valeric acid
Antibiotic I.C.I. 13,599 (ref. 13)	*Paecilomyces* species	Anti-trypanosome	—	—	α-aminobutyric, leucine, β-alanine, γ-methyl-proline, β-hydroxy-leucine	
Aspartocin (ref. 14)	*Streptomyces griseus* *Streptomyces violaceus*	Gram + bacteria	—	—	Aspartic, glycine, proline, valine, α,β-diamino-butyric, β-methyl-aspartic	D-α-pipecolic; (+)-12- and 13-methyl-3-tetra-decenoic acids
Bacillomycins (ref. 15)	*Bacillus subtilis*	Fungi and yeast	1,000	—	Aspartic, glutamic, tyrosine, leucine, valine, serine, proline	
Bacitracins (ref. 16)	*Bacillus subtilis*	Gram + bacteria	1,400	Ornithine, aspartic, glutamic	Lysine, histidine, cysteine, isoleucine, aspartic, valine, phenylalanine, leucine	
Bottromycins (ref. 17)	*Streptomyces bottropensis*	Gram + bacteria Acid-fast bacteria	750	—	Glycine, valine, β-(2-thiazole)-β-alanine, α-amino-β-phenyl-butyric acid	
Brevin (ref. 18)	*Bacillus brevis*	Gram + bacteria	—	—	Aspartic, glycine, tyrosine, serine	Unknown 'base'
Cephalosporin *C* (ref. 16)	*Cephalosporium acremonium*	Gram + bacteria Gram − bacteria	350	α-aminoadipic	—	
Cephalosporin *N* (ref. 16)	*Cephalosporium synnematum*	Gram + bacteria Gram − bacteria	350	α-aminoadipic Penicillamine	—	
Cinnamycin (ref. 19)	*Streptomyces cinnamoneus*	Gram + bacteria Acid-fast bacteria	—	—	Glutamic, lanthionine, aspartic, valine, proline, arginine, phenylalanine, β-methyllanthionine	
Circulin (ref. 20)	*Bacillus circulans*	Gram − bacteria	825	Leucine	α,γ-diaminobutyric, threonine, isoleucine	(+)-6-methyl-octanoic acid
Colistin (ref. 21) (polymycin)	*Bacillus colistinus*	Gram − bacteria	925	Leucine	α,γ-diaminobutyric, threonine, leucine	(+)-6-methyl-octanoic acid
Comirin (ref. 22)	*Pseudomonas antimycetica*	Fungi and yeasts	—	—	Serine, aspartic, glycine, α,γ-diaminobutyric, lysine, valine, threonine, leucine, arginine, glutamic, isoleucine, alanine	
Duramycin (ref. 23)	*Streptomyces cinnamoneus*	Gram + bacteria Fungi and yeasts	—	—	Lanthionine, β-methyl-lanthionine, aspartic, glutamic, glycine, valine, proline, phenyl-alanine, ornithine, hydroxyproline	
Enniatins (ref. 24)	*Fusarium* species	Acid-fast bacteria	840	—	*N*-methylvaline	D-α-hydroxy-isovaleric acid
Etamycin (ref. 25) (viridogrisein)	*Streptomyces lavendulae*	Gram + bacteria	800	Leucine, allo-hydroxyproline	α-phenylsarcosine, threonine, sarcosine, β-*N*-dimethylleucine, alanine	3-Hydroxypicolinic
Fungistatin (ref. 26)	*Bacillus subtilis*	Fungi and yeasts	2,400	—	Aspartic, lysine, serine, threonine, proline, alanine, isoleucine, valine, tryptophan, tyrosine	Unidentified acid
Gramicidins (ref. 27)	*Bacillus brevis*	Gram + bacteria	1,350–2,000	Leucine, valine, phenylalanine	Tryptophan, valine, alanine, leucine, glycine, tyrosine, isoleucine	Ethanolamine
Ilamycins (ref. 28)	*Streptomyces islandicus*	Acid-fast bacteria	1,170	—	Alanine, leucine, *N*-methylleucine, γ-formyl-methyl-norvaline, 3-nitro-4-hydroxy-phenylalanine, trypto-phan, 2-amino-4-hexenoic acid	
Licheniformins (ref. 29)	*Bacillus licheniformis*	Acid-fast bacteria	3,800–4,800	—	Aspartic, glycine, serine, proline, arginine, phenylalanine, valine, lysine	
Matamycin (ref. 30)	*Streptomyces matansis*	Gram + bacteria Gram − bacteria	—	—	Cysteine, glycine, serine, alanine, arginine (?)	
Micrococcin *P* (ref. 31)	*Bacillus pumilis*, *Micrococcus* species	Gram + bacteria	—	—	Threonine	Propionic acid, 2-propionylthiazole-4-carboxylic acid, 2(1-amino-2-methyl-propyl)thiazole-4-carboxylic acid
Mycobacillin (ref. 32)	*Bacillus subtilis*	Fungi and yeasts	1,525	—	Aspartic, proline, glutamic, tyrosine, serine, leucine, alanine	
Nisins (ref. 33)	*Streptococcus lactis*, *Streptococcus cremoris*	Gram + bacteria	7,000	—	Leucine, isoleucine, methionine, valine, proline, alanine, glycine, serine, aspartic, histi-dine, lysine, lanthionine, β-methyllanthionine	
Penicillins (ref. 34)	*Penicillium* species, *Aspergillus* species	Gram + bacteria	350	Penicillamine	—	Phenylacetic acid, valeric acid
Polymyxins (ref. 35)	*Bacillus polymyxa*	Gram − bacteria	1,225	Phenylalanine	Leucine, threonine, α,γ-diaminobutyric	(+)-6-methyl-octanoic acid

[*Continued overleaf*

Table 1.—*Continued*

Name	Antibiotic-producing organisms	Antibiotic spectrum (organisms inhibited)	Est. mol. wt.	Amino-acids reported		Other components
				D-Amino-acids	L-Amino-acids	
Polypeptin (ref. 36)	*Bacillus krzemieniewski*	Gram + bacteria Gram − bacteria Fungi and yeasts	1,150	Valine, phenylalanine	Threonine, leucine, isoleucine, α,γ-diaminobutyric	Unidentified acid
Quinoxaline, antibiotics (ref. 37) (echinomycin, quinomycins, triostins, actinoleukin, levomycin)	*Streptomyces echinatus*, *Streptomyces aureus*	Gram(+) bacteria Gram(−) bacteria Acid-fast bacteria Antineoplastic	850	Serine	Alanine, *N*,*N*′-dimethylcystine, *N*-methylvaline, *N*-methylisoleucine, *N*-dimethylleucine	Quinoxaline-2-carboxylic acid
Saramycetine (ref. 38)	*Streptomyces saraceticus*	Fungi and yeasts	14,000	—	Glycine, threonine, proline, cysteine, aspartic	
Sideromycine (ref. 39)	*Streptomyces griseoluteus*	Gram + bacteria	1,100	—	—	1-amino-5-hydroxylaminopentane
Streptothricins (ref. 40) (streptolin, geomycin, mycothricins, racemomycin, roseothricins)	*Streptomyces lavendulae*, *Streptomyces racemochromogenes*, *Streptomyces xanthophaeus*	Gram + bacteria Gram − bacteria Acid-fast bacteria Fungi	550	—	β-lysine	D-gulosamine, streptolidine
Stendomycins (ref. 41)	*Streptomyces* species	Yeast and fungi	1,850	—	Alanine, glycine, isoleucine, leucine, proline, serine, threonine, valine	
Subtilin (ref. 42)	*Bacillus subtilis*	Gram + bacteria	3,200	—	Glycine, alanine, valine, lysine, leucine, glutamic, proline, isoleucine, lanthionine, phenylalanine, tryptophan, β-methyllanthionine	
Telomycin (ref. 43)	*Streptomyces* species	Gram + bacteria	1,000	—	*cis*-3-hydroxyproline, *trans*-3-hydroxyproline, glycine, alanine, threonine, aspartic, β-hydroxyleucine, serine, allo-threonine, tryptophan, β-methyltryptophan	
Thiostreptons (ref. 44) (siomycin, bryamycin)	*Streptomyces azureus*, *Streptomyces hawaiiensis*	Gram + bacteria	1,650	Cysteine	Threonine, alanine, isoleucine	Thiostreptoic acid, thiostreptine
Tyrocidines (ref. 45)	*Bacillus brevis*	Gram + bacteria	1,200	Phenylalanine	Tyrosine, phenylalanine, tryptophan, aspartic, glutamic, ornithine, leucine, proline, valine	
Valinomycin (ref. 46)	*Streptomyces fulvissimus*	Acid-fast bacteria Fungi and yeasts	100	Valine	Valine	D-α-hydroxyiso-valeric acid, lactic acid
Vernamycins (ref. 47) (staphylomycins, mikamycins, ostreogrycins)	*Streptomyces loidensis*, *Streptomyces virginiae*, *Streptomyces ostreogryceus*, *Streptomyces mitakaensis*	Gram + bacteria	700	Aminobutyric	Proline, phenylalanine, threonine, phenylglycine, *N*-methylphenylalanine, *N*-methyl-*p*-dimethylaminophenylalanine	4-oxopipecolic
Viomycin (ref. 48)	*Streptomyces floridae*, *Streptomyces vinaceus*, *Streptomyces puniceus*	Gram + bacteria Gram − bacteria Acid-fast bacteria	475	—	Serine, β-lysine, α,β-diaminopropionic	Viomycidine

one. On the other hand, the size of such a specific surface limits the number of components which can be assembled. For large peptide antibiotics, the independent coding systems should be postulated.

Is there a need to make this unlikely visualization of a coding system? Do large-size peptide antibiotics exist? Apart from cellular proteins and toxins produced by micro-organisms containing the common amino-acids, there are two compounds reported which have large molecular weights and unusual amino-acids: nisin[33] and saramycetine[38]. Both contain 55 'amino-acid' components and have molecular weights of 6,300 and 14,000 respectively. As shown in Tables 3 and 4, alternative compositions and molecular weights can be proposed for these two antibiotics. If the new proposals are confirmed by experimental investigations, this would eliminate the need for the hypothetical and unlikely coding system for peptide antibiotics. The proposals in Table 3 seem justified on the basis that the presence of three rather than nine β-lanthionine residues allows an easier interpretation of Cheeseman and Berridge's[33] finding that from 20 peptide fragments, obtained during the partial hydrolysis of nisin, only two contained β-lanthionine. In saramycetine, a molecular weight of about 1,750, rather than 14,000, leads to a molar absorption coefficient of 48,000 at 275 mμ and 77,000 at 227·5 mμ instead of the very unusual values of 385,000 and 615,000 respectively.

Of course, caution should be used in such speculative interpretation of published experimental data. Molecular weight measurements by diffusion constant in the case of nisin and by diffusion and sedimentation constants in the case of saramycetine were used to support the high molecular weights proposed for these two antibiotics. On the other hand, the extremely pertinent warning from Battersby and Craig[49] should be kept in mind: "The problem of determination of molecular size approximately 1,000 or more has never been satisfactorily solved. Such approaches as freezing-point depression, diffusion and sedimentation in the ultracentrifuge have not given results in agreement. With these methods, the state of aggregation of the molecule probably is a confusing factor. In general, this type of substance shows a strong tendency to associate and tenaciously holds solvent of crystallization."

Less equivocal molecular weights can be calculated from the examination of partially substituted products. Partial dinitrophenylation of nisin and partial esterification or salt formation of saramycetine could provide the necessary compounds and data.

With the elimination of the exceptionally high molecular weight antibiotics, we cannot only re-state our contention that peptide antibiotics are not small proteins, but also conclude that the methods used for the elucidation of structure of these compounds should be different from the methods useful in the case of proteins.

In the chromatographic methods of amino-acid analysis, elution volumes or R_F values of the components of a mixture are compared with the known elution volume

Table 2. AMINO-ACIDS FOUND IN PEPTIDE ANTIBIOTICS

Amino-acids found in antibiotics and in 'normal proteins'*	Amino-acids found in antibiotics but not reported from 'normal proteins'
L-alanine	L-β-alanine; *N*-methyl-L-alanine
L-arginine	*N*-hydroxy-L-ornithine; D-ornithine; L-ornithine
L-aspartic acid	D-aspartic acid; L-β-methylaspartic acid; L-α-amino-butyric acid; L-α,γ-diaminobutyric acid; D-α,γ-diaminobutyric acid
L-cysteine	L-lanthionine; L-β-methyllanthionine; L-*N*-*N*′-dimethyl-cystine
L-glutamic acid	D-glutamic acid; D-α-aminoadipic acid
Glycine	Sarcosine
L-histidine	
L-hydroxyproline	Allo-D-hydroxyproline; *trans*-3-hydroxy-L-proline; *cis*-3-hydroxy-L-proline
L-isoleucine	Allo-D-isoleucine; *N*-methyl-L-isoleucine; L-β-dimethyl-leucine
L-leucine	D-leucine; *N*-methyl-L-leucine; L-β-hydroxyleucine; L-2-amino-4-hexenoic acid
L-lysine	L-β-lysine
L-methionine	
L-phenylalanine	D-phenylalanine; L-phenylglycine; L-α-phenylsarcosine *N*-methyl-L-phenylalanine; *N*-methyl-L-*p*-dimethyl-phenylalanine; L-3-nitro-4-hydroxyphenylalanine
L-proline	L-γ-methylproline; β-methyl-L-proline
L-serine	D-serine
L-threonine	
L-tryptophan	
L-tyrosine	
L-valine	D-valine; *N*-methyl-L-valine; D-penicillamine; L-γ-formyl-methylnorvaline
——	L-β-(2-thiazole)-β-alanine; L-α-amino-β-phenylbutyric acid; L-α,β-diaminopropionic acid

* Amino-acids for which a genetic code triplet has been assigned (Ochoa, ref. 2).

Table 3. AMINO-ACID COMPOSITION OF NISIN

Amino-acid	Reported[33]	Proposed alternative
Isoleucine-leucine	9	3
Methionine	3	1
Valine	3	1
Proline	2	1
Alanine	4	1
Glycine	6	2
Serine	1	
Aspartic acid	3	1
Histidine	3	1
Lysine	9	3
β-Lanthionine	9	3
Lanthionine	3	1
Total	55	18

Table 4. AMINO-ACID COMPOSITION OF SARAMYCETINE

Amino-acid	Reported[38]	Proposed alternative
Aspartic acid	8·50	1
Glycine	8·18	1
Threonine	7·30	1
Proline	8·33	1
Cystine	23·09	3
Total	55·0	7

values or R_F values of the 20 amino-acids. This comparison is fully justified if unusual components can be excluded with reasonable certainty. This is the case in the analysis of amino-acid mixtures from hydrolysates of proteins or peptides of animal origin. It is certainly not the case in the analysis of hydrolysates from peptide antibiotics. In addition to the possibility of erroneous identifications, the quantitative analysis of amino-acid content of microbial products is beset also with the danger of miscalculation, especially if the calculation is based on the ninhydrin yield of a single component. If the latter is not a common amino-acid, but was identified by mistake with a common amino-acid, or if it is a common amino-acid formed as a secondary degradation product from an unusual component in less than theoretical amounts, then the whole calculation becomes erroneous. The only reliable way out of this dilemma is the isolation of the individual building stone from the hydrolysates in amounts which allow elemental analyses, measurement of specific rotation, and comparisons by infra-red and/or nuclear magnetic resonance spectra with authentic material. This approach was used in the studies on tyrocidin, bacitracin, and micrococcin *P*.

After completion of amino-acid analysis and sequence investigations, a structure can be proposed for a peptide antibiotic.

Independent evidence for the correctness of a proposed structure can be obtained by synthesis and subsequent bioassay of the peptide. Unfortunately, the synthesis of peptide antibiotics has been an arduous task and achieved in only a few cases. An alternative method of confirmation of proposed structures is the detailed examination of the nuclear magnetic resonance spectrum of the peptide antibiotic. Supporting evidence for the 'amino-acid' composition and to some extent for the structure of peptide antibiotics can be found in these spectra.

The nuclear magnetic resonance spectra of vernamycin $B\alpha$[47] and of thiostrepton[44] taken in deuterioacetic acid (CD_3COOD) gave very useful information. Not only is CD_3COOD a general solvent for polar compounds, but it is usually not destructive. Trifluoroacetic acid, the solvent which is commonly used in nuclear magnetic resonance spectroscopy of amino-acids, can lead to radical structural changes in antibiotic peptides[44]. Since very polar compounds such as amino-acids are also soluble in CD_3COOD, a direct comparison can be made between the spectra of an antibiotic and those of its components. The chemical shifts observed in CD_3COOD are very close to those found in $CDCl_3$ and permit a comparison with literature values. On the other hand, the chemical shifts obtained in CF_3COOH are quite different from those in $CDCl_3$. CD_3COOD is also desirable since all the exchangeable hydrogens are exchanged for deuterium and this leads to simplification and less complicated interpretation of the spectrum.

On the basis of the foregoing discussion, we conclude that antibiotic peptides are not small proteins. Their biogenesis should be investigated using conventional enzymatic techniques without complete reliance on the applicability of the genetic code to the formation of large and small peptides. The methods used for the elucidation of protein structures are not always applicable to the study of peptide antibiotics.

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DISCHARGE PATTERNS OF ELEMENTS IN CAT CEREBELLAR CORTEX, AND THEIR RESPONSES TO IONTOPHORETICALLY APPLIED DRUGS

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IN an earlier paper[1] it was indicated that cells in the cerebellar cortex are excited by iontophoretically applied acetylcholine. Excitable neurones were found most often in the deeper areas of cortical folia and were identified as granule layer cells on the basis of their electrical activity. Other units, identified as superficially located Purkinje cells, could not be fired by iontophoretically applied acetylcholine. Purkinje cells located more deeply had not been defined in these earlier experiments. Further experiments to be described in this paper have led to a more accurate definition of the cell types concerned.

Purkinje cells can be identified by the antidromic invasion of an action potential following stimulation of their axons or axonic terminals in the cerebellar white matter or deep nuclei[2]. Cells which fired with a short, constant latency to antidromic volleys and followed 100/sec antidromic stimulation frequencies were included as Purkinje cells in this survey. Figs. 1*A* and *B* illustrate the extracellularly recorded action potentials of two Purkinje cells which were identified by antidromic invasion. Both cells were invaded by short-latency action potentials after stimulation of their axons with an electrode situated in the fastigial nucleus. Both would follow repetitive stimulation in the deep nucleus at 100/sec and both fired in response to the application of glutamic acid from one barrel of the multi-barrelled micropipette. However, they differed in their responses to iontophoretically applied acetylcholine, since the cell illustrated in Fig. 1*A* fired 15 sec after the onset of application of acetylcholine by a current of 80 n.amp, whereas the second cell failed to fire even after several applications of this drug. In a series of experiments, a number of neurones, which have been identified as Purkinje cells by their responses to stimulation in the deep nuclei or white matter, has been tested with acetylcholine and, of these, less than a quarter were considered to be excited by acetylcholine. The micropipettes routinely contained an anticholinesterase (prostigmine or tensilon) in one of their barrels and this was frequently applied before testing the cells with acetylcholine. The majority of acetylcholine-sensitive Purkinje cells initially required prolonged application of large amounts of acetylcholine before they commenced to discharge, a finding that has also been reported by Crawford *et al.*[3].

An understanding of the relationship of electrical activity to cerebellar topography has been gained with a technique for making lesions of 100–200μ diameter in the cerebellum[4]. These are formed by passing currents of 2–10 μamp for brief periods through a microelectrode barrel containing 0·1 N hydrochloric acid at sites where anatomical-electrophysiological correlations are required. The cerebellar area under investigation (tuber vermis in these experiments) is then serially sectioned and the position of the lesions ascertained. In these experiments cerebellar folia were traversed at right angles to accentuate the differences between regions.

Lesion placements have demonstrated that the following electrical recordings are characteristic of the different cortical regions. (The micropipettes used had a tip diameter of 8–12μ.) As the microelectrode proceeds through a molecular layer the record is dominated by positive deflexions of 100–300 μV amplitude and 1–3 msec duration. These tend to disappear if the electrode tip is left at a particular site and are probably the result of injury to the Purkinje cell dendrites. Similar potentials can also be evoked by the application of glutamic acid, and this is illustrated in Fig. 1*C*. Simultaneous recording of d.c. potential changes has shown that these positive deflexions are associated with the development of a local negativity with respect to an indifferent electrode on the neck muscles of the cat; this may be of the order of 10 mV. Unlike the characteristically rapid onset and cessation of glutamate action on neurone soma spikes, the positive deflexions and negative potential shift develop and recover slowly, with comparable time courses. It seems likely that the positive deflexions are the result of Purkinje cell soma discharges generated by the dendritic depolarization.

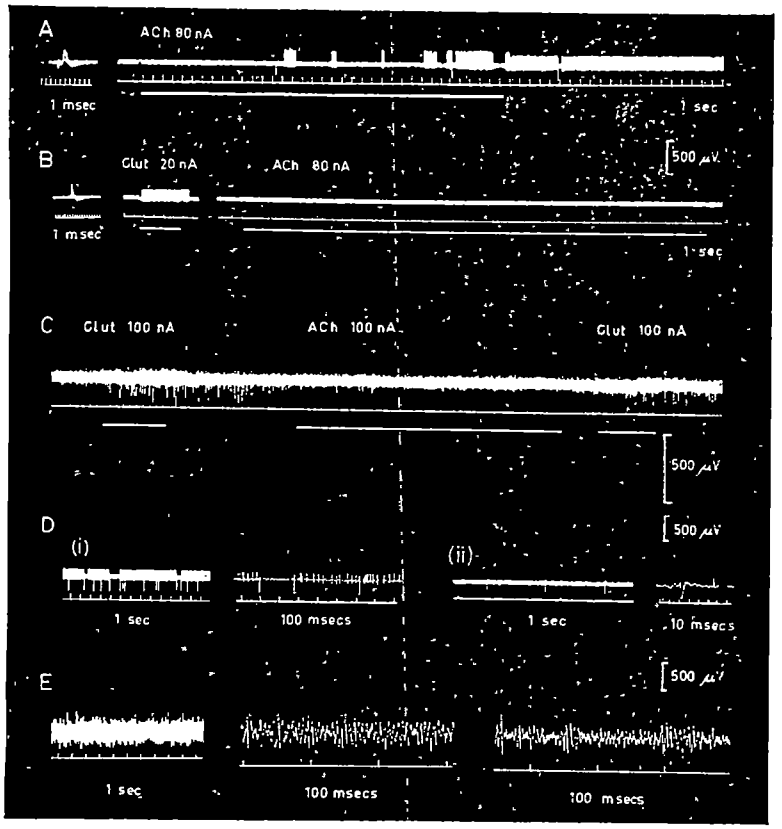


Fig. 1. Potentials (negative upwards −) recorded with a five-barrelled micropipette in cat cerebellar cortex. Solid bars indicate duration of application of drugs. *A*, Purkinje cells: successful antidromic invasion; excited by acetylcholine. *B*, Purkinje cell: successful antidromic invasion; excited by glutamate; no response to acetylcholine. *C*, Molecular layer: positive-going deflexions induced by glutamate, not by acetylcholine. *D*, Purkinje cells; positive-going potentials (i) associated with initiation and cessation of firing; (ii) regularity of firing; negative spike, superimposed on positive deflexion. *E*, Granule cell layer; firing pattern showing discharge at 160–170/sec and variations in amplitude

Neither of these phenomena could be elicited by acetylcholine.

Positive deflexions can be recorded through much of the molecular layer, but as the microelectrode tip approaches a Purkinje cell layer they frequently give way to small (about 100 μV) positive spikes which, in turn, reverse to become the characteristic negative Purkinje cell spikes (0·5–1 mV). Both positive and negative spikes are greatly accelerated by glutamic acid, the onset and cessation of action corresponding closely to the onset and cessation of application. A phenomenon characteristically recorded from this area of Purkinje cell bodies is a large (up to 1 mV) positive deflexion, which, if the cell is firing spontaneously, may frequently be associated with the initiation or cessation of firing (Fig. 1*D* (i)). Fig. 1*D* (ii), recorded from another cell, illustrates the regularity often associated with these discharges, which generally have one or more negative-going spikes superimposed on them.

A similar type of potential has been described by Granit and Phillips[2], who showed that it was associated with an intracellular depolarization. The potential can, therefore, be attributed to a relatively synchronous depolarization of the Purkinje cell dendrite tree by climbing fibres or parallel fibres. Thirty-seven of the 97 (38 per cent) Purkinje cells identified in this topographical survey were excited by acetylcholine; these were more frequently the cells that had been approached from the granule cell layer. Deeply located Purkinje cells showed a greater tendency to respond to acetylcholine. Excitation of Purkinje cells was often preceded by a discharge of adjacent units and these often fired when the Purkinje cell failed to discharge. These cells had small action potentials, and were probably situated in the granule cell layer.

Within the granule cell layer the microelectrode recorded activity that was principally the summed action potentials of groups of cells, though occasionally isolated spikes would predominate. Potentials ranged in magnitude from little more than the baseline noise-level to about 500 μV. There was a definite tendency for the amplitude of granule cell-layer activity to increase as the electrode approached a Purkinje cell layer that had not yet been penetrated.

Using the lesion technique, we have verified our earlier report that cells responding to acetylcholine occur throughout the granule cell layer; these were more frequently detected in the area of larger potentials described above. Fig. 1*E* illustrates, on slow and expanded records, the typical form of activity in these areas. The frequency of discharge in this record is 160–170/sec, and its amplitude underwent periodic modulation (7–8/sec). The activity evoked by acetylcholine on more susceptible groups of cells closely resembled the rapid discharge illustrated in Fig. 1*E*. However, there was a range of responses among sensitive cells, extending from a just demonstrable excitation to frantic activity.

The induced activity and some of the spontaneous firing could be inhibited by dihydro-β-erythroidine and hexamethonium. In areas where acetylcholine-sensitive cells were present, anticholinesterases had a powerful excitatory effect far exceeding that which might be expected of a direct action.

During these experiments it has been confirmed that acetylcholine sensitivity is more readily detectable in the depths of the cerebellar cortex. Fig. 2 shows the acetylcholine sensitivity of a population of 929 cells in the cerebellar cortex derived from experiments in which the micropipette entered the cerebellum in a series of parallel tracks commencing with one at right angles to the anterior portion of the tuber vermis. The tracks, therefore, will pass through one or more folia. The slope of the regression line is significant ($\chi_1^2 = 49 : P < 0{\cdot}01$).

Examination of the distribution of cholinesterase in the cerebellar cortex[5,6] has revealed that acetylcholinesterase is concentrated in the granule cell layer of cat cerebellum,

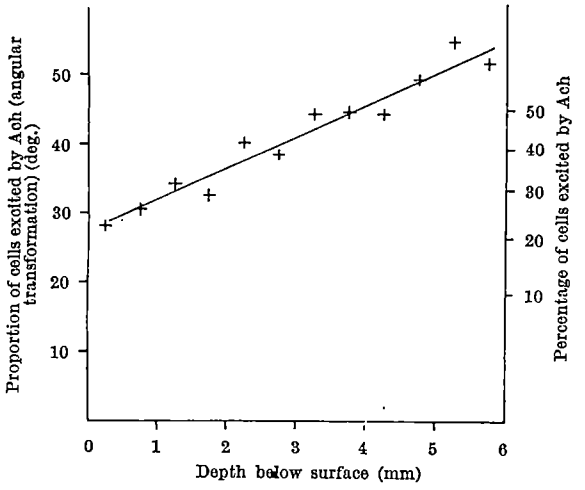


Fig. 2. Increase in proportion of cells excited by acetylcholine with increasing depth below the cerebellar surface. Depths recorded by micromanipulator; grouped into 0·5 mm classes

occurring on the surface of many granule cells and within the glomeruli. Experiments in which the middle and inferior peduncles were stained for acetylcholinesterase have shown that stained fibres were present in both peduncles, and it has been shown, using the technique of tract transection[7,8], that these fibres are running centripetally into the cerebellum. The cerebellar peduncles are known to contain choline acetylase[9,10], and, taken in conjunction with the distribution of cholinesterase, this indicates that some afferent fibres to the cerebellum are cholinergic.

The experiments described here have shown that some cells in the granule cell layer are excited by acetylcholine. This fact and the pattern of cholinesterase distribution make it likely that these may be granule cells and that a proportion of the mossy fibres terminals release acetylcholine. Since Golgi cells in the cat occur in a layer close to the Purkinje cell layer and have synaptic connexions with the mossy afferent fibres[11], it may be that these larger cells are responsible for both the greater amplitude of the discharges and the increased acetylcholine sensitivity that occurs in the area deep to the Purkinje cell layer.

Excitation of Purkinje cells with cholinergic substances has been reported previously[3], an action on dendritic receptors having been postulated. Evidence presented here suggests, rather, that Purkinje cells are being activated either as a result of stimulation of cells in the granule cell layer or by a direct action of acetylcholine on excitatory receptors on the Purkinje cell soma.

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EFFECT ON TUMOUR GROWTH IN SYNGENEIC RECIPIENTS OF ANTIBODIES AGAINST TUMOUR-SPECIFIC ANTIGENS IN METHYLCHOLANTHRENE-INDUCED MOUSE SARCOMAS

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SPECIFIC antigens characterizing tumour tissues and absent from normal cells have been demonstrated in tumours induced by various chemical carcinogens[1-5] and by different viruses[6-8], while analogous investigations with spontaneous mammary carcinomas and fibrosarcomas failed to reveal such antigens[2,3]. Transplantation of tumours containing tumour-specific antigens under conditions where their progressive growth is precluded (for example, by X-rays, sub-threshold isografting, etc.) may lead to immunization of syngeneic recipients. As a result, subsequent and otherwise lethal isografts of the same tumour may be inhibited to various degrees. The antigenic specificity of carcinogen-induced tumours is individually distinct and cross-reactivity between different neoplasms of the same ætiological group is rare or absent[8-10]. In contrast, tumour-specific antigens of virus-induced neoplasms appear to be identical in all tumours induced by a given virus[8]. The transplantation resistance induced by carcinogen-induced tumours in syngeneic recipients is immunological in nature, as indicated by its successful passive transfer to untreated syngeneic hosts by lymphoid cells taken from mice pre-treated with the tumour[4,9]. So far, the antigens in these neoplasms have only been demonstrated by transplantation methods, while serological techniques *in vitro* (for example, cytotoxicity) and *in vivo* (neutralization) have been negative[4,9].

Immunological enhancement has been successfully induced with leukæmias in non-*H*-2 systems[11] by passive transfer of antisera which were not cytotoxically active against otherwise sensitive target cells, such as lymphoid cells. Thus, enhancement appeared to be a sensitive test for demonstrating the presence of weak antibodies and it was attempted to apply it for the study of tumour-specific antigens in the present work.

Immune sera against tumour-specific antigens were prepared in syngeneic animals. In all experiments care was taken to avoid immune reactions against sex-determined antigens; tumours which originated in males were always transferred only into males, whereas tumours of female origin were inoculated into both sexes. Immunization was performed by inoculating trypsinized but otherwise untreated methylcholanthrene-induced tumour cells of recent origin (7th–20th transfer generation) subcutaneously into syngeneic recipients for three consecutive times. If progressively growing tumours appeared they were removed by operation. The animals were bled 10–30 days after the last inoculation.

In order to induce enhancement of the tumours, 0·1 ml. undiluted antiserum was injected intraperitoneally 30 min before the tumour inoculation and thereafter every third or fourth day until palpable tumours developed. Tumour cells were brought into suspension by exposure to 0·25 per cent trypsin for 1 h. A determined number of trypan-blue unstained cells was inoculated subcutaneously into the antiserum-treated recipients and into controls, which were untreated or treated as described with antiserum prepared in *C57BL*/10 mice against another, non-cross-reacting methylcholanthrene-induced sarcoma (*MBA*) of *C57BL*/10 origin.

The methylcholanthrene-induced sarcoma *MALA* of $(A \times C57L)F_1$ hybrid female origin, strongly antigenic according to transplantation tests[4], was inoculated subcutaneously (10^5 viable tumour cells from a trypsinized suspension) into two groups of four $(A \times C57L)F_1$ females. One control group was given 0·1 ml. immune serum directed against an unrelated methylcholanthrene-induced sarcoma (*MBA*), while the experimental group received 0·1 ml. $(A \times C57L)F_1$ anti-*MALA* serum. Both sera were injected 14 times during the first 44 days. It was found (Fig. 1) that all recipients treated with specific immune serum developed progressively-growing neoplasms, while two of four controls did not develop any palpable tumours at all. The other two controls developed progressively-growing sarcomas, which appeared later and grew slower, however. Thus, specific antiserum considerably facilitated growth in the strain of origin.

Attempts were also made to enhance the methylcholanthrene-induced sarcoma *MDAY* of $(A \times DBA/2)F_1$

Table 1. Growth of methylcholanthrene-induced sarcoma *MDAY* of $(A \times DBA/2)F_1$ origin in syngeneic recipients treated with $(A \times DBA/2)F_1$ anti-*MDAY* serum

Exp. No.	Tumour	Recipient	Treatment of recipients with:	Mean tumour diameter at the following days after transplantation: 10	14	18
1	*MDAY*	$(A \times DBA)F_1$	$(A \times DBA)F_1$ Anti-*MDAY* serum	3·0, 3·0, 3·0 *3·0*	3·0, 5·4, 6·9 *5·1*	6·0, 7·8, 9·5 *7·8*
	MDAY	$(A \times DBA)F_1$	Nothing	0, 0, 5·0 *1·7*	0, 4·8, 4·5 *2·9*	0, 5·5, 7·5 *4·3*
2	*MDAY*	$(A \times DBA)F_1$	$(A \times DBA)F_1$ Anti-*MDAY* serum	0, 0, 3 *1·0*	3·0, 4·5, 6·3 *4·6*	6·7, 7·1, 7·4 *7·1*
	MDAY	$(A \times DBA)F_1$	Nothing	0, 0, 0 *0*	0, 0, 0 *0*	0, 0, 4·5 *1·5*

Figures in italics represent mean values.

origin by exposure to tumour-specific antiserum. Serum was taken from $(A \times DBA/2)F_1$ hybrids pre-treated as described. Two experiments were performed (Table 1). In the first, six injections of antiserum were given at intervals of 3–4 days and in the second nine injections at the same intervals. Controls were untreated in the first experiment and received nine injections of anti-*MBA* serum in the second: 10^5 trypsinized *MDAY* cells were inoculated subcutaneously into groups of three $(A \times DBA/2)F_1$ hybrids. In both experiments all antiserum-treated hosts developed progressively growing neoplasms, which had a shorter latency period and higher growth rate compared to the controls (Table 1).

Analogous experiments were performed with the methylcholanthrene-induced sarcoma *MC57G* of *C57BL* origin. Sera of immunized *C57BL* or $(A \times C57BL)F_1$ recipients were tested for their possible enhancing effect. Two groups of three $(A \times C57BL)F_1$ hosts received 10^5 trypsin-treated *MC57G* sarcoma cells subcutaneously. One group was treated with repeated injections of anti-*MC57G* serum while the other was untreated. In contrast to the results

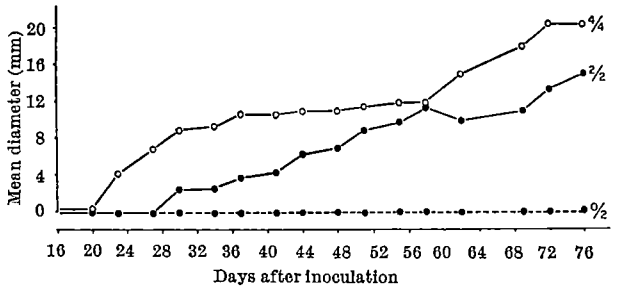


Fig. 1. Growth of 10^6 *MALA* cells of $(A \times C57L)F_1$ origin in $(A \times C57L)F_1$ hybrids repeatedly injected with $(A \times C57L)F_1$ anti-*MALA* serum (Group *A*) and in controls treated with *B*10 anti-*MBA* serum (Group *B*). The figures denote the number of mice killed by progressively growing tumours as related to the total number inoculated. Group *B* is represented by two curves: the solid line represents the mean tumour diameter in mice with palpable tumours, whereas the broken line represents mice which did not develop palpable tumours. This denotation will be followed also in the following figure. O—O, Group *A*; ●—● and ●- -●, Group *B*

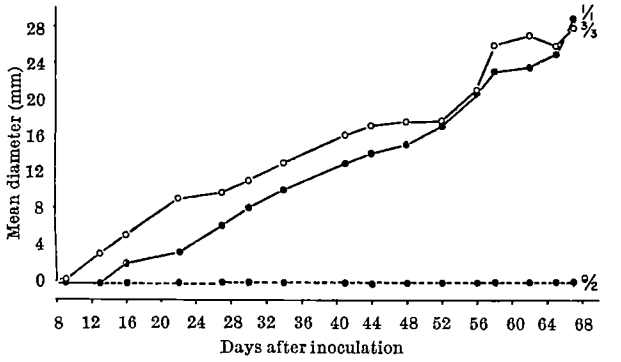


Fig. 2. Growth of 10^5 *MC57G* sarcoma cells of *C57BL* origin in $(A \times C57BL)F_1$ recipients repeatedly treated with *C57BL* anti-*MC57G* serum (Group *A*) and in untreated controls (Group *B*)

with the other sarcomas described above, the growth of this tumour was clearly inhibited in the antiserum-treated hosts as compared to untreated controls (Fig. 2) and only one of three antibody-treated recipients developed a progressively growing tumour, which showed a prolonged latency period in comparison with the three progressively growing control neoplasms.

A second attempt was made to enhance *MC57G* by using larger inoculum doses (3×10^6 cells per recipient). One group of three *C57BL* mice received eight injections of the same anti-*MC57G* serum with intervals of 3–4 days, while three others were untreated. In spite of the large inoculum dose the antibodies were clearly inhibitory as revealed by a delayed latency period, although the effect was less pronounced than with the lower cell dose.

The results presented suggest that humoral antibodies directed against tumour-specific antigens in methylcholanthrene-induced mouse sarcomas exist and can be demonstrated by their capacity to enhance or inhibit tumour growth in syngeneic recipients. Alternative explanations of the results, based on the presence of antibodies directed against isoantigens, because of residual heterozygosity of the mouse strains, or against the transplantation antigen characterizing male cells, are highly unlikely. The strains used in the experiments recorded here are all antigenically homozygous as revealed by uniform acceptance of intra-strain skingrafts and the incompatibility reactions against male antigens were excluded by the experimental design.

Attempts were made to demonstrate the presence of humoral antibodies against the tumour-specific antigens in the sera used for the above experiments by *in vitro* techniques. The cytotoxic test of Gorer and O'Gorman[12] was negative with all three sarcomas studied. The indirect fluorescent antibody technique performed on living tumour cells in suspension[13] gave positive staining reactions with *MALA* sarcoma cells treated with specific antiserum prepared in syngeneic hosts, whereas *MDAY* and *MC57G* cells gave negative reactions. The positive staining reaction with *MALA* cells was similar in nature to that seen with anti-*H2* sera: the membrane of living cells was clearly outlined by a fluorescent 'ring', whereas controls were unstained or stained with the homogenous reaction characterizing dead cells. It could be excluded that the 'ring' reaction with the *MALA* sarcoma cells was caused by antigens revealed by the trypsin treatment[8], since trypsinized sarcoma cells of another tumour (*S3A*) were entirely unstained with serum strongly efficient against *MALA* cells. The latter cells were not reacting with another immune serum prepared in syngeneic hosts against a methylcholanthrene-induced sarcoma of *C57BL*/10 origin (*MBA*). It would appear, therefore, that the positive staining reaction with *MALA* cells in the fluorescent antibody technique was caused by the presence of antibodies directed against tumour-specific antigens.

This work was supported by grants from the Swedish Cancer Society and the Damon Runyon Memorial Fund, and by grant *C*–3700 from the National Cancer Institute, U.S. National Institutes of Health. I thank Prof. G. Klein for his help and interest during the investigation, and Miss Lena Lundin for skilful technical assistance.

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PANGLOBINOPENIA

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THE term 'panglobinopenia' designates a hypothetical hereditary inhibition of hæmoglobin synthesis distinct from thalassæmia. Whereas in α-thalassæmia the synthesis of hæmoglobin α-chains and in β-thalassæmia the synthesis of hæmoglobin β-chains are specifically inhibited, in panglobinopenia the inhibition is non-specific and involves simultaneously all hæmoglobin polypeptide chains.

Microspectrophotometric measurements of total hæmoglobin and of fœtal hæmoglobin were performed by G. Matioli on individual erythrocytes from a patient clinically characterized as a case of 'thalassæmia major'. The results of this work have been tentatively interpreted by Matioli and myself[1] as revealing a heterozygous panglobinopenia, superimposed on a heterozygous β-thalassæmia. Because of circumstances, this analysis could for the time being be based on only one single 'thalassæmic' individual. The notion of panglobinopenia had, however, been derived from an analysis of other results to be found in the literature. It is desirable to discuss these results, which furnish a background to the aforementioned publication.

While the existence of a panglobinopenia locus as distinct from the loci of α-thalassæmia and of β-thalassæmia is not demonstrated, the circumstantial evidence appears to justify the proposal of the concept. The results to be found in the literature and to be recalled here suffer from the considerable disadvantage (a disadvantage fully overcome for the first time by Matioli) that they offer only mean values relative to total cell populations. Even then conclusions of interest can be drawn, provided the data are expressed in the form of average content per cell of the different individual hæmoglobin chains, the α-, β-, γ- and δ-chains. This approach is adopted in the present article. The value for mean cellular hæmoglobin (MCH), based on the concentration of hæmoglobin in the blood and the red cell count, is used in conjunction with the percentage of the different hæmoglobin components that are present. The approximate percentage is usually established by the combined techniques of electrophoresis and alkali denaturation.

Thalassæmia or panglobinopenia ? The family, originally examined by Neel, Wells and Itano[2] and re-examined by Cohen, Zuelzer, Neel and Robinson[3] in 1959, presents a special problem. There are two normal offspring from a marriage between a normal person and a person doubly heterozygous for sickle-cell trait and what appeared to the authors to be thalassæmia trait. If the diagnosis of normality of the three offspring were genetically correct, if paternity were ensured, and if the thalassæmia were of the β-type, the findings would prove non-allelism between the β-thalassæmia gene and the β-chain gene, in opposition to the conclusion drawn from the great majority of available results[4].

The results of this family investigation, converted to mean absolute amounts of hæmoglobin chains per cell, are presented in Table 1. A third abnormal gene is present, that of hereditary spherocytosis, which appears, however, to have no influence on the amounts per cell of different hæmoglobin chains.

The siblings decisive for establishing independent segregation of the 'thalassæmia' and the β-chain genes are subjects II-5 and II-9. The presence of the sickle-cell gene allows one partly to clarify the situation. Table 2 reproduces the figures for the β^A- and β^S-chains relating to the individuals considered as thalassæmic (groups 2 and 4 of Table 1) and adds to it a column for the total β-chain. In the heterozygotes for HbS and the supposed thalassæmia (I-2 and II-3) the contribution of the β^A-chain gene is on the average 7·3 and 8·0 μμg of β^A-chain per cell. These would be good and, in the case of subject II-3, even high values for the synthetic activity of a single normal β^A-gene if it were associated with a normal allele. No known thalassæmic β-chain gene controls the production of polypeptide chain in such high amounts[5]. (The value for β-chain in the 'normal' group 1 of Table 1 is the result of the activity of the two allelic genes; it is here in general somewhat below the average normal value.)

If any β-chain gene were specifically inhibited, it would rather appear to be the β^S-gene. However, in the same family, the HbS heterozygotes without 'thalassæmia' (Table 1, group 3) produce normal amounts of β^S-chain, and the HbS-gene involved must be the same. A familial constancy of the synthetic activity of a given structural hæmoglobin chain gene is a reasonable assumption in view of the findings of Itano[6] and of the situation that prevails in thalassæmics[5]. The normal mean value for β^S-chain per cell in the peripheral circulation is 5·3 ± 1·1 μμg (ref. 5). With respect to this mean the inhibition of the β^S-chain production per cell in subjects I-2 and II-3 is seen to amount to roughly 50 per cent. Characteristically, the synthetic activity of the β^A-allele of the same subjects undergoes vicissitudes in this family quite like those observed for the β^S-gene.

When in association with the β^S-gene, the synthetic activity of this β^A-gene is normal. But when associated with another β^A-gene (subjects II-8 and III-1) its activity must be assumed to be depressed, because the total synthetic activity of the two β^A-alleles results in the average production of only about 10 μμg/cell, unless it were not the β^A-chain gene found in the 'thalassæmic' double heterozygotes, but its β^A-allele that were to be considered as 'thalassæmic' in subjects II-8 and III-1, a paradoxical assumption. Most likely both β^A-alleles display a reduced synthetic activity in subjects II-8 and III-1. The particular β^A-chain gene as well as the β^S-chain gene found in the supposedly thalassæmic individuals are, therefore, able to display a normal synthetic activity in some members of this family. Thus the presence in this family of a β-thalassæmia not only is in doubt because of the absence of an increase in Kunkel's HbA_2 component, as pointed out by Ingram and Stretton[7], but is ruled out by the behaviour of the critical β^A-chain gene itself on the defendable assumption that a given structural hæmoglobin chain gene that is thalassæmic in one individual will not be uninhibited in another[5].

The most significant observation on the present family, in the author's opinion, is the constancy of the mean total amount of β-chain in the four 'thalassæmic' individuals belonging to three generations, listed in Table 2. This total amount is the same, irrespective of whether it arises from the contributions of two β^A-chain genes or of one β^A- and one β^S-chain gene. Apparently only the limited mean total amount of β-chains that is found can be produced, and the control of this amount is distributed over whatever β-chain genes are present. In the competition for a compound that must be assumed to limit the total synthesis to the observed value, the product from the β^S-chain gene is, as usually, at an apparent disadvantage, and even more so than on the average. Yet there is a general depression of β-chain production in the individuals listed in Table 2. The amount of α-chain to be found in

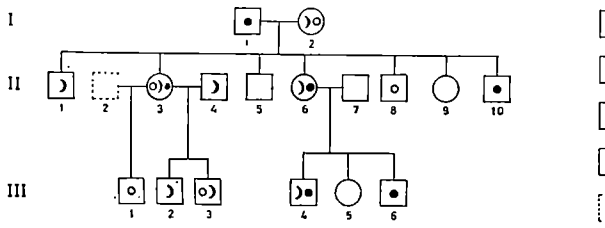
* On leave from the Centre National de Recherche Scientifique, Paris.

Table 1. Data on Family R. (Cohen *et al.*[3]) converted to μμg/cell of Mean Amount of Different Haemoglobin Chains

Subject		Hbg%	αA chain	βA chain	βS chain	γF chain	δA$_2$ chain	Her. spher.*
(1) Subjects with no HbS nor considered to carry the 'thalassæmia' gene								
I	1	12·5	12·4	12·0	0	0·12	0·25	+
II	5	11·9	11·4	11·1	0	0·11	0·17	−
II	7	15·0	14·2					
II	9	11·0	14·9	14·4	0	0·15	0·37	−
II	10	13·5	13·4	13·0	0	0·13	0·32	+
III	5	11·4	12·9	12·5	0	0·13	0·32	−
III	6	9·2	13·1	12·8	0	0·13	0·23	+
(2) Subjects with no HbS but carrying the 'thalassæmia' gene								
II	8	12·2	9·9	9·6	0	0·10	0·25	−
III	1	11·9	10·5	10·2	0	0·10	0·16	−
(3) HbS heterozygotes without 'thalassæmia'								
II	1	15·9	12·5	7·9	4·4	0·13	?	−
II	6	11·9	13·9	8·7	5·0	0·14	?	+
(4) HbS and 'thalassæmia' double heterozygotes								
I	2	12·5	10·3	7·3	2·9	0·10	?	−
II	3	12·4	10·4	8·0	2·3	0·10	?	+

* Hereditary spherocytosis.

Pedigree



the peripheral circulation is decreased correspondingly. It is reasonable to assume that this decrease in the amount of α-chain is a secondary effect[8]. We note, in any event, that in the cases under consideration, no non-α-chain gene appears to be inhibited specifically.

Table 2. Constancy of the Total Amount of β-chain per Cell in the 'Thalassæmic' Subjects listed in Table 1

Subject	βA-chain μμg/cell	βS-chain μμg/cell	Total β-chain μμg/cell
II-8	9·6	0	9·6
III-1	10·2	0	10·2
I-2	7·3	2·9	10·2
II-3	8·0	2·3	10·3

Can an α-chain gene be inhibited specifically? This is questioned by Ingram and Stretton[7], who consider the family re-analysed by Cohen *et al.*[3] as carrying a gene for α-thalassæmia. This interpretation cannot at present be ruled out with finality. It appears to me, however, to be of limited probability. It would seem reasonable to assume that in a heterozygote for α-thalassæmia the condition of which is not complicated by the presence of some other mutant gene, either the non-thalassæmic α-chain allele is able to make up for the thalassæmic allele to an extent sufficient for nearly all non-α-chains which are produced in normal amounts to combine with α-chains; that this can be the case is demonstrated by the α-thalassæmia—α^{I}-heterozygote investigated by Atwater *et al.*[9] (cf. Table 6, ref. 5)—or the synthetic powers of the α-chain gene fall short of this goal, and significant amounts of HbH and/or Hb 'Barts' (β_4 and/or γ_4) are produced. In the first case, and in the absence of a specifically inhibited β-chain gene, the total β-chain production would not be limited to a sub-normal level, as, in fact, it is. In the second case, HbH should have been observed by Cohen *et al.*[3]. In spite of the instability of HbH, which is liable to lead to under-estimates in quantitative assays, total mean amounts of β-chain per cell in HbH patients are occasionally found to be normal or sub-normal[5]. If α-thalassæmic, subjects I-2 and II-3 reported on by Cohen *et al.*[3] should either contain more HbA and notably more HbS per cell, or should contain in addition to what is found a mixture of HbH and Hb Augusta I (refs. 10, 11) ($= \beta_4^S$).

It seems likely, therefore, that the condition described by Cohen *et al.*[3] as a thalassæmia is neither a β- nor an α-thalassæmia. This family certainly cannot be quoted, as was done up to the time of its re-interpretation by Ingram and Stretton[7], as offering evidence in favour of independent segregation between a gene for thalassæmia and the structural hæmoglobin chain gene which shows the thalassæmic inhibition.

The family of Cohen *et al.*[3] appears not to be unique in kind. The case of Larry W., a HbC/HbA heterozygote described by Zuelzer and Kaplan[12], seems to be analogous to Cohen's *et al.* cases I-2 and II-3, except that the general inhibition of hæmoglobin chain synthesis in Larry is still more pronounced. Larry has, on the average, in μμg per cell, 7·0 α-chain, slightly less than 4·9 β^A-chain, 2·0 β^C-chain, and less than 0·14 γ-chain. The normal mean amount of β^C-chain per cell in β^C/β^A heterozygotes is 5·5 ± 2·0 μμg[5]. An α-thalassæmic individual with only 7 μμg of α-chain per cell will almost certainly possess significant amounts of HbH. The patient's blood was tested by Tiselius electrophoresis for abnormal hæmoglobins at *p*H 6·5 as well as by paper electrophoresis (*p*H unspecified), and was found to contain hæmoglobins A and C. If HbH had been present in significant amounts, it should have been detected, although in 1954 HbH would not yet have been identified as such. From the results we have good reason to believe that HbH was absent. Consequently, it appears unlikely that we are dealing with an α-thalassæmia; nor is the condition likely to be β-thalassæmia, since not only the β^A-chain gene, but also the β^C-chain gene are significantly inhibited in Larry. This implies that the β^A-chain gene is not inhibited specifically. Finally, the condition cannot be attributed to a simple deficiency anæmia, both because treatment failed and because of red-cell morphology. Let us emphasize the dual cell population noted by the authors, which includes a proportion of normal-appearing red cells—a fact of significance and in accord with the findings of Matioli[1]. (In the family investigated by Cohen *et al.*[3], discussed here, no similar phenomenon is noted. Perhaps spherocytosis, in that case, interferes with its expression.)

Expression of panglobinopenia and its relation to the synthesis of HbF and HbA$_2$. The analysis of the cases listed here, as well as of the one on which Matioli and I have reported, lead one to suspect the existence of a gene the action of which results in a general inhibition of hæmoglobin synthesis. The occurrence of such cases was predicted by Itano[6]. Whether the synthesis of all proteins is partly inhibited (as might occur in the case of a deficiency of ribosomes, in RNA-polymerase, or in other constituents) or only that of hæmoglobin polypeptide chains cannot be gathered from the available data, although the latter appears more probable in the case examined by Matioli. I therefore refer here provisionally to a panglobinopenia rather than to a panprotidopenia. Both conditions may actually exist.

The expression of the panglobinopenia gene and perhaps of the β-thalassæmia gene may be subject to variegation (cf. Lewis[13]). Matioli has indeed observed a mosaic condition of the degree of hæmoglobinization of the red cells in the case examined by him[1]. If this finding can be generalized, as the appearance of published photographs of red cell smears from 'thalassæmic' patients suggests, the clinical expression of the genomes may vary a great deal according to the proportion of affected red cells.

Homozygous panglobinopenia may be thought to be mostly a lethal condition since practically no hæmoglobin should be produced in the affected cells. The proportion (not the absolute amount per cell) of HbF should be very high because of the prevalent 'stress' condition[1,14], and the mean quantity per cell of HbA$_2$ should be below normal.

A heterozygous panglobinopenia would be characterized by a blood picture resembling that of thalassæmia, but

Table 3. Predicted Mean Amounts per Cell of HbF and HbA_2 in Thalassæmia, Panglobinopenia and Mixed Conditions

	Genetic constitution	HbF	HbA_2
1	$Th_\beta^N/Th_\beta^N.Pgl^N/Pgl^N$	Norm.	Norm.
2	$Th_\beta^T/Th_\beta^N.Pgl^N/Pgl^N$	Norm. or slightly incr.	Incr.
3	$Th_\beta^T/Th_\beta^T.Pgl^N/Pgl^N$	Strongly incr.	Incr.
4	$Th_\beta^N/Th_\beta^N.Pgl^P/Pgl^N$	Norm. or slightly incr.	Decr.
5	$Th_\beta^N/Th_\beta^N.Pgl^P/Pgl^P$	Absent or incr. Perhaps not observed	Decr. (mostly lethal *in utero*)
6	$Th_\beta^T/Th_\beta^N.Pgl^P/Pgl^N$	Incr.	Decr., norm. or incr.
7	$Th_\beta^T/Th_\beta^N.Pgl^P/Pgl^P$	Probably not observable (lethal *in utero*)	
8	$Th_\beta^T/Th_\beta^T.Pgl^P/Pgl^P$	Probably not observable (lethal *in utero*)	

Norm. = normal; incr. = increased; decr. = decreased; Th = β-thalassæmia locus; Pgl = panglobinopenia locus; superscript N = normal allele; superscript T = thalassæmia allele; superscript P = panglobinopenia allele.

HbA_2 would not be elevated. HbF, according to the degree of severity of the anæmia, would or would not be moderately elevated. Consequently this condition, on the basis of the criteria at present used, could easily be confused with an α-thalassæmia, except for the absence of HbH. This absence, however, does not rule out α-thalassæmia. Cases of heterozygous panglobinopenia may commonly be classified as 'thalassæmia minor'.

A pure heterozygous β-thalassæmia without panglobinopenia would mostly appear phenotypically normal or only slightly thalassæmic, but would in all cases be characterized by an increase in absolute amounts per cell of HbA_2. There would mostly be no increase or a moderate increase in the HbF component and the clinical picture would be most commonly that of 'thalassæmia minima'.

A pure homozygous β-thalassæmia would show significant increase not only in the HbA but also in the HbF component. True homozygous thalassæmia is expected to represent always a very severe, 'major' condition. The latter statement is made in view of the absence of any evidence for the existence of moderately inhibited thalassæmic β-chain genes (cf. ref. 5, Table 7).

A double heterozygote for β-thalassæmia and panglobinopenia would show elevated values for HbF because of the 'severity' of the condition, while the mean cellular amounts of HbA_2 would be variable, in part, according to the degree of variegation that may be observed at the level of the individual red cells. The clinical characteristics may be those of 'thalassæmia major'.

These predicted relationships are summarized in Table 3. Conditions 7 and 8 would be expected to occur only if the thalassæmia and panglobinopenia loci are not closely linked.

It is interesting that Table 3 predicts a decrease in absolute amounts per cell in the HbA_2 component in some cases which will be clinically characterized as thalassæmia major, namely in double heterozygotes for panglobinopenia and thalassæmia. The mean amount per cell of δ-chain is increased in heterozygous thalassæmia, but in a number of cases is less than doubled. If a heterozygosity for panglobinopenia is superimposed on such a situation, it is to be expected that the mean average amount of δ-chain per cell will be slightly below normal.

In conclusion, the multiplicity of the phenotypic manifestations in thalassæmia can, in part, be accounted for if the two following assumptions are justified:

(1) There are cases of pure thalassæmia, of pure panglobinopenia, and of mixed thalassæmia and panglobinopenia, each of these presenting a different picture.

(2) Within each of these categories, further variation is brought about by a variability in the mosaic condition (variegation) that seems to be observed, the mutant genes being expressed, within different individuals and/or different families, in different proportions of the cell population.

Although panglobinopenia should occur in conjunction with α-thalassæmia as well as with β-thalassæmia, the fragmentary topical data that are available do not warrant a discussion of this matter here.

This work was supported by grant *GM* 04276–09 from the U.S. National Institutes of Health.

I thank Prof. R. D. Koler and Prof. E. B. Lewis for their advice.

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FACILITATION OF HOST LYMPHOID TISSUE DEVELOPMENT IN NEONATALLY THYMECTOMIZED MICE BY INJECTION OF ALLOGENEIC DISPERSED THYMUS CELLS

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THE growth failure, early mortality and immunological deficiency of neonatally thymectomized mice may be prevented by several procedures: grafting of syngeneic or allogeneic thymus[1,2], injection of adult syngeneic spleen or lymph node cells[2–4], and intraperitoneal implantation of 'Millipore' chambers containing thymic tissue[5,6]. More recent investigations have established that thymus cells obtained from new-born or adult thymus can also provide immunological reconstitution of neonatally thymectomized mice[7,8]. Analysis of the immunological characteristics of the spleen and lymph node cells of the mice restored with allogeneic thymus cells has heretofore shown that these are of donor competence[7] and that the restoration is apparently not a function of the humoral factor capable of reconstituting host cell competence following intraperitoneal implantation of allogeneic thymus in 'Millipore' diffusion chambers.

The purpose of this article is to present evidence that administration of large numbers of fully dispersed neonatal thymus cells can facilitate, to some extent, the host cell development of the spleens of mice thymectomized in the new-born period.

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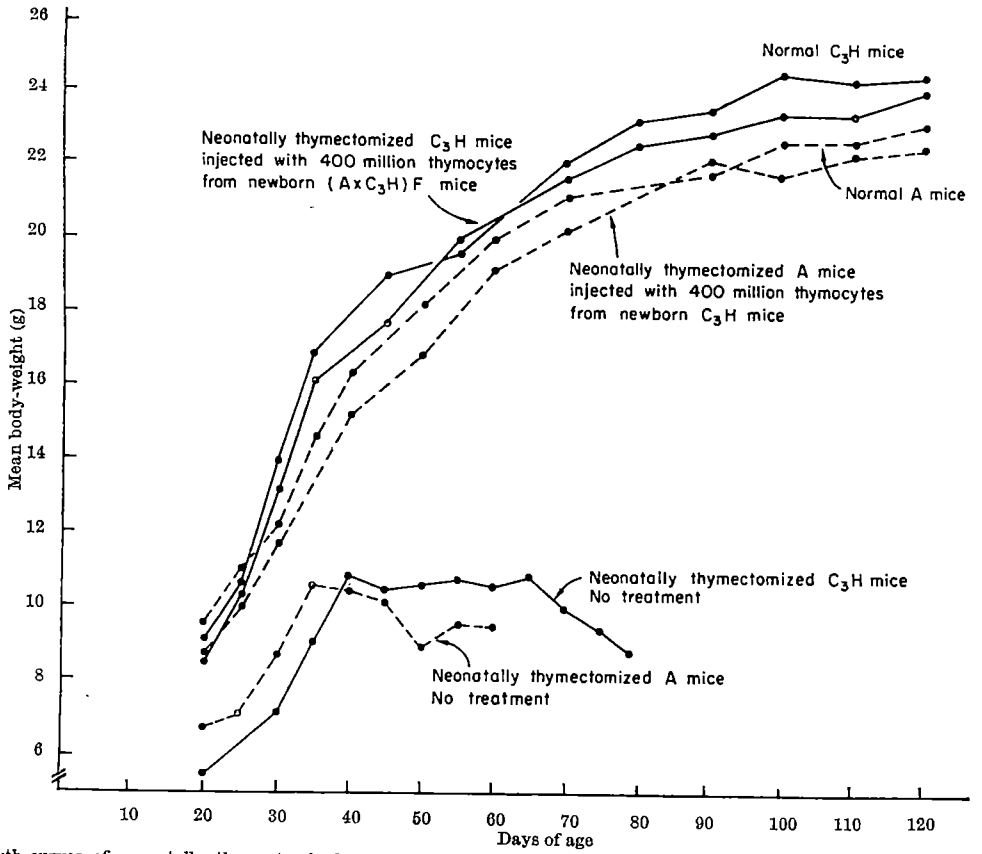


Fig. 1. Growth curves of neonatally thymectomized mice injected with allogeneic or hemi-allogeneic new born thymocytes, compared with intact and neonatally thymectomized animals of both strains. The points on the curves are mean values

Mice of the *C3H* and *A* strains of both sexes were thymectomized within 24 h after birth by the method routinely used in this laboratory[9]. Thymectomized *A* and *C3H* strain mice were given intraperitoneal injections of isolated thymic cells obtained from new-born mice of *C3H* or $(A/C3H)F_1$ strains. Two intraperitoneal injections of 50 million viable cells were given each week over a four-week period, beginning on the fifth day of life. Suspensions of viable, fully dispersed cells from thymus were prepared in Ringer's lactate saline solution, using a loose-fitting Potter–Elvehjem glass homogenizer. Further dissociation of the cells was completed by passing the suspension gently in and out of 24-, 27- and 30-gauge needles. Another group of neonatally thymectomized mice (*C3H*) were grafted with hemi-allogeneic new-born thymus $(A/C3H)F_1$ five days following thymectomy. The entire thymus from the new-born donors was introduced into the subcutaneous tissue of the dorsal region of the recipients.

Neonatally thymectomized mice were evaluated by the following parameters: changes in body-weight, survival to 120 days of age, capacity to reject skin homografts, and the capacity of spleen cells derived from the treated mice to produce splenomegaly after injection into appropriate F_1 hybrids. The immunogenetic characteristics of the spleen cells from thymectomized *A* strain mice given *C3H* thymus cell suspensions were examined by the discriminating spleen assay of Simonsen and Jensen[10]. In this assay 30 million spleen cells from 2–3-month-old animals of both the experimental and control groups were used.

In one group of experimental animals (*C3H* mice neonatally thymectomized and treated with thymus cells or thymus grafts from new-born $(A/C3H)F_1$ donors), the discriminating spleen assay of host component was used[10]. Ten million spleen cells from experimental and control groups were administered intraperitoneally to 8-day-old $(C3H/C57BL)F_1$ mice. Litters of 6–8 animals were injected, in groups of one or two, with spleen cells from: (*a*) 2-month-old intact *C3H* mice (positive control); (*b*) 2–3-month-old $(C3H/C57BL)F_1$ animals (negative control); (*c*) 2–3-month-old $(A/C3H)F_1$ mice (negative experimental control); (*d*) 2–3-month-old *C3H* neonatally thymectomized mice treated with either 400 million $(A/C3H)F_1$ thymus cells or $(A/C3H)F_1$ thymus grafts from new-born donors (experimental group). Eight days after cell administration the hybrids were killed, their spleen and body-weights determined, and relative spleen weights (mg/100 g body-wt.) calculated. Finally, spleen indices were calculated. The mean of the log of each relative spleen weight was determined and the differences between the experimental group and the positive and negative control groups evaluated by the '*t*' test. The experimental spleen index was calculated using the experimental control as the divisor, because these cells caused slightly greater splenomegaly in the $(C3H/C57BL)F_1$ hybrids than did syngeneic cells, perhaps a reflexion of host versus graft activity.

At two months of age, a group of *A* and *C3H* thymectomized mice treated with allogeneic or hemi-allogeneic thymus cells were grafted[11] with skin from mice of the same strain as the donor of the thymocytes and with skin of an unrelated strain (*BALB/c*). All reconstituted neonatally thymectomized mice were killed at 120 days of age. Extensive search was made in each thymectomized mouse with a stereoscopic microscope and sample serial

Table 1. Survival of Neonatally Thymectomized Mice treated with Allogeneic Dispersed Thymus Cells from New-born Donors

Thymectomized recipient strain	Treatment	No. of thymus cells	Survivors at 120 days
A	50×10^6 *C3H* thymus cells intraperitoneally at 5, 8, 12, 15, 18, 20, 22 and 24 days of age	400×10^6	9/10
	None	None	0/17*
C3H	50×10^6 $(A \times C3H)F_1$ thymus cells intraperitoneally at 5, 8, 12, 15, 18, 20, 22 and 24 days of age	400×10^6	12/12
	None	None	0/24†

* All mice died before 70 days of age. † All mice died before 89 days of age.

Table 2. Immunogenetic Composition of Spleen Cells from Neonatally Thymectomized *A* Mice restored with Thymus Cells from New-born *C3H* Donors

Assay for donor component (*C3H*): test litters—$(C3H \times C57BL)F_1$				Assay for host component (*A*): test litters—$(A \times C57BL/1)F_1$			
Experimental group (neonatally thymectomized *A* restored with *C3H* thymus cells)*	Negative control group (Intact *A*)*	Mean log experimental–Mean log negative control	*P*	Experimental group (neonatally thymectomized *A* restored with *C3H* thymus cells)*	Negative control group (intact *A*)*	Mean log experimental–Mean log negative control	*P*
2·887	2·748	0·139	0·01	2·710	2·769	−0·059	*N.S.*
2·874	2·712	0·162	0·01	2·738	2·758	−0·020	*N.S.*
2·643	2·690	−0·047	*N.S.*	2·770	2·848	−0·078	*N.S.*
2·949	2·675	0·274	0·001	2·852	2·816	0·036	*N.S.*
3·057	2·724	0·333	0·001	2·833	2·806	0·027	*N.S.*

* Each number represents the mean of the log of the relative spleen weight (mg/100 g body-wt.) obtained from 2–3 mice of $(C3H \times C57BL/1)F_1$ or $(A \times C57BL/1)F_1$.

Table 3. Graft versus Host Assay in $(C3H \times C57BL)F_1$ Litters of Host Component of Neonatally Thymectomized Mice restored with Thymus Grafts or Thymus Cells from New-born $(A \times C3H)F_1$ Donors

Experimental group (neonatally thymectomized *C3H* restored with $(A \times C3H)F_1$ thymus cells)*	Experimental control (intact $(A \times C3H)F_1$)*	Mean log experimental–Mean log experimental control	*P*	Experimental group (neonatally thymectomized *C3H* grafted with new-born $(A \times C3H)F_1$ thymus*	Experimental control (intact $(A \times C3H)F_1$)*	Mean log experimental–Mean log experimental control	*P*
2·775	2·683	0·092	0·10	3·037	2·630	0·408	0·001
2·616	2·598	0·018	*N.S.*	3·066	2·617	0·446	0·001
2·968	2·696	0·272	0·0005	2·965	2·672	0·293	0·01
2·886	2·726	0·160	0·10	2·880	2·679	0·201	0·01
2·889	2·611	0·278	0·0005				0·01†
2·743	2·571	0·172	0·01				
2·845	2·594	0·251	0·0005				
2·889	2·670	0·219	0·0005				
2·766	2·673	0·093	*N.S.*				
2·674	2·586	0·088	*N.S.*				
			0·005†				

* Each number represents the mean of the log of the relative spleen weight (mg/100 g body-weight) obtained from 3 to 6 mice of two different $(C3H \times C57BL/1)F_1$ litters.
† This figure represents the *P* value ('*t*' test) of the differences between the mean relative spleen weights of the experimental groups and the experimental control groups.

sections obtained to rule out thymic remnants. In none of the animals included here could remnants of thymus tissue be found.

The results of these experiments show, first of all, that neonatally thymectomized mice injected with thymus cells obtained from new-born hemi-allogeneic or allogeneic mice show steady increase of weight, do not develop wasting disease, and survive for prolonged periods (Fig. 1) (Table 1).

Assessment by discriminating spleen assay of the immunogenetic composition of the spleens of thymectomized *A* strain mice injected with thymus cells from new-born *C3H* mice showed that host *A* cells did not contribute significantly to the immunological reconstitution (Table 2). This result was similar to an earlier study of this same strain combination in which 300 million adult *C3H* thymocytes were used in the inoculum[7].

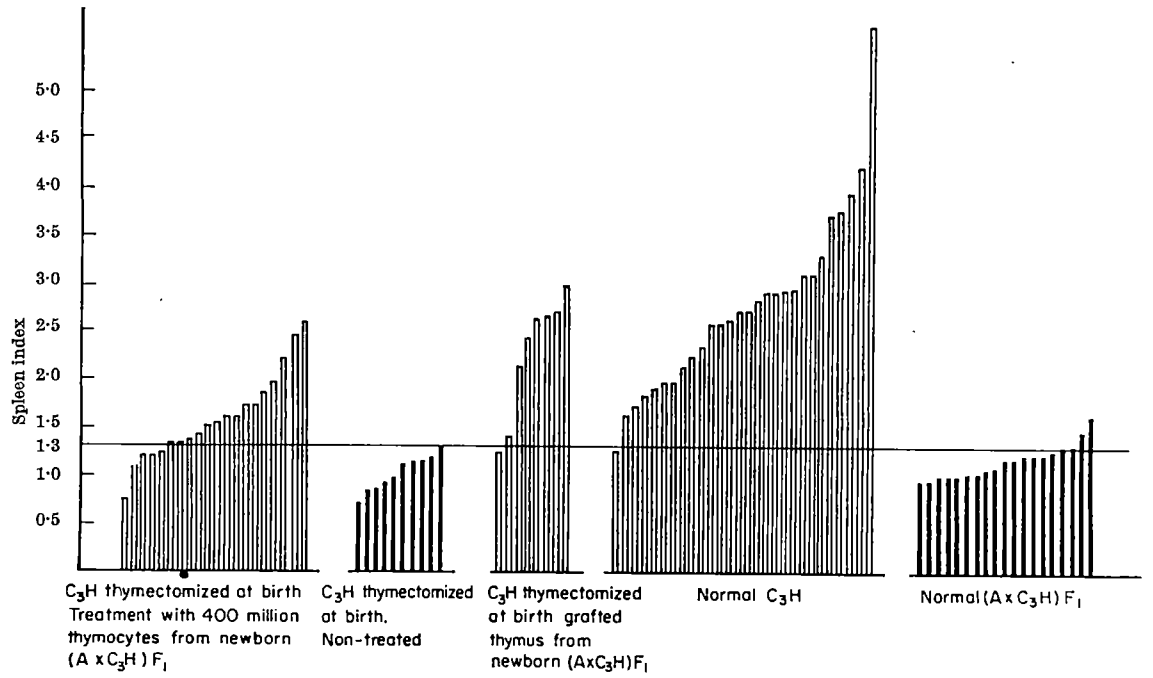


Fig. 2. Graft versus host assays of spleen cells from neonatally thymectomized *C3H* mice treated with either a thymus graft or thymocytes from new-born $(A \times C3H)F_1$ donors. The solid bars represent spleen indices calculated with the relative spleen weights of syngeneic cell-injected $(C3H \times C57BL/1)F_1$ animals as the divisor. The other indices were calculated with the relative spleen weights of $(A \times C3H)F_1$ cell-injected $(C3H \times C57BL/1)F_1$ mice as the divisor. They show the relative activity of the positive controls (normal *C3H*), of the neonatally thymectomized *C3H* animals grafted with neonatal $(A \times C3H)F_1$ thymus, and of the neonatally thymectomized *C3H* animals treated with new-born $(A \times C3H)F_1$ thymus cells. There was significant restoration of host reactivity in most of the thymus-grafted animals, and in about half the thymus cell injected mice

The discriminating spleen assay of host component in the spleens of neonatally thymectomized *C3H* mice grafted with $(A/C3H)F_1$ thymus showed a predominant host component (Table 3), consistent with the earlier observations of Dalmasso *et al.*[2]. This assay also showed significant host reactivity in the spleens of some *C3H* animals receiving new-born $(A/C3H)F_1$ thymus cells, as shown in Table 3. As indicated earlier, administration of spleen cells from adult $(A/C3H)F_1$ mice to the test $(C3H/C57BL)F_1$ hybrids resulted in a slight increase of the relative spleen weights; hence the mean relative spleen weights of the test hybrids receiving $(A/C3H)F_1$ cells were used as the divisor in calculating the spleen indexes shown in Table 3 and Fig. 2. Of the 10 experimental neonatally thymectomized animals injected with hemi-allogeneic $(A/C3H)F_1$ cells, five were capable of inducing significant splenomegaly in the $(C3H/C57BL)F_1$ hybrids. The levels of statistical significance varied from 0·01 to 0·005. The values of the positive controls were statistically significant at a level between 0·0001 and 0·01.

The restoration of immunological capacity of the neonatally thymectomized mice injected with new-born thymocytes was also revealed in the skin-grafting studies, summarized in Table 4. All the mice tested accepted skin from the same strain as the thymocyte donors, and rejected skin from a third strain.

These results demonstrate that mice thymectomized at birth can be immunologically reconstituted by injections of isolated thymus cells obtained from allogeneic new-born donors. The results with neonatally thymectomized *A* mice injected with new-born *C3H* cells corroborate our previous results which showed that reconstitution is entirely attributable to donor cells when the neonatally thymectomized *A* strain mice were treated with *C3H* thymus cells obtained from adult donors. The experiments on neonatally thymectomized *C3H* mice injected with large numbers of fully dispersed $(A/C3H)F_1$ thymus cells show that some of the animals develop immunologically competent cells of host characteristics in their spleens. The effect observed could, of course, be consequent to seeding of thymic stromal cells to other parts of the body where they can exercise their hormone-secreting function. Alternatives exist, however, and it seems entirely possible that a trephocytic function of the donor cells or other forms of cell–cell interaction could account for development of immunological capacity in host cells under the conditions of these experiments. Whatever the basis, it seems clear that permission for development of immunological capacity of host cells may be provided by fully dispersed allogeneic thymus cells in at least some strain combinations.

Table 4. SKIN HOMOGRAFT SURVIVAL IN NEONATALLY THYMECTOMIZED MICE INJECTED INTRAPERITONEALLY WITH THYMUS CELLS FROM NEW-BORN MICE

Group	Skin grafts*		
	BALB/c	$(A \times C3H)F_1$	*C3H*
C3H mice injected intraperitoneally with 400×10^6 thymocytes from new-born $(A \times C3H)F_1$ mice	1/6 (16·6%)†	12/12 (100%)	—
A mice injected intraperitoneally with 400×10^6 thymocytes from new-born *C3H* mice	0/5 (0%)	—	5/5 (100%)

* Number of mice taking the graft for 30 days or more/number of mice grafted.
† This mouse rejected the skin 35 days after grafting.

We thank Mr. K. Sjodin for performing the thymectomies in the neonatal mice.

This work was supported by grants from the U.S. Public Health Service (*HE*–02085, *C*–3511, *AI*–00798). American Heart Association, Minnesota Heart Association, American Cancer Society, and the National Foundation.

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SPECIFICITY OF THE INTERACTIONS BETWEEN HISTONES AND DEOXYRIBONUCLEIC ACID

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RECENT experiments have shown that DNA free from histone can act as a template for the synthesis of RNA, under the influence of the RNA polymerase demonstrated by Weiss[1] and Hurwitz *et al.*[2], while the native nucleoprot ein has been shown to have little ability in this direc tion[3]. Furthermore, the addition of histone to DNA in amounts approaching those present in the native complex markedly reduced the ability of DNA to promote RNA synthesis[4,5].

These observations support the view originally expressed by Stedman and Stedman[6] that histones control gene function; but they do not provide a mechanism whereby the DNA of active genes may be uncovered and that of inhibited genes covered by histones in differentiated cells. In fact, few differences have been found so far between histones from different tissues of the same or even of widely different animals, although little precise knowledge exists of the relative amounts of the various histone fractions[7].

The question whether there are specific interactions between the DNA of different genes or classes of genes and particular kinds of histone molecules is thus of considerable interest. A number of distinct histone fractions of well-defined composition (designated *F1*, *F2a*, *F2b*, *F3*) have been obtained in this laboratory. Although these are known to be capable of further resolution, their differences of composition and character are considerable[8], and they may be expected to be sufficiently distinctive to yield information about the specificity of DNA–histone interactions. The experiments described here were all carried out with DNA and histones obtained from calf thymus. The DNA was prepared by the method of Kay, Simmons and Dounce[9] and the methods of preparing the histone fractions have been recorded[8].

(1) *Precipitation of DNA from solution with increasing amounts of histone.* The concentration of histone at which the first precipitation occurs depends on the salt concentration, and at low ionic strengths higher proportions of histones have to be added to cause precipitation. However, in previously reported experiments[10] it was found that each histone fraction, in sufficient amount, is capable of precipitating almost the whole of the DNA in 0·9 per cent sodium chloride. The lysine-rich fraction, *F1*, precipitates the DNA at a lower concentration than



Fig. 1. Starch-gel electrophoresis patterns of histones present in nucleoproteins prepared by the successive addition of DNA to excess histone. *a*, Whole histone; *b*, first precipitate; *c*, second precipitate; *d*, third precipitate; *e*, fourth precipitate

the other fractions, which are similar in their precipitating power. Thus, almost complete precipitation of DNA is obtained with *F*1 when the ratio of histone to DNA (w/w) is about 0·8, but with the other fractions the ratios required for complete precipitation are about 1·5 or higher.

(2) *Addition of histone fractions to excess of DNA.* It was suggested by Huang, Bonner and Murray[11] that if an opportunity of equilibration is given by mixing DNA and histone in a strong salt solution (in which the complex is dissociated) and reducing the concentration of salt slowly by dialysis, the histones will find their positions of 'maximum stability' on the DNA. If this is true, when specific histone fractions are added to excess DNA in sufficient quantity to precipitate the major part of it, one might expect to find that the DNA remaining in solution has distinctive properties which might be demonstrated by a variation in the base ratios.

To test this, about 15 mg of histones *F*1 or *F*3 were added to 50 mg of DNA in 2 M sodium chloride. The solution was dialysed to 0·14 M sodium chloride and the precipitated nucleoprotein removed. Under these conditions, using a deficiency of histone, *F*1 gives a fibrous precipitate, while *F*3 forms a gel. The base ratios of the DNA remaining in solution (about 20 per cent in the case of *F*1 and 10 per cent in the case of *F*3) were determined. The results shown in Table 1 indicate that only slight fractionation of the DNA resulted, much less than can be produced by differential extraction of DNA from the denatured nucleoprotein by salt solutions[12].

(3) *Addition of DNA to excess of whole histone.* If different portions of DNA combine specifically with particular fractions of histone, the addition of DNA to an excess of the homologous whole histone should result in the precipitation of nucleoprotein containing histones in the proportions in which they are present in the native nucleoprotein.

Successive portions of DNA (50 mg in 10 per cent sodium chloride) were added to a quantity (400 mg) of whole histone from calf thymus dissolved in 10 per cent sodium chloride. The mixture was dialysed against water

Table 1. Base Ratios in DNA remaining after Precipitation with Histone Fractions *F*1 and *F*3

	G	*A*	*C*	*T*
Original DNA	23·8	28·0	21·8	26·6
DNA remaining after precipitation with *F*1	26·0	25·7	23·4	24·8
DNA remaining after precipitation with *F*3	24·0	27·2	23·5	25·4

The bases were determined by the method of Wyatt[13] and are expressed as a percentage of all bases recovered.

to 0·9 per cent salt concentration and the precipitate collected. After bringing the salt concentration up to 10 per cent again, a similar addition of DNA was made and the dialysis and collection of precipitate repeated. This was carried out twice more and the histones present in the precipitates were extracted with 0·25 N hydrochloric acid and examined by starch-gel electrophoresis at *p*H 2·3 as previously described[14] (Fig. 1). It can be seen that DNA does not combine with the histone fractions in their 'natural' proportions. The first two precipitates contain histones *F*2*a*, *F*2*b* and *F*3; the third precipitate contains *F*2*a*, *F*2*b*, *F*1 and a little *F*3 in proportions which cannot be exactly evaluated, and the fourth contains *F*1 only, which is also the only histone present in the solution after the whole series of additions. Table 2 gives information about the approximate amounts of histone and DNA in the different precipitates.

Table 2. Composition of Precipitates formed on Successive Addition of DNA to whole Histone

Successive additions of 50 mg DNA to solution containing 400 mg tota histone

Addition of DNA	Composition of precipitate (mg) DNA	Histone	Ratio *H*/*D* in precip.	Nature of histones in precipitate
1	47	107	2·3	*F*2*a*, *F*2*b*, *F*3
2	45	70	1·55	*F*2*a*, *F*2*b*, *F*3
3	40	40	1·0	*F*2*a*, *F*2*b*, *F*1 and trace *F*3
4	40	25	0·62	*F*1 only

(4) *Addition of excess of histone fractions to DNA.* It is of interest to determine the amounts of the histone fractions which can combine with DNA. An excess (180 mg) of each histone fraction in 10 per cent sodium chloride was added to 45 mg of DNA in the same medium. The mixtures were dialysed against water until the salt concentration was 0·9 per cent (0·14 M). The precipitates, which contained nearly all the DNA, as judged by the extinction of the supernatant at 260 mμ, were collected, washed twice with 80 per cent EtOH, three times with acetone and dried. The ratios of histone to DNA (*H*/*D*) in the precipitates were determined in two ways: (1) by the weights of the precipitates obtained knowing that all the DNA had been precipitated; (2) by nitrogen and phosphorus analyses. The results given in Table 3 are averages obtained by these two methods, which did not differ by more than 10 per cent. It can be seen that under these conditions much less of fraction *F*1 is required to precipitate the DNA than the other three fractions.

Table 3. Ratios of Histone to DNA in Precipitates of DNA with Different Histone Fractions

Histone fraction	*H*/*D*
*F*1	0·9
*F*2*a*	2·3
*F*2*b*	1·4
*F*3	1·5

These experiments do not provide any evidence for specific interactions between specific histones and parts of the DNA which are different in composition, even when, by slow dialysis from a concentrated salt solution, an opportunity is given for the histone molecules to find positions of maximum stability. While each of the histone fractions is capable of precipitating all the DNA, there are, however, marked differences in the modes of combination of the different histones. Much less of the lysine-rich fraction *F*1 than of the other fractions is required to precipitate a given quantity of DNA. This indicates that the mode of combination of *F*1 is different from that of the other fractions and this may explain the greater effectiveness of the fraction in inhibiting RNA synthesis[4,11]. The differences between *F*1 and the other histones are

too great to be accounted for in terms of its rather greater proportion of basic groups. On the other hand, *F*1 is less strongly bound in the natural nucleoprotein than the other fractions, as is shown by the fact that it is more easily extracted by dilute acids[15], and also, as shown previously, in a mixture of histones, *F*1 is the last to be precipitated by DNA. It is therefore unlikely that experiments with reconstituted nucleohistones can throw any light on the parts of the DNA covered by particular histones in the native state.

This work was supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research: Royal Cancer Hospital) from the Medical Research Council and the British Empire Cancer Campaign for Research, and by U.S. Public Health Service research grant *CA*–03188–08 from the National Cancer Institute.

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VIRUS SPECIFIC RIBONUCLEIC ACIDS IN BABY HAMSTER KIDNEY CELLS INFECTED WITH FOOT-AND-MOUTH DISEASE VIRUS

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IT is now generally accepted that double-stranded DNA replicates by a progressive separation of the two strands and the formation on each of a complementary strand. The demonstration by Sinsheimer *et al.*[1] that the single-stranded DNA of bacteriophage φ × 174 becomes double-stranded during replication raised the question of whether double-stranded structures are also produced during the multiplication of the single-stranded RNA viruses. Montagnier and Sanders[2] have provided evidence that a double-stranded infective RNA is produced in Krebs II ascites cells infected with mouse encephalomyocarditis virus, and more recently Darnell *et al.*[3] have demonstrated a double-stranded RNA in HeLa cells infected with poliovirus, although in these experiments the RNA was not examined for infectivity. Double-stranded RNA molecules have also been found during the multiplication of the bacteriophages MS_2 (ref. 4) and R_{17} (ref. 5).

In the work described here it is shown that infection of cultured baby hamster kidney cells with foot-and-mouth disease (FMD) virus leads to the appearance in the cells of 3 distinct ribonucleic acids which can be separated by centrifugation in sucrose gradients. All 3 are infective but only one is destroyed by ribonuclease.

Monolayers of a cloned strain (*C* 13) of a baby hamster kidney cell line *BHK* 21 (ref. 6) were infected with FMD virus of type O (strain 1) which had been given 10–15 passages in *BHK* cells. After washing free from phosphate, the cells were incubated in a low-phosphate medium containing carrier-free phosphorus-32-labelled phosphate and 1 μg/ml. actinomycin *D*. The actinomycin was included in the medium to prevent the uptake of phosphorus-32 into ribosomal RNA. This concentration of the inhibitor has no effect on FMD multiplication in *BHK* cells[7]. After incubation for 6 h at 37° the cell sheets were washed in Eagle's medium to remove as much unincorporated phosphorus-32 as possible and then extracted with phenol at 2°. The aqueous layer containing the RNA was then extracted twice with ether and centrifuged immediately on a sucrose gradient. Fractions collected from the bottom of the tube were assayed for radioactivity and ultra-violet absorbancy at 260 mμ. The details of the fractionation and the results of a typical experiment are shown in Fig. 1. The distribution of radioactivity and ultra-violet absorbancy from the fractionation of the RNA from uninfected *BHK* cells incubated in phosphorus-32 and actinomycin *D* is shown for comparison. The results show the presence in the RNA from infected cells of 3 radioactive peaks which are not present in the RNA from uninfected cells.

As the RNA prepared in this way is contaminated with phosphorus-containing substances of low molecular weight, it was important to establish that the material in the radioactive peaks was acid-insoluble RNA. Aliquots of each fraction from the sucrose gradient from one experi-

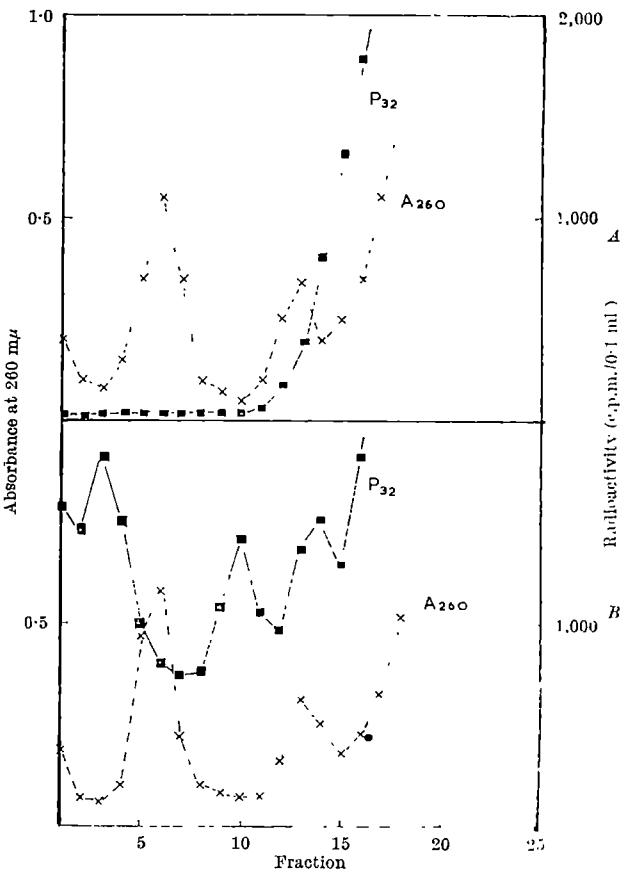


Fig. 1. Sedimentation in sucrose gradient (5–25 per cent) of RNA extracted from actinomycin-inhibited *BHK* cells (*A*) uninfected (*B*) 6 h after infection with FMD virus (16 h 24,000 r.p.m. *SW* 25 in *tris*-hydrochloric acid, 0·005 M, EDTA 0·02 M, *p*H 7·2)

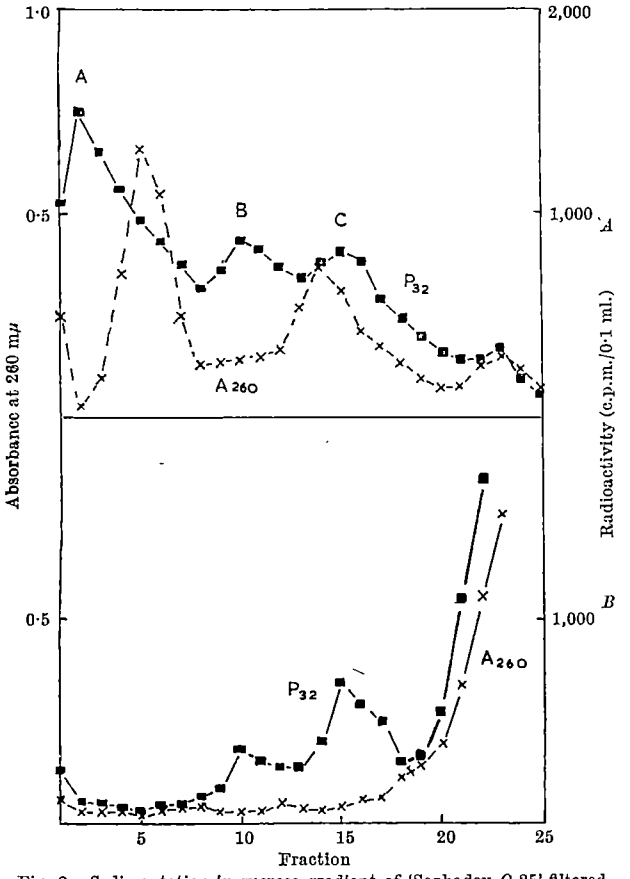


Fig. 2. Sedimentation in sucrose gradient of 'Sephadex G-25' filtered RNA from actinomycin-inhibited *BHK* cells 6 h after infection with FMD virus (*A*) untreated (*B*) after incubation with 0·01 μg RNase/ml.

ment were therefore mixed with cellular RNA and then precipitated with cold 5 per cent trichloroacetic acid (final concentration) and the radioactivity contained in the washed precipitate was measured. These experiments confirmed the presence of 3 additional radioactive peaks in the RNA prepared from infected cells. In further experiments the phenol extracts were filtered through a column of 'Sephadex *G*-25' (41 cm × 1·5 cm) before centrifugation on the sucrose gradient. Fig. 2*A* shows the distribution of radioactivity and ultra-violet absorbancy when RNA which had been purified in this way was centrifuged in a sucrose gradient under the same conditions as described for Fig. 1. The relative amounts of radioactivity in the 3 peaks *A*, *B* and *C* were usually as shown in Figs. 1 and 2, but in some experiments the radioactivity in peaks *B* and *C* exceeded that in peak *A*.

When the RNA from infected cells was incubated with 0·01 μg RNase/ml. for 10 min at 20° prior to centrifugation, all the ultra-violet absorbing material was then found at the top of the tube (Fig. 2*B*). In contrast, the radioactivity in peaks *B* and *C* still sedimented to approximately the same positions as previously, but peak *A* was lost and the radioactivity from this peak was then located at the top of the tube.

Preliminary experiments showed that the RNA extracted from *BHK* cells 6 h after infection was highly infective for *BHK* cells. For the purpose of this assay, which has recently been developed in this laboratory[8], monolayers of *BHK* cells were washed twice in phosphate-buffered saline, once in 1 M sodium chloride in 0·04 M phosphate, *p*H 7·6, and the RNA added in 2 M magnesium sulphate in 0·1 M *tris*, *p*H 7·6. After absorbing for 15 min at 20°, the sheets were washed with phosphate-buffered saline and then overlaid with nutrient agar. After 2 days at 37°, the plates were stained with neutral red to reveal the plaques. A small proportion of the RNA from infected cells resisted the action of RNase at a concentration of 0·01 μg/ml. Determination of the infectivity of the individual fractions from a sucrose gradient centrifugation of infected cell RNA showed that the bulk of the infectivity was present in peak *A* but small amounts were present in the region of peaks *B* and *C*. Treatment of the individual fractions from the gradient with 0·01 μg RNase/ml. destroyed the infectivity of peak *A*, but the fractions in peaks *B* and *C* were still infective, although the infectivity was lower than in the untreated fractions. The lower infectivity in the region of peaks *B* and *C* following RNase treatment may be due to destruction of the trailing edge of peak *A* which would be present in these fractions prior to enzyme hydrolysis.

These experiments show that, as with the multiplication of encephalomyocarditis[2] and polio[3] viruses, RNase-resistant RNA molecules are produced during FMD virus replication. The experiments described here for FMD virus replication differ from those with encephalomyocarditis and polio viruses in that a third RNA molecule, which is specific to virus infection, is observed by centrifugation in sucrose gradients. The question of whether the RNA molecules in peaks *B* and *C* are double-stranded awaits the results of accurate base ratio analyses. The role of these molecules in the replication of FMD virus is being investigated.

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PLANT POPULATION AND CROP YIELD

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UNDER the above title, Holliday[1] has described the relationship between population per unit area and crop yield. He defined two basic types of relationship and expressed them graphically. One form gave a curve which was approximately parabolic in shape and was typical of the yield response to population change when yield was a function of the fertilization process. The second relationship defined was asymptotic and was claimed to be typical of the yield response to population change when yield was a function of vegetative growth.

At Ibadan, Western Nigeria, population investigations have been carried out over a number of years with the white yam, *Dioscorea rotundata* Poir, the yield of which is a tuber and thus a function of the vegetative growth of the plant.

Yams are grown from tuber cuttings, termed setts, and in this note 'sett' will refer to the planting material used for an individual plant whereas 'seed rate' will refer to the total weight of setts used per unit area.

In much previous work on this subject seed rate was directly proportional to the population per unit area because sett weight was kept constant. Yam setts, however, may weigh anything from a few ounces to six pounds[2], and in the present investigation treatments were

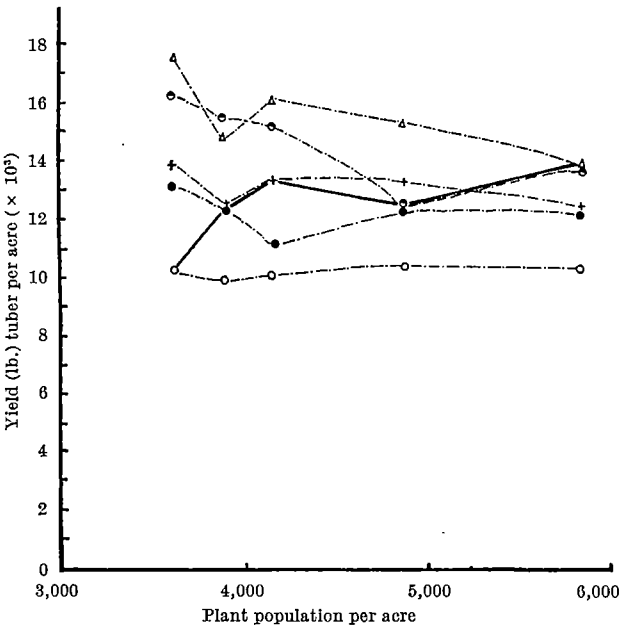


Fig. 1. Responses to population change at different seed rates per acre as yield per acre (lb. tuber). Seed rate per acre: ○, 1,815 lb.; ●, 1,936 lb.; +, 2,074 lb.; ◓, 2,420 lb.; △, 2,904 lb.; ○—●—◓—△, constant sett weight (8 oz.)

so designed that the effect of population on yield could be determined when sett weight was constant at eight ounces, and when sett weight varied but seed rate remained constant. The full range of treatments is given in Table 1 and the design and layout of the experiments is fully discussed by Freeman[3].

Thus the effects of population on yield when seed rates were constant could be obtained from treatments along the row in Table 1, while the effect of population on yield when sett weight was constant could be obtained from treatments along the leading diagonal (underlined in Table 1).

The results, shown graphically in Fig. 1, indicated that an asymptotic curve was only obtained when sett weight was constant and seed rate was proportional to population. When seed rate was maintained constant the yield response to population change was approximately linear and the slopes of the curves for the different seed rates were themselves in linear relationship to the seed rate per unit area. Regression coefficients for the five curves relating population to yield per acre at the different seed rates are given in Table 2.

Table 1. Experimental Treatments

Seed rate per acre	Sett weight in lb.				
2,904 lb.	0·500	0·600	0·700	0·750	0·800
2,420 lb.	0·417	0·500	0·583	0·625	0·667
2,074 lb.	0·357	0·428	0·500	0·536	0·571
1,936 lb.	0·333	0·400	0·467	0·500	0·533
1,815 lb.	0·312	0·375	0·437	0·469	0·500
Population (No. of plants per acre)	5,808	4,840	4,149	3,872	3,630

Table 2. Regression Coefficients for Population against Yield at Different Seed Rates per Acre

Seed rate (lb.)	b
1,815	+0·0631
1,936	−0·1498
2,074	−0·3359
2,420	−1·3783
2,904	−1·2038
b'	−0·001297

Although the individual regression coefficients, b, were not significantly different from zero, the linear by linear component of the interaction between population and seed rate was significant at the 5 per cent level in the analysis of variance. The significance of this interaction was fully supported by b', the regression coefficient of the values of b on seed rate. This regression coefficient was highly significant:

$$(b' = -0{\cdot}001297 = 0{\cdot}000405).$$

With the variety of *Dioscorea rotundata* used in these experiments, cultivar 'Olonko', only one shoot per sett was produced and large setts may be expected to produce more vigorous plants than small setts[4]. The change in the slope of the curves relating population to yield, reflected by b', was a measure of the increased intensity of competition experienced by the more vigorous plants produced from the larger setts at the higher seed rates.

Holliday[5] has stated that the relationship between yield and population, when yield is a function of vegetative growth, may be simply given in terms of the reciprocal of the yield per plant. Expressed in this form the relationship between yield and population is approximately linear and is written:

$$\frac{1}{Y_p} = a + bx \qquad (1)$$

where Y_p is the yield per plant and a and b are constants at population x per unit area.

Fig. 2 shows the curves obtained for the different seed rates per acre. There was no difference between the slopes of the curves, but the intercept at the y axis of each curve was related to seed rate. Thus the value of a in equation (1) was no longer constant but varied with seed rate. A further term must therefore be introduced into the equation to allow for the effect of seed rate, w, on yield. Thus:

$$\frac{1}{Y_p} = (a + cw) + bx \qquad (2)$$

where c is the regression of $\frac{1}{Y_p}$ on seed rate.

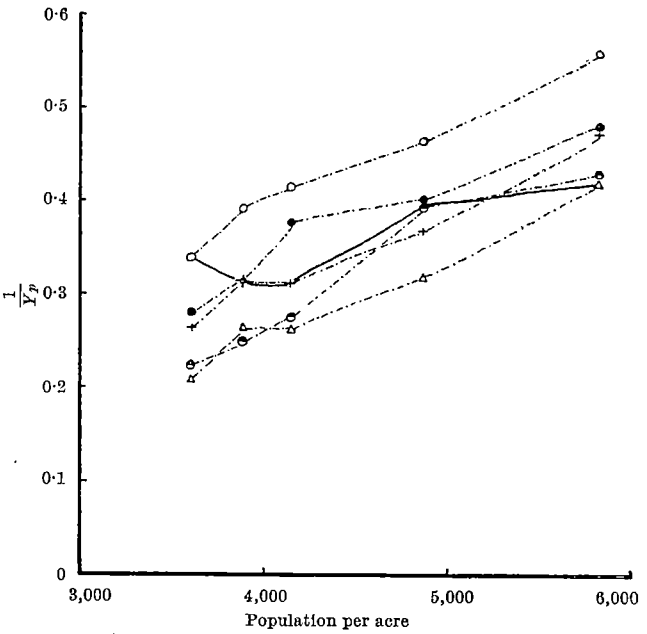


Fig. 2. $\frac{1}{Y_p}$ Response to population change for the different seed rates per acre. Seed rates per acre, same as Fig. 1

It is considered that equation (2) will be applicable in cases where the crop yield is influenced, as in the yam, by the size of the individual sett.

A more detailed account of the work carried out at Ibadan will be published elsewhere.

I thank Dr. C. A. Puddy, chief agricultural research officer, and Mr. G. E. Hodnett of the Department of Mathematics, University of Ibadan, for their assistance.

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EFFECT OF CERTAIN CONDITIONS ON YIELD OF CROP PLANTS

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Plant Density : Soil Water : Yield Relationships

THE results of recent wheat seeding trials carried out in southern New South Wales[1] have indicated that, under a relatively wide range of rainfall conditions during the growing season (11–29 in.), varying the seeding rate from 20 to 60 lb. per acre had negligible effect on grain yield.

Similar results have been obtained on chernozem-like soils of high natural fertility in the Narrabri district of New South Wales, provided the plants were not subjected to a period of water stress sufficiently severe to impair ear development prior to anthesis. However, where such water stress conditions had occurred, and where stem rust damage was negligible, grain yields were markedly reduced with plant densities above optimum. Some results of seeding-rate trials carried out in the 1961–62 seasons are presented to illustrate these effects.

Exp. 1. A 7 × 2 factorial combination of the following treatments was used: seeding rate, 5, 10, 15, 20, 30, 40 or 80 lb./acre; row spacing, 7 or 14 in.; replication, 3; plot size, 7 ft. × 70 ft; five sites were selected to give a range in the depth of wet soil at sowing (about 9–30 in.); variety, *Triticum aestivum* cv. 'Mengavi'. All sites were sown during June 19–29, 1961, and gathered during November 9–15, 1961. Rainfall during the growing season was similar at all sites and ranged from 4·4 in. to 5·3 in. No basal fertilizers were applied. At gathering, two quadrats (each 7 ft.^{2} in area) were taken from each plot using a method of sampling described by McIntyre[2].

As the row spacing treatments did not have any significant effect on grain yield, the results have been combined for each seeding rate treatment.

Exp. 2. A 2 × 5 × 4 factorial combination of the following treatments was used: variety, *Triticum aestivum* cv. 'Mengavi' or 'Spica'; seeding rate, 'Mengavi', 5, 10, 20, 40 or 80 lb./acre; 'Spica', 6, 12, 24, 48 or 96 lb./acre; sub-soil water at sowing, 4 levels; replication, 3; plot size, 7 ft. × 70 ft.; sown, July 31, 1962; gathered, December 6–10, 1962; basal fertilizer, superphosphate and ammonium sulphate, each at 100 lb./acre; rainfall during the growing season, 7 in. The seeding rates for 'Spica' were adjusted in order to obtain plant densities comparable to those for 'Mengavi'. The four levels of sub-soil water at sowing were effected by growing grain sorghum during the fallow period for 0 (bare fallow), 8, 11 or 13 weeks; the sorghum treatments are referred to as S_0, S_8, S_{11} and S_{13} respectively. For the S_0 treatment, the soil was wet to a depth of about 40 in. at sowing. In both experiments, the depth of wet soil refers to the depth where soil consistency changed from a plastic to a non-plastic state. At gathering, the plots were divided into four strata and one quadrat (7 ft.^{2}) taken at random from each stratum.

The relationships between grain yield and plant density are shown in Fig. 1. It should be noted that the 'Mengavi' in 1962 was affected by stem rust, damage (as indicated by 100 grain weights and bushel weight estimates) increasing with decreasing plant density.

The yield curves for the 'Spica' S_0 and S_8 treatments, as well as those for the 'Mengavi' S_{11} and S_{13} treatments (Exp. 2), are examples of the typical flat-topped parabolic type described by Hudson[3] and Holliday[4], in that increases in plant density from about 5 to 20 plants/ft.^{2} resulted in relatively small changes in yield. In contrast the yield curves for both 'Spica' S_{11} and S_{13} treatments, and at all sites in 1961, fell sharply with increasing plant densities above the optimum, the range of near-optimum densities being particularly restricted. The tendency for optimum plant densities to become less with reduced levels of sub-soil water is in agreement with previous findings[4].

Because of stem rust damage, the 'Mengavi' grain yields (Exp. 2) for the lower plant densities at all soil moisture

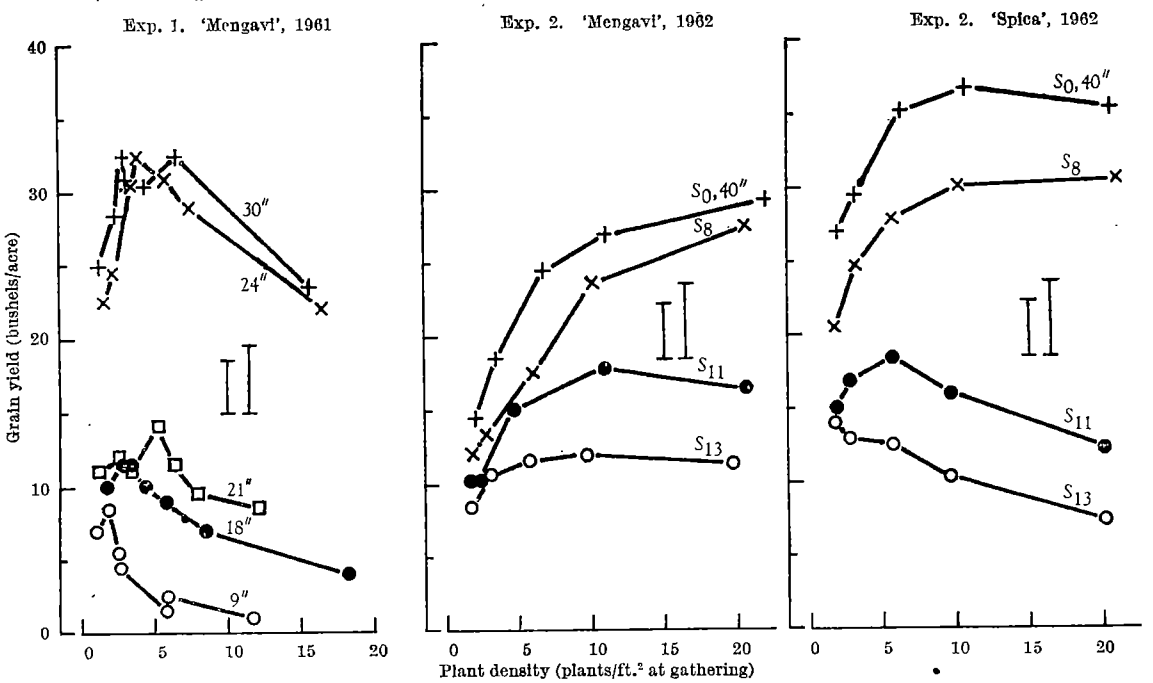


Fig. 1. Effect of plant density and sub-soil water at sowing on grain yield. The numbers refer to the depth of wet soil at sowing (in.), and the symbols S_0, S_8, S_{11} and S_{13} refer to the period (weeks) for which sorghum was grown during the fallow period prior to sowing. The short vertical lines indicate *L.S.D.* values at $P < 0{\cdot}05$ and $P < 0{\cdot}01$

Table 1. CLIMATIC DATA

1961: Narrabri West Post Office

	June	July	Aug.	Sept.	Oct.	Nov.*
Mean temp. (° F) at 1500 h	64·4	60·8	62·5	75·8	84·8	89·7
Mean min. temp. (° F)	31·9	30·6	38·8	56·1	58·8	63·7
Mean V.P.D. at 1500 h (mm mercury)†	8	8	9	14	19	22
Rainfall (in.)	0·55	1·96	2·35	0·07	2·18	0·45

1962: Site of trial

	July	Aug.	Sept.	Oct.	Nov.	Dec.*
Mean max. temp. (° F)	63·0	62·7	69·6	78·6	89·8	89·0
Mean min. temp. (° F)	35·5	35·8	39·0	47·6	56·0	57·5
Mean max. V.P.D. (mm mercury)†	9	9	11	17	26	24
Rainfall (in.)	1·00	2·37	1·04	2·88	0·74	1·83

* Values until November 15 and December 10, the last day of gathering for the respective years.

† Vapour pressure deficit; the values for 1962 are approximate, being estimated from thermohygrograph recordings.

levels were less than may have been expected and the yield–plant density relationships were modified accordingly.

The overall grain yields for each site or fallow water treatment generally decreased with decreasing levels of sub-soil water.

The pattern of climatic conditions during both growing seasons was generally similar (Table 1). In both seasons a relatively dry period, during which less than an inch of rain fell, lasted for 6–7 weeks prior to anthesis (early October 1961 and late October 1962). Air temperature and vapour pressure deficit values began to increase rapidly during these dry periods.

'Ear tipping', an effect where the development of the upper florets is impaired by a period of severe plant water stress prior to anthesis, was not evident on plants of the S_0 and S_8 treatments (Exp. 2). The incidence of ear tipping for the S_{11} and S_{13} treatments in 1962, and for all sites in 1961, while low at plant densities up to the optimum for maximum grain yield, increased markedly with further increases in density. At the driest sites in each year, most of the ears were affected at the highest plant density. The plants sown at densities below optimum were, therefore, subjected to less water stress prior to anthesis than those at the higher densities, presumably because of a greater reserve of available soil water. The incidence of ear tipping was a significant factor with regard to the reduction in grain yields where plant densities were greater than optimum.

Where stem rust damage was negligible, environmental conditions during the period of grain development were sufficiently favourable in both seasons for the formation of well-filled grain, irrespective of plant density.

It is concluded that the range of near-optimum plant densities for wheat with regard to the efficient utilization of soil water (as measured by grain yield) may under certain environmental conditions be particularly restricted. Evidence from the present trials has provided information on some aspects of these environmental conditions.

In recent years, there has been a trend among many wheat growers on fertile chernozem-like soils in north-west New South Wales to reduce seeding rates to within the range of 25–35 lb./acre. The present results suggest that where the depth of wet soil at sowing is less than 2 ft. even lower seeding rates could be advantageous.

[1] Colwell, J. D., *Austral. J. Exp. Agric. Anim. Husb.*, **3**, 114 (1963).

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Plant Arrangement : Yield Relationships

EVIDENCE so far indicates that grain yields of cereals and some other grain crops tend to be greater the closer the planting pattern is to a square grid[1,2]. Holliday concluded that, at constant seed rate, decreasing the row width less than 7–8 in. had, in most cases, given a small increase in the grain yield of cereals (5–7 per cent), whereas yields tended to decrease with row widths greater than standard.

The effect on grain yield of varying the spatial arrangement of wheat plants has been examined on fertile chernozem-like soils near Narrabri, New South Wales, in order to provide some information on the magnitude of these effects under local environmental conditions.

Exp. 1. A 4 × 5 factorial combination of the following treatments was used: seeding rate, 10, 20, 40 or 80 lb./acre; row spacing, 7, 14, 21, 28 or 35 in.; replication, 3; plot size, 7 ft. × 70 ft.; variety, *Triticum aestivum* cv. 'Mengavi'; basal fertilizer, superphosphate and ammonium sulphate each at 100 lb./acre; sown, June 11, 1962; gathered, November 20–22, 1962.

At gathering the plots were divided into four strata and one quadrat was taken at random from each stratum. It should be noted that the effective area sampled per quadrat was 7 ft.^{2} for the 7-, 14- and 21-in. row spacings but only 4·7 and 5·8 ft.^{2} for the 28- and 35-in. row spacings, respectively. The yield estimates are, therefore, less

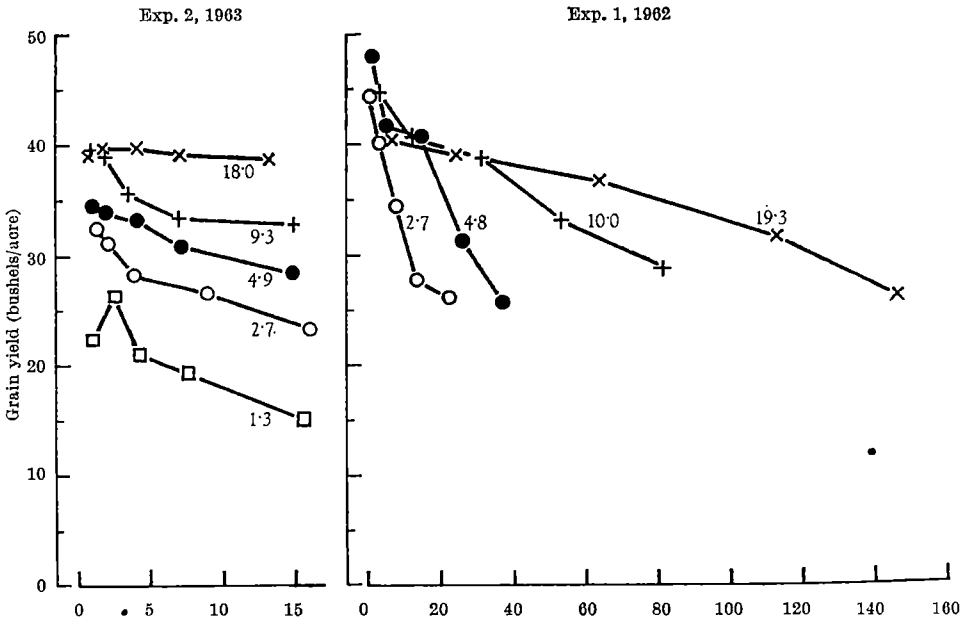


Fig. 2. Effect of plant arrangement on grain yield. The numbers refer to the mean plant density at gathering (plants per sq. ft.)

accurate for the latter spacings. Plant counts were made on each sample before threshing.

Exp. 2. Row spacings were adjusted to confine the values of r/p (ratio of the distance between the rows to the mean distance between the plants within the row at gathering) within the range of approximately 1–16 for each seeding rate treatment.

A 5 × 5 factorial combination of the following treatments was used: seeding rate, 5, 10, 20, 40 or 80 lb./acre; value of r/p (approx.), 1, 2, 4, 8 or 16. Details of row spacing in relation to seeding rate and values of r/p are given in Table 2. Replication, 4; plot size, 8 ft. × 9 ft.; variety, *Triticum aestivum* line No. 52, a line derived from the cross 'Mengavi' × 'Koda' × 'Spica' × 'Gabo'; basal fertilizer, superphosphate and ammonium sulphate each at 150 lb./acre; sown, July 25–26, 1963, into a dry seed bed; germination was delayed until late August when sufficient rain was received to wet thoroughly the seed bed; gathered during December 16–20, 1963.

Table 2

Row width (in.); Exp. 2

r/p (approx.)	Seeding rate (lb./acre) 5	10	20	40	80
1	11·0	7·5	5·5	4·0	2·5
2	15·0	11·0	7·5	5·5	4·0
4	21·5	15·0	11·0	7·5	5·5
8	30·0	21·5	15·0	11·0	7·5
16	43·0	30·0	21·5	15·0	11·0

At gathering two quadrats were taken from each plot and plant counts made on each sample before threshing. The effective area sampled per quadrat was approximately 7 ft.² for all row spacings except the 30-in. spacing (10 lb. seeding rate) for which the effective area was approximately 5 ft.². Growing conditions were relatively favourable in both seasons.

The overall results are in agreement with the conclusions of Donald and Holliday in that at constant seed rate the closer the planting pattern was to a square grid, that is, the closer values of r/p were to unity, the higher the grain yield per unit area (Fig. 2). This effect was more pronounced at the lower plant densities, and the use of row spacings greater than 7 in. also brought about substantial reductions in grain yield.

Although the 1963 results suggest that grain yields may be higher where the use of row spacings less than 7 in. give values of r/p closer to unity, the practical value of such row spacings has yet to be established.

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EVIDENCE FOR THE PARTICIPATION OF CHLOROPHYLL *b* IN THE PRIMARY REACTION OF PHOTOSYNTHESIS

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BY the method of sensitive flash photometry it has been possible to derive a reaction scheme for the electron-transport system in photosynthesis of green plants[1–15]. TPN is reduced by transfer of electrons originating from water through at least eight intermediates (Fig. 1). The electron transfer between Cyt-*f* and *Z* and between *Y* and *Q* (both endergonic in the dark, energy gaps ≈20 kcal) is made possible by excitation of Chl-a_I and Chl-a_{II} with $h\nu_I$- and $h\nu_{II}$-light. In earlier experiments[1–6] it was shown that the electron acceptor of Chl-a_{II} is an unknown substance *X* (absorption changes at 478 and 513 mμ). Later[7–13], evidence was given that *X* is very closely related to, or identical with, plastoquinone *Q* (absorption changes at 254 mμ):

(*a*) The absorption changes of *X* and *Q* have the same action spectrum (long wave-length limit at ≈700 mμ)[9,12,13].

(*b*) X/X^- and Q/Q^- have the same redox potential (≈0 V)[8].

(*c*) The absorption changes of both *X* and *Q* disappear after extraction of plastoquinone and reappear in full after recondensation of plastoquinone[8,12].

(*d*) The difference spectrum of both *X* and *Q* can be separated from that of the other intermediates (Chl-a_I and Cyt-*f*) by addition of DPIP and ferricyanide in excess. Ferricyanide oxidizes via DPIP Chl-a_I chemically and traps via DPIP the electrons from *Q*. In this way the further flow of electrons from *Q* to TPN is prevented[12].

(*e*) The magnitude as well as the time-course of the absorption changes of *Q* (at 254 mμ) and *X* (at 515 mμ) correspond to each other under different conditions. This is shown to be true by exciting photosynthesis with light flashes longer than 10^{-2} sec (ref. 12).

These similarities lead to the assumption that the reaction of *Q* (absorption changes at 254 mμ) influences physically the absorption of the surrounding pigments *X* which results in additional absorption changes at 478 and 513 mμ.

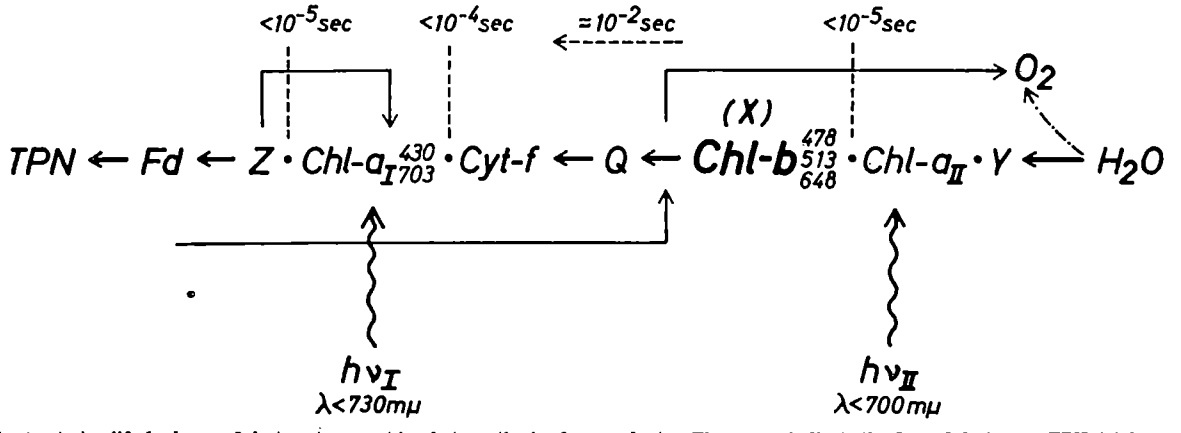


Fig. 1. A simplified scheme of electron transport in photosynthesis of green plants. The arrows indicate the flow of electrons. TPN, triphosphopyridinenucleotide; *Fd*, ferredoxin; Cyt-*f*, cytochrome-*f*; *Q*, plastoquinone; Chl-a_I, chlorophyll *a* absorbing at 430 and 703 mμ; Chl-a_{II}, special chlorophyll *a* (ref. 9); Chl-*b*, chlorophyll *b* absorbing at 478 and 648 mμ, which on illumination absorbs at 513 mμ (formerly this Chl-*b* was symbolized by *X*. Whether Chl-*b* is a true intermediate in the electron-transport or an indicator for an unknown substance in this position is an open question). *Z* and *Y* are unknown substances. The 'feed-back' of electrons from *Z* to Chl-a_I may include Cyt-*f*

Recent measurements (unpublished), however, show that, under special conditions, for example by exciting photosynthesis with flashes shorter than 10^{-2} sec, one can distinguish between the kinetic behaviour of the absorption changes of X and Q. These experiments lead to the assumption that X indicates an intermediate in the electron-transport system between Q and Chl-a_{II}.

In this work evidence is given that X is obviously identical with a chlorophyll b (designated Chl-b-478-513-648).

The difference spectrum of X (and Q) can be separated chemically by addition of DPIP and ferricyanide in excess[12]. In this case changes of absorption of X could be observed about 478 and 513 mμ; but no changes due to X could be observed in the region between 600 and 800 mμ. Changes of X in this region may be compensated by changes which occur by the redox reaction of DPIP. Therefore, an attempt was made to separate the difference spectrum of X physically. This was done in whole *Chlorella* cells by different intensities and qualities of background light.

Fig. 2 (top) shows the flashing light-induced difference spectrum under conditions of weak detecting light (incident intensity $\leqslant 380$ ergs/cm² sec) and background light $\lambda = 728$ mμ (incident intensity 7,100 ergs/cm² sec). The far-red background light stimulates only light reaction $h\nu_I$. The observed changes at 478 and 513 mμ are caused by X and those at 703 mμ by Chl-a_I. Fig. 2 (bottom) shows the result of the same experiment with background light $\lambda = 650$ mμ (incident intensity 2,200 ergs/cm² sec). This light stimulates preferably the light reaction $h\nu_{II}$. In this case the absorption changes of Chl-a_I about 703 mμ are strongly reduced. The same result can be obtained without any background light. So under conditions where light reaction $h\nu_I$ is not continuously stimulated a difference spectrum can be separated which consists of the two bands of X at 478 and 513 mμ and, in addition, a band at 648 mμ. The disappearance of the absorption changes of Chl-a_I at 703 mμ with decreasing far-red background light is demonstrated in more detail in Fig. 3 (top). (Fig. 3 (bottom) shows the influence of far-red background light on the absorption changes of 515 mμ; this will be discussed later.)

First, an explanation is given for the disappearance of the difference spectrum of Chl-a_I by the assumption of a Z-Chl-a_I-Cyt-f-complex and, secondly, it will be shown that the remaining difference spectrum characterized by

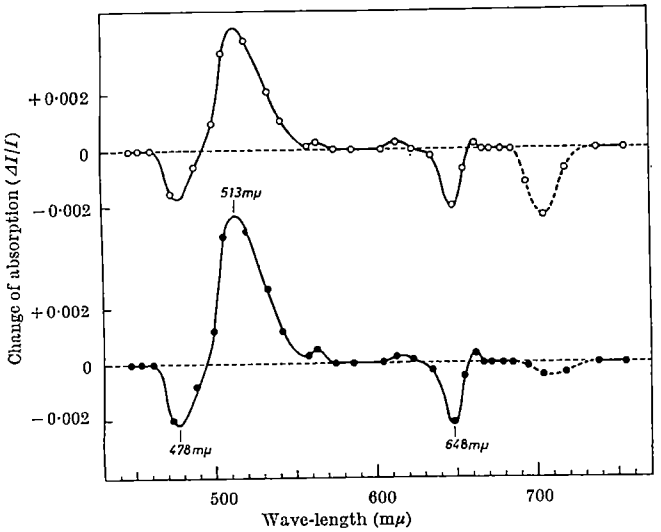


Fig. 2. Difference spectra in *Chlorella* cells. The changes were induced by flashing light: duration 3×10^{-5} sec, wave-length 620–720 mμ (range $\lambda < 575$ mμ) and 390–490 mμ (range $\lambda > 575$ mμ), saturating intensity. Detecting light: intensity $\leqslant 380$ ergs/cm² sec, half band-width ≈ 10 mμ (two Schott interference filters). Background light: wave-length 728 mμ, intensity 7,100 ergs/cm² sec (top); wave-length 650 mμ, intensity 2,200 ergs/cm² sec (bottom); half band-width ≈ 20 mμ (Schott interference filter). Temperature 20° C, pH value 9 (0·04 M/l. carbonate), chlorophyll content $1{\cdot}7 \times 10^{-5}$ M/l., optical path 15 mm

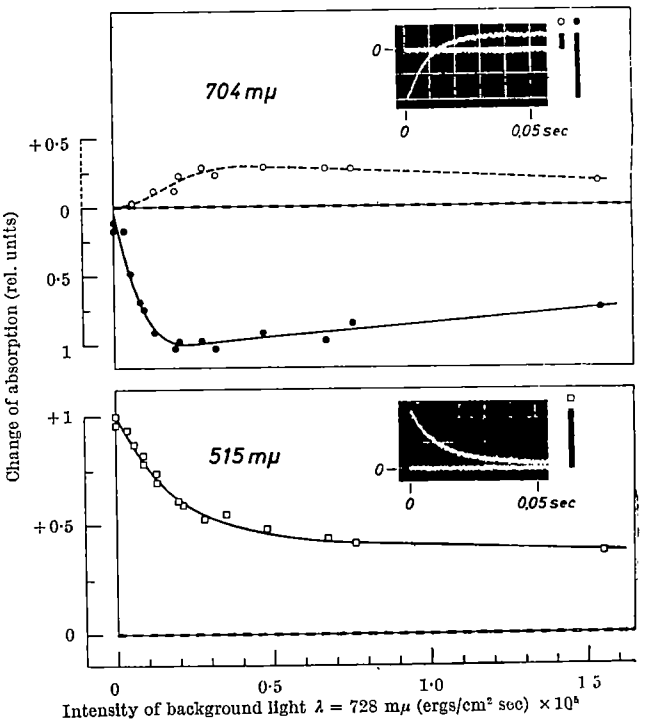


Fig. 3. Changes of absorption in *Chlorella* cells at 704 mμ (and 515 mμ as a function of the intensity of background light $\lambda = 728$ mμ. The changes were induced with flashing light: duration 3×10^{-5} sec, wave-length 620–720 mμ (515 mμ) and 390–490 mμ (704 mμ), saturating intensity. Detecting light: intensity 170 ergs/cm² sec (515 mμ) and 270 ergs/cm² sec (704 mμ), half band-width ≈ 10 mμ (two Schott interference filters). Background light: half band-width 24 mμ (Schott interference filter). Temperature 20° C, pH value 9 (0·04 M/l. carbonate), chlorophyll content $\approx 2 \times 10^{-5}$ M/l., optical path 15 mm. Top right, time-course of the changes

the absorption changes at 478, 513 and 648 mμ is caused by one and the same substance, from which it can be concluded that the substance is chlorophyll b.

Z-Chl-a_I-Cyt-f-complex. When light reaction $h\nu_I$ is continuously stimulated by far-red background light of medium intensity ($\approx 7{,}000$ ergs/cm² sec at $\lambda = 728$ mμ) one has to assume that Cyt-f is kept in the oxidized form, but not Chl-a_I. The following flash oxidizes Chl-a_I. Afterwards Chl-a_I^+ can be reduced only as fast as Cyt$^+$ is reduced, this is within $\approx 10^{-2}$ sec (Fig. 1). Therefore, absorption changes of Chl-a_I at 703 mμ with a life-time of $\approx 10^{-2}$ sec should occur. This has been observed. When light reaction $h\nu_I$ is not stimulated continuously (no far-red background light) Cyt-f remains in the reduced form. The following flash oxidizes Chl-a_I, and Chl-a_I^+ is immediately reduced by Cyt-f. When the electron transfer from Cyt-f to Chl-a_I^+ is a very fast one ($< 10^{-4}$ sec) the corresponding absorption changes at 703 mμ should not be detectable. Since this is the case, we have to assume a very fast electron transfer between Chl-a_I^+ and Cyt-f which is possible only when a complex exists between Z, Chl-a_I and Cyt-f. This is in accordance with the fact that at low temperatures (−150° C) an irreversible oxidation of Chl-a_I as well as of Cyt-f can be observed[3]. This is also only explainable by the assumption of a fast electron transfer within a Z-Chl-a_I-Cyt-f-complex. (These results imply a correction of the formerly derived reaction time of $\approx 10^{-3}$ sec for the reaction between Chl-a_I^+ and Cyt-f.)

In all our earlier experiments absorption changes at 703 mμ were observed also without additional far-red background light. In all these experiments the detecting light at 703 mμ has already acted as far-red background light. Therefore, for the experiments reported here detecting light of low intensity was used.

When light reaction $h\nu_I$ is stimulated by far-red background light of strong intensity ($> 10{,}000$ ergs/cm² sec at 728 mμ) Cyt-f and Chl-a_I should be kept in the oxidized form. Then the following flash can only act on light

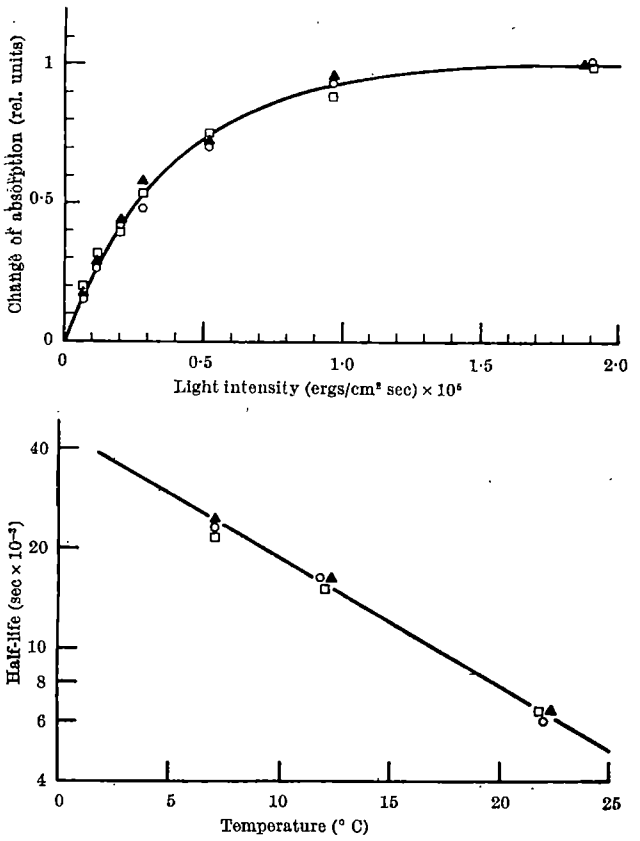


Fig. 4. Top, Absorption changes in *Chlorella* cells at 473 (○), 513 (□) and 648 (▲) mμ as a function of actinic light intensity. Actinic light: duration 1 sec, wave-length 686 mμ, half band-width 21 mμ (Schott interference filter). Detecting light: intensity 350 ergs/cm² sec, half band-width ≈10 mμ (two Schott interference filters). Temperature 20° C, *p*H value 9 (0·08 M/l. carbonate), chlorophyll content $\approx 2 \times 10^{-5}$ M/l., optical path 15 mm. Bottom, half-life of the absorption changes in *Chlorella* cells at 473, 513 and 648 mμ as a function of temperature. Qualities of actinic light (see Fig. 2). Intensity of detecting light: 500 ergs/cm² sec. Pre-illumination (10 sec) with red light ($\approx 10^6$ ergs/cm² sec). Temperature 20° C, *p*H value 9 (0·08 M/l. carbonate), chlorophyll content $\approx 2 \cdot 10^{-5}$ M/l., optical path 15 mm. (From the measurements it follows an energy of activation of ≈15 kcal/mol.)

reaction $h\nu_{II}$ which provides electrons from water for the reduction of Cyt-f^+ and Chl-$a_I{}^+$. Under these conditions positive absorption changes of Chl-a_I at 430 and 703 mμ should be observed. This is indeed the fact and has already been described in detail[4,10,12]. (With respect to *Chlorella* cells the enrichment of Chl-$a_I{}^+$ by far-red background light depends on the condition of the cells. Positive absorption changes up to 50 per cent of the full absorption changes at 703 mμ have been observed.)

Identification of chlorophyll b. The absorption changes of the separated difference spectrum (Fig. 2, bottom) were measured as a function of the intensity of the actinic light (Fig. 4, top). The half-life of these absorption changes was measured as a function of the temperature (Fig. 4, bottom). In all these cases (and in others not published here) the behaviour of the absorption changes at 478, 513 and 648 mμ is identical. From this it follows that the difference spectrum characterized by these absorption changes is caused by one and the same substance. The decreases of absorption at 478 and 648 mμ occur just within the two absorption bands of chlorophyll *b in vivo*. This indicates that very probably a chlorophyll *b* is in action which is transformed in light in a product with a band at 513 mμ. (Therefore, we replace the symbol *X* by Chl-*b*-478-513-648.) This conclusion is in agreement with the fact that the absorption changes take place only in green plants, which are the only ones which contain chlorophyll *b*. In earlier experiments it was shown that the extinction coefficient of *X* at 475 mμ is $2 \cdot 10^4$ 1/M cm[18]. This is also in agreement with the assumption that *X* is a chlorophyll *b*.

All properties of *X*[10,18] which were derived from the behaviour of the absorption changes at 478 and 513 mμ are now guilty for Chl-*b*-478-513-648: reaction in light within $< 10^{-5}$ sec, relaxation within $\approx 10^{-2}$ sec, long wave-length limit of the action spectrum at ≈700 mμ, concentration ≈1/1,000 of the bulk of chlorophyll, redox potential ≈0 V, *p*H region 5–8, temperature of inactivation 45° C (5 min), high sensitivity to ageing, high sensitivity to the poison chlorophenyl-dimethylurea (CMU).

Feed-back. The limit of the action spectrum at ≈700 mμ is observed only when light reaction $h\nu_I$ is separated chemically from light reaction $h\nu_{II}$ (refs. 9 and 12). In the whole system the limit of the action spectrum is extended to ≈730 mμ (refs. 9, 16 and 17). But on the whole system only half the full amplitude of the absorption changes of Chl-*b* at 515 mμ can be induced by far-red illumination. This is demonstrated in Fig. 3 (bottom). Both effects can be explained when one assumes a feed-back of electrons from light reaction $h\nu_I$ to Chl-*b* as indicated in Fig. 1.

I thank Prof. H. T. Witt for his advice.

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MORPHOLOGY OF SYNTHETIC FIBRES; A STUDY ON DRAWN POLYETHYLENE

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IDEAS on the nature of a crystalline polymer have undergone profound changes during recent years. Whereas previously it was believed that long-chain molecules by their very nature form bundle-like crystals intermeshed with an amorphous matrix, more recent research has revealed that against all expectations the crystalline units are distinctly lamellar and built of folded chains (cf. refs. 1 and 2). This was first established in the case of solution-grown crystals; the relevance of this finding to melt crystallized material has been disputed for a long time in spite of steadily accumulating experimental material in its favour obtained on surfaces—free and

fracture[1]. Obviously, while the nature of the standard melt crystallized materials was still in dispute, the new developments had little impact on the existing concepts on drawn fibres. An important turning point has been, therefore, the recent discovery by Palmer and Cobbold[2] that bulk polyethylene could be dispersed by digestion with nitric acid. This demonstrated incontestably the lamellar nature of the melt crystallized material[3]. Further, by this technique many close similarities to chain-folded solution-grown crystals have been revealed[4]. In addition, layer-shaped disintegration products were also demonstrated in the drawn form of polyethylene[5] which opens up a new line of enquiry concerning the nature of fibres. In this connexion we have observed that on treatment with nitric acid drawn polyethylene breaks up in a characteristic fashion.

Strips of square cross-section were cut from sheets 6 mm thick of low- and high-pressure polyethylenes, 'Alkathene' and 'Rigidex' respectively. Such strips extended 4·5 times when drawn at room temperature. The X-ray patterns showed the normal highly oriented fibre diagrams. On exposure to 70°–85° C—the temperature of the subsequent nitric acid treatment—these fibre patterns remained unaffected except for a slight disorientation in 'Alkathene' at 85° C which nevertheless is of no consequence for the conclusions to be drawn.

The main features of the nitric acid treatment were as follows. At 70° C in both 'Alkathene' and 'Rigidex' deep transverse cracks were produced, the effect being more

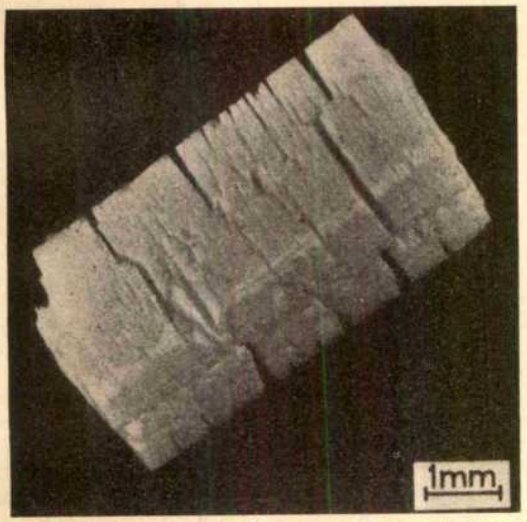


Fig. 1. Linear polyethylene ('Rigidex') drawn 5 times, after 72 h nitric acid treatment at 70° C

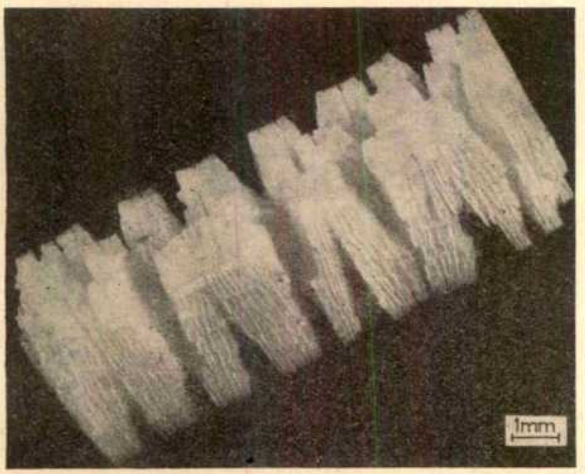


Fig. 2. Branched polyethylene ('Alkathene') drawn 4·5 times, after 12 h nitric acid treatment at 70° C

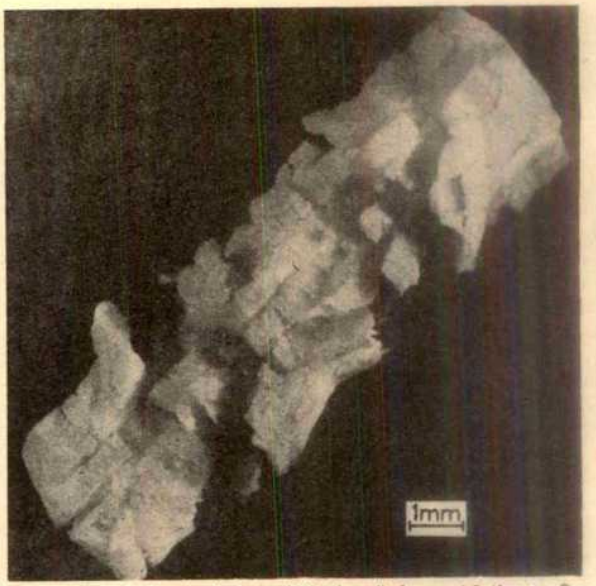


Fig. 3. Branched polyethylene ('Alkathene') drawn 4·5 times, after 3·5 h nitric acid treatment at 85° C

conspicuous in 'Alkathene' (Figs. 1 and 2). These cracks develop in 'Alkathene', for example, after some 8 h, and become very pronounced after 12 h. Further treatment up to 100 h does not appear to sever the specimens completely. On the contrary, shrinkage of the specimen occurs, closing up the cracks. The effects on 'Rigidex' are similar but less pronounced.

At 85° C longitudinal cleavage occurred on 'Alkathene' after about 1·5 h (Fig. 3). Some perpendicular cracking also occurred, and this developed on longer treatment. The plane of the longitudinal cracks was preferentially parallel to that of the plane of the original sheet. Initial cracks of this type were already apparent in the 70° C samples (Figs. 1 and 2) even in 'Rigidex', where in contrast to 'Alkathene' they did not develop further at 85° C. Neither X-ray nor birefringence revealed any orientation with respect to the surface of the sheet (before or after drawing). Accordingly some lamination must have occurred on extrusion which escapes the usual structural tests, yet it gives rise to planes of weakness which are revealed by the nitric acid digestion.

As seen, the nitric acid treatment gives rise to spontaneous internal crack surfaces in a rather striking manner. The study of such surfaces raises the possibility of exploring the fibre interior without externally introduced fracture, a technique already utilized by Palmer and Cobbold[2] in connexion with unoriented material. In the case of fibres in particular this should provide an alternative to the examination of the dispersed material with the important added advantage that it can give information not only on the basic building units but also on the manner in which they are arranged in the fibre interior.

For this purpose the transverse surfaces were exposed by snapping pieces as in Figs. 1 and 2—they broke at the lightest touch—and these were replicated for electron microscopy by means of a one-stage carbon replica process. Figs. 4 and 5 show results on 'Rigidex' and 'Alkathene' respectively. Both reveal lamellæ as seen flat on, the structure in 'Rigidex' being the more regular. Replication of longitudinal cleavage surfaces was not successful owing to their extreme roughness. Meaningful results, however, were obtained by first cutting the samples in a plane parallel to the draw direction and then taking a two-stage replica of this surface after a short exposure (2–4 h) to nitric acid. These revealed pronounced striations transverse to the direction of draw (Fig. 6). These we interpret as layers seen edge-on with very deep grooves etched in between them. In fact, Fig. 5 can be regarded as incipient stages of what is seen in Figs. 1 and 2 but on a much

smaller scale. It is not possible to make an accurate assessment of the lamellar thickness but as seen it is between 100 and 500 Å. Similar structures in 'Alkathene' were indicated. In one case where the periodicity was particularly clear it was in the 100 Å range. This is all in broad agreement with the periodicity revealed by low-angle X-rays which is ~200 Å for 'Rigidex' and ~100 Å for 'Alkathene' before treatment. After treatment, low-angle X-ray information was much more difficult to obtain owing to intense diffuse scatter; but where this was achieved, no spacing change of consequence for the present was observed (in fact there was a decrease of about 25 per cent on prolonged treatment, to be published elsewhere). However, whenever the reflexions were originally split (four point diagram), corresponding to spacings oblique to the fibre axis, they became meridional on the nitric acid treatment in line with the finding that the lamellæ were seen perpendicular to the draw direction.

Accordingly, drawn polyethylene should consist of lamellæ stacked one on another. As the lamellæ are perpendicular to the draw direction the molecules must be perpendicular to the lamellæ. While some changes might have occurred during the nitric acid treatment—see previous remark on the low-angle X-ray patterns—this is unlikely to alter the basic conclusion on the morphology. The action of the nitric acid is not clear in all details. It is known, nevertheless, that it removes some disordered material, that it severs links between lamellæ and that it may cut folds[3].

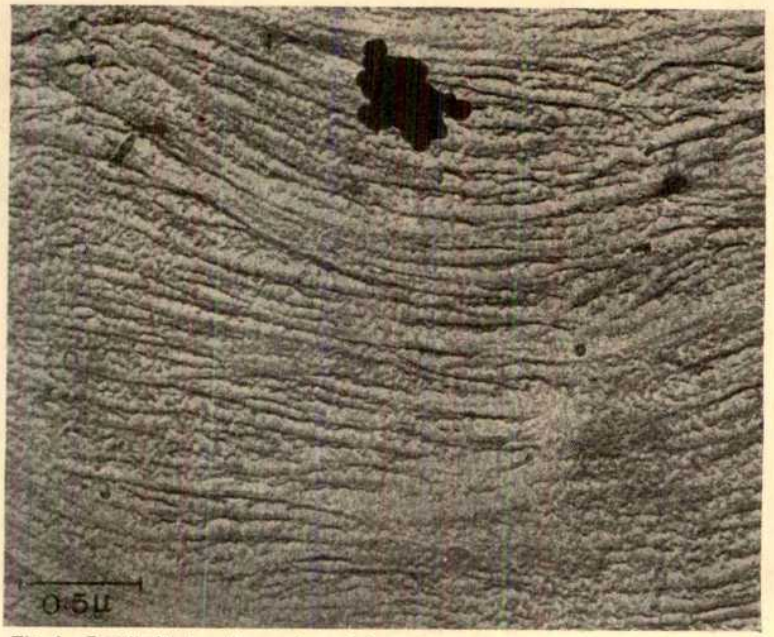


Fig. 6. Longitudinal section surface of drawn linear polyethylene ('Rigidex') etched with nitric acid at 70° C for 4 h. Draw direction vertical. Electron micrograph; replica

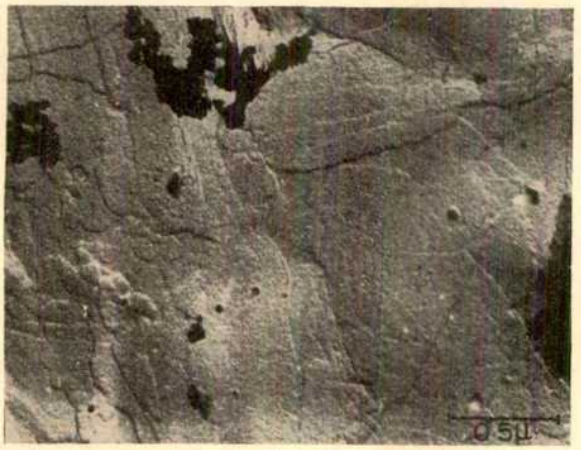


Fig. 4. Transverse cleavage surface of drawn and nitric acid treated (70° C, 100 h) linear polyethylene ('Rigidex'). Electron micrograph; replica



Fig. 5. Transverse cleavage surface of drawn and nitric acid treated (70° C, 44 h) branched polyethylene ('Alkathene'). Electron micrograph; replica

From the earliest, solution-grown crystals were sedimented in forms of oriented mats for X-ray examination[5,6]. It was remarked there that so far as the diffraction patterns were concerned—both wide and low angles—such mats could have been transverse sections of a fibre. Now this analogy can be taken further; a fibre is in fact seen to consist of a stack of lamellæ, as is a mat of sedimented solution-grown crystals contrary to all accepted concepts on fibre structure[7]. In addition, in a different line of work[8] the crystalline units (which by the work recorded here should be lamellar) were found to slide past each other revealing an unexpected individuality in contrast to the intimate molecular connectedness between consecutive crystallites usually envisaged.

Based on these findings we suggest that the molecules in the lamellæ are to a large extent folded. Some molecular ties naturally must exist to provide cohesion. The possibility of folded chains within a drawn fibre has already been raised[2,9] and theoretical evaluation of X-ray patterns on the basis of a mixture of folded molecules and non-folded ones passing through crystals has already been presented[10]. The distinctness of the lamellar morphology and the close similarity to single crystals revealed now are surprising even so. We do not claim universal validity for the chain folded lamellar concept for fibres in general, certainly not for extreme elongations. Nevertheless, it is noteworthy that this has now been found in materials which in normal technical usage would be considered as drawn fibres.

We thank R. P. Palmer and A. J. Cobbold, Imperial Chemical Industries, Ltd., for advanced communication of their technique and results and G. A. Bassett for his advice on electron microscopy.

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LETTERS TO THE EDITOR

RADIO ASTRONOMY

Microwave Observations of the Galactic Centre Region

THIS communication describes a new set of observations of the region surrounding the radio source Sagittarius *A*, at present assumed to be the galactic centre. The observations were made at a frequency of 5 Gc/s, and the results are summarized in the form of a contour map showing the various components of radiation in this region. The position of the Sagittarius *A* source appears to differ slightly from the zero of the $^{III}b^{II}$ co-ordinate system. The spectral index of the source, derived by comparing the flux density at 5 Gc/s with other measurements in the microwave band, indicates that it is non-thermal.

The observations recorded here were made with a parametric receiver connected to a paraboloid antenna, 85 ft. in diameter, giving a circular beam-width of 10·8 arc min to half power. The receiver was a modified Dicke type, switched electronically between the main receiving antenna and a comparison antenna directed at the zenith. The parametric amplifier, operating in the degenerate mode, was followed by a zero intermediate frequency system of band-width 100 Mc/s. An output time constant of 1 sec set the minimum detectable temperature for any individual scan at 0·25° K. Forty-five drift scans, spaced 3·6 arc min apart, were taken in an area bounded by galactic longitudes −358·5° to +1·5° and galactic latitudes −1° to +1°. The peak antenna temperature for Sagittarius *A* was approximately 20° K. The survey was made at night, during the summers of 1963 and 1964, at times when the galactic centre region was at meridian transit ±2 h. At transit, the region was at an elevation of 30° above the horizon.

Table 1. CO-ORDINATES OF SAGITTARIUS *A* (1950·0)

Frequency (Gc/s)	Antenna beam (arc min)	Right ascension	Declination
5 (ref. 1)	10·8′	17h42m27s ±2s	−28°58·0′ ±0·5′
3 (ref. 2)	6·7	17 42 30 ±2	−28 58·7 ±0·5
14·5 (ref. 3)	5·9	17 42 26 ±6	−28 58·2 ±1·0
Galactic centre as defined by $^{III}b^{II}$ system		17 42 26 ·5	−28 55·2

The precise position of Sagittarius *A*, the bright central component, was determined by comparison with position measurements at meridian transit of 10 other radio sources, spanning a declination range −45° to +58°. This position is shown on the first line of Table 1. The second and third lines of Table 1 show other position measurements made with pencil beams at 3 Gc/s and 14·5 Gc/s. All the observations agree within the quoted error limits. The fourth line of the table gives the equatorial co-ordinates for the zero in longitude and latitude of the $^{III}b^{II}$

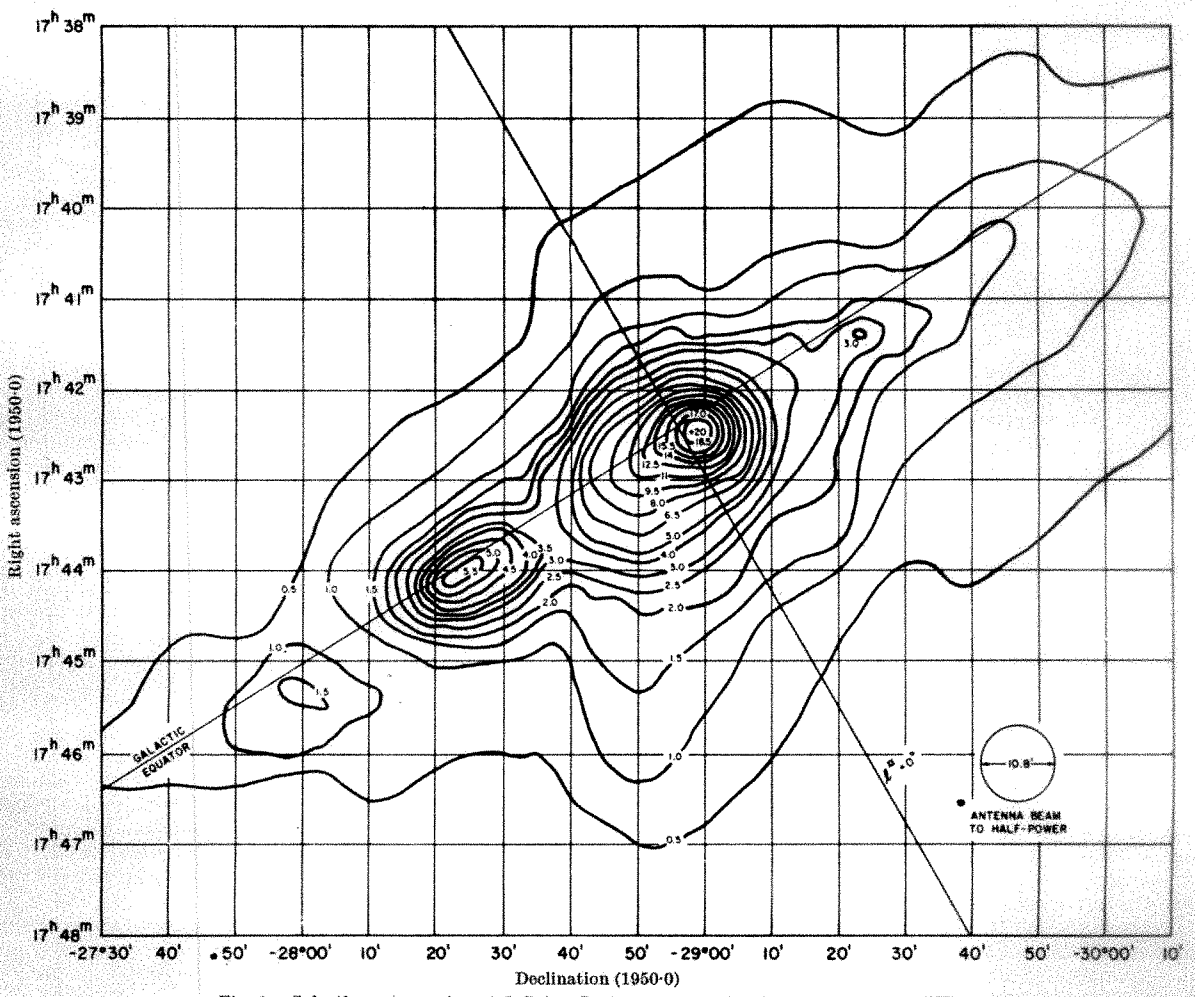


Fig. 1. Galactic centre region at 5 Gc/s. Contours represent antenna temperature (°K)

co-ordinate system. The existing data indicate that in right ascension the position of the central radio component agrees with the zero of the $l^{II}b^{II}$ system, but that in declination it differs by approximately 3 arc min.

The temperature contours determined from the present set of observations are given in Fig. 1. The contour map shows the main central source and the two well-known sources of somewhat lesser intensity, situated on the galactic plane on each side of the galactic centre and separated from it by about 0·5°. The small spur source adjacent to the galactic centre, first distinguished by Drake[4], is not resolved with the present 10·8 arc min beam-width, but appears as an asymmetric extension along the galactic plane of the contours surrounding the centre. These sources lie on a line parallel to, but 2 arc min south of, the galactic equator in the $l^{II}b^{II}$ system. A source has also been detected at right ascension 17h 45m 20s and declination −28° 00′.

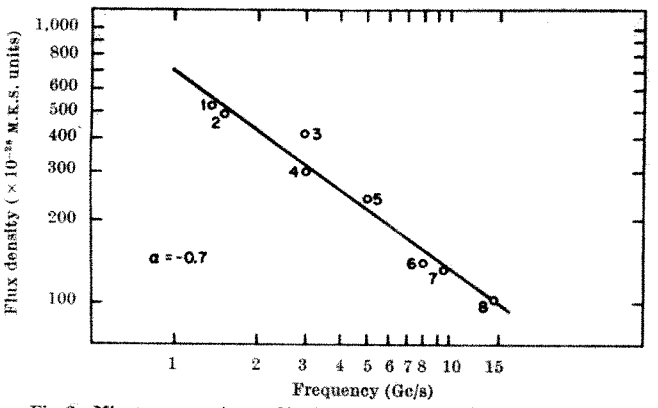


Fig. 2. Microwave spectrum of Sagittarius *A*. The flux values have been corrected to an assumed source diameter of 3·5 arc min. Corrections have also been made for the background ridge component. (1) Davies and Jennison[7]; (2) Kerr[8]; (3) Sloanaker and Nichols[9]; (4) Cooper and Price[2]; (5) Maxwell and Downes[1]; (6) Williams[10]; (7) Haddock and McCullough[11]; (8) Hollinger[3]

The flux from the source Sagittarius *A* at 5 Gc/s is estimated as 240 × 10^{-26} W m^{-2} $(c/s)^{-1}$, on the assumption that the flux density of *M* 87 at the same frequency is 70 × 10^{-26} units[5]. The diameter of the source was assumed to be 3·5 arc min[2,6], and a 10 per cent correction was made for the ridge of background radiation near the galactic centre. Under these assumptions, the estimated uncertainty in the flux value is about 10 per cent. The flux value at 5 Gc/s has been compared with measurements of flux densities made at other frequencies in the microwave band, as shown in Fig. 2. Results plotted in the figure are confined to measurements made in the frequency range 1·36–14·5 Gc/s with paraboloid antennæ of beam-widths within the limits 6–18 arc min. Measurements made with antennæ of greater beam-widths or with fan beams have been rejected, since, in comparing flux measurements to obtain a spectrum for a complex source, it is advisable to compare data taken with antennæ the beam-widths of which are reasonably similar. All the results on the graph have been corrected to a source diameter tentatively assumed to be 3·5 arc min[2,6]. Where necessary, further corrections to the published flux values have been made for radiation from the background ridge; for most frequencies in the microwave band, this correction does not exceed 10 per cent. Corrections have not been made for antenna skirt responses to the small spur source 10 arc min from Sagittarius *A*; for most cases this effect is estimated as being only a few per cent. The solid line in Fig. 2 is a linear least square solution fitted to the plotted data points. Its slope gives the microwave spectral index for the Sagittarius *A* source and this index is −0·72 ± 0·05. Such a spectral index does not uphold previous suggestions that the source is thermal[2,4,6,12], but strongly indicates that it is non-thermal in nature.

This work was supported by the U.S. National Science Foundation. We thank Dr. D. R. W. Williams and Dr. F. J. Kerr for permission to refer to unpublished data.

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ASTROPHYSICS

Lunar Luminescence

Following the report of the characteristic red luminescence of enstatite achondrite meteorites under proton excitation[1], Kopal and Rackham photographed the Moon through interference filters with passbands near the maximum and minimum of the enstatite emission profile, in a search for regions which might show this characteristic emission. They reported finding such a region around the crater Kepler[2], and concluded that this crater might therefore have been produced by a large enstatite achondrite meteorite. However, a difficulty is that after an impact the material spread around would probably be largely lunar surface material, with only a small proportion—perhaps one part in a thousand[3]—of material from the actual meteorite. This view is fairly widely held, but does not seem to have been mentioned specifically in the correspondence which has followed these observations.

An alternative explanation is that the lunar surface may in parts consist of minerals which are capable of luminescence, but it also seems likely that they would have become so damaged by prolonged exposure to radiation that no appreciable luminescence would be seen. A coating of mainly non-luminescent dust of micrometeoritic origin would have the same effect. However, any disturbance to the surface, for example by the arrival of a large enough meteorite of any type, or even by volcanic activity, would distribute and expose undamaged material from just below the original surface. If the disturbance occurred in a region consisting of luminescent material, luminescence might then be observed at times of sufficiently intense excitation. Cameron[4] has suggested that such times might be around full Moon, because of an anti-solar 'tail' of charged particles trapped by the Earth's magnetic field. The luminescence would continue to be possible for a time depending on the rate of damage and of dust arrival; this time might easily be of the order of 10^4–10^5 years. Regions observed to luminesce could thus be regarded as the sites of geologically recent disturbances. This explanation has the advantage that it does not require a large specimen of a rather rare type of meteorite to produce large luminescent patches of the type seen by Kopal and Rackham—any large enough meteorite, or volcanic disturbance, will do. It is also possible that the very small patches of red luminescence observed by Greenacre and Barr[5] are of the same type. A disturbance in the terraced rim of Aristarchus would tend to throw material preferentially along the terrace and produce the shape of one of the patches they observed.

It is interesting that Urey[6] and, more recently, Cameron[7] have suggested that enstatite is probably a constituent of the lunar surface.

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GEOPHYSICS

Mass of the Canyon Diablo Meteoroid

Nordyke[1] has derived empirical scaling laws for the apparent radius R and the apparent depth D of craters produced by both chemical and nuclear explosions in desert alluvium at the Nevada Test Site. The scaling equations are $R = R_s W^{1/3.4}$ and $D = D_s W^{1/3.4}$, in which R_s and D_s are the appropriate dimensions for a 1 kt (kiloton) explosion of TNT and W is the yield in kt of the explosion in question.

If the dimensions of the Canyon Diablo meteorite crater scale in accordance with the same empirically derived equations as the dimensions of the explosion craters examined by Nordyke, then his equations can be used for calculating the energy required for production of this meteorite crater. The apparent crater for the Canyon Diablo meteorite is generally quoted as having a 4,000-ft. diameter and a 570-ft. depth[2]. The ratio of apparent radius to apparent depth is then 3·5. A value of 3·5 for the ratio of apparent radius to apparent depth occurs in Nordyke's plots close to the surface (at a scaled depth of not more than 20 ft./kt$^{1/3.4}$) and again at a scaled depth of about 200 ft./kt$^{1/3.4}$.

If the energy used in producing the Canyon Diablo crater was expended near the surface of the Earth, Nordyke's scaled apparent crater depth indicates that $W^{1/3.4} = 570/30$ kt, such that the energy necessary to produce the crater is $W = 2{\cdot}2 \times 10^4$ kt. A comparable calculation, using the apparent crater radius, gives $W^{1/3.4} = 2{,}000/110$ kt, such that $W = 1{\cdot}9 \times 10^4$ kt. Thus, if the energy which produced the Canyon Diablo meteorite crater was expended at a scaled depth less than 20 ft./kt$^{1/3.4}$, it amounted to a yield equivalent to about 20 megatons of TNT. At a yield of 20 megatons, a scaled depth of 20 ft./kt$^{1/3.4}$ is a depth of 400 ft., so, when we say that the equivalent point of detonation is at a scaled depth less than 20 ft./kt$^{1/3.4}$, we are saying that the explosion occurred somewhere between the surface and a depth of 400 ft.

At a scaled depth of 200 ft./kt$^{1/3.4}$ the equivalent yield of TNT calculated from the apparent crater dimensions is about $1{\cdot}1 \times 10^4$ kt. For a yield of 11 megatons of TNT a scaled depth of 200 ft./kt$^{1/3.4}$ is more than 3,000 ft.—a value which appears to be in excess of the depth to which the meteorite penetrated, hence will not be considered further in this communication.

Based on this information we can calculate the mass of the meteoroid that would have had to hit the Earth to release an amount of energy comparable to 20 megatons of TNT. An energy of 1 kt is[3] 10^{12} calories, or $4{\cdot}2 \times 10^{19}$ ergs, so 20 megatons is comparable to $8{\cdot}4 \times 10^{25}$ ergs. If we assume that the energy W comes solely from the kinetic energy gained from gravitational attraction, $W = 8{\cdot}4 \times 10^{25} = G\frac{mM}{r} = (6{\cdot}26 \times 10^{11})\ m$ ergs, in which $G = 6{\cdot}67 \times 10^{-8}$, the mass M of the Earth is $5{\cdot}98 \times 10^{27}$ g and the Earth's radius r is $6{\cdot}38 \times 10^8$ cm. The mass of the meteoroid is then $m = (8{\cdot}4 \times 10^{25})/(6{\cdot}26 \times 10^{11}) = 1{\cdot}34 \times 10^{12}$ g. The magnitude of this mass could have been less if the meteoroid had a measurable velocity relative to the Earth prior to its entry into the gravitational field of the Earth. Since retrograde motion of objects in solar orbit are essentially unknown, a maximum velocity of 30 km/sec for the meteoroid relative to the Earth appears to be a reasonable assumption. With a kinetic energy produced by a relative velocity of 30 km/sec added to the gravitational energy, a meteoroid mass of $1{\cdot}6 \times 10^{11}$ g is needed to provide an energy of $8{\cdot}4 \times 10^{25}$ ergs at the time of contact with the surface of the Earth.

The mass found by this empirical method is intermediate between those theoretically predicted by Öpik[4] and by Bjork[5]. In general, the empirically determined mass appears to be in better agreement with Öpik's predictions. The higher mass value of $1{\cdot}34 \times 10^{12}$ g overlaps Öpik's predictions whereas the lower limit of $1{\cdot}6 \times 10^{11}$ g corresponds closely to the upper limit of Bjork's predictions.

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MINERALOGY

Pyrochlore Minerals as a Potential Source of Reactor-grade Niobium

For many years the chief source of niobium has been the mineral 'columbite', a member of the columbite–tantalite series (Fe, Mn) $(Nb\ Ta)_2O_6$. As the name of the series implies, niobium (columbium) and tantalum occur in varying ratios, but even at the columbite end the proportion of tantalum is still appreciable, the ratio of niobium to tantalum being generally of the order of only 6 : 1.

An important application of niobium is in nuclear power production, where what is known as 'nuclear-grade' niobium must contain less than 0·15 per cent by weight of tantalum (0·05 per cent in the United States). This requirement is based partly on physical properties and partly on the nuclear characteristics of the metal. In the production of pure niobium metal an essential step, therefore, is the careful separation of niobium and tantalum. In current industrial practice this is achieved by liquid/liquid extraction from an aqueous acid solution, using hexone (methylisobutylketone)[1].

Attention has been increasingly directed during the last decade to another source of niobium, 'pyrochlore', essentially a calcium niobate $(Na, Ca)_2\ Nb_2\ O_6\ (OH, F, O)$. Significant sources of this mineral have been discovered in several countries, but so far most of these are still the subject of intensive mineral processing research. Like columbite, pyrochlore is a member of an isomorphous, although not necessarily continuous, series, the end-members of which are pyrochlore and microlite. There is, however, an important distinction between the two series, columbite–tantalite and pyrochlore–microlite, in the way they occur in Nature. While the commonly occurring columbites always contain appreciable concentrations of tantalum, most analyses of the pyrochlore–microlite series, on the other hand, are close to one end or the other, so far as the niobium and tantalum contents are concerned[2]. This finding, supported by extensive data published recently[3], appeared to us to be of special significance, because of the prospect that high-purity niobium might be produced in future from a suitable pyrochlore source, without the expensive niobium–tantalum liquid–liquid separation step.

We have therefore examined a number of samples from sources which were either producing commercial quantities of pyrochlore concentrate already, or which might become of commercial interest in the future as a source of niobium.

The niobium in the samples was determined by an adaptation of the method described by Pickup[4], in which the sample materials were decomposed by digestion with hydrofluoric acid, the precipitated fluorides removed, and the niobium solutions evaporated with sulphuric acid. The determinations were completed spectrophotometrically, using the colour given by niobium with hydrogen peroxide in a sulphuric–phosphoric acid solution.

The tantalum determination, based on the procedure of Atkins and Smales[5], involved measuring the activity of the separated tantalum after irradiation of the sample materials in *Bepo* reactor for 12 days at a neutron flux of 2×10^{12} n/cm² sec. Following irradiation, an inactive tantalum solution was added as carrier, and decomposition effected by evaporation with hydrofluoric acid. The tantalum was precipitated from a sulphuric–tartaric acid solution with tannin at *p*H 2·3, and converted to oxide. This was dissolved in a hydrofluoric–nitric acid mixture, the tantalum present extracted into methylisobutylketone, back-extracted into hydrogen peroxide solution and reprecipitated with tannin at *p*H 2·3. The tannate was ignited to oxide, weighed and the activity of the tantalum-182 isotope measured.

The results are given in Table 1

Table 1

Sample	Ta (p.p.m.)	Nb (%)	Nb/Ta
Pyrochlore concentrate, Mrima, Kenya	565	28·6	506
Pyrochlore soil, Mrima, Kenya	61	1·27	208
Pyrochlore, picked crystals, Sukulu, Uganda	17,060	46·6	27
Pyrochlore, Nkombwa, Rhodesia	405	51·7	1,277
Pyrochlore, soil, Araxa, Brazil	232	3·29	142
Barium pyrochlore, Araxa, Brazil	559	38·7	692
Pyrochlore, Chilwa, Nyasaland	73	42·4	5,808
Pyrochlore crystals, Leushe, Kivu, Congo	446	48·2	1,081
Pyrochlore, Panda Hill, Tanganyika	6,300	40·5	64
Pyrochlore crystals, Lokupoi, Uganda	444	46·3	1,043
Pyrochlore concentrate "A", Oka, Quebec, Canada	4,170	35·9	86
Pyrochlore concentrate "B", Oka, Quebec, Canada	1,563	40·5	259
Pyrochlore concentrate "C", Oka, Quebec, Canada	1,453	41·5	286
Pyrochlore concentrate, Busumbu, Uganda	31,690	29·3	9·2
Pyrochlore concentrate, Søve, Norway	4,210	38·8	92

On the face of it, the interest might be restricted to pyrochlores with niobium/tantalum ratios of around 700 : 1 (2,000 : 1 in the United States), corresponding to the desirable concentration of 0·15 per cent tantalum in the metal. However, this need not be the case, in that in processing the ore, from concentrate to metal, some preferential depletion of tantalum occurs, even without the hexone separation step. Thus, niobium/tantalum ratios as low as 500 : 1 may be satisfactory for reactor grade metal and also for alloy production[6]. Specifications for other applications of pure niobium metal (for example, niobium-base super-conducting alloys) are less stringent concerning tantalum concentration in the metal. It is expected that for such applications most of the pyrochlores listed in Table 1 would be suitable without the hexone extraction step. Thus, the availability of pyrochlores as a source of niobium metal can be expected to make an important contribution to an eventual reduction in the cost of this element for industrial applications.

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PHYSICS

Gravitational Collapse

Hoyle and Narlikar[1] have recently considered the gravitational contraction of large spherically symmetric masses. They claim that according to the field equations of general relativity a sufficiently large mass must collapse to a space–time singularity, provided that the equation of state of the matter is realistic. Much of their argument is based on the alleged absence of suitable static spherically symmetric interior solutions.

I wish to point out that a relativistic solution exists for a static pressure-free sphere of arbitrary size and mass provided that the matter carries an appropriate electric charge. This is the counterpart of the classical solution in which gravitational attraction and electric repulsion just balance. The metric outside the sphere can be put in the form:

$$ds^2 = -\left(1 + \frac{m}{r}\right)^2 (dr^2 + r^2 d\theta^2 + r^2 \sin^2\theta d\varphi^2) + \left(1 + \frac{m}{r}\right)^{-2} dt^2, \quad (1)$$

and the electrostatic potential is $e/(r + m)$, where $|e| = m$ in relativistic units, or $|e| = m\sqrt{G}$ in dimensional units, G being the gravitational constant. It is known[2] that there exist interior solutions which: (*a*) satisfy the correct boundary conditions at the surface; (*b*) are regular and have positive mass density throughout the interior. The internal electric charge density is everywhere proportional to the mass density, which is arbitrary. It will be noted that the metric (1) has no Schwarzschild singularity.

The total electric charge of the sphere is fairly small compared with the mass: a sphere of neutral hydrogen which had lost a fraction 10^{-18} of its electrons could satisfy the conditions.

There also exist static solutions for a sphere carrying no total charge but inside which there is a separation of positive and negative charge. In this case the exterior field is a Schwarzschild one, but the interior would carry, say, an excess of positive charge near the centre balanced by an envelope of negative charge. Although solutions satisfying the boundary conditions exist[2], it is not known whether they permit an isotropic positive pressure which may be a desirable feature from the physical point of view.

These considerations suggest that charged interior solutions may be relevant to the problem of gravitational collapse.

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Non-thermal Biological Effects of Laser Beams

Investigating the effects of pulsed and continuously emitting lasers, we were able theoretically and experimentally to establish that non-thermal effects exist which have to be considered biologically more important than the thermal effects, such as the discrete coagulation of tissue which is therapeutically used, for example, in eye surgery.

We calculated that the laser field has an important electrical vector which has to be considered responsible for causing biological effects of the following classification: (*a*) thermal; (*b*) specific-thermal; (*c*) specific-electric; (*d*) chemical; (*e*) kinetic.

If the output of a laser is focused optically on an absorbing area, it is possible to produce a very high

radiant energy density. To a first order, the diameter of the focal spot is given by $d = f\,\Theta$, where f is the focal length of the optical element and Θ the angle of the beam. For $\Theta = 0{\cdot}01$ radian (about 0·5° angle), and $f = 1$ cm, then d is equal to 0·01 cm. The area of the focal spot is then of the order of 10^{-4} cm². Much smaller spots can be obtained by using high-quality optical elements such as are used in microscopes (as low as 10^{-8} cm²). For a 1-kW ruby laser output, the focused power density will reach about 10^{7} W/cm². At this concentrated power density, the electrical field E V/m is very high. Because $E = \sqrt{\eta I}$, where η is the impedance of free space and I is the intensity of the beam in W/cm², we set for our example $I = 10^{15}$ W/m², then E will be of the order of 6×10^{8} V/m or about 360 V across a red corpuscle. This has to be considered as a very high electrical gradient, even much higher in stronger laser devices, which is doubtless capable of initiating chemical effects, such as polarization of molecules, resonance effects, Debye relaxation, and many more. We call these effects 'specific electric' because they are of non-thermal nature. We see also kinetic effects such as the crater-effect where matter is pushed up around the area where the laser beam impinges.

Considering the laser to be a coherent microwave emission, it occurred to us that this principle should produce

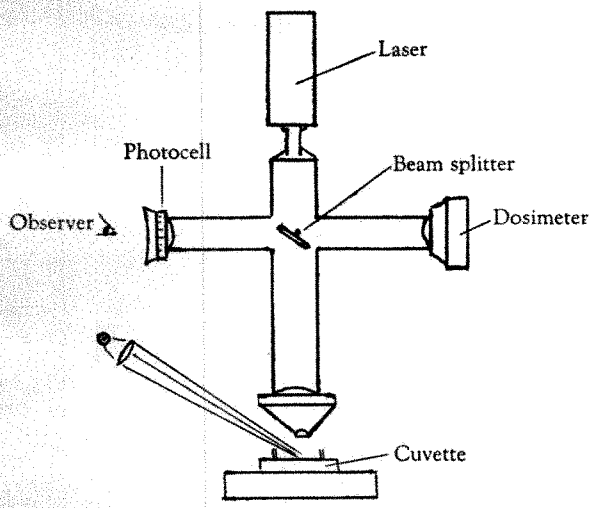


Fig. 1. The laser microscope. Microscope device for irradiation of samples with concentrated laser beams. At the top, a laser head is attached. Two side tubes connected optically through a rotating beam splitter serve for observation and dosimetry respectively. A side lamp with a hair cross optics illuminates the sample in a cuvette and indicates the spot where the laser beam will hit. For coagulation studies, a photocell can be attached to the side tube; this permits opacity changes to be recorded in a sample

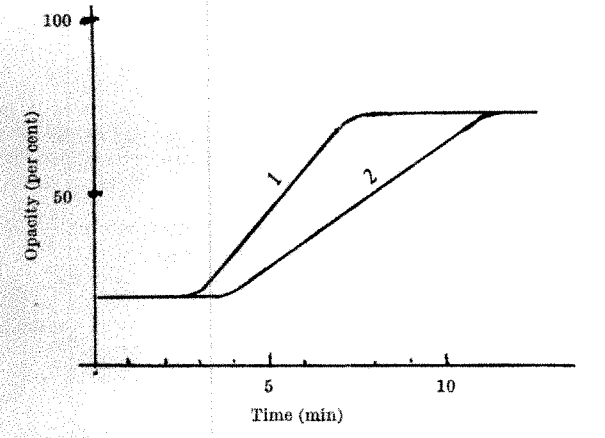


Fig. 2. Two curves of coagulation of citrated blood plasma. Curve No. 1 is a reference sample; curve No. 2 is a sample irradiated with a laser beam

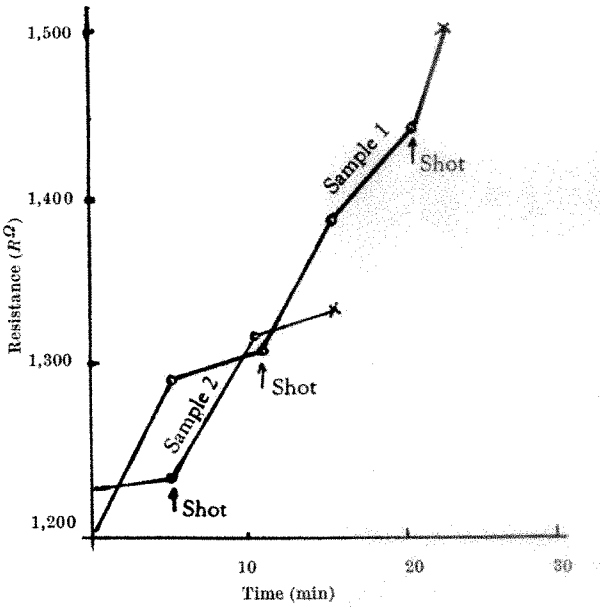


Fig. 3. Blood plasma (citrated) irradiated with a laser. The increase of resistance is shown in the two samples, when a shot (0·5 ms) of the laser is applied

the same biological effects and interactions as microwave beams. We investigated, therefore, the specific-thermal and specific-electric effects of lasers[1,7,8].

One of the appropriate tests, as I found, is the change of the dielectric constant, the change of the electrical conductivity and the specific change of the coagulation rate of plasma[7].

We used the principle of an inverted microscope (Fig. 1) introduced earlier for laser-welding purposes[1]. At the side of the ocular the laser head is attached. Then, there are two side tubes, one going to a dosimeter, the other going to an ocular by which an observer is able to see the object on the slide holder. The optical axis of the two side tubulars crosses a rotating beam splitter inside the microscope tube. First a sample of material is fixed on a substrate and placed on the slide holder. Then the beam splitter is manually rotated to the side of the observer ocular tube and the microscopic adjustment is used to focus the laser head on the right spot of the sample to be irradiated. A side lamp with a lens and a hair cross is set in such a way that the area where the laser will hit is indicated. Then the beam splitter is rotated in the direction of the dosimeter tube. When the laser is fired, the dosimetric device (electric calorimeter or radiometer) indicates simultaneously the power obtained.

We irradiated a sample of citrated plasma in this way. Then we added calcium chloride solution in order to initiate slowly progressing coagulation characterized by a gradual opacity. The rate of it was observed photometrically, by attaching to the side tubular a photocell with diaphragm and filter, and using the technique described elsewhere[3,4]. We obtained the pattern as indicated in Fig. 2. Then a xenon lamp, adjusted through an optical system, filter (7000 Å) and shutter, was used to give the same power output as the pulsed ruby laser beam. Both coagulation rate curves were compared and showed that the non-coherent light source did not produce the same effect of the coagulation process as was obtained using a laser. Therefore, we conclude that the coherent light of the laser is able to produce chemical or electro-chemical effects which are of a non-thermal nature.

In other experiments we measured, by the same technique of comparison, the electrical conductivity changes in proteins. In Fig. 3 are shown the results obtained but which could not be obtained by using ordinary light of the same power and wave-length. In Fig. 4 is shown the crater

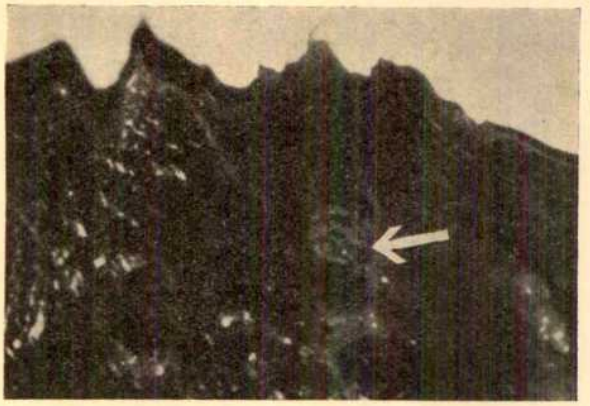


Fig. 4. Crater effect of a concentrated laser beam. The photograph shows a slice of tissue with a laser coagulation mark on it. Through the kinetic effect of the light, the walls around the mark are pushed up

effect produced by a ruby laser on a sliced tissue layer. The output of the beam was 0·2 joules, the pulse time about 0·5 msec (ref. 7). Here also it must be concluded that the concentrated laser beam is able to produce a kinetic effect which may have biological consequences. These mechanical effects are similar to that observed on paddle wheels of radiometers which are turned by the kinetic effect of light.

These results show that laser radiations behave in a similar manner to electromagnetic waves when the interaction with matter is specifically considered. Furthermore, mechanical effects due to the kinetic effect of light are obtained when a high-power output is used. These kinetic effects may also be responsible for some biological actions.

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Molecular Excitations in Liquid Helium

Cell theories of liquid helium have been proposed by several investigators[1]. They distinguish successfully between the statistics of helium-3 and helium-4 but are unable to reconcile the measured energy gap with the distance of the observed nearest neighbour. Co-operative motion of the cells accounts without difficulty for the phonon spectrum, but the assumption of weak coupling between the diatomic rotors and the acoustical vibrations is inadequate. The analysis recorded here is a new attack on the nature of the excitations which account for the normal component.

In the normal co-ordinate representation of Born[2] the Schroedinger equation of a normal mode is:

$$\frac{\partial^2\psi}{\partial q_i^2} + (\lambda - \alpha^2 q_i^2)\,\psi = 0$$

where $\lambda = 8\pi^2 m W/h^2$ and $\alpha = 4\pi^2\, m\, \nu_0/h$. The wave function of the whole system in n dimensions is:

$$\Psi = \psi_1(q_1)\,\psi_2(q_2)\,\psi_3(q_3)\,\ldots\,\psi_n(q_n)$$

and the eigenvalues will be:

$$W = \left(v + \frac{n}{2}\right) h\nu_0$$

where the zero point energy is $n/2$. The quantum weight of each of these states is:

$$\omega = \frac{(v + n - 1)\,!}{v\,!\,(n - 1)\,!}$$

The partition function is:

$$f = \sum_{v=0}^{n} \omega e^{-(v+n/2)\,h\nu_0/kT} = e^{-nh\nu_0/2kT}\,(1 - e^{-h\nu_0/kT})^{-n}$$

The energy then is:

$$E = \frac{\partial \log f}{\partial\theta} = \frac{nh\nu_0}{e^{-h\nu_0\theta} - 1} + \frac{h\nu_0}{2}\left(n + \theta\,\frac{\partial n}{\partial\theta}\right) - \log\left(1 - e^{h\nu_0\theta}\right)\frac{\partial n}{\partial\theta}$$

where $\theta = -1/kT$.

If n were Avogadro's number, N_0, the theory would reduce to the usual expression for the Debye theory, and the zero-point energy would be a constant. The present proposal identifies n as the number of rotationally excited atomic pairs:

$$n = \frac{\sum_{2,4\ldots}(2J + 1)\,e^{-J(J+1)B/kT}}{\sum_{0,2,4\ldots}(2J + 1)\,e^{-J(J+1)B/kT}}\,N_0$$

The rotational constant B has the usual value $B = h^2/8\pi^2\,\mu r^2$ with r the nearest neighbour distance of 3·7 Å. The summation over $J = 0, 2$ is sufficient to approximate the system below the λ point. The energy then becomes:

$$E = N_0\,\frac{5e^{-6B/kT}}{1 + 5e^{-6B/kT}}\left[\frac{h\nu_0}{e^{h\nu_0/kT} - 1} - \frac{6B}{1 + 5e^{-6B/kT}}\left(\log\,(1 - e^{-h\nu_0/kT}) + \frac{h\nu_0}{kT}\right) + \frac{h\nu_0}{2}\right]$$

in which the first term only is of importance. It is noted that the terms associated with the zero-point energy are dependent on temperature and become zero at low temperature.

The internal energy observed in the laboratory is well represented below 2° K by:

$$U = \frac{4\pi V}{c^3}\left(1 + \frac{300e^{-6B/kT}}{1 + 5e^{-6B/kT}}\right)\int \frac{h\nu}{e^{h\nu/kT} - 1}\,\nu^2 d\nu$$

in which the first term measures phonons and the second term the normal component. The coefficient of the normal component is sixty times that provided by theory and will be justified later. In the low-temperature approximation the internal energy becomes:

$$U = \frac{4\,\pi^5\,V\,k^4\,T^4}{15\,c^3\,h^3}\left(1 + \frac{300e^{-6B/kT}}{1 + 5e^{-6B/kT}}\right)$$

Fig. 1 compares the heat capacity, the normal component, and the density for the Landau theory, the present theory, and experiment. The theoretical heat capacity does not deviate from experiment below 1·9° K. Landau has derived a relation between internal energy and density of the normal component. Phonon and roton excitations are treated as separate components using $\rho_n = 4\,U/3\,c^2$ with $c_1 = 237$ m/sec and $c_2 = 18{\cdot}5$ m/sec. Normal component measurements by different laboratories do not agree. The theory fits the most recent measurements in the region of greatest reliability. The density is derived from a theory of perfect solution of normal and Debye components having the same expansion coefficients but differing by 1·2 per cent in intrinsic volume. (In liquid hydrogen the *ortho* form has a density 0·5 per cent greater than the *para* form.) The variation in density is, therefore,

a combination of expansion and transformation from superfluid to normal component of greater density.

Ice shows analogous anomalies in heat capacity and density. The Debye theory is adequate to 3° K where an anomaly begins. The anomaly is most pronounced at 10° K. Since independent *ortho* and *para* forms have been demonstrated, molecular rotation can be assumed[3]. The lowest *ortho–ortho* transition in the vapour ($1_{-1} \rightarrow 1_{+1}$) has an energy of 18·6 cm^{-1}, equivalent to 27·8° K. The transition has nuclear degeneracy but no rotational degeneracy. Assuming a transition energy of 15 cm^{-1} the proposed theory fits the data through the maximum of the anomaly. In water, two hydrogen atoms are unambiguously identified with one oxygen atom. If this was not true, *ortho* and *para* forms could not endure. In helium, however, each helium atom is surrounded by about eight nearest neighbours and a rotating pair can be formed with any of these. Isihara[4] has recently calculated the pair distribution function of a dilute hard sphere Boson gas. He finds the number of pairs to be very nearly n^2 or a little more than the required factor of 60 for helium.

The number of excitations may be counted:

$$N = \frac{4\pi V}{c^3} \frac{300e^{-6B/kT}}{1 + 5e^{-6B/kT}} \int \frac{\nu^2 d\nu}{e^{h\nu/kT} - 1}$$

If N is taken as Avogadro's number, a temperature may be calculated equivalent to the Debye temperature. At this temperature the number of excitations or quasi-particles equals the number of molecules. This occurs at 2·7° K, the λ point of the theory. The counting is in error by a factor of two, since $N_0/2$ would give 2·2° K. In the case of ice, the Dulong-Petit limit is reached before the number of excitations reaches Avogadro's number and no λ point is observed.

In helium-3 – helium-4 solutions, only ^{4}He–^{4}He pairs show the quantum properties required by the theory. If this pairing is random, the number of pairs of interest will

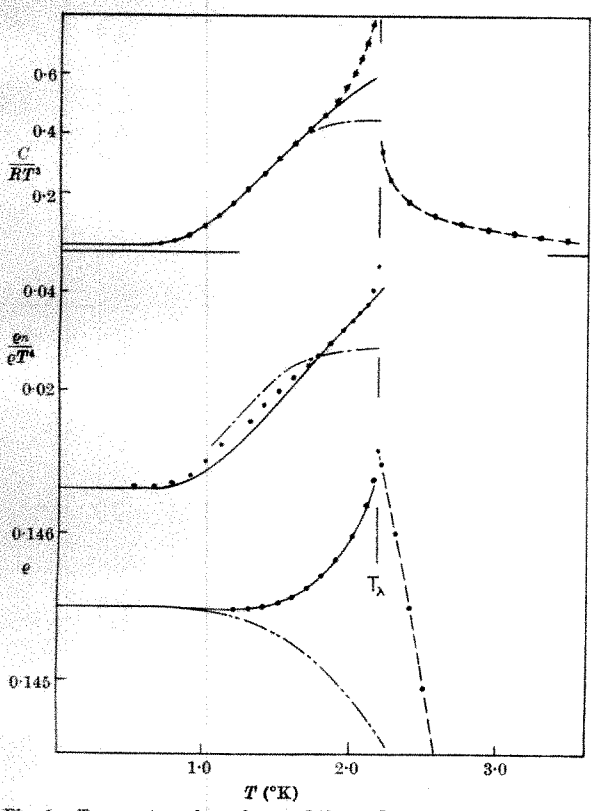


Fig. 1. Temperature dependence of thermodynamic variables. Continuous line, proposed theory; dot-dash, Landau theory; dash-double-dot, Debye theory. Top, heat capacity; centre, normal component; bottom, density

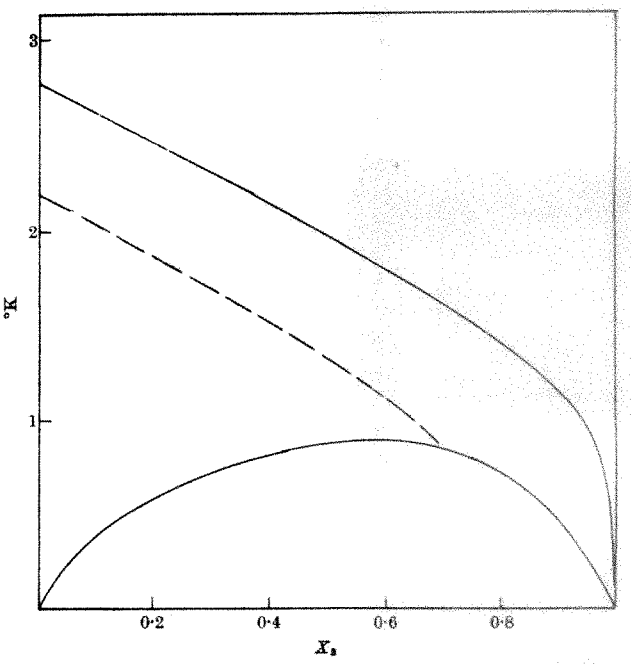


Fig. 2. Phase diagram helium-3–helium-4 solutions. Atomic fraction helium-3 plotted horizontally. Upper line, calculated lambda temperature; dashed line, observed lambda temperature, Lower line, phase separation boundary

be proportional to the square of the atomic fraction of helium-4. The calculated λ temperatures (Fig. 2) show the correct concentration dependence but are 0·5° K above the experimental data.

The character of the excitations of which the normal component is composed is quantitatively described by the normal co-ordinate representation of Born if restricted to rotationally excited atomic pairs. The superfluid (more accurately the Debye liquid) is a substrate within which the excitations exist as a distinguishable component.

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CHEMISTRY

Seven Co-ordinate Vanadium Complexes with Metal-to-metal Bonds

IN contrast with the dimeric carbonyls of transition metals with odd atomic number, hexacarbonylvanadium, with the electron configuration d^5, is mononuclear and paramagnetic with one unpaired electron. It is of interest to enquire, therefore, whether: (1) by partial substitution of the carbonyl groups, dimeric compounds containing vanadium–vanadium bonds can be stabilized; (2) complexes containing M-V(CO)$_6$ groups (M = Cu, Ag, Au), analogous with other complexes recently described[1], can be prepared. The corresponding halides are unknown.

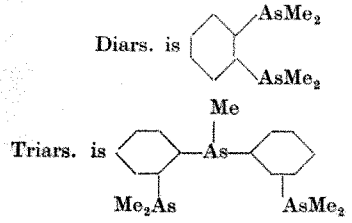
Hieber and Winter[2] have reported that the complex $\{V(CO)_4[P(cyclohexyl)_3]_2\}_2$ is diamagnetic and evidently contains a vanadium–vanadium bond. Other phosphines used gave rise to monomeric, paramagnetic compounds, for example, V(CO)$_4$(PPh$_3$)$_2$. We now report the isolation of the dimeric compound [V(CO)$_4$(diars)]$_2$, [diars = *o*-C$_6$H$_4$(AsMe$_2$)$_2$]. This is formed readily by reaction of the hexacarbonyl with the di(tertiary)arsine in light petroleum at room temperature. It is diamagnetic in the solid state

and in solution, implying a metal–metal linkage; other properties are shown in Table 1. Spectrophotometric titration of the complex with iodine indicates the formation of the product $V(CO)_4$(diars)I, one equivalent of iodine per vanadium being consumed.

Table 1

Complex*	Colour	Molecular weights† Found	Calc.	C—O stretching freq. (I.R., cm^{-1})
$[V(CO)_4(Diars)]_2$	Light yellow	863‡	898	2,085 (*w*), 1,970 (*m*), 1,890 (*sh*), 1,870 (*s*)‡
$Ph_3PAu—V(CO)_6$	Yellow-orange	670§	678	2,043 (*s*), 1,991 (*m*), 1,960 (*m*), 1,925 (*vs*), 1,890 (*sh*), 1,870 (*m*)¶
(Triars)Cu—$V(CO)_6$	Light yellow	710§	734	2,020 (*m*), 1,925 (*m*), 1,885 (*sh*), 1,858 (*s*)

* All are analytically pure and diamagnetic in the solid state.
† Measured osmometrically (concentrations ≃ 0·01–0·05 M).
‡ In CH_2Cl_2.
§ In C_6H_6.
¶ In *cyclo*hexane.



Reaction of the salt $[Na(diglyme)_2][V(CO)_6]$ with the compounds Ph_3PAuCl and (triars) CuBr {triars = $[C_6H_4(AsMe_2)]_2AsMe$} in tetrahydrofuran yields the products Ph_3PAu-$V(CO)_6$ and (triars)Cu-$V(CO)_6$ respectively. Physical data, presented in Table 1, fully support these formulations. Iodine oxidations, using two equivalents of the halogen, yield the complexes Ph_3PAuI or (triars)CuI and hexacarbonylvanadium. Attempts to prepare an analogous compound containing a silver–vanadium bond have led, so far, to reduction to metallic silver. The possible use of substituted carbonyl anions of vanadium is being investigated together with other systems containing vanadium-heavy metal linkages. These metal–metal bonded complexes provide examples of seven co-ordinate vanadium(0), a co-ordination number which was, until recently, very uncommon in the first transition series.

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Iodine as a Scavenger for Hydrogen Atoms in Irradiated Ethanol

As with most organic compounds, one of the principal end-products resulting from the effect of high-energy ionizing radiation on pure ethanol is hydrogen gas. Much interest has been shown recently in the radiolysis of alcohols, and this paper presents data on hydrogen atom yields, which help to distinguish the various possible mechanisms.

The yield of hydrogen gas is decreased, but not reduced to zero, by the presence during radiolysis of a radical scavenger. This leads to the concept of molecularly detached hydrogen gas produced from entities which decompose without the formation of reactive intermediate species. Schematically, the reaction for the production of hydrogen may be represented as:

$$C_2H_5OH \rightsquigarrow H + H_2 + \ldots$$

where H_2 represents molecularly detached hydrogen and H the hydrogen atom, which may react to give hydrogen molecules in the pure solvent, or react with a scavenger when such a suitable radical trap is present.

By isotopic labelling of the ethanol molecule with deuterium, Burr[1] has concluded that the hydrogen atoms formed during radiolysis undergo an abstraction reaction of the type:

$$H\cdot + C_2H_5OH \rightarrow \cdot C_2H_4OH + H_2$$

which leads to the production of one molecule of hydrogen gas for each hydrogen atom taking part in the abstraction reaction.

The kinetics of two competing reactions has been used[2] to measure the hydrogen atom yield, assuming that:

$$C_2H_5OH \rightsquigarrow H + H_2 + \ldots \quad (1)$$

is followed by:

$$H\cdot + C_2H_5OH \xrightarrow{k_1} \cdot C_2H_4OH + H_2 \quad (2)$$

where alternatively the hydrogen atoms in the presence of a scavenger react as follows:

$$H\cdot + S \xrightarrow{k_2} HS \quad (3)$$

in which (2) and (3) are the competing reactions.

Using benzoquinone (Q) as a scavenger, and from a plot of $\frac{1}{G(H_2)_{max} - G(H_2)}$ against $\frac{1}{[Q]}$, Adams, Baxen-

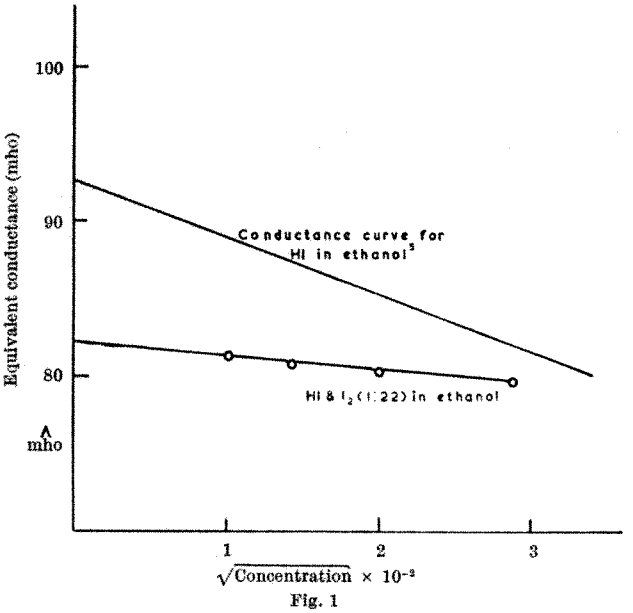


Fig. 1

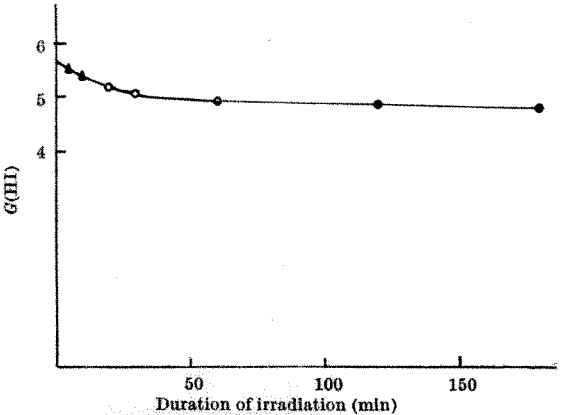


Fig. 2. Source: Cs-137, ●, *G*(HI) determined by conductance method, ○, *G*(HI) determined by spectrophotometric method; cobalt-60, ▲, *G*(HI) determined by spectrophotometric method

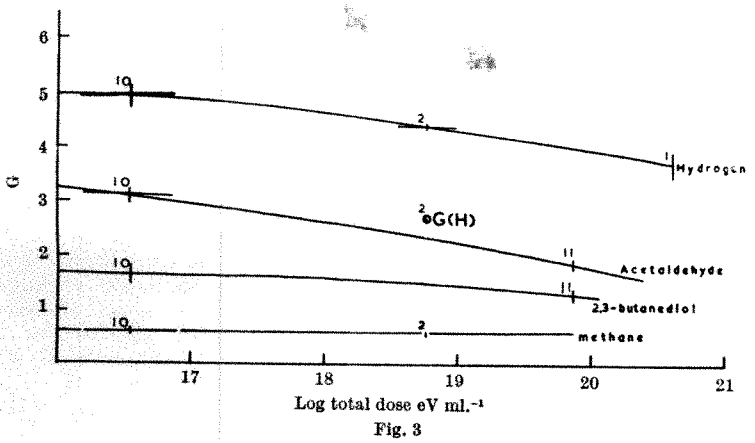


Fig. 3

dale and Sedgwick[2] have found the intercept on the $\frac{1}{G(H_2)_{max} - G(H_2)}$ axis to be 0·37, which, assuming the kinetics of the foregoing reactions, gives a value for $G(H)$ of 2·7.

An alternative scavenging agent is iodine, and we have used two methods to measure the quantity of hydrogen iodide formed. The first depends on the fact that, in a polar solvent such as ethanol, the hydrogen iodide ionizes to form solvated protons and iodide ions, the latter forming an equilibrium mixture of triiodide ions with excess iodine.

$$H\cdot + I_2 \rightarrow HI + I\cdot \quad (4)$$

$$HI \rightleftharpoons H^+ + I^- \quad (5)$$

$$I^- + I_2 \rightleftharpoons I_3^- \quad (6)$$

Equilibrium (6) has been investigated spectrophotometrically in water[3] and methanol[4].

Similar results in ethanol show the triiodide ion to have two well-defined absorption peaks at 292 mμ and 360 mμ which lie between the ethanol–iodine interaction peak at 445 mμ and the charge-transfer band at 224 mμ. If the concentration ratio of I^- to I_2 is maintained below 1 : 20, then equilibrium (6) is sufficiently displaced to the right to produce a constant concentration of I_3^- ions for varying ratios of I^- to I_2. Under these conditions ε_{max} at 360 mμ for I_3^- ions is 24,400 while at the same wave-length ε for I_2 is 84. Hence, by observing the increase in optical density at 360 mμ in irradiated solutions of iodine in ethanol, the concentration of I_3^- ions may be calculated. From this value the concentration of HI may be calculated.

The second method utilizes the ionic dissociation of hydrogen iodide in ethanol and the variation of equivalent conductivity (Λ) with change of concentration, which has been investigated by Goldschmidt[5]. Fig. 1 shows the modification to the conductance curve for HI in ethanol by the addition of a 20-fold excess of iodine over the concentration of HI. This modification is caused by the difference in mobility between the I^- and I_3^- ions. Thus, by measuring the change in resistance of irradiated solutions of iodine in ethanol a measure of the HI produced may be obtained.

Solutions of iodine in pure dehydrated ethanol were prepared in light-screened vessels. Measurements showed that visible light initiated the decomposition of iodine–ethanol solutions. Dehydration and purification of ethanol were carried out as for conductance experiments[6] when the alcohol showed a specific resistance greater than 10^8 ohms. The iodine–ethanol solutions were de-aerated by vacuum techniques and irradiated by γ-rays either from a 200-c. cæsium-137 source or a 300-c. cobalt-60 source.

The total dose variation was 4×10^{17} to $2{\cdot}4 \times 10^{18}$ eV ml.$^{-1}$. The dose rate was varied from $1{\cdot}4 \times 10^{16}$ to $6{\cdot}8 \times 10^{16}$ eV ml.$^{-1}$ min^{-1}. The initial iodine concentration range was 7×10^{-4} to 10^{-2} molar. The irradiation time varied from 5 min to 3 h. The results showed that $G(HI)$ depended on the duration time of irradiation and also the total absorbed dose as shown in Fig. 2.

The time dependence is consistent with results obtained by Meshitsuka and Burton[7] for HI production in irradiated iodine–*cyclo*-hexane solutions. The variation in $G(HI)$ with time has been attributed to the scavenging properties of HI (ref. 7). Fig. 2 shows that extrapolation to zero dose zero time gives $G(HI) = 5{\cdot}6$.

In order to correlate $G(HI)$ at zero dose with $G(H_2)$, a knowledge of the variation of $G(H_2)$ with total energy input is required. This has been investigated in the case of the bombardment of ethanol with 28-MeV helium ions by Newton and McDonell when it was found $G(H_2)$ was an inverse function of the total energy input[8]. Johnsen reports an inverse relationship between $G(H_2)$ and the total energy input when ethanol is irradiated with cobalt-60 γ-rays from a total dose of 10^{18}–10^{22} eV ml.$^{-1}$ (ref. 9). Table 1 shows various yields of $G(H_2)$ obtained using cobalt-60 γ-radiation.

Table 1

Reference	$G(H_2)$	Total dose eV ml.$^{-1}$
10	$4{\cdot}87 \pm 0{\cdot}2$	$1{\cdot}6 - 8{\cdot}0 \times 10^{18}$
2	4·35	$3{\cdot}8 - 8{\cdot}9 \times 10^{18}$
1	$3{\cdot}66 \pm 0{\cdot}26$	$3{\cdot}9 \times 10^{20}$

Fig. 3 shows these results to lie on a smooth curve when log total dose in eV ml.$^{-1}$ is plotted against $G(H_2)$. Also in Fig. 3 are the yields of acetaldehyde 2, 3-butanediol and methane plotted against log of the total dose as obtained by various workers. There are insufficient data to extrapolate accurately the curve for $G(H_2)$ to zero dose, but an approximation may be made at $G(H_2) \simeq 5{\cdot}6$, the same value for $G(HI)$ at zero dose. $G(H)$ at zero dose may not be estimated as only one value is available.

Primary processes may only be postulated in the knowledge of radical and molecular yields at zero dose. Further experimental work is in progress to measure hydrogen gas yields in the presence and absence of iodine and to establish the nature of the energy input effects.

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Circumstances of Multi-spot Paper Chromatograms of Sodium Orthophosphates: *in situ* Identification of the Species

The formation of more than one zone containing phosphate when the sodium orthophosphates are chromatographed on paper suggests that these spots contain different molecular species originating in proton transfer equilibria of the kind:

$$H_2PO_4^- + B^{-n} = HPO_4^{=} + HB^{-(n-1)}$$

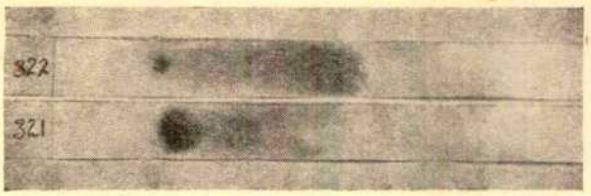


Fig. 1. Paper chromatograms of Na_3PO_4 (321) and NaH_2PO_4 (322) formed with butanol/water

where B^{-n} is a base[1,2]. The speed of proton transfers, for example, the half-life for the reaction of hydronium and hydroxyl ions is 10^{-8} sec[3], argues against this as such equilibria yield multiple spots only if the transition time is comparable with the time required for the formation of the chromatogram[4]. It is natural to question next if the zones are actually of different species since discontinuities in either the immobile or mobile phases can also produce the result[4]. Chromatograms of orthophosphoric acid and of the primary, secondary, and tertiary sodium salts were formed on 55 cm × 2·8 cm strips of Whatman No. 1 filter paper, acid washed, which had also been washed in 2 M acetic acid followed by three successive rinsings in distilled water[5]. The chromatograms were formed either with pyridine/ethyl acetate/water (the faster solvent) or butanol/water. Fig. 1 is a photograph of 10 μl. of 0·01 M trisodium phosphate (No. 321) and 10 μl. of 0·01 M sodium dihydrogen phosphate (No. 322) formed simultaneously with BuOH/15 per cent H_2O. The origin was the vertical pencil line adjacent to the numbers. When revealed, the colour process was that employing ammonium molybdate and benzidine hydrochloride[6]. In certain select cases, the multiple spots were irradiated in the University's *Triga* reactor[7,8]. Both sodium and phosphorus undergo (n,γ)-reactions to form radioactive isotopes which decay as follows[9,10]:

$$^{24}Na = {}^{24}Mg + \beta^- \ (1{\cdot}39 \ \text{MeV}) + \gamma \ (1{\cdot}38,\ 2{\cdot}76 \ \text{MeV}) \ (\text{half-life} = 14{\cdot}97 \ \text{h})$$

$$^{32}P = {}^{32}S + \beta^- \quad (1{\cdot}712 \ \text{MeV}) \quad (\text{half-life} = 341{\cdot}3 \ \text{h})$$

The nuclear cross-sections, the natural existence of each element as a single isotope, the absence of interfering reactions[11], and the difference in half-lives, make the pair ideal for simultaneous determination of the two when mixed. Applied to known salts without chromatography, the resolution of the activity versus time curve gave $Na/P = 1{\cdot}0, 1{\cdot}2$ for NaH_2PO_4; $Na/P = 2{\cdot}0, 2{\cdot}0$ for Na_2HPO_4; and $Na/P = 3{\cdot}1, 3{\cdot}0$ for Na_3PO_4. Table 1 gives the pertinent results from the chromatograms. R_F-values are not given since, in order to obtain clear unambiguous separation, it was necessary that the mobile phase flow from the end of the chromatogram. With Pyr-EtAc/H_2O, the fast spot has a composition nearly that of orthophosphoric acid while the slow spot has one approximately that of primary sodium orthophosphate regardless of the solution applied. The circumstances of double spot formation with a mobile phase of BuOH/H_2O are different, but again there is a spot of composition very much different from the solution applied with a much higher degree of protonation.

Table 1. Na/P Ratio of Zones of Paper Chromatograms of Phosphoric Acid and the Sodium Orthophosphates

Solution applied	Forming solvent	Na/P	
		Slow	Fast
0·01 M H_3PO_4	Pyr-EtAc/15 per cent H_2O	—	0·03
0·05 M H_3PO_4	Pyr-EtAc/15 per cent H_2O	—	0·14
0·10 M H_3PO_4	Pyr-EtAc/15 per cent H_2O	—	0·05
0·01 M NaH_2PO_4	Pyr-EtAc/15 per cent H_2O	—	0·24
0·01 M Na_2HPO_4	Pyr-EtAc/15 per cent H_2O	0·75	0·19
0·01 M Na_3PO_4	Pyr-EtAc/15 per cent H_2O	1·2	0·19
0·01 M Na_3PO_4	Pyr-EtAc/24 per cent H_2O	1·3	0·39
0·01 M NaH_2PO_4	BuOH/10 per cent H_2O	1·2	0·38
0·01 M Na_3PO_4	BuOH/10 per cent H_2O	0·80	—

Multiple-spot formation depends on a number of factors. The first of these is prewashing the paper with acid. With water alone or a solution of disodium dihydrogen ethylenediamine tetraacetate (EDTA), an excellent chelating agent for rare-earth cations, the phosphate does not migrate from the origin or else produces a smear. With acetic acid, acetic acid and EDTA, or hydrochloric acid, migration occurs and multi-spots can be produced. Since the effect of the acid overwhelms that of the EDTA, it seems that removal of the rare earths is not as important as exchange of protons for cations, principally sodium. Neutron activation studies of the sodium content of the paper showed that washing with acetic acid reduced the sodium content by a factor of 7–8 while chromatography further reduced it by a factor of 12 below that of the washed paper. The forming solvent must contain water. With 5 per cent or less water in the Pyr-EtAc solvent, there was no migration from the point of application. Between 5 per cent and saturation (24 per cent) water enhanced zone disengagement. Multi-spot formation depended on the total phosphate concentration and the Na/P ratio. These were adjusted by adding sodium acetate. As a rule, the slow spot appeared at 0·02 M phosphate when $Na/P = 1$ and 0·01 M phosphate when $Na/P = 2$. The size of the second spot increased with the Na/P ratio at 0·01 M. This is independent of the acidity of the solution applied. Identical double spots formed from Na_3PO_4 for *p*H-values of 2·1–12·3. The secondary salt is critical, for both single spots and double spots could be obtained. This did not depend on the *p*H. The primary salt and the acid gave a single spot at *p*H-values from 2·2 to 12·0. In all these cases the *p*H was adjusted with ammonia and hydrogen chloride.

The phenomena can be explained if the system consists of phosphate species, hydronium ion, the carboxyl groups of the paper, and their conjugate bases, all in competition for protons. The forming solvent, in passing from the reservoir to the point of application, passes through paper from which it extracts protons, placed there by the acid prewash, to give an acid front. The acidity drops sharply a small distance behind the advancing front much like the water concentration in chromatograms formed with absolute ethanol[12]. This pulse of acid, on entering the solute zone, converts part of the phosphate to approximately phosphoric acid, with advances at a higher rate than less-protonated phosphate ions. This conversion stops once the acid front passes the origin and the remainder of the phosphate proceeds as a less-protonated species approximating NaH_2PO_4.

The Na/P ratios were determined at positions within the spots where the phosphate had the greatest activity. In some cases shoulders were observed on the radiographic profiles which decayed swiftly, indicating a far higher sodium content than in other portions of the same spot. A region of high sodium activity between the solvent front and the phosphate spot was observed in chromatograms of 0·01 M Na_3PO_4 formed with BuOH/H_2O, which indicates a separation of cation from the original anion[13]. The Na/P ratio in the phosphate spot was 0·80, which suggests that in this case the normal salt was sufficiently protonated to produce the NaH_2PO_4 but not the fast H_3PO_4. The exchanged sodium moved away from the phosphate.

This work was supported by the National Institutes of Health and the National Science Foundation. One of us (M. A. R.) was the 1963 Koppers Company Fellow and the 1964 Continental Oil Company Research Fellow. We also thank Dr. L. E. Weaver and his colleagues in the

Department of Nuclear Engineering for their co-operation.

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Rapid Determination of D_2O Content in Water using a Vapour Pressure Osmometer

I HAVE carried out determinations of D_2O content in water using the vapour pressure osmometer 'Mechrolab, Model 301 *A*', designed to perform molecular-weight determinations. Briefly, the principle of this instrument is as follows[1,2]: two thermistors, charged with a drop of solvent and arranged in a d.c. Wheatstone bridge, are at the same temperature in an ambient atmosphere saturated with the vapour of the selected solvent. The bridge is equilibrated, and a drop of a solution of the same solvent is placed on one of the two thermistors (called the sample thermistor). Solvent condenses on this drop of solution until vapour pressure equilibrium is approached, and the temperature of the thermistor increases. Its resistance variation depends on the concentration of the solution and it is determined by rebalancing the bridge.

Vapour pressures of heavy and light water are also different and, in principle, it is possible to use the vapour pressure osmometer for carrying out heavy-water analysis. But in this case a complication arises from the deuterium exchange between the heavy-water drop and 'light' water vapour and between D_2O vapour and the 'light' water drop placed on the reference thermistor. Because of the usual inertia of thermal instruments it is not possible to shorten the time of measurement in order to reduce the exchanged amount of deuterium to a negligible value. Indeed, about 2 min are necessary to reach the stationary condition in the standard measurements of molecular weight with this vapour pressure osmometer. The difficulty can be overcome by determining the progressive decrease of resistance difference (ΔR) between the reference and sample thermistor during the course of the measurement. If $\log \Delta R$ is plotted against time a straight line is obtained. The exchange reactions are described by the following relationship:

$$\frac{C_1}{C_0} = \frac{S_1}{S_1 + S_2} + \frac{S_2}{S_1 + S_2} \exp\left\{ - \rho t \left(\frac{1}{S_1} + \frac{1}{S_2} \right) \right\} \quad (1)$$

$$\frac{C_2}{C_0} = \frac{S_1}{S_1 + S_2} - \frac{S_1}{S_1 + S_2} \exp\left\{ - \rho t \left(\frac{1}{S_1} + \frac{1}{S_2} \right) \right\}$$

where C_i is the concentration of label in compartment i at the time t; S_i is the amount of exchangeable material in compartment i; and ρ the exchange rate. In the relationship (1) the following initial conditions are used: at $t = 0$, $C_1 = C_0$ and $C_2 = 0$.

Table 1

D_2 molar fraction	ΔR_0 (ohms)	ΔR_0 normalized to $2{\cdot}91 \times 10^3$	$\Delta P/P_{H_2O}$ calculated
0·9980	330 ±3	0·113	0·112
0·6921	226 ±2·5	0·0777	0·0781
0·4499	147 ±2	0·0505	0·0511
0·2783	91·2 ±1·2	0·0313	0·0318
0·2478	82·6 ±0·2	0·0284	0·0283
0·1952	65·4 ±0·1	0·0225	0·0223
0·1196	40·3 ±0·2	0·0138	0·0137
0·0475	16·2 ±0·2	0·0056	0·0054
0·0343	11·5 ±0·2	0·0039	0·0039
0·0118	4·00 ±0·06	0·0014	0·0014

In our case C_1 and C_2 can be replaced by P_1 and P_2 (vapour pressure of water placed on the reference and sample thermistors). The vapour pressure difference (ΔP) is given by:

$$\Delta P = \Delta P_0 \exp\left\{ - \rho t \left(\frac{1}{S_1} + \frac{1}{S_2} \right) \right\} \quad (2)$$

ΔP_0 can be determined by the extrapolation of a linear plot of $\ln \Delta P$ against t at zero exchange time, that is, the time at which the heavy-water sample was placed on its thermistor. The half-exchange time ($T_{1/2}$) is not only dependent on ρ but also on $(1/S_1 + 1/S_2)$ and, therefore, on the drop sizes. Variations of $T_{1/2}$ values are expected, but this fact does not affect the determination of Δp_0.

For our purpose formula (2) can be re-written as follows:

$$\Delta R = \Delta R_0 \exp\left\{ - \rho t \left(\frac{1}{S_1} + \frac{1}{S_2} \right) \right\} \quad (2')$$

ΔP being proportional to ΔR.

Water samples with different D_2O contents were prepared by dilution of a heavy-water sample (99·8 per cent D_2O) supplied by CISE Laboratories of Milan. The vapour pressure osmometer 'Mechrolab Model 301 *A*' was equipped for determinations at 37° C. The measurements were

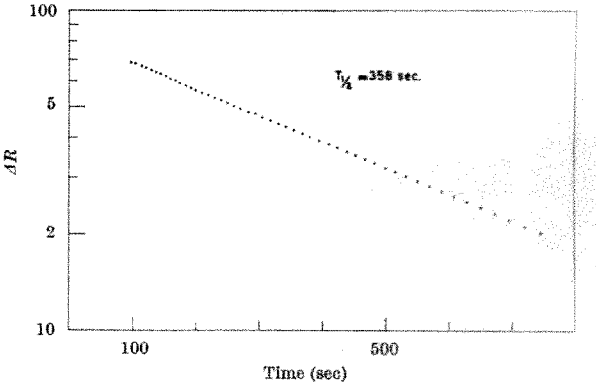


Fig. 1. Plot of ΔR against time

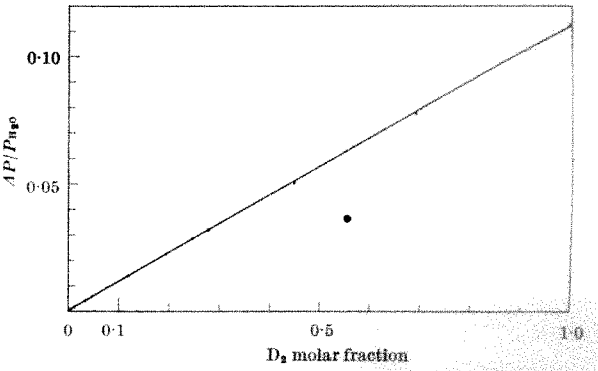


Fig. 2. Behaviour of vapour pressure of heavy water samples against D_2 molar fraction

carried out as follows: as soon as possible, four drops of heavy water were successively placed on the sample thermistor and the moment at which the fourth drop was placed on the thermistor was chosen as zero time. Readings started after about 90 sec and the decrease of ΔR was followed for about 2 times $T_{1/2}$. A typical plot is shown in Fig. 1. The reproducibility of $T_{1/2}$ is about ± 10 per cent with a mean value of 350 sec, as was foreseen, but the reproducibility of ΔR_0 readings is 1·0–1·5 per cent (Table 1).

The theoretical values of the vapour pressure of heavy-water mixtures P_m are deducible from the following relationship:

$$P_m = P_{H_2O}\,\gamma_{H_2O} + P_{HDO}\,\gamma_{HDO} + P_{D_2O}\,\gamma_{D_2O} \qquad (3)$$

where γ_i is the molar fraction of compound i and P_i its vapour pressure. The value of γ_i can be deduced from the mole percentage of deuterium (x) with respect to the total hydrogen, and the value of the equilibrium constant of the reaction: $H_2O + D_2O \rightleftarrows 2HDO$.

Therefore:

$$P_m = P_{H_2O}(1-x)^2 + 2P_{HDO}x\,.\,(1-x) + P_{D_2O}x^2 \qquad (3')$$

Using the P_i values reported in ref. 3, we obtain for our case:

$$\frac{\Delta P}{P_{H_2O}} = -0{\cdot}003\,x^2 + 0{\cdot}115\,x$$

where $\Delta P = P_{H_2O} - P_m$

This equation is plotted in Fig. 2 together with experimental ΔR_0 values, normalized to $2{\cdot}91 \times 10^3$ (see also Table 1). The agreement between calculated and experimental data is good, so that the method described may be considered practicable, especially in view of its rapidity and the small volume of sample required.

I thank Prof. M. Silvestri for advice.

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Carbonium Ion Mechanism and Results of Solvolysis of (+)-2-Bromo-octane

The remarkable prevalence of re-arrangement in simple alkyl groups during the formation of alkyl halides, and the concomitant loss in optical purity have been loosely ascribed to the intermediate formation of a carbonium cation[1]. The conversion of an alkyl halide into the alcohol, ester, or ether must therefore be scrutinized for points of correlation. There are few published examples of alkyl rearrangement in the solvolysis of straight chain alkyl halides. Isopropanol was formed in the mercury catalysed solvolysis of *n*-propyl bromide in aqueous formic acid, and the carbonium ion mechanism was postulated[2].

By a mechanism designated 100 per cent S_N1 (that is, the carbonium ion mechanism) (+)-2-bromo-octane was solvolysed in 60 per cent aqueous ethanol at 80°, resulting in the formation of (−)-2-octanol showing some 60 per cent loss in optical purity[3,4]. It remained to be seen if rearrangement of the alkyl group also occurs in this system. We find by gas-liquid chromatography[5] that isomerically pure 2-octanol and ethyl 2-octyl ether were obtained when 2-bromo-octane (M/40) was heated under reflux (72 h) in 60 per cent aqueous ethanol. In another example, with (+)-2-bromo-octane, α_D^{20} + 40·24° (l = 1) (0·31 molar instead of M/40) the (−)-2-octanol, b.p. 77·5°/10 mm, n_D^{20} 1·4256, α_D^{18} −3·62° (l = 1) showed 51 per cent loss in optical purity, but was accompanied by only < 0·5 per cent rearranged products. It is true that hydrogen bromide is a product of the solvolysis; but (+)-2-octanol lost no optical purity when it was heated under reflux (72 h) with 60 per cent aqueous ethanol containing hydrobromic acid (0·155 molar).

We have therefore an example of a system involving considerable loss in optical purity, previously attributed entirely to the carbonium ion mechanism, yet showing no rearrangement in the alkyl group.

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Photodimerization in Crystalline 9-Cyanoanthracene

Robertson[1] has pointed out that the type of crystal structure adopted by an aromatic molecule appears to depend on the molecular shape. Thus, those in the form of elliptical planes crystallize with an anthracene-type (A) structure on which weak coupling between molecules in the electronically excited state is responsible for a slight shift in the fluorescence spectrum with respect to the molecular spectrum, and the vibrational structure is largely preserved. Disk-shaped molecules such as pyrene and coronene, on the other hand, exhibit strong coupling with a close-packed type B structure in which pairs of molecules present as a structural 'unit' or as an imperfection are responsible for the emission of the characteristic excimer band, red-shifted by some 6,000 cm^{-1} from the molecular spectrum and devoid of vibrational structure[2].

It has been shown[3] that a change in the molecular shape of anthracene by the substitution of a 9-cyano group or phenyl groups in the 9,10 positions produces a change from the molecular to the excimer fluorescence spectrum which is undoubtedly due to a corresponding change in the crystal structure from type A to type B (ref. 4). In the former case the replacement of the green structureless-fluorescence by a blue structured 'molecular' fluorescence spectrum during exposure to ultra-violet radiation was attributed[3] to the photochemical consumption of dimeric exciton traps, the logical alternative photomorphic change being difficult to visualize.

We have now taken X-ray diffraction photographs of powdered 9-cyanoanthracene in its original green-fluorescent form and of the blue-fluorescent photoproduct together with that of the photodimer precipitated from a saturated outgassed solution in toluene during exposure to ultra-violet radiation. From a comparison of the Guinier photographs shown it is clear that crystalline 9-cyanoanthracene undergoes photodimerization which may be promoted by strong interaction in the lowest excited singlet state of the close-packed type B structure. The blue, structured fluorescence of the photoproduct must originate from residual molecules of 9-cyanoanthracene dispersed in the photodimer matrix.

Although the photodimerization of anthracene itself in solution is well known[5], there is no reported evidence for this process in its (type A) crystal form. It therefore appears that in so far as molecular shape determines the type of lattice adopted by an aromatic molecule, this may also influence its photochemical behaviour in the crystalline state. It must also be concluded that, since photodimerization competes with excimer fluorescence, these two processes cannot be associated with parallel and

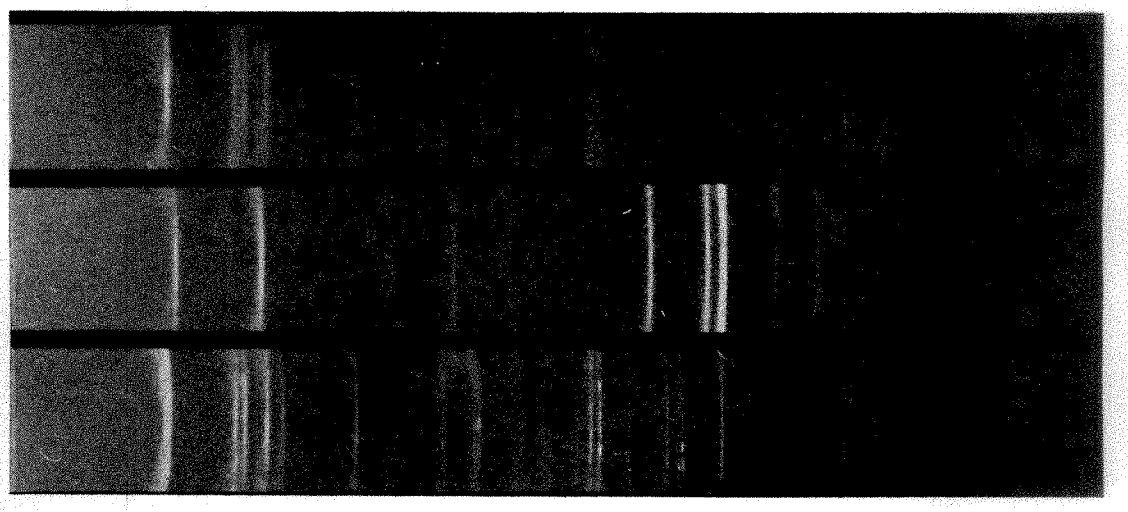


Fig. 1. Guinier photographs of 9-cyanoanthracene powders. (*a*) Blue-fluorescent product produced by ultra-violeti rradiation of original green-fluorescent crystals (*b*); (*c*) photodimer

anti-parallel configuration of the photo-associated state as previously suggested for 9-methylanthracene[6].

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Nonaboronnonachloride

The recent preparation of the borane B_9Cl_8H (ref. 1) prompts us to report some of our mass spectral work on the products arising from the thermal decomposition of diboron tetrachloride. When diboron tetrachloride decomposes spontaneously at temperatures more than 0° the main product is boron trichloride with lesser amounts of yellow B_4Cl_4 (ref. 2), red B_8Cl_8 (ref. 3) and dark red $B_{12}Cl_{11}$ (ref. 4); a dark purple solid has also been detected but in amounts too small to allow accurate classical analysis[4].

The diboron tetrachloride used in this work was prepared by reducing boron trichloride in a mercury discharge[5] and had been allowed to decompose slowly during a period of 20 months at room temperature. A series of fractional condensations and sublimations performed in a thoroughly baked-out, all-glass vacuum apparatus allowed separation of the several species; if the baking-out process had not been carried out sufficiently well traces of a permanganate-coloured hydrolysis deposit appeared on the vessel walls. A deep blue crystalline solid, volatile at about 40° (10^{-2} mm) was isolated in a virtually pure state and evaporated into a mass spectrometer at 49°; an intense group of mass peaks (407–428) was observed corresponding to the ion $B_9Cl_9^+$; the cracking pattern contained all the peaks for the ions $B_nCl_x^+$ where $n \geqslant x$ (Table 1). The amounts of this blue solid were too small to allow other analytical techniques to be used, but due to the similarity of the volatility and cracking pattern to B_9Cl_8H we suggest the solid is a new boron sub-chloride, nonaboronnonachloride, B_9Cl_9.

Table 1. Relative Intensities of Several Peaks in the Cracking Pattern of B_9Cl_9

Mass No. for $^{11}B_n{}^{35}Cl_x^+$		Relative intensities, $B_7Cl_5^+ = 100$
B_9Cl_9,	414	6·1
B_9Cl_8,	379	0·5
B_9Cl_7,	344	7·9
B_8Cl_8,	368	16·6
B_8Cl_7,	333	0·25
B_8Cl_6,	298	43·7
B_8Cl_5,	263	2·8
B_7Cl_7,	322	0·45
B_7Cl_6,	287	10·7
B_7Cl_5,	252	100·0
B_7Cl_4,	217	3·9
B_7Cl_3,	182	13·1
B_7,	77	0·3
B_6Cl_6,	276	0·4
B_6Cl_5,	241	7·2
B_6Cl_4,	206	16·7
B_6Cl_3,	171	50·0
B_5Cl_5,	230	*ca.* 1·5
B_5Cl_4,	195	*ca.* 1·5
B_5Cl_3,	160	25·6
B	55	0·9

Forstner, Haas and Muetterties[1] describe a group of peaks in the mass spectrum of B_9Cl_8H due to the ion $B_{10}Cl_{10}^+$ which they believe arose from recombination reactions in the mass spectrometer. The mass spectrum of one of our nonaboronnonachloride samples also showed peaks due to $B_{10}Cl_{10}^+$, $B_{10}Cl_9^+$ and $B_{10}Cl_8^+$. This spectrum was obtained at a lower pressure than those not showing $B_{10}Cl_{10}^+$ peaks, which would appear to rule out the possibility of recombination reactions since they would be expected to occur most readily at the highest pressures. There is therefore a distinct possibility that decaboron-decachloride, $B_{10}Cl_{10}$, exists as a stable entity and was present as an impurity in one sample of nonaboron-nonachloride. Products containing boron and chlorine in a ratio 1 : 1 are to be expected since diboron tetrachloride produces BCl radicals during its thermal decomposition[6].

We thank Dr. W. L. Mead and Dr. R. A. Saunders for recording the mass spectra on A.E.I. instruments (*MS*9 and *MS*8, respectively).

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Auxiliary Electrodes in Mosaic Membrane Systems: Porosity Mosaics

Any heterogeneity in the pore structure of an ionic membrane, such as differences in pore width, charge density at the pore walls, or the sign of these charges, renders the membrane electrochemically a mosaic[1]. When such a mosaic membrane is interposed between two electrolytic solutions of different composition its different parts will ordinarily give rise to different potentials. Consequently, the various parts of the mosaic interact electrically; spontaneous local circuits arise, somewhat analogous to those causing metal corrosion[1-3].

The small dimensions of the component parts of true mosaic membranes impede their direct physicochemical examination. Macromosaic models have largely overcome this difficulty[1,4-7]. The examination of macromodels can be greatly furthered by the introduction of a pair of current-carrying symmetrical auxiliary electrodes which do not contribute to the effective electromotive force of the system. This method has been applied recently to the measurement of the electro-osmotic water transport in 'sign of charge' mosaic systems[7]. We want to point out that such auxiliary electrodes are a tool of general usefulness in studying mosaic membranes, and demonstrate this in an additional instance, water transport investigations in 'width of pore' mosaics.

The general approach, technique, and apparatus used were the same as described in detail for a 'sign of charge' mosaic model[7]. The only difference was that, instead of two membranes of opposite charge, two membranes of the same charge but of different porosities were used. The model examined was:

Ag	AgCl	dilute electrolyte	dense membrane	concentrated electrolyte	porous membrane	dilute electrolyte	AgCl	Ag

←

the arrow indicates the flow of the positive current when electronegative, preferentially cation permeable membranes are used. This model is based on the fact that the water transport per faraday is larger across porous membranes than across denser ones[8]. The anticipated effect in this scheme is the difference between the larger flow (per faraday) across the porous membrane into the concentrated solution and the rather small outward movement across the dense membrane.

In such 'width of pore' models, we determined the rate of volume change of the concentrated solution on open and on closed circuit, and the current; the difference between the two rates is the expected electro-osmosis.

The membranes used were (electronegative) polystyrene sulphonic acid-collodion membranes[9], the denser ones being permselective and permeable to non-electrolytes having molecular weights less than 100. The more porous ones, prepared by swelling a permselective membrane in 97 per cent ethanol[10], were permeable only to molecules with molecular weights lower than 100,000. The concentration potential 0·1 M/0·01 M across such permselective membranes is +54 to +55 mV for KCl or LiCl; across the more porous membranes it is about +10 to +15 mV with KCl, and differs not much from zero with LiCl. LiCl was chosen as the electrolyte for the model experiments because of the large hydrated size of the Li^+. In electro-osmosis Li^+ carries more water per faraday than other univalent cations[7]; also, with LiCl there is a larger difference between the potentials arising across the dense membrane and across the porous membrane than with other uni-univalent salts. The solute concentrations were the same as previously[7]; LiCl was 0·2 M in the more concentrated solution, and 0·002 M in the dilute solution with 0·25 M sucrose added to minimize the effect of normal osmosis.

In one experiment with such a 'width of pore' mosaic model, a current of 1·3 m.amp was obtained; the concomitant electro-osmotic flow was 34 moles of water/faraday into the compartment of the concentrated solution. In another test the current was 1·9 m.amp accompanied by an electro-osmosis of 42 moles of water/faraday.

The results obtained with the 'width of pore' model give additional support, more convincing than that furnished before[1,4], to the concept that anomalous osmosis in ordinary membranes is due to heteroporosity[2]. In a true micromosaic the differences in pore size are likely to be considerably less than in our model; this would reduce the electro-osmotic effect. On the other hand, as stressed previously[7], the linear pathways of the current in micromosaic systems are smaller by two or more orders of magnitude, and the sum of the local currents and the concomitant water transport are correspondingly higher. This view of the mechanism of anomalous osmosis is not necessarily incompatible with Schlögl's very ingenious idea concerning this problem[11]. It does not seem improbable that the two mechanisms might be component parts of the basic mechanism which still awaits quantitative elucidation.

Auxiliary electrodes have now been applied successfully in the study of three different mosaic membrane systems, two previously reported[5,7] and one presented here. Thus it seems most probable that a variety of other effects in mosaic systems might be studied to advantage by the use of such electrodes. Obvious problems include: the transport of both electrolytes and of co-existing non-electrolytes in 'sign of charge' mosaics with various combinations of permselective and more porous membranes; the transport of electrolytes and of co-existing non-electrolytes in 'width of pore' mosaics.

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BIOPHYSICS

An Investigation of Soluble Ribonucleic Acid by Zone Electrophoresis

Structural and physical chemical investigations of transfer RNA, whether fractionated or not, in general require that the material be reasonably pure and essentially free from contaminant RNAs of different kinds. Most of the methods at present used for preparation of *s*RNA from the cell supernatant involve only removal of the proteins, and as Brown has pointed out in a recent review[1], the presence of contaminants in the form of other relatively low molecular weight RNA without transfer function is not to be ruled out. Nor has any method been available for the detection of such impuri-



Fig. 1. Polyacrylamide-gel electrophoresis of 'Sephadex G-100' fractions of yeast *s*RNA; *a–d* are successively eluted fractions from the column, and *e* is the unfractionated sample. The start line is indicated

ties, although an apparent sedimentation heterogeneity in a number of preparations has been noted[2,3]. It therefore appeared desirable to evolve techniques both for observing the purity of *s*RNA preparations, and of purifying them on a preparative scale if necessary.

The technique which we have developed to meet the first of these requirements is zone electrophoresis in acrylamide gels. High resolution of protein mixtures has been achieved in this medium, in virtue of a molecular sieve action, whereby larger molecules are retarded more than smaller ones[4,5]. In addition it is possible to take advantage of zone sharpening effects by selection of buffer systems containing ions of appropriate mobilities relative to each other[4].

We have used the disk electrophoresis procedure of Ornstein and Davis[5] with a *tris*-barbital buffer system of *p*H 8·9, and an acrylamide concentration of 10 per cent w/v. Staining was effected with a precipitating solution containing acridine orange, lanthanum acetate and acetic acid, and the stain was removed from the background electrophoretically. The resultant gels show orange zones in a clear background, and the relative proportions of the various components may be estimated by densitometry. A large number of samples have been examined, in particular of yeast *s*RNA; these were prepared by the now standard phenol extraction procedure of Holley[6]. A typical result with one such preparation by General Biochemicals, Inc., is shown in Fig. 1*e*. Many components are clearly present: in the absence of any evidence to suggest that different amino-acid specific transfer RNAs differ in size or shape, one must suppose that the electrophoretic separation is largely unrelated to amino-acid specificity. Extraction of the zones, followed by their examination in the analytical ultracentrifuge (Spinco model *E*, equipped with ultra-violet absorption optics), has shown that the migration rate through the gel is correlated with sedimentation coefficient.

Extraction from the polyacrylamide gel is possible; but this method does not lend itself to the purification of large quantities. We have therefore used 'Sephadex' gel filtration. The best results so far have been achieved by the use of 'Sephadex *G*-100'. The column-length is 150 cm, and the eluent 0·1 M sodium chloride. The flow rate with a column of 5 cm diameter is maintained at about 25 ml./h, and 250 mg of RNA may be fractionated in each experiment without much loss of resolution. The elution curve indicates partial resolution of a lower molecular weight component from most samples. The fractions making up the different regions of the elution profile were pooled, the RNA recovered by ethanol precipitation or dialysis and lyophilization, and aliquots were examined by polyacrylamide gel electrophoresis.

The results for the four major fractions are shown in Fig. 1, together with that for the unfractionated material. Fraction *c* represents the major portion from the centre of the peak, and it is seen to be very largely homogeneous, with the contaminants suppressed to a level which is probably acceptable for most purposes. Assays for arginine and serine acceptor activity revealed that the activity was predominantly concentrated in the main portion of the peak, fraction *c*. Fraction *d* shows substantial enrichment in the low molecular weight components, and the earlier fractions *a* and *b* are enriched in a remarkable series of higher-molecular-weight components which have been reproducibly present in all preparations of phenol-extracted yeast *s*RNA so far examined. The nature of these contaminants is not clear: the small components may be transfer RNA degraded by endogenous nuclease[7], or indeed similarly degraded ribosomal or messenger RNA. The higher molecular weight components may again be messenger or ribosomal degradation products; but the sharpness of these zones strongly indicates that they are each monodisperse, and may therefore represent other RNA species of at present unknown function. At least one such species has been previously reported[8], and it may be profitable to search among such minor nucleic acid components of the cell supernatant for specific inhibitor molecules, or perhaps ribosomal RNA precursors.

Fig. 1 shows clearly the separation which may be achieved by 'Sephadex' filtration, and at the same time emphasizes its limitations of resolution, when confronted with a mixture of relatively small molecules of rather narrow polydispersity. It is important in this connexion to emphasize that high molecular weight ribosomal RNA migrates very slowly under our conditions, and remains near the gel interface.

Very recently Schleich and Goldstein[9] have reported that 'Sephadex' fractionation of *E. coli* *s*RNA reveals the presence of inactive *s*RNA aggregates; on treatment with urea, disaggregation occurs, together with restoration of acceptor activity. We have found no evidence for such a phenomenon in our samples of yeast *s*RNA: the electrophoresis has been performed in the presence of 6 M urea, without heating, and the distribution of components remains unchanged. Similarly, heating the RNA in the presence of 4 M urea for 15 min at 70° C produced no change in the electrophoresis pattern. It is not yet clear whether the apparent absence of aggregation depends on the preparation of the samples, or in our use of yeast, rather than *E. coli*, *s*RNA.

The polyacrylamide gel electrophoresis technique, of which full details will shortly be published[10], would seem to have wider applications, and is at present being used for the investigation of degradation products of both transfer and ribosomal RNA.

We thank Miss Jacqueline Large, Mr. J. A. Coll and Mr. G. Forte for their assistance.

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BIOCHEMISTRY

Infra-nuclear Distribution of Transaminases

It has previously been shown[1] that isolated calf thymus nuclei prepared by the sucrose-$CaCl_2$ procedure[2] contain a free amino-acid pool. Of the free amino-acids, glutamic acid represents about 40 per cent. The presence of such a relatively large amount of glutamic acid suggests its rapid synthesis, or its formation by metabolic conversion from other substances, such as those resulting from transaminase-catalysed reactions. The intracellular distribution of transaminase activity has been extensively investigated, particularly in rat liver[3–6], and most of the enzymatic activity has been found in the mitochondrial fraction. Little is yet known, however, about the intranuclear distribution of transaminases. Techniques for separating nuclear proteins into various morphologically-defined fractions made possible the present investigation, undertaken to assay the calf thymus cells for two transaminase activities. It will be shown that the cell nucleus contains significant transaminase activities, mostly localized in the nuclear sap, resolvable by chromatography.

Calf thymus was homogenized in sucrose-$CaCl_2$ medium following the procedure of Allfrey *et al.*[2]. The cytoplasmic suspension was clarified by centrifuging twice at 2,500*g*, and the mitochondria were collected in the pellet centrifuged at 1,500*g*. The supernatant was ultracentrifuged to give the microsomes and the soluble fraction[7]. All cellular fractions were purified by repeated suspension and centrifugation.

Fractionation of nuclear proteins was carried out as previously described[7]. Briefly, the nuclei were extracted with buffered physiological saline and the resulting nuclear extract was ultracentrifuged. The pellet obtained from this centrifugation was treated with sodium deoxycholate to give the nuclear ribosomes and the deoxycholate-soluble (DOC-sol) fraction[7]; the supernatant, which contained the nuclear-soluble proteins and which constituted the bulk of the nuclear sap[8,9], was further separated into a *p*H 5 fraction and a *p*H 5 supernatant[7,10] by acid precipitation. The remaining nuclear residue, after washing with buffered saline, was afterwards extracted with 1 M NaCl to yield an NaCl extract and residual protein, the latter consisting of nucleoli and residual chromatin[9,11–13]. The 1 M NaCl extract was then diluted to 0·14 M with respect to NaCl to precipitate the DNA-histones, which are the chromatin substances[9,14]. The supernatant solution, designated as NH-sol[7], was lyophilized.

Cytoplasmic and nuclear fractions were obtained in this way and assayed for glutamic-oxaloacetic transaminase (GOT) activity according to the method of Lowry *et al.*[15], and for glutamic-pyruvic transaminase (GPT) activity by Lowry's method as described by Rosen *et al.*[16].

Table 1. Intra-cellular and Nuclear Activities of Transaminases of Calf Thymus

Fraction	Specific activity (μmoles/h/mg protein) Glutamic-oxaloacetate	Glutamic-pyruvate
Homogenate	7·7	0·47
Nuclei	2·3	0·13
Mitochondria	14·3	0·70
Microsomes	3·3	0·39
Soluble fraction	8·2	0·24
Nuclear fractions		
Soluble proteins	19·0	0·24
*p*H-5 fraction	0·0	0·0
*p*H-5 supernate	28·0	0·32
Ribosomes	1·5	0·17
DOC-soluble	2·1	0·13
NH-sol	2·6	0·02
Residual protein	2·4	0·09

* Average of two determinations.

Table 1 shows the intracellular and nuclear specific activities of GOT and GPT of the calf thymus cells. As can be seen, GPT activity is on the average 1/20 that of GOT among the cellular components. Mitochondria clearly have the highest specific activities. The activities of GOT and GPT of the nuclear fraction are 16 per cent and 19 per cent, respectively, of those represented by mitochondria. Transaminase activity of the nuclear fraction has generally been attributed to contamination by mitochondria, but nuclei containing 33 per cent of the total cellular GOT activity has also been reported[6]. Since the nucleus represents a greater part of the thymus cell, it is unlikely that most of the nuclear transaminase activities could be due to cytoplasmic contamination.

Unlike the microsomes, the cytoplasmic soluble fraction exhibited high GOT activity. High GOT activity in the soluble fraction of rat liver has been reported[3,4], although the contrary has also been noted[6]. Since the release of the enzymatic activity from mitochondria is possible only after considerable structural damage[3], the transaminase activities in the thymus soluble fraction may be regarded as significant.

Because the cytoplasmic soluble fraction contains considerable GOT and GPT activities, it seemed probable that topographically most of the nuclear transaminases might be localized in the nuclear sap. The assay results of GOT and GPT activities of the various nuclear fractions indicate that such is the case. It can be seen that the nuclear *p*H 5 fraction is devoid of either enzyme, and all the GOT and GPT activities present in the nuclear sap are located in the *p*H 5 supernate.

We have previously reported a number of enzymes associated with the nuclear ribosomes[17], like ribosomes from other sources. The presence of some GOT and GPT activity in the nuclear ribosomes (and the DOC-sol fraction) may similarly be interpreted as suggesting that the ribosomes are the site of protein synthesis. This may also include the residual protein fraction the possible involvement of which in protein synthesis has been demonstrated and suggested[7,12,18].

Like the residual protein, the DNA-associated NH-sol fraction contains low GOT and GPT activities. It has previously been shown that this nuclear protein fraction differs from the nuclear soluble proteins in preparation[19], in metabolic activities[7], and in chromatographic behaviour[20]. The present work further emphasizes their distinction by the localization of the transaminase activities. Since most of the GOT and GPT activities are associated with the nuclear soluble fraction, the nuclear soluble proteins were subjected to DEAE-cellulose fractionation[20], and the enzymatic activities of the column fractions were determined. Fig. 1 shows

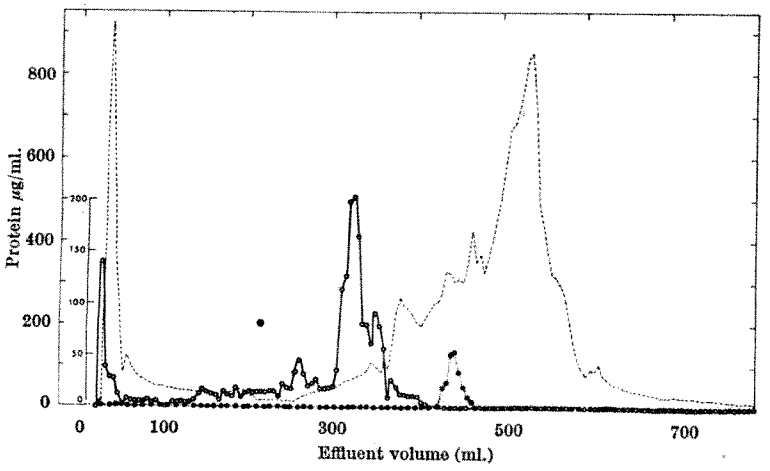


Fig. 1. Transaminase activities of nuclear soluble proteins chromatographed on DEAE-cellulose. Open circles: GOT activity, in μmoles/h/mg protein; closed circles: GPT activity, in × 10^{-2} μmoles/h/mg protein

such a chromatogram. It can be seen that all GOT and GPT activities are eluted before the major peak. GPT is completely separated from GOT as a single peak at higher salt elution, while GOT activity appears in several locations eluted with very low salt concentration, including the unadsorbed peak. Similar experiments made on the NH-sol fraction showed that the transaminase activities were localized only in the unadsorbed buffer peak. Such separation of GOT activity may be indicative of the existence of different molecular species of the enzyme (isozymes), or of the combination of the enzyme with other proteins.

This work was aided by U.S. Public Health Services grant *RG*–7923, the National Science Foundation grant *G*–19573 (T. Y. W.), and St. Louis University Cancer Research Institutional Award 24–19–708 (K. M. W.).

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Failure of Physiological Concentrations of Epinephrine to affect Glycogen-levels in the Isolated Rat Liver

ALTHOUGH it is generally believed that the hyperglycæmia which follows the administration of epinephrine is due, at least in part, to hepatic glycogenolysis, attempts to demonstrate a direct effect of epinephrine on liver glycogen-levels have led to conflicting conclusions. In the rat, for example, Cori, Cori and Buchwald[1] found that a decrease in liver glycogen occurred during the first 15 min after subcutaneous epinephrine injection, and considered this to represent a direct action of epinephrine on the liver. On the other hand, Sherlock[2] found that portal veining infusions of epinephrine did not affect liver glycogen- or blood glucose-levels and concluded that the hormone did not exert a direct effect on hepatic glycogen metabolism of rats. We repeated and extended the experiments of Cori, Cori and Buchwald and also observed the transient hepatic glycogenolysis which they had described[3]. However, we presented evidence that this represented an indirect rather than a direct effect of epinephrine and suggested that it might be due to stimulation of endogenous glucagon release.

Because of the difficulty in distinguishing between primary and secondary events in experiments with intact animals, we have investigated the effects of different concentrations of epinephrine on glycogen-levels of the isolated perfused rat liver. This experimental system permits the investigation of hepatic glycogenolysis under precisely controlled conditions, uncomplicated by secondary hormone secretion, lactate release from muscle, or other events which often make interpretation of animal experiments difficult. It is superior to liver slice techniques for the study of glycogenolysis[4]. In previous work with Miller[4,5] we demonstrated that both glucagon and epinephrine were active in this system; however, the concentrations of these hormones then tested were far above any encountered in Nature.

Livers from well-fed male Sprague–Dawley rats were excised and perfused with approximately 120 ml. of diluted heparinized rat blood to which antibiotics had been added, using techniques and apparatus similar to those described by Miller *et al.*[6]. Blood entered the liver by free flow through a cannula in the portal vein, at a pressure of 13–15 cm of blood. A cannula in the inferior vena cava returned blood to the reservoir, whence it was recirculated through the liver. In all experiments, an equilibration period of at least 30 min was allowed after initiation of the perfusion. At various intervals thereafter, minor hepatic lobes were removed for glycogen determination, and a final specimen was obtained from the right lobe at the conclusion of the experiment. It was usually possible to obtain 4 or 5 liver samples for analysis. Samples of the perfusing blood were withdrawn at regular intervals for glucose determination. Solutions of synthetic epinephrine or crystalline glucagon were added to the blood reservoir after baseline liver and blood samples had been obtained. This technique was used rather than continuous infusion into the system[4,5] so that the peak concentrations of these agents reaching the liver could be known with certainty. Thus, when glycogenolysis was observed, it was possible to affirm that it was initiated by a specific concentration of hormone. The volume of blood in the system was large enough to maintain hormonal levels near this original concentration for 8–15 min before inactivation in the liver exerted a major effect. Epinephrine was diluted in rat plasma rather than saline to protect it from oxidation, and the dilute solutions were prepared just before use.

In control experiments, the blood glucose concentration, which had risen initially to about 200 mg per cent because of glycogenolysis during the operative procedure and establishment of the perfusion, remained stable or declined slowly after the equilibration period. Liver glycogen values ranged from 1 per cent to 4 per cent (wet weight basis) and remained quite stable during 3 h of perfusion. Variations in glycogen concentration among successive samples from the same liver averaged 3 mg/g (0·3 per cent) and rarely exceeded 5 mg/g.

Various amounts of epinephrine were added to the blood reservoir after appropriate baseline samples had been obtained for glycogen and glucose determinations. Single or multiple additions of 0·1–3·0 μg (yielding peak plasma concentrations of approximately 1·4–40 μg/l.) exerted no measurable effect on either liver glycogen-levels or the glucose concentration of the perfusing blood (Fig. 1). Slight to moderate vasoconstrictor effects were observed at the higher concentrations, however; in one perfusion, for example, the hepatic blood flow dropped from 11 ml./min to 6 ml./min after the addition of 3 μg of epinephrine.

When 10 μg of epinephrine was added to the blood reservoir (resulting in a peak concentration of approximately 140 μg/l. of plasma) small increases in blood glucose (20–40 mg per cent) and borderline decreases in liver glycogen concentration (2–5 mg/g) were seen. Multiple additions resulted in cumulative effects (Fig. 2). This concentration also produced substantial reductions of the blood flow rate. In order to differentiate between a specific effect of epinephrine on glycogen metabolism and a non-specific effect due to reduction of blood flow and hepatic anoxia, experiments were performed in which

phentolamine was added to the blood reservoir before this amount of epinephrine. This virtually abolished the vasoconstrictor effect, but slight glycogenolysis was still observed. Higher concentrations of epinephrine could not be tested without the use of a blocking agent because blood flow virtually ceased. With phentolamine, however, it proved possible to test the effect of 30 μg (peak plasma concentration, approximately 400 μg/l.). This induced distinct glycogenolysis, as is seen in Fig. 3.

To confirm the sensitivity of this test system, experiments were performed in which small amounts of glucagon were added to the blood reservoir. These investigations, which will be reported in detail elsewhere[7], indicated that concentrations of glucagon within the range which has been observed *in vivo*[8] are glycogenolytic for the isolated rat liver. Fig. 4 presents the data from one perfusion of this series. It is evident that glycogenolysis induced by 0·3 μg of glucagon (peak plasma concentration, approximately 4 μg/l.) was as intense as that following the 100-fold greater amount of epinephrine.

These experiments confirm the conclusions previously reached from *in vivo* investigations[3] that epinephrine can exert a direct glycogenolytic effect on the liver, but that very high concentrations of this hormone are required for such action. The threshold plasma level needed for minimal hepatic glycogenolysis in this investigation appeared to be about 140 μg/l. This is far higher than any concentration known to occur spontaneously in mammalian species, and also well above values observed after intense stimulation of adrenomedullary secretion. Heiffer, Mundy and Mehlman[9] found that the baseline arterial plasma epinephrine concentration of rats was 0·7 μg/l., and observed peak levels of about 50 μg/l. after pharmacological stimulation with cystamine. The resting level of epinephrine in the circulation of dogs[10] and humans[11] is also less than 1 μg/l. of plasma. It is elevated to concentrations of 2–3 μg/l. during the stress of insulin-induced hypoglycæmia[10,12] and during infusions of epinephrine at a rate rapid enough to induce cardiovascular effects and distress symptoms[13]. The highest systemic plasma epinephrine concentration ever recorded in man, to our knowledge, was 14 μg/l., observed by Goldfien *et al.*[11] in a patient with a pheochromocytoma, during its surgical removal. Although there are species differences in sensitivity to epinephrine, it seems unlikely that dog and human livers should be 50 times more sensitive than that of the rat to the glycogenolytic effect of this hormone. Thus, it is probable that the direct contribution of epinephrine to hyperglycæmia, in these species also, is limited to its peripheral effects.

We conclude that while glucagon may serve as a physiological glycogenolytic agent for the liver, epinephrine cannot. Furthermore, it is unlikely that even moderate pharmacological doses of this hormone would

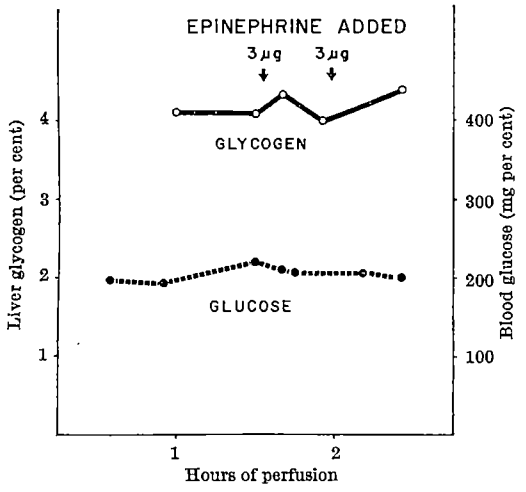


Fig. 1. Failure of two additions of 3 μg of epinephrine (yielding peak concentrations of 40 μg/l of plasma) to stimulate glycogenolysis in the isolated liver

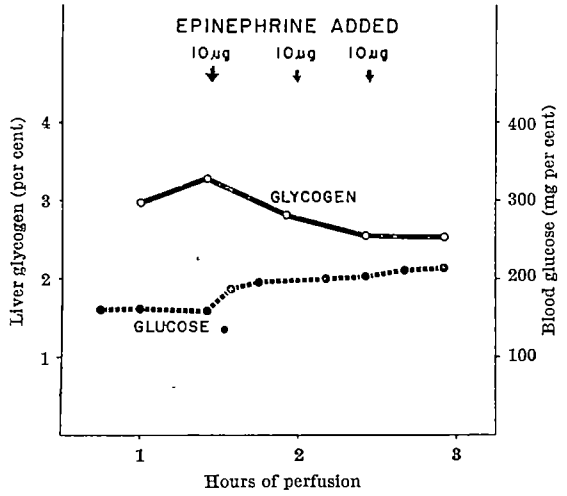


Fig. 2. Slight glycogenolytic effect of 10 μg of epinephrine (peak plasma concentration, 140 μg/l.)

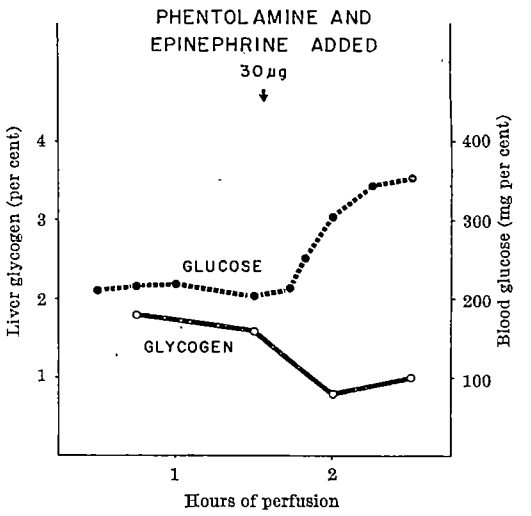


Fig. 3. Glycogenolytic effect of 30 μg of epinephrine (peak plasma concentration, 400 μg/l.). Phentolamine was added before epinephrine in order to reduce the intense vasoconstriction caused by this concentration of the hormone

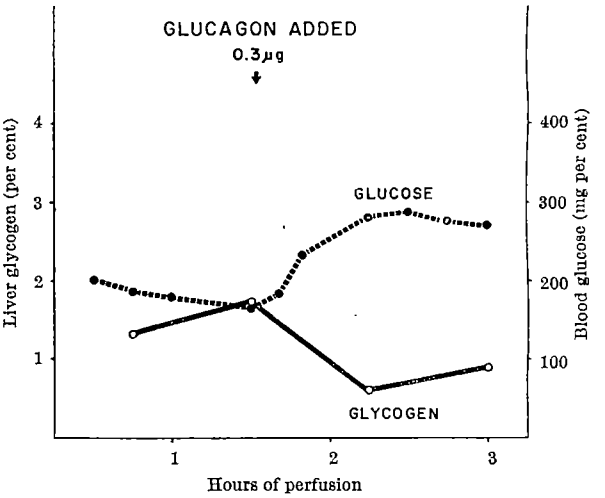


Fig. 4. Glycogenolytic effect of 0·3 μg of glucagon (peak plasma concentration, 4 μg/l.), demonstrating the sensitivity of this test system

result in sufficiently high plasma concentrations to exert a direct effect on hepatic glycogen metabolism.

This work was supported in part by grant *A*–1646 from the National Institute for Arthritis and Metabolic Diseases, U.S. Public Health Service.

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Lactic Dehydrogenase Isozymes and Ageing of Erythrocytes

DURING the life span of erythrocytes several of their enzymes undergo some modification[1–3]. Lactic dehydrogenase (LDH) is of special interest, because of the knowledge we have of its isozyme composition[4,5]. To investigate whether ageing affects each isozyme to the same degree, the electrophoretic pattern of LDH isozymes of young and old red cells were compared.

Young and old red cells from rabbits were separated by centrifugation in the presence of bovine serum albumin. In this procedure the upper layer consists of young cells, whereas the lower layer contains the heavier, older red cells. In one experiment, leucocytes (the density of which is similar to that of young red cells) were eliminated by agglutinating the red cells with phyto-agglutinins followed by slow centrifugation to remove the leucocytes. The clotted red cells were then hæmolysed after washing.

Both layers of hæmolysed cells were submitted to starch-gel electrophoresis, in borate buffer according to Smithies[7]. Isozymes were visualized by nitro blue tetrazolium salts and phenazine methosulphate, according to Markert[8] and Nachlas[9].

Under these conditions, two main anodic bands were found for both old and young red cells; they moved faster than hæmoglobin, and the faster one was the broader: these fractions seem to correspond to fractions 4 and 5.

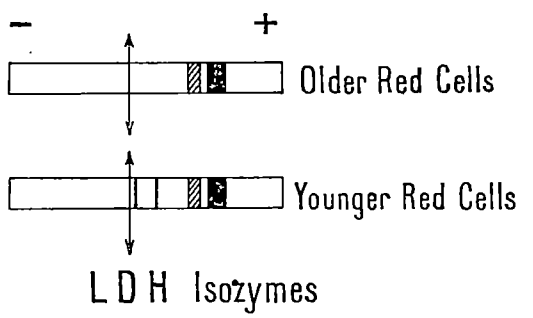


Fig. 1. LDH isozymes in older and younger red cells

In younger cells, two additional faint bands were observed; these were also anodic, but slower than the main bands. The migration rate of one of them was close to that of hæmoglobin. The same pattern was seen in the experiment where white cells were removed by centrifugation after agglutinating the red cells. Vesell[5] has pointed out that in many preparations of human hæmolysates, he found a faint supplementary band of slower mobility.

It seems that this band, which does not always occur, corresponds to the bands which we see only in younger cells. Cahn and Kaplan[10] have proposed that the various bands of lactic dehydrogenase are hybrids of four subunits of two different types. We have seen that there are two bands found in younger red cells which do not appear in older ones; it seems that these isozymes are preferentially inactivated or destroyed during the life span of the red cells.

This work was supported by the Institut National d'Hygiène, Caisse Nationale de Sécurité Sociale, Centre national de la Recherche Scientifique, Délégation Générale à la Recherche Scientifique et ses Comités Scientifiques (Fonds de Développement), France, The National Institute of Arthritis and Metabolic Disease, and The Division of General Medical Science, U.S. Public Health Service (grants *AM*–02773 and *GM*–06016).

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Electrophoresis of an Insect Inorganic Pyrophosphatase

IN our work on the properties of inorganic pyrophosphatase in the boll weevil, *Anthonomus grandis* Boheman[1], we used the enzyme present both in whole homogenates and in an acetone powder. We have since attempted electrophoresis of the enzyme to determine whether activity was present in one or more resolvable components.

All preparations and electrophoretic studies were made at 2° C. Approximately 1,000 frozen adult weevils were homogenized with cold acetone, and an acetone powder was prepared. After removal of the solvent and drying *in vacuo* in the refrigerator, the dry filter cake was extracted with demineralized water for 0·5 h and filtered through glass wool. The filtrate was centrifuged (20,000*g*) to remove particulate debris and other matter, and the clear supernatant was lyophilized. The lyophilized powder was used as the enzyme source.

Separation of the soluble components was achieved by paper electrophoresis, by means of a Durrum-type hanging paper strip electrophoresis cell (Spinco electrophoresis system).

The following conditions were found to give excellent separation in 4 h at 2° C: Buffer, veronal–HCl *p*H 8, 0·02 M; buffer volume, 1,100 ml. (550 ml. each in the (+) and (−) compartments); 3·5 m.amp, at 100 V; sample sizes, 1–3 mg of lyophilized powder in 10 μl.

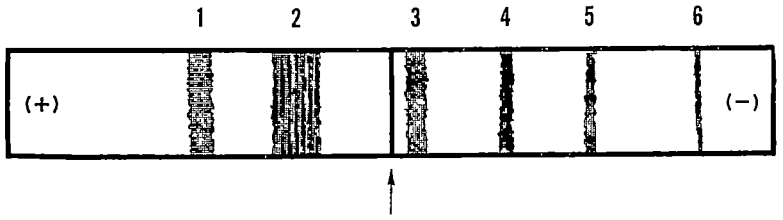


Fig. 1. Diagrammatic sketch of the ninhydrin-positive components in water extract of the boll weevil acetone powder. The arrow marks the line of application

of demineralized H_2O; 1·6 mg satisfactorily combined resolvability of the enzyme activity and colour intensity on the sprayed strips.

After 4 h of electrophoresis the strips were removed. Half of them were dried at 110° C for 0·5 h, sprayed with 0·2 per cent ninhydrin in acetone, and re-dried for 10 min. The remaining half, used for enzyme analysis, were frozen immediately after electrophoresis and stored at −20° C. The enzyme was assayed by incubating appropriate portions of each strip in a reaction mixture consisting of 0·2 ml. of 0·01 M $MgCl_2$, 0·5 ml. of 0·0025 M pyrophosphate, and 0·3 ml. of veronal–HCl buffer, 0·02 M, *p*H 8, for 2 h. Zero time control and termination of the reaction were achieved with boiled samples and also with certain sulphydryl inhibitors.

Fig. 1 illustrates the resolvable ninhydrin-positive components. Two bands migrated toward the anode (+), and four toward the cathode (−). The development of the bands was very consistent and reproducible. More than 100 separate analyses were made with negligible variation. In several instances electrophoresis was conducted for 6 h, but no further fractionation was observed. The same six bands were detected, but were spread over a wider area, and their centres had migrated a distance 50 per cent farther away from the starting application line than those strips run for 4 h.

The darkest ninhydrin-coloured band was band 2, while cathodic bands 5 and 6 were very pale. Colour intensity of all bands was in direct proportion to the amount of extract applied.

Table 1. Electrophoretic Separation of Inorganic Pyrophosphatases from Lyophilized Water Extract of Boll Weevil Acetone Powder
Enzyme activity: μg phosphorus released/ml. of reaction mixture/2 h at 35° C

Band No.	Charge	Enzyme activity	Percentage of total activity
1	(−)	0·092	2·53
2	(−)	3·097	85·24
3	(+)	0·276	7·60
4	(+)	0·193	5·31
5	(+)	−0·025*	Nil
6	(+)	—	Nil

* Uptake of free orthophosphate.

Once reproducible separations were achieved, it was necessary to determine which band(s) would hydrolyse, inorganic pyrophosphate. By incubating the cathodic and anodic halves of the strips in pyrophosphatase reaction mixture and afterwards analysing for orthophosphate[2] we determined that the enzyme was migrating electrophoretically to the anode; but we still did not know whether it was in band 1 or band 2, or whether any of the cathodic bands had a slight pyrophosphatase activity.

By separate analysis of each band, its position determined by comparison with alternate ninhydrin-developed strips, we were able to show that only band 2 consistently possessed enzyme activity. Pyrophosphatase activity was also found occasionally in bands 1, 3 and 4 (Table 1); but these bands sometimes yielded no activity. Band 5 was negative and actually used up small amounts of the free phosphate present at the start of the reaction. Band 6 was also negative. The high pyrophosphatase activity in band 2 accounted for approximately 85 per cent of the total detectable activity.

Four inhibitors of inorganic pyrophosphatase (iodoacetate, fluoride, alloxan, and Cu^{++} ions) were used in place of boiling water to obtain zero time and 2-h readings. All four were effective in blocking the enzyme action of all the bands.

These data suggest that the boll weevil enzyme extract has four alkaline inorganic pyrophosphatases capable of producing orthophosphate from pyrophosphate, but that the majority of this activity is located in an anionic protein (band 2).

The presence of a complex assortment of phosphatases is not unusual. Others have shown that phosphatases in general, including inorganic pyrophosphatases[3,4], can exist as a group of several isoenzymes capable of acting on a single substrate. To determine whether or not these four active bands represent four distinct enzymes will require a complete description and comparison of their individual characteristics.

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Metabolic Fate of 2,4-Dichlorophenoxyacetic Acid in the Stem Tissue of *Phaseolus vulgaris*

In this Department previous investigations[1,2] of the metabolic fate of a series of chlorinated phenoxyacetic acids in stem tissues of *Avena sativa* have demonstrated that the formation of glucose esters or hydroxylation followed by conversion to a glucoside are major pathways. In this tissue 2,4-dichlorophenoxyacetic acid (2,4-D) is converted principally into 1-0-(2,4-dichlorophenoxyacetyl)-β-D-glucose. In contrast, in the stems of *Phaseolus vulgaris* the principal metabolite isolated was seemingly a glucoside of a phenolic acid[3] which was not a hydroxy-2,4-dichlorophenoxyacetic acid[3–5]. Further investigation has identified the presence of a mixture of 2,5-dichloro-4-hydroxyphenoxyacetic acid and 2,3-dichloro-4-hydroxyphenoxyacetic acid.

The following procedures were used for the isolation of the aglycones. The whole of the aerial portions of seedlings of *P. vulgaris* var. 'Canadian Wonder', grown in a greenhouse for 12 days, were gathered and the cut ends immersed in a solution of 2,4-D-^{14}C (30 mg/l.) at *p*H 5 for 12 h in the light. After a further 24 h in the dark the stem and petiolar tissue was macerated and extracted with hot 20 per cent aqueous ethanol. The solvent was removed and the residue partitioned between hydrochloric acid (5 per cent) and ether. The ether solution contained about 85 per cent of the carbon-14 and was shown to consist almost entirely of 2,4-D. The dilute acid solution after saturation with ammonium sulphate was extracted with butanol and the residue, following the removal of the butanol, dissolved in a minimum quantity of methanol. A considerable amount of material containing very little carbon-14 was precipitated from the methanol solution by the addition of four volumes of ether; the supernatant liquid was evaporated to dryness and treated with β-glucosidase in aqueous solution at *p*H 5. The resulting ether soluble aglycone was freed from non-radioactive material by paper chromatography using isopropanol/ammonia (*d*, 0·88)/water (10 : 1 : 1) and then chloroform saturated with 50 per cent aqueous acetic acid as developing solvents. In this way 11 mg of a crystalline solid was obtained from 4·5 kg of stems.

There was some evidence that this product was not homogeneous, and thin-layer chromatography on silica

gel using ether/light petroleum (b.p. 60°–80° C)/formic acid (50 : 50 : 2) effected a separation of two phenolic acids having R_Fs 0·45 and 0·3 present respectively in a ratio of approximately 10 : 1.

While this work was in progress it was shown by Faulkner and Woodcock[6] that 2,5-dichloro-4-hydroxyphenoxyacetic acid was a product of the metabolism of 2,4-D in *Aspergillus niger*. Comparison of the major constituent of the phenolic acid mixture from *Phaseolus* with authentic 2,5-dichloro-4-hydroxyphenoxyacetic acid showed that they were identical.

The minor constituent proved to be identical with authentic 2,3-dichloro-4-hydroxyphenoxyacetic acid prepared from 2,3-dichlorohydroquinone[7].

The criteria for verification of the identity of these two acids with authentic materials are given in Table 1.

Table 1

Compound	R_F	Diazodye colour	λ_{max} *p*H 2	λ_{max} *p*H 11	M.p. of methyl-ether	Spectra
2,3-Dichloro-4-hydroxyphenoxy-acetic acid	0·3	burgundy red	295 mμ	318 mμ	—	ultra-violet
2,5-Dichloro-4-hydroxyphenoxy-acetic acid	0·45	beige brown	298 mμ	315 mμ	165–6°C	ultra-violet and infra-red

It is now apparent that the intriguing shift of the chlorine atom in 2,4-D by *Aspergillus* can be brought about by a higher plant. In *Phaseolus*, however, the products are accumulated as the β-glucosides so that the possibility of the occurrence of secondary reactions during the extraction processes is unlikely. Again the possibility of any considerable contamination of the 2,4-D used with 2,3-dichlorophenoxyacetic acid or 2,5-dichlorophenoxyacetic acid is remote. These impurities have been specifically sought for and are not present in amounts exceeding 0·5 per cent, which is the limit of resolution of the methods used.

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Rose Bengal De-iodination *in vitro*

Rose bengal as well as bromsulphalein and indocyanin green are utilized for the study of liver chromo-excretion. Changes of these dyes in the organism were intensively studied and it was ascertained that bromsulphalein is conjugated with glutathione and excreted in the bile. Similar metabolites were not proved for rose bengal and indocyanin green. In earlier work we described the isolation of the pure tetra-chloro-tetra-iodo-fluorescein from the commercial preparations of rose bengal[1,2]. We ascertained further that up to 8 per cent of ^{131}I is split off after the intravenous application of rose bengal, labelled with ^{131}I, to rats, and that it is excreted as a colourless compound in the urine. We have since studied the de-iodination of rose bengal-^{131}I *in vitro* by homogenates of heart, liver and bone muscle in conditions described by Georgi and Hartmann[3] for thyroxine and di-iodotyrosine de-iodination. Our work was stimulated by a certain similarity in chemical structure between rose bengal and these compounds. We homogenized the

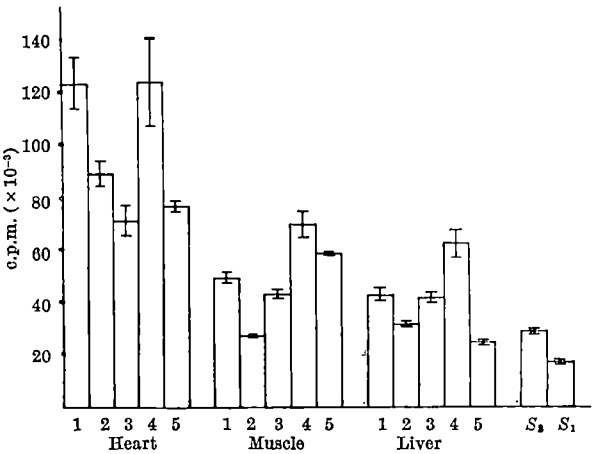


Fig. 1. Counting rates of supernatants from individual homogenates. 1, 3 ml. of homogenate + 0·5 ml. of rose bengal-^{131}I, incubated for 24 h at 37° C. 2, 3 ml. of homogenate + 0·5 ml. of rose bengal-^{131}I, not incubated. 3, 3 ml. of homogenate + 0·5 ml. of rose bengal-^{131}I + sodium pyruvate (its final concentration in solution is 1 mmole/ml.), incubated for 24 h at 37° C. 4, 3 ml. of homogenate heated to 100° C + 0·5 ml. of rose bengal-^{131}I, incubated for 24 h at 37° C. 5, 3 ml. of homogenate heated to 100° C + 0·5 ml. of rose bengal-^{131}I, not incubated. S_2, 0·5 ml. of rose bengal-^{131}I + 3 ml. of phosphate buffer, incubated for 24 h at 37° C. S_1, 0·5 ml. of rose bengal-^{131}I + 3 ml. of phosphate buffer, not incubated

organs (heart, liver, muscle) from 10 rats by pulverizing with liquid air and mixing with 0·2 phosphate buffer at *p*H 8·0 so that the total organ concentration was 0·03 g/ml. calculated for the wet organ weight.

The homogenates were distributed in 3-ml. portions into centrifuging tubes, and 0·5 ml. solution of rose bengal-^{131}I with specific activity 12 μc./ml., was added. The homogenates of each organ were then distributed between 5 groups. The first group was incubated for 24 h at 37° C, the second group was not incubated, and the third group contained sodium pyruvate in concentration 1 mmole/ml. and was incubated for 24 h at 37°. The fourth and fifth groups contained homogenates previously heated to 100° C. Rose bengal and proteins were precipitated with concentrated hydrochloric acid and separated by centrifugation. The radioactivity of 2 ml. aliquots of the supernatant was measured by the scintillation counter with the NaI (Tl) well-crystal. The results are expressed as the mean of 10 samples with introduced middle square deviations (Fig. 1). It is clearly demonstrated that more ^{131}I is split off by tissue homogenates than by the buffered solution of rose bengal-^{131}I alone. The quantity depends on the organ, and decreases in the order: heart, muscle, liver. Boiling the homogenates before mixing with the rose bengal effects no reduction, and even enhances the effect in the case of the muscle and liver homogenates. The addition of sodium pyruvate affects the ^{131}I splitting inhibition, particularly in the case of heart homogenate. Thus, rose bengal behaves similarly to thyroxine or mono-iodoacetic acid in contrast to di-iodotyrosine, the de-iodination of which is increased by the admixture of pyruvate[3]. The mechanism of rose bengal de-iodination is not entirely clear. From the fact that it is produced by the untreated and by boiled homogenates, and since the effect of the boiled homogenate is even higher in some cases, it cannot be assumed that de-iodination is controlled by a sensitive enzymatic system. These results suggest that a thermostable factor is present in the organs, in different concentrations. It is in accord with some earlier studies, which demonstrate that de-iodination takes place also in the presence of flavine mononucleotide[4–6], or of the other low-molecular fluorescent substances (fluorescein, eosine, acriflavine)[5]. It has also been demonstrated[6] that some proteins have a stimulative effect on the de-iodination. Differences in the effect of flavine mononucleotide, in the different tissues studied, are perhaps to be explained by the

activating or inhibiting effects of different proteins within these tissues.

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Influence of Red and Far-red Light on the Photo-oxidation of Ascorbic Acid and Photoreduction of its Oxidized Forms

Photo-oxidation of ascorbate and photoreduction of its oxidized forms have been demonstrated in leafy tissues[1,2]. In these investigations evidence was obtained that photoreduction was a separate photochemical reaction from photo-oxidation, being differentiated from the latter by: (1) a different time sequence in its initiation by light; (2) its insensitivity to cyanide; (3) its more heat-labile nature; (4) the ability of red light (710–720 mμ) to activate photo-oxidation but not photoreduction.

Our attention was first directed to the possibility that these reactions might be influenced by previous exposure of the leaf to light of different wave-lengths, when we observed that both reactions occurred at a much greater rate on illumination of darkened leaves if these had previously been exposed to daylight rather than to incandescent light. The rate of photo-oxidation of ascorbate stimulated by the activating light immediately following a dark period (10–30 min) after daylight was five times greater than the corresponding rate observed in leaves which had been exposed to a short period (5 min) of light from a tungsten lamp (5,000 lux) prior to the dark period.

We have now demonstrated with the use of suitable filters that these photochemical reactions, activated by light energy of the visible spectrum, are themselves conditioned by the previous exposure of the leaf to either red or far-red light. When strawberry leaves after a period in the dark (30 min) following daylight illumination are exposed to the green activating light (100 lux of wave-length 500–550 mμ) complete photoreduction of the dehydro-L-ascorbate in the darkened leaf occurs within a period of 4–5 sec, and this is followed by a rapid photo-oxidation of ascorbate until a new steady-state level is reached. If, however, following a dark period (30 min) after removal from daylight the leaf is illuminated for 5 min with far-red light (700–900 mμ, light source, tungsten) and this again followed by a further dark period (10 min), on subsequent illumination with green activating light only slight photoreduction and no photo-oxidation is observed. It might be noted that unfiltered incandescent light gives total inhibition of both processes. In fact the leaf in this condition behaves entirely like a darkened leaf. Conversely, if the far-red light is replaced by red light (600–690 mμ; light source fluorescent) the leaf responds normally and shows a rate of photoreduction and oxidation equal to that observed after exposure to daylight (Fig. 1). Similar effects were found with unfiltered fluorescent light.

We were also able to show that the inhibition of photoreduction and oxidation induced by exposure of the leaf

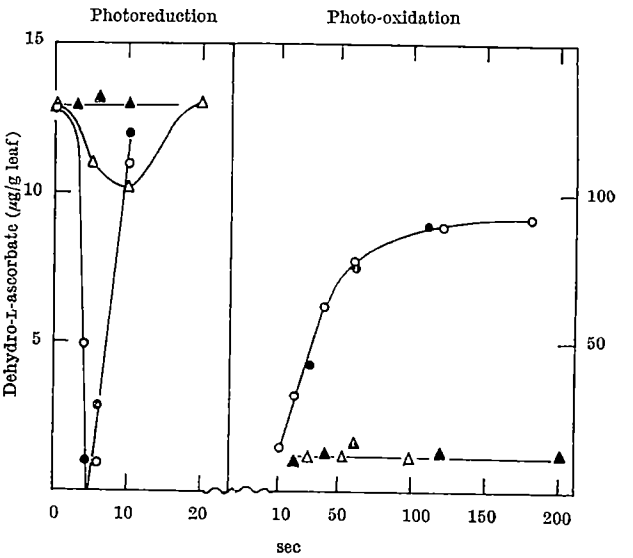


Fig. 1. Inhibition of photoreduction and photo-oxidation by far-red light. Pre-treatment of leaf, ▲, *DL*→*D*–30→*TL*–5→*D*–10→*AL*; △, *DL*→*D*–30→*FR*–5→*D*–10→*AL*; ○, *DL*→*D*–30→*R* or *FL*–5→*D*–10→*AL*; ●, *DL*→*D*–30→*FR* or *TL*–5→*D*–5→*R* or *FL*–5→*D*–10→*AL*. *DL*, Daylight; *R*, red light (600–690 mμ); *D*, dark; *FR*, far-red light (700–900 mμ); *TL*, incandescent light (5,000 lux); *AL*, activating light (500–550 mμ) 100 lux; *FL*, fluorescent light (5,000 lux)

to far-red light may be easily reversed by subsequent exposure of the leaf to red light (Fig. 1), and the converse is equally true, namely, that the activating effect of red light may be reversed by subsequent exposure to far-red light. Similarly, the effect of tungsten light can be reversed by fluorescent light.

Finally, we were also able to show that the photoreduction, which alone may be observed in leaves poisoned with cyanide (5×10^{-5} M) (ref. 1), is also inhibited by prior exposure to far-red light, but promoted with prior exposure to red light (Fig. 2).

These results suggest some connexion between the reactions of the photosynthetic electron transfer chain, the ascorbate system, and phytochrome. At present the results suggest that visible light energy activated by the photosynthetic or other photochemical system can drive

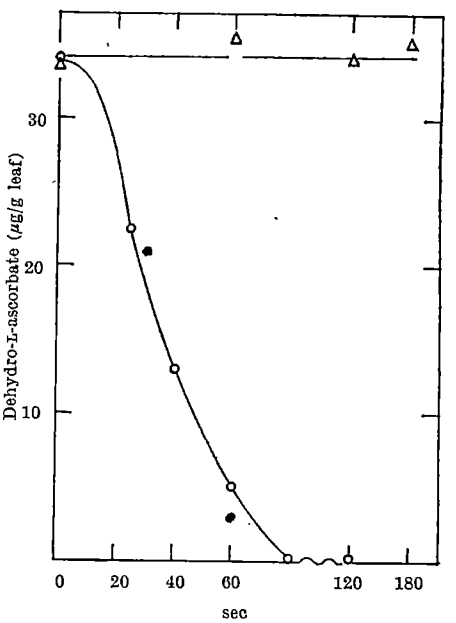


Fig. 2. Photoreduction in cyanide-poisoned leaves. Pre-treatment: ○, *DL*→*D*–30→*AL*; △, *DL*→*D*–30→*TL*–5→*D*–10→*AL*; ●, *DL*→*D*–30→*FL*–5→*D*–10→*AL*

reactions only if an active phytochrome or similar system is present.

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Determination of 2-Phenylethanol in Cider

Methods used for the determination of 2-phenylethanol in fermented beverages have utilized solvent extraction of the beverage or of a distillate, followed by measurements of absorption spectra[1–3] or by gas chromatography[4–8]. The compound is not included in the determination of fusel oils by the colorimetric procedures usually adopted[8,9]. The methods described have certain disadvantages: preliminary extraction may be incomplete unless prolonged, absorption measurements may involve previous purification of the fraction containing 2-phenylethanol, and in the gas chromatographic methods described the retention time of the compound may exceed 1 h with column temperatures above 100°. The method to be described is simple in operation and has been found satisfactory for cider: by the use of a column of low capacity of the type described by Hishta *et al.*[10] the retention time of 2-phenylethanol has been reduced to 8 min.

Cider (50 ml.) was distilled without fractionation and 25 ml. of distillate collected; 25 ml. of water was added to the flask and a further 25 ml. distillate collected. The process was repeated and the third distillate containing only traces of 2-phenylethanol collected separately. To the third distillate was added sodium chloride (3 M) and it was then extracted at about 5° C with two volumes each of 15 ml. ethyl chloride. These extracts were used to extract the main distillate after addition of sodium chloride; it was then extracted twice further with 10 ml. ethyl chloride. The combined ethyl chloride solutions were washed with minimal volumes of water, freed from water droplets by passage through a layer of sodium sulphate (anhydrous) and concentrated to one-third volume by distillation of the solvent. The solution was transferred to a weighed tube, 2 ml. of 50 per cent aqueous ethanol added and the ethyl chloride removed by a slow stream of dry air. The solution, of known weight, was used directly for gas chromatography, using 5 μl. injections.

The apparatus used for gas chromatography was built in this laboratory and consisted of a 122 cm × 4·5 mm. internal diam. glass column packed with 0·2 per cent 'Apiezon *L*' on 60 mesh (A.S.T.M.) glass micro-beads, run isothermally at 80° with flame ionization detector. The flow-rate was approximately 15 ml./min with nitrogen–hydrogen in 1 : 1 mixture as carrier gas. Under these conditions 2-phenylethanol gave a reasonably narrow peak so that the amount present could be estimated from peak height taking as standards solutions of 2-phenylethanol in 50 per cent aqueous alcohol that gave peaks of comparable size.

The retention times relative to benzene of the main fusel oil components found in cider[11,12] were, as shown below, well separated from that of 2-phenylethanol; they all emerged within 2 min, whereas the latter gave a peak maximum at 7 min 42 sec.

Table 1. Fusel Oil Components of Cider and their Retention Times (relative to benzene)

Methanol	1·32
Ethanol	1·37
1–Butanol	1·59
1–Hexanol	5·05
2–Methyl-1-propanol	1·39
3–Methyl-1-butanol	2·90
β–Methyl-propyl ethanoate	1·75
γ–Methyl-butyl ethanoate	8·45
2–phenylethanol	44·85

The chromatograms showed two main peaks, the more volatile components forming one unresolved peak, the second being that corresponding to 2-phenylethanol. There is as yet no evidence for the presence of other cider components contributing to the second peak. The chromatograms were readily reproducible and estimates of the amount of 2-phenylethanol present did not vary by more than ± 5 per cent.

Extraction with ethyl chloride gave recoveries of 2-phenylethanol exceeding 90 per cent from synthetic fusel oil solutions corresponding to the composition of cider, or from distillates of the solution. Continuous extraction with *n*-pentane required more than 14 h for completion; direct extraction with ether or ether–pentane mixtures gave recoveries below 80 per cent. Persistent emulsions were formed when cider itself was shaken with solvents. The extraction of cider distillates with ethyl chloride was therefore adopted, this being the most efficient and selective solvent.

As the method of determination is based solely on a comparison of retention time with that of a standard, a semi-quantitative isolation of 2-phenylethanol from cider was made using a sample estimated to contain 100 p.p.m. The distillate from cider (1,560 ml.) was extracted with ether (5 vol. of 200 ml.) and the ether extract washed with N caustic soda and with water. The ether was removed, the liquid residue (10 ml.) extracted with ethyl chloride and the extract washed with water and dried. After removal of the solvent the liquid residue (3·1 g) was distilled in a slow air stream at 24 mm and 50° C until the residue had no odour of amyl alcohols. On raising the temperature to 70° a fraction with an odour of 2-phenylethanol condensed in the distillation head, from which it was removed with ether; 90 mg of this material was collected. 50 mg converted to the 3 : 5 dinitrobenzoate gave an 88 per cent yield of crude product, giving 62 mg of m.p. 105° C, raised by re-crystallization to 107°; mixed with an authentic specimen of m.p. 107° C the m.p. was unchanged. The infra-red spectra of the two specimens were identical.

Table 2. 2-Phenylethanol Content of Ciders

No.	Fermentation	p.p.m.
1	Yeasted	9
2	,,	7
3	,,	9
4	,,	15
5	Not yeasted	100
6	,, ,,	102

The amounts of 2-phenylethanol found in ciders were found to vary widely (as shown in Table 2); the amounts in yeasted fermentations were below 20 p.p.m., whereas sulphited juices fermented by members of the natural yeast flora (Nos. 5, 6) gave products containing about 100 p.p.m. A figure of 121 p.p.m. for a cider has been found by Morgan[13].

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PHYSIOLOGY

Participation of Rat Liver Nuclei in Movements of Sodium

In vitro investigations of movements of sodium with muscular tissue seem to have shown that the non-extracellular sodium belongs to more than one phase[1]. The existence of either a membranous and a cellular phase[2] or of two cellular phases with different degrees of order[3] has been discussed. We wish to present some experimental findings which may well contribute to the interpretation of sodium transfer processes in animal tissue.

Previously, it was reported[4] that in rat liver nuclei, isolated by the non-aqueous technique[5], the sodium concentration is 10 times that of the cytoplasm: 160 μmoles Na^+/g dry nuclei or 72 μmoles Na^+/g fresh nuclei, compared with 16 μmoles Na^+/g dry cytoplasm or 6 μmoles Na^+/g fresh cytoplasm. Since nuclei comprise about 10 per cent of the liver cell, total intranuclear sodium roughly equals total cytoplasmic sodium.

Relations between extracellular and intranuclear sodium now were investigated with the aid of sodium-22. Rats which were deprived of food and drinking water for 16 h received 5 μc. sodium-22 in 0·1 ml. saline by injection into the jugular vein. After different times, whole blood, serum and liver were obtained from each of 50 animals per group. The livers were processed for nuclei as already described[5]. Sodium concentrations were determined by flame photometry, and the radioactivity by means of a scintillation counter. The experimental conditions, especially adapted for the work with nuclei, neither permit a shorter time-interval than 10 min nor a calculation of transport rates. The error in timing for taking blood and liver samples is about 15 per cent with the 10 min experiment but less than 2 per cent with all other time-intervals. Due to a breakdown of a scintillation counter during the experiments, another instrument had to be used for the 10 min readings which appear to be too low; a correction was not made. However, at each given time, the figures for blood, serum and nuclei are quite comparable with each other.

Table 1. Specific Activities of Blood, Serum, and Liver Nuclei of Rats after Administration of Sodium-22

1	2	3	4
10	710	820	860
30	910	985	1,125
60	810	840	855
120	685	670	710
240	650	590	610
480	585	555	575

Each experimental group consisted of 50 rats. The figures for nuclei are mean values of two separate determinations in the pooled livers. The figures for blood and serum are mean values from 25 samples, obtained of each two rats, determined twice.

1, Time-interval between injection of ^{22}Na and death (min). 2, Specific activity of sodium in blood (μc./μequiv Na^+). 3, Specific activity of sodium in serum (μc./μequiv Na^+). 4, Specific activity in isolated nuclei (μc./μequiv Na^+).

As can be seen in Table 1, the specific gravity of intranuclear sodium is found at the same level as the specific activity of serum sodium during the whole time-interval of 10 min–8 h after injection of sodium-22. Therefore, extracellular and intranuclear sodium reach their equilibrium within a surprisingly short time. It is rather improbable that this rapid equilibration occurs by a passage of extracellular sodium through the cellular membrane–cytoplasm–nuclear membrane since a remarkable difference in potential at the nuclear membrane has been described[6]. More likely, extracellular sodium enters the nucleus via channel-like structures which directly connect the extracellular with the intranuclear space, such as the endoplasmatic reticulum.

Obviously, it is not possible from these experiments with liver nuclei to draw definite conclusions with respect to muscle nuclei. However, in the interpretation of sodium fluxes in muscular tissue, it is worth considering muscle nuclei as possible separate compartments which actually contain much of the intracellular sodium and the link of which with the extracellular space may well be a more direct one than that of the cytoplasm proper.

This work was supported by the Deutsche Forschungsgemeinschaft.

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PHARMACOLOGY

Lowering Effect of Neostigmine on Plasma Free Fatty Acids

The actions of a number of drugs provide evidence that the sympathetic nervous system functions in the mobilization of free fatty acids. Epinephrine, norepinephrine and isoproterenol cause an elevation of free fatty acid levels whereas blocking drugs such as dichloroisoproterenol[1], pronethalol[2] and *N*-isopropyl methoxamine[3,4] have been reported to block the elevation of free fatty acid due to injection of one or more of these sympathomimetic substances. The present communication describes the ability of another type of agent, the cholinesterase inhibitor neostigmine methylsulphate, to lower fasting levels of plasma free fatty acid in the conscious dog and to antagonize the epinephrine-induced release of free fatty acid from the rat epididymal fat pad *in vitro*.

The dogs used were fasted for about 20 h. Blood samples were taken according to the times noted in Table 1 and plasma free fatty acids were measured by the method of Dole[5]. The same dogs were used for treatment and control investigations. At least two weeks were allowed to elapse between experiments on individual animals. As shown in Table 1, each of the animals under control conditions demonstrated the moderate elevation of plasma free fatty acid which is usual in fasted dogs subjected to repeated

Table 1. Effect of Neostigmine on Fasting Levels of Plasma Free Fatty Acid in the Conscious Dog

Dog No.	Treatment	Neostigmine methylsulphate (μg/kg s.c.)	FFA (μequiv./l.) change from control level 1 h	2 h	3 h
KC–4	Saline	—		+210	+435
KC–4	Neostigmine	3·5	−163	−219	−417
KC–5	Saline	—	—	+172	+257
KC–5	Neostigmine	3·5	−162	−368	−306
C–6	Saline	—	+30	+150	+274
C–6	Neostigmine	7·0	−200	−266	+234
C–6	Neostigmine	28·0	−266	−532	+190
C–12	Saline	—	−43	+197	+197
C–12	Neostigmine	7·0	−397	−311	−243
C–12	Neostigmine	28·0	−134	−588	+30
C–14	Saline	—	+118	+260	+288
C–14	Neostigmine	14·0	−303	−119	−199
C–33	Saline	14·0	+129	+271	+361
C–33	Neostigmine	14·0	−380	−66	+172

The values at the various time-intervals represent the change in plasma free fatty acid levels from a control value obtained separately for each dog and for each experiment by averaging the values from the first and second blood samples. These samples were taken 30 min apart. Neostigmine and saline were injected at 0 time immediately after taking the second blood sample.

venipunctures. When neostigmine was administered to these animals, it caused in each instance a significant fall in plasma free fatty acid. Although there is evidently a rather wide variability in the response of individual animals to neostigmine it is noteworthy that dogs C-6 and C-12 experienced a larger fall from 28 μg/kg than from 7 μg/kg. No signs of parasympathetic stimulation, such as defæcation or salivation, were noted in any of the dogs receiving neostigmine.

The *in vitro* studies were done on epididymal fat pads removed from rats weighing approximately 180 g. The results are shown in Table 2. It is evident that neostigmine is much more active than carbachol in blocking the epinephrine-induced release of free fatty acid. A comparison of the present results with previous experiments done in the same manner in these laboratories[3] indicates that neostigmine prevents the release of free fatty acid in much lower concentrations than dichloroisoproterenol, pronethalol or *N*-isopropyl methoxamine.

Table 2. Effect of Neostigmine and Carbachol on the Epinephrine-induced Release of Free Fatty Acid from the Rat Epididymal Fat Pad

Exp. No.	Blocking drug*	Molar concentration of blocking drug	Epinephrine induced release of FFA†	Inhibition of FFA release (%)
1	None	—	3·43 ± 0	—
	Neostigmine methylsulphate	10^{-7}	0·58 ± 0	91
		10^{-9}	1·16 ± 0	72
2	None	—	3·05 ± 0·06	—
	Carbachol	10^{-6}	1·68 ± 0·20	55
		10^{-7}	2·29 ± 0	31

* All incubations contained $2·7 \times 10^{-5}$ M L-epinephrine.

† Free fatty acid release is expressed as μmoles/fat pad/h of incubation ± *S.E.* for 3 incubations. Release of free fatty acid in both experiments in absence of epinephrine and blocking drug was 0·44 ± 0·10. The release of free fatty acid by tissue incubated only with neostigmine (10^{-7} M) was 0·40 ± 0·1 and 0·84 ± 0·02 for tissue incubated only with carbachol (10^{-5} M).

It would seem unlikely, considering the known actions of neostigmine, that its effects in the present study are due to sympathetic blockade. The same would hold true for the *in vitro* effects of carbachol. A more likely explanation would appear to be that some sort of cholinergic mechanism is involved. In this connexion it should be noted that carbachol, although its primary action is on postganglionic parasympathetic effector sites, can also be demonstrated to possess anticholinesterase activity[6]. Cholinesterase, largely in the form of pseudocholinesterase, has recently been found to occur in high concentrations in fat depots[7]. It is of interest also that the parasympathetic blocking drugs, atropine and methantheline, will prevent the fall in fasting levels of plasma free fatty acid in the conscious dog caused by *N*-isopropyl methoxamine. An interpretation which would explain these effects of atropine and methantheline and which would also be consistent with the effects of neostigmine and carbachol in the work recorded here is that there are cholinergic receptors associated with the release of free fatty acid and that, when these receptors are stimulated, inhibition of release occurs.

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Urethane Hyperglycæmia and Hypothalamic Activation

An unexpectedly high blood glucose-level was found in rats which had been anæsthetized with ethyl urethane in the course of routine biochemical assays. The blood glucose-level of fasting rats rose from 58 ± 1 mg per cent (mean ± *S.E.*; $n = 40$) to 167·7 ± 7·6 mg per cent 1 h after intraperitoneal injection of 1·25 g/kg urethane.

Intraperitoneal or intravenous injection of anæsthetic doses of urethane produced a similar degree of hyperglycæmia in rabbits and cats. In dogs, only a small and short-lasting rise in blood glucose was found; this is obviously due to the known fast metabolism of urethane in this species, which is also the reason for the absence of an anæsthetic effect.

The fact that urethane produces hyperglycæmia is not widely known. Underhill[1] suggested an unusual sensitivity towards the hyperglycæmic action of adrenaline as the cause of the glycosuria, and Bang[2] claimed pancreatic degeneration to be responsible for the hyperglycæmia.

The wide use of urethane as an anæsthetic in physiological and pharmacological experiments warranted, it was felt, further experimental studies to elucidate the mode of action of the urethane hyperglycæmia. Anæsthetics commonly used in animal experiments such as hexobarbitone, 'Nembutal' and chloralose did not produce hyperglycæmia (Fig. 1).

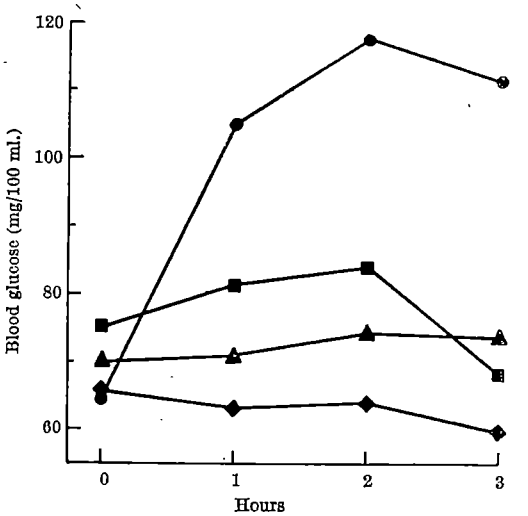


Fig. 1. Blood glucose-levels after administration of ●, urethane (1·25 g/kg intraperitoneally; $n = 5$), ■, hexobarbitone (50 mg/kg intravenously; $n = 3$); ▲, 'Nembutal' (40 mg/kg intravenously; $n = 3$); ◆, chloralose (80 mg/kg intraperitoneally; $n = 3$) to fasting rabbits

The urethane hyperglycæmia could be due to a number of mechanisms such as: direct action on glycogenolysis; increased sensitivity to adrenaline; direct stimulation of the adrenal medulla; increase of central sympathetic activity.

No effect of urethane on *in vitro* glycogenolysis could be found. The urethane hyperglycæmia is absent in rabbits after pretreatment with dibenamine or hexamethonium and in cats after adrenalectomy, cutting of the splanchnic nerves or despinalization. Intramuscular administration of 15 mg/kg guanethidine to rabbits for two weeks reduced but did not abolish the hyperglycæmia (Fig. 3). Guanethidine administered under identical conditions will decrease the noradrenaline concentration in peripheral tissue but not the catecholamine of the adrenal medulla or the noradrenaline of the hypothalamus[3]. Pre-treatment with 2·5 mg/kg reserpine injected intramuscularly 48 and 24 h before anæsthetic doses of urethane abolishes the hyperglycæmia (Fig. 2). Pretreatment with reserpine at this dose-level decreases the amine levels of the adrenal medulla, the central nervous system and the peripheral

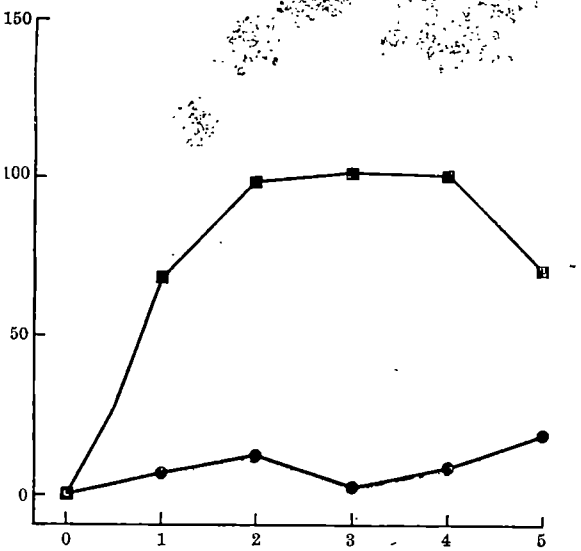


Fig. 2. Rabbit. Ordinate: percentage change of blood glucose from pre-dose level after 1·25 g/kg urethane (intraperitoneal). Abscissa: h. ■, Urethane ($n=7$); ●, after pretreatment with 2·5 mg/kg reserpine intramuscularly 48 and 24 h before urethane

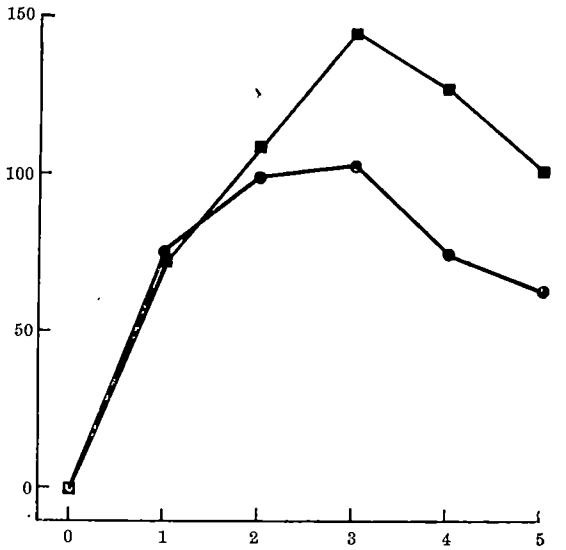


Fig. 3. Rabbit. Ordinate: percentage change of blood glucose from pre-dose level after 1·25 g/kg urethane (intraperitoneal). Abscissa: h. ■, Urethane ($n=4$); ●, after pretreatment with 15 mg/kg guanethidine intramuscularly on alternate days for 2 weeks

tissues. These results exclude an indirect action (sensitization to circulating amine) or a direct action (release of amine) as causes of the hyperglycæmia, but they imply an action via a centrally mediated adrenal catecholamine release. Vogt[4] demonstrated that hypothalamic activation with certain drugs is reflected in a decrease of hypothalamic noradrenaline as well as adrenal catecholamine. If urethane acts via hypothalamic (or general limbic) activation, then, to accord with Vogt's view, hypothalamic noradrenaline and adrenal catecholamine should decrease.

Reserpine (5 mg/kg), urethane (1·25 g/kg), sodium diethyl barbiturate (225 mg/kg) and saline were injected intraperitoneally into different groups of rats 16 h before they were killed by decapitation. The noradrenaline content of the hypothalamus was determined using the method described by Merrills[5]. The results obtained are shown in Fig. 4 and clearly reflect the decrease of hypothalamic noradrenaline after urethane and reserpine while no change from the control-level was seen after sodium diethyl barbiturate. The adrenal catecholamine content after urethane was 63·5 per cent of the control value and 42·5 per cent after reserpine. If it is accepted that the decrease of hypothalamic noradrenaline reflects enhanced central sympathetic activity, then an increased activity in efferent sympathetic neurones should be measurable.

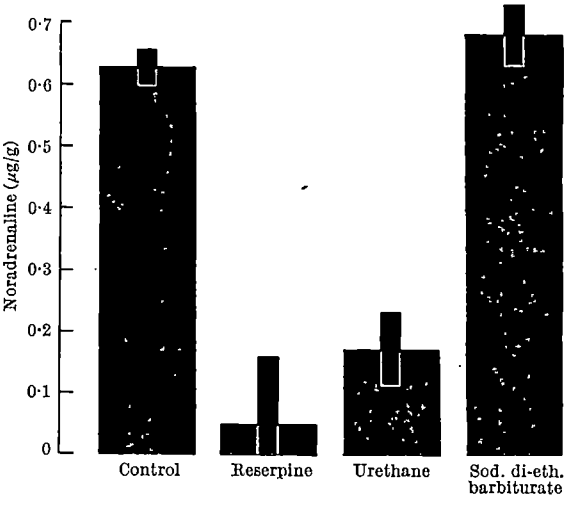


Fig. 4. Hypothalamic noradrenaline-levels (μg/g) of rats 16 h after intraperitoneal injection of saline ($n=20$); 5 mg/kg reserpine ($n=8$); 1·25 g/kg urethane ($n=8$) and 225 mg/kg sodium diethyl barbiturate ($n=8$)

The spontaneous spike discharge in filaments of the preganglionic cervical sympathetic of cats under 'Fluothane', N_2O–O_2 anæsthesia was recorded using Iggo and Vogt's[6] procedure and criteria. In numerous experiments a progressive increase of spike activity was found after 0·2–0·8 g/kg urethane (intravenous). The firing rate of the preganglionic neurones increases immediately after injection and, like the hyperglycæmia, remains raised for several hours (Fig. 5). The intravenous injection of 0·2–0·8 g/kg urethane results in a fall in blood pressure lasting 5–10 min. After this short and initial fall a progressive increase of the blood pressure follows. This is possibly due to continuous recruitment of vasoconstrictor activity through increased central sympathetic discharge. Baroreceptor reflex mechanisms are, therefore, not the cause of the increased preganglionic sympathetic activity. The activation by an initial small dose can be further increased by subsequent injections of the same or larger amounts of urethane.

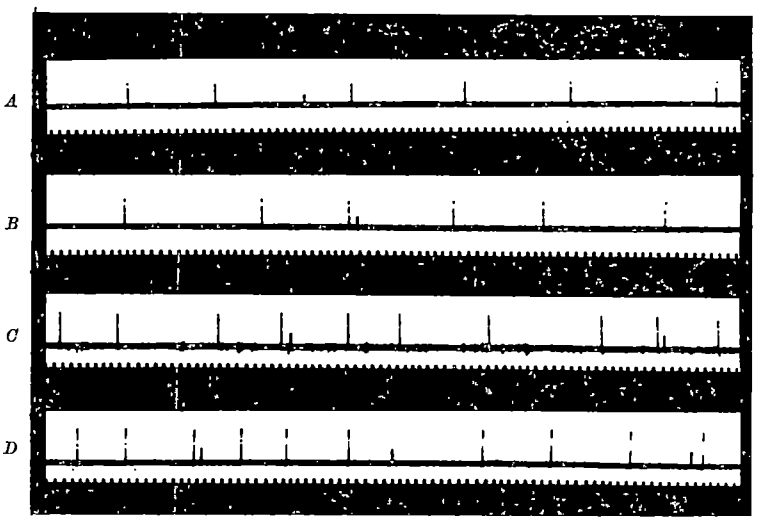


Fig. 5. Cat. 'Fluothane', N_2O/O_2 anæsthesia. Record of spontaneous spike discharge from filament of the cervical sympathetic. *A*, Control period; *B*, 10 min after intravenous injection of 0·2 g/kg urethane; *C*, 30 min later; *D*, 60 min later. Time signal, 100 msec

The hyperglycæmic action of urethane can, therefore, be envisaged as hypothalamic (or general limbic) activation resulting in increased descending sympathetic discharge and enhanced catecholamine secretion from the adrenal medulla. The activating effect of urethane on sympathetic areas in the central nervous system may be relevant for the interpretation of neurophysiological, neuro-endocrine or cardiovascular experiments when this compound is used as a general anæsthetic.

I thank Mr. J. Offerman for the biochemical noradrenaline assays.

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Prevention of Cerebral Œdema induced by Triethyltin in Rabbits by Cortico-steroids

PATIENTS receiving cortico-steroid therapy who underwent craniotomy were observed to have a smoother post-operative course than would have been expected[1]. Surgeons were thus led deliberately to administer cortico-steroid preparations before and after craniotomy, in order to ease the patient's course. In particular, patients diagnosed as having cerebral œdema, either from a tumour or more diffuse cause, seemed to benefit[2], and the improvement is explained on the assumption that this treatment reduces the cerebral œdema. The most recent preparation used has been dexamethasone since it is supposed to have minimal water- and sodium-retaining properties.

The assumption that patients improve because the cerebral œdema is reduced is based on analogy with the systemic anti-inflammatory properties of these hormones. Little experimental work exists to verify this hypothesis. One pathological and chemical investigation showed no effect of cortisone acetate on local brain œdema caused by cold injury[3]. Experimental focal œdema due to implantation of *Psyllium* seeds did not respond to steroids[4], nor did the anoxic ischæmic œdema due to carotid ligation[5]. The effects of triethyltin (TET) on the electrolyte content and fine structure of cerebral white matter have been extensively investigated[6,7].

The present experiment is in the nature of a pilot investigation to see whether large doses of cortico-steroid (dexamethasone) could modify the expected diffuse cerebral œdema from injections of triethyltin sulphate.

New Zealand white rabbits weighing between 2·5 and 3·5 kg were used. Six rabbits received TET, 0·5 per cent solution, in a dosage of 1 mg/kg daily for six days by intraperitoneal injection, together with dexamethasone 0·4 per cent solution, 0·75 mg/kg daily by intramuscular injection. This dosage of dexamethasone corresponds to cortisone acetate 25 mg/kg, a maximal dose. Six rabbits received dexamethasone alone during the same period. Also for controls, normal rabbits and rabbits receiving TET alone were examined.

After six days these animals were killed by means of exsanguination. The brains were removed intact, and immediately placed in a high humidity chamber (relative humidity 90 per cent). Four samples of grey matter and four samples of white were dissected under a dissecting microscope. Each was weighed on a microbalance, using weighed aluminium foil planchets, to give the wet weight of the sample. The fragments were then desiccated overnight at 100° C and reweighed to give dry weights. Digestion with conc. HNO_3 preceded flame photometry (with a lithium standard) to estimate Na and K values. The Na/K ratio was then calculated. Samples of serum were withdrawn from each rabbit at death and their electrolyte content and osmolarity were also measured. A Fiske osmometer was used.

The most striking feature was the clinical condition of the animals receiving both TET and steroid. Usually, animals treated with TET are progressively more lethargic, with quadriparesis, and roughening and discoloration of their fur by the fifth day of drug administration. The rabbits in this experiment remained quite lively, with smooth white fur, and two animals showed only very slight weakness. The animals on steroid alone appeared perfectly healthy.

At death, the brains from the rabbits receiving simultaneous steroid and TET appeared slightly œdematous. One brain from an animal on steroid alone appeared quite swollen while the others had normal gross appearance.

The most remarkable changes in brain electrolytes produced by TET intoxication are in the white matter (Table 1). As has been previously reported[6], the water content of the white matter was increased by 109 per cent (dry weight 28·4 per cent in normals and 16·9 per cent in TET animals, converted to g H_2O/100 g dry white matter). In addition, the sodium content of the white matter was increased by 174 per cent (56·3 m.equiv./kg in normals to 91·7 m.equiv./kg in TET animals, converted to m.equiv./100 g dry white matter). The potassium content in the white matter showed only a 14·8 per cent decrease on similar dry weight calculation. The sodium/potassium ratio was changed from 0·685 in normal white matter to 2·21 in TET intoxicated animals. The grey matter showed no significant changes (Table 1).

Table 1. EFFECTS OF GLUCOCORTICOID ON BRAIN ELECTROLYTES IN NORMAL AND TRIETHYLTIN-TREATED RABBITS

	Normal		Steroid treated		TET intoxicated		Simultaneous TET and steroids	
	Grey	White	Grey	White	Grey	White	Grey	White
No. animals/samples	10/23	10/23	5/20	5/20	3/12	3/12	6/24	6/24
Dry/wet × 100	18·7*	28·4	18·2	28·6	18·9	16·9	19·0	24·7
Na m.equiv./kg wet	58·9	56·3	54·4	51·3	47·4	91·7	54·4	71·9
K m.equiv./kg wet	91·0	82·3	86·7	73·0	93·8	41·8	87·1	60·3
Na/K	0·643	0·685	0·629	0·673	0·506	2·21	0·673	1·24

* Mean.

Table 2. RECALCULATION OF ELECTROLYTE DATA WITH DRY WEIGHT REFERENCE

	g H_2O/100 g dry white matter	m.equiv. Na/100 g dry white matter	m.equiv. K/100 g dry white matter
TET intoxicated	491*	54·3	24·7
Simultaneous TET	304	29·1	24·4
Difference	187	25·2	0·3
Per cent change	38·2	46·5	1·2

* Mean.

The administration of dexamethasone alone produced no significant changes in electrolyte content of the brain (Table 1). Simultaneous administration of dexamethasone and TET produced less change in the water and electrolyte content of the white matter than TET alone (Table 1). In comparing the TET-treated rabbits with those receiving simultaneous TET and dexamethasone, there was a 38·2 per cent reduction in water content and 46·5 per cent reduction of sodium content in the white matter (Table 2) calculated on basis of g of water or m.equiv. Na/100 g dry white matter. The potassium showed no significant changes. Again, all the changes were limited to white matter.

Perusal of the results reveals that simultaneous administration of TET and dexamethasone prevents the accumulation of sodium and water in white matter usually seen in TET intoxication. This accumulation provides criteria for describing the resulting brain œdema. Using these criteria, it is permissible to state that the dexamethasone has (partially) prevented the occurrence of cerebral œdema.

The sodium and water are probably retained largely within the intramyelinic vacuoles of white matter[6]. It is

clear that the systemic anti-inflammatory properties of glucocorticoids have not affected their suppression of œdema. Further work is required to find out how the steroid molecule has acted on the neural tissue, or if its action has been on TET excretion.

In conclusion, rabbits were treated with triethyltin sulphate intraperitoneally, concurrently with dexamethasone intramuscularly. A group of rabbits received the dexamethasone solely, as controls.

The clinical condition of the animals receiving both drugs compared very favourably to rabbits intoxicated by TET alone.

The water and sodium content of white matter from simultaneously treated rabbits was considerably less than that in rabbits intoxicated by TET.

Dexamethasone alone caused no significant changes in sodium or water content.

The expected cerebral œdema was partially prevented by the use of dexamethasone.

This work was supported by grant *MS* 303 from the National Multiple Sclerosis Society and grants *CA*–06264 and *NB* 1450 from the National Institutes of Health.

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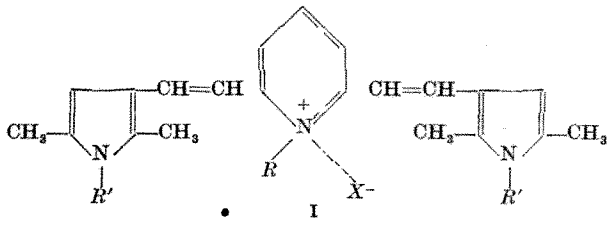
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Dipyrrylvinyl Pyridines as Trichuricides

Whipworm infections in man and dog have been, until recent years, quite difficult to eradicate. During the past decade several compounds have been found to have activity against these worms. These compounds are 'Phthalofyne' orally[1] or intravenously[2], dithiazanine iodide[3], stilbazium iodide[4,5], and 'Glycobiarsol'[6]. However, most of these drugs require large doses in mg/kg or a series of doses, or they have undesirable side-effects, or the worm loss is not high enough. Among the numerous modifications of stilbazium iodide[4] which were prepared, a number of members of the dipyrryl vinyl pyridines were found to have activity against the dog whipworm, *Trichuris vulpis*.

The compounds have the following basic structure (I):



I

Numerous variants of the 2,6-*bis* pyrrylvinyl pyridine quaternary ammonium salts, (I), have been made in which R = CH_3—C_5H_{11}, benzyl, etc., and R' = various alkyl, substituted alkyl, aryl, and substituted aryl groups. Details of the syntheses and activities of all these products will be published elsewhere.

In general, the most active members of this type are those in which R is C_2H_5 to C_4H_9–*n* and R' is C_2H_5 or $CH(CH_3)_2$. These compounds, when given in single doses of 15–25 mg/kg, have eliminated whipworms from most of the dogs treated, have been quite active against the mouse pinworm, *Syphacia obvelata*, and the dog ascaris, *Toxocara canis*, but have had little or no activity against three species of dog and cat hookworms.

Four of these compounds are being used extensively in the treatment of dog whipworm and other concomitant infections, for these four proved to be among the most active and least toxic of the series. When compared with the activity of stilbazium iodide against *T. vulpis* infections[6], these require fewer and smaller doses.

Inasmuch as rats tolerate single doses of nearly 2 g/kg and the usual dose for a dog or cat is between 15 and 25 mg/kg, there is a wide margin of safety. The compounds are tasteless, and emetic activity is slight.

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HÆMATOLOGY

A Second Example of – – –/– – – Blood or Rh_{null}

The blood of a white female (Mrs. L. M.) was submitted for testing following the birth of her first infant, who was slightly anæmic at the age of 2–3 weeks. The maternal serum contained anti-e. Her red cells failed to react with 5 anti-D, 5 anti-C, 13 anti-E, 14 anti-c, 9 anti-e, 1 anti-f, 2 anti-C^w, 1 anti-C^x, 3 anti-Ce(rh_i), 1 anti-hr^s, and 1 anti-G. The red cells also failed to react with the sera of four immunized –D–/–D– individuals. Her phenotype can be represented as – – –/– – – or as suggested by Ceppellini[1] as "Rh_{null}". The other antigens—A_1, N, s, k, Fy(a + b –), Jk(a –), Le(a – b +)—appeared to be normal.

This blood represents the second example of the phenotype – – –/– – –. The first one was described by Vos, Vos, Kirk and Sanger[2] in an Australian Aborigine who had no living relatives.

Fortunately the propositus is a member of a large family and all bloods were of the probable genotypes R^1R^1 and R^1r. Available members of the family tree are given in Fig. 1, which shows that 12 out of 16 members of both sides of the family exhibited weak reactivity for Rh antigens, D, C, c and e by titration scoring values as compared with appropriate control bloods. The depressed reactivity applied also to the compound antigens ce(f) and Ce(rh_i). Only four of the 16 members gave normal Rh reactivity. These include two paternal uncles II–6, II–8, one maternal uncle II–15, and one sibling (III–1) of the propositus.

For sake of brevity the titration scoring values of the red cells of the parents, their four offspring including the propositus, her husband and their child are presented in summarized form in Table 1. Normal activity is represented by 'N' and suppressed activity by 'w'.

Table 1 shows that only one sibling III–1 and the husband gave normal reactivity. All others, excluding the propositus, exhibit weak reactions. These include the parents, who are not related to each other. The Rh_{null} propositus like her three sibs could be either R^1R^1 or R^1r. Her genotype is represented as [R^1R^1] or [R^1r], the brackets indicating the absence of detectable Rh antigens.

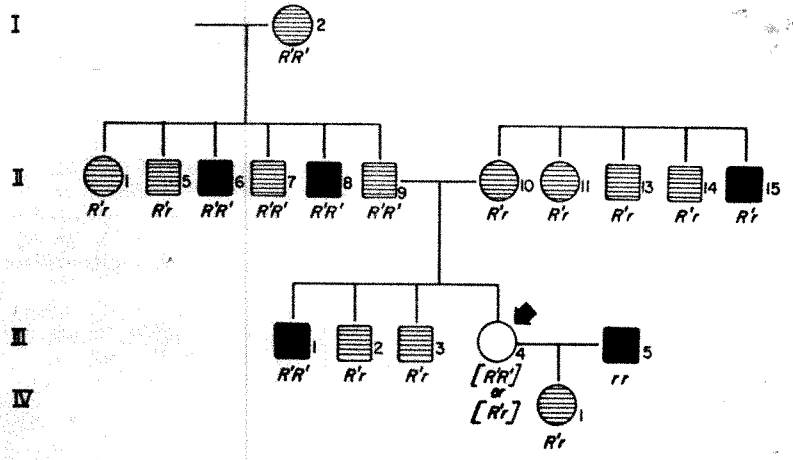


Fig. 1. Selected members of family. Black, normal; hatched, suppressed

Family analysis strongly suggests that the infant's genotype must be R^1r. The R^1 chromosome could be derived only from the Rh_{null} propositus, and r is transmitted by the normal husband. The expression of all Rh antigens was diminished in the infant and this applied especially to antigen C. With some anti-C sera the infant's blood and one maternal aunt (II–11), failed to react, and both bloods were at first mistyped as R^0r.

Since the infant is of the probable genotype R^1r, the Rh_{null} propositus could have produced anti-D, anti-C, anti-G, anti-c and anti-ce (if her underlying genotype is R^1R^1) and anti-e. In fact, the propositus produced only anti-e. In any event, her capacity to produce anti-e would further indicate the complete absence of this antigen. Her failure to produce additional antibodies does not alter the fact that the propositus is, indeed, of the phenotype Rh_{null}.

In order to obtain a more precise measure of the degrees of reactivity of the red cells of the various members of the family, quantitative haemagglutination by probit analysis was carried out by M. E. Gibbs *et al.*[4]. On the whole the results are in agreement with the scoring values in that the same four members had normal activity while the others tested showed varying degrees of suppressed reactivity. These investigations also confirmed that the C antigen was very weak in II–11 and IV–1, and confirmed the observation that chromosome r' was not present in the family.

The propositus as well as the Australian Aborigine were both LW negative while all other members of the large family were LW positive. LW is the name assigned by Levine *et al.*[5] to the blood factor discovered by Landsteiner and Wiener[6].

In their description of the Australian Aborigine blood, Vos, Vos, Kirk and Sanger[2] speculate that the phenotype – – –/– – – "may reflect the absence of a very frequent gene necessary for the production of some Rh precursor substance". It is more than a coincidence that the Rh_{null} Australian blood[3] and the blood of the propositus are LW negative. We believe that both LW and Rh require the same precursor substance to produce their normal phenotypic expression[7]. In the presence of a theoretical and almost universal gene in the homozygous state X^1rX^1r, sufficient precursor substance is available to be acted on by *LW* and *Rh* genes to produce complete expression of their respective antigens. This applies to the four members who showed normal Rh reactions.

Table 1. Summary of Scoring of Titration Experiments

		D	C	c	e
II–9	father R^1R^1	w	w		w
II–10	mother R^1r	w	w	w	w
III–1	sib R^1R^1	N	N		N
III–2	sib* R^1r	w	w	w	w
III–3	sib R^1r	w	w	w	w
III–4	propositus†	0	0	0	0
III–5	husband rr			N	N
IV–1	infant* R^1r	w	w	w	w

*These reactions were not titrated and are based on qualitative tests with several sera. Control bloods showed normal (N) activity.
†$[R^1R^1]$ or $[R^1r]$; phenotype Rh_{null}.

To account for the diminished reactivity of Rh antigens in the 12 members of this family, the data presented suggest the presence of a mutant heterozygote X^1rX^0r, which exerts its effect by producing less precursor substance. In other words, one X^1r gene is replaced by a very rare amorph X^0r. Since all members of the family except the propositus have LW antigen which is apparently not suppressed, it is assumed that *LW* gene successfully competes for precursor substance at the expense of *Rh*. Perhaps *LW* genes have prior claim to precursor substance because they are older from the point of view of evolution as indicated by their wide distribution in monkey bloods[8].

In the homozygous mutant X^0rX^0r—the propositus—genes *Rh* and *LW* are present but no precursor substance is available for production of their respective antigens. Evidence that *Rh* and *LW* genes are present in the propositus is provided by transmission to her infant of R^1 gene complex and probably also *LW*. In short, the genetic explanation for the occurrence of Rh_{null} is analogous to that given for the homozygous suppressor *xx* genes which prevent expression of H, A or B antigens in the Bombay bloods[9]. It differs from the Bombay blood in that *Xx* of the latter cannot be differentiated from the normal *XX* while X^1rX^0r is readily distinguished from the normal X^1rX^1r.

It is difficult, however, to envisage that a mutation at the X^1r locus could explain the rare occurrence of LW negative individuals with apparently normal Rh antigens, some of whom have produced anti-LW[5,10]. The simplest explanation for these cases would seem to be an independent suppressor gene operating at the *LW* locus itself so that only *Rh* genes utilize precursor substance.

In view of the findings reported, the term Rh_{null} for the phenotype of the propositus is preferable to – – –/– – –, which indicates a genotype and implies deletion as underlying the genetic mechanism. The expected genotype can then be represented as usual but characterized by being placed in brackets.

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Ultrastructure of Human Leucocytes synthesizing DNA

THE application of autoradiographic techniques in the localization of radioactively labelled compounds in cells by electron microscopy has been developed by a number of workers[1]. The direct coating of ultra-thin sections with photographic emulsion has the disadvantage that long exposure times are required to obtain positive autoradiographs. Furthermore, it is difficult to locate labelled cells in the electron microscope by this method when their frequency is low. In the experiments recorded here the characteristics of the ultrastructure of cells in DNA synthesis were examined by using a 'thick–thin' technique[2]. Two examples of proliferation in atypical lymphocytes were investigated by this method: normal human lymphocytes cultured with phytohæmagglutinin[3–5], and freshly isolated leucocytes from patients suffering from infectious mononucleosis (glandular fever). The cells were incubated with thymidine (^{3}HT) (10 mc./ml., specific activity 1·9 c./mmole) for 30 min and then fixed with osmic acid and embedded in methacrylate. A thick section (about 0·25μ) was cut from a suitable part of the block and mounted on a glass slide. The contiguous ultra-thin section was then cut and mounted on an electron microscope grid. The methacrylate was removed from the thick section with chloroform and the section coated with Ilford *L4* emulsion and stored at 4° C. Three weeks later it was developed in Kodak *D19B* and stained with toluidine blue. A map of the whole section was made from a mosaic of light photomicrographs and the cells with positive autoradiographs noted. This map was used to locate the position of the DNA-synthesizing cells in the ultra-thin section, which were then examined and photographed in the electron microscope.

In general, cells in DNA synthesis (*S* cells) tended to be larger than other transformed cells and their ultrastructure differed in several respects. The nuclear double membrane showed a far greater degree of irregularity in the cells synthesizing DNA. The separation of the two components was variable and resulted in a beaded appearance. In some cases the outer membrane showed evaginations, and in others it was fragmented. It seems likely that this represents a very early stage in the breakdown of the nuclear membrane which is known to precede mitosis. Exceptionally large-diameter nuclear pores were also present in these cells. The chromatin of these nuclei was loosely packed, especially in the central zone of the nucleus; nucleoli were frequently seen and often had a 'bobbined' appearance. The resting transformed cells had more densely packed chromatin and the nuclear membrane was more regular but contained some smaller pores. Nucleoli were less frequently seen in these cells. A few cells with negative autoradiographs were seen to have loose chromatin and highly irregular cell membranes which were breaking down; these were considered to be nuclei in early prophase.

The cytoplasm of the *S* cells had three major features which distinguished it from the organization of the resting transformed cells. The most noticeable was the far higher concentration of free ribosomes and larger number of dense bodies, possibly containing glycogen, scattered in the cytoplasm of the DNA-synthesizing cells. Endoplasmic reticulum was poorly developed in the transformed lymphocytes, but these cells had a more pronounced quantity of smooth endoplasmic reticulum than the resting transformed cells. Among the DNA-synthesizing cells there was considerable evidence of elaboration of mitochondria, especially in the Golgi zone. The other features of the transformed cells—micropinocytosis, the multivesicular bodies, lipid-containing bodies and Gall bodies—were present in the cytoplasm with equal frequency in resting and in DNA-synthesizing cells.

A general ultrastructural description of the characteristic cells of infectious mononucleosis has been given[6,7]. This work suggests that the differences between DNA-synthesizing and resting cells in this cell line were very similar to those described here for phytohæmagglutinin-transformed cells. The chromatin density and nuclear membrane differences were present, but nucleoli were infrequently seen in both resting and DNA-synthesizing cells. The greatly increased cytoplasmic free ribosome concentration in the *S* cells was equally marked, as was the increase in elements of the smooth endoplasmic reticulum. The other cytoplasmic differences, though present, were less marked than in the phytohæmagglutinin-transformed cells.

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Allotypes of Hen Serum Proteins

THE allotypic differences in antigenic specificity of rabbit serum proteins were discovered by Oudin[1] in 1956. Oudin's results were confirmed by Dray and Young[2]. Serum groups in rabbits were also described by Dubiski, Dudziak and Skałba[3]. Immunological differences in serum proteins have also been demonstrated in the guinea-pig[4] and the mouse[5].

This paper presents the results of investigations which showed the allotypic specificity of hen serum proteins. The experiments were carried out on four breeds of hen: Rhode Island Red, Sussex, White Leghorn and Greenleg. The isoprecipitins were obtained by immunizing the hens with bacteria mixed with corresponding immune antibacterial hen serum. For the production of antibacterial sera hens were immunized with heat-killed bacterial suspensions of *Proteus vulgaris* (*OXl9*). 0·5 ml. of antibacterial serum was mixed with 0·05 ml. of *Proteus* suspension containing approximately 10^9 cells. The mixture was incubated for 60 min at 37° C and re-suspended in 1 ml. of saline. The inoculum thus prepared was injected intravenously. The injections were made three times a week. During the whole period of immunization (7–10 months) each hen was inoculated with one antibacterial serum only. The birds were bled eight days after the final injection.

The immune sera were tested in 1·5 per cent agar gel by the method of Ouchterlony. The concentration of sodium chloride was 8 per cent in agar gel.

Table 1. DISTRIBUTION OF THE ALLOTYPES GA1, GA2 AND GA3 IN DIFFERENT HEN RACES

Breed	Number of hens	Allotypes GA1	GA2	GA3
Rhode Island Red	112	112	40	41
		1·000	0·357	0·366
Sussex	113	78	65	68
		0·690	0·572	0·602
White Leghorn	120	74	68	48
		0·617	0·567	0·400
Greenleg	117	111	60	37
		0·949	0·513	0·316

Three precipitating antisera were obtained. Three distinct antibodies were identified and the corresponding allotypes of serum proteins. In accordance with the agreed notation for allotypy[6], the allotypic specificities of hen serum proteins were marked GA1, GA2 and GA3.

It is theoretically possible to foresee eight different combinations of the three allotypes, if they were independent.

These eight serum groups could be distinguished in Sussex and White Leghorn hens. Among the sera of Sussex, White Leghorn and Greenleg hens there were some in which no allotypes were detected. The frequency of this allotype formula, GA(1–2–3), was very low in the sera of Sussex and Greenleg hens; it reached 9 per cent in the sera of White Leghorn hens. The sera of Rhode Island Red hens could be divided among four groups only.

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IMMUNOLOGY

Techniques for the Measurement *in vitro* of Tissue Sensitization in Immunized Pigs

CYTOPATHIC tests have been used to detect cytophilic antibodies on monocytes and in sera derived from guinea-pigs immunized with *Cl. welchii* toxin[1], and similar tests were made to investigate cytophilic antibodies on leucocytes and in sera from chickens infected with *Salm. gallinarum*[2]. Further experiments have been made to record the development of tissue sensitization in pigs immunized against ovalbumin, and to predict the time at which an additional antigenic stimulation would induce a typical anaphylactic reaction.

Preliminary experiments showed that cultures of *ERK* cells adsorbed antibody from pig sera. They were used in these experiments to provide a constant and homogeneous cell suspension for the detection of serum cytophilic antibody. In addition cytophilic antibody produced by leucocytes was detected by growing washed leucocytes from immunized pigs for five days, and then testing the supernatant growth media for antibodies by absorbing with *ERK* cells. After removal from the culture media, and washing, the *ERK* cells were mixed with albumin, and the cytopathic indices[2] were recorded. Suitable controls were run in parallel with each experiment, using fresh unused growth medium and medium that had been used for growing leucocytes from non-immunized pigs.

Fig. 1 shows cytophilic antibody production in a pig of 25 kg weight, after intravenous inoculation of 1 g ovalbumin. The antibody titre showed a marked decline from the fourth to the seventh day, and the pig was given an intravenous challenge dose of 1 g of ovalbumin on the seventh day. Within 5 min this produced mild symptoms

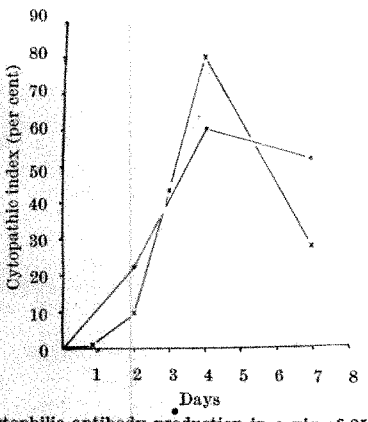


Fig. 1. Cytophilic antibody production in a pig of 25 kg, after i.v. inoculation of 1 g ovalbumin. ×——×, serum, cytophilic antibodies; ●——●, *in vitro* cytophilic antibody production by leucocytes

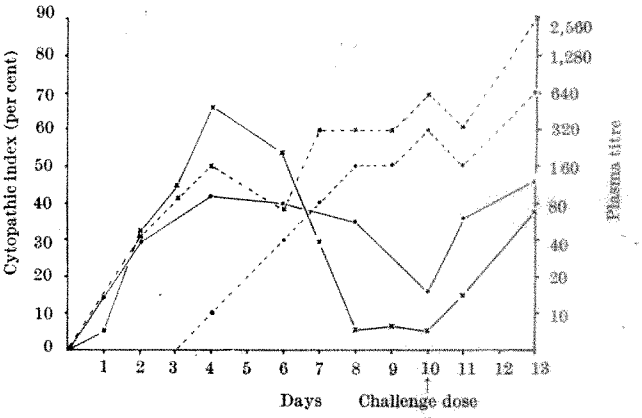


Fig. 2. Cytophilic antibodies and hæmagglutinins in a pig after i.v. inoculation of 1 g ovalbumin, followed by a challenge dose of ovalbumin on day 10. ×——×, serum cytophilic antibodies; ●——●, *in vitro* cytophilic antibody production by leucocytes; × — — — ×, antiglobulin hæmagglutinins; ● — — — ●, hæmagglutinins

of anaphylaxis, lasting for approximately 2 h, similar to those described by Thomlinson and Buxton[3].

The supernatant media from primary cultures of leucocytes were removed after five days' growth and stored at −25° C until all samples had been collected. These were collectively tested and compared with a control medium for the presence of cytophilic antibodies. The results are recorded in Fig. 1 and show that *in vivo* antibody production was continuing despite the decline in the amount of detectable circulating cytophilic antibodies.

A further attempt was made to relate serum antibodies to tissue sensitization and anaphylaxis, by giving an intravenous dose of 1 g of ovalbumin to a pig of approximately 20 kg weight. The concentration of serum cytophilic antibodies reached a peak on the fourth day after immunization and fell steeply until the eighth day (Fig. 2). This decrease would be expected with a cytophilic antibody which was being fixed to tissue cells, particularly when the rate of antibody production by leucocytes had reached a maximum and was also declining. At this state in the immune response it was assumed that extensive sensitization of the pig's tissues had occurred, and that the animal was therefore in a hypersensitive state. For this reason the pig was given a challenge dose of 1 g of ovalbumin intravenously on the tenth day. A moderately severe anaphylactic reaction developed in which congestion of the skin and cyanosis were marked and the respiratory rate was increased; other characteristic symptoms included coughing, muscular tremors, restlessness, straining, vomiting, defæcation and unsteadiness of gait. The pig recovered during the following 18 h, and the serum cytophilic antibody level increased markedly from the tenth to fourteenth day.

Figs. 1 and 2 show that in both cases cytophilic antibodies were being produced by leucocytes and were also detectable in the sera of these animals following the initial inoculation of antigen. During the first four days after inoculation antibody concentrations detectable by both methods increased rapidly. Thereafter the concentration of serum cytophilic antibodies declined more rapidly than antibody production by the leucocytes *in vitro*.

Buxton and Allan[4] have shown that antiglobulin hæmagglutinins in chickens acutely infected with *Salm. gallinarum* were cytophilic. However, Fig. 2 shows that from the sixth day onwards there is a striking divergence in the relative concentration of antiglobulin hæmagglutinins and cytophilic antibodies, and that simultaneously the former follow the same pattern as hæmagglutinins. The antiglobulin hæmagglutination technique does not enable any qualitative distinction to be made between cytophilic antibodies and concentrations of hæmagglutinins which can only be detected by an antiglobulin

system. It is probable that immediately after injection of antigen the antiglobulin hæmagglutination test will detect cytophilic antibodies only, and later the detection of this type of antibody will become masked by the higher titres of hæmagglutinins which are only observed by this antiglobulin method.

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New Cellulose Derivatives for the Isolation of Biologically Active Molecules

The search for the relationship between structure and function of antibodies has gone through different phases. Much information has been accumulated concerning the interactions of antibody with antigens, particularly with respect to haptens of related and different structures. These data have given rise to a multitude of speculations concerning the chemical and physical structure of antibodies and the mechanism of antibody reactions. At present, major questions awaiting answers are: (1) Does antibody to a particular antigen have a unique primary structure? (2) Do all antibodies have the same primary structure but they are folded differently? (3) Are the antibody combining sites for a particular antigen the same, but are other parts of the antibody molecule modified for other functions? (4) What is the composition and structure of the active site?

The evidence that antibody produced against protein antigens is heterogeneous, and the possibility that antibody to haptens is heterogeneous makes it mandatory that any investigations concerning antibody structure or interaction be made on all the antibody from a selected quantity of serum, unless it can be shown that a representative sample has been obtained during the isolation process. Conclusions based on comparisons of peptide or amino-acid analyses, and other antibody components are particularly sensitive to fractionation of antibody during its isolation from serum.

For these reasons we have concentrated our present efforts on investigating methods for isolating the maximum possible quantity of antibody from a serum sample, in as pure a form as possible, measured by the fraction precipitable with specific antigen. Available methods have drawbacks which include incomplete separation from antigen, low overall yield, low purity, lack of generality and very involved procedures. The most promising approach is that of chemically coupling the antigen to an insoluble derivative of a polymeric carrier such as an aromatic or aliphatic resin, a protein or a cellulose derivative. In principle, contamination of antibody by antigen should be negligible, and separation from other serum proteins should be complete, if conditions are properly controlled. A review of this and other isolation techniques has recently been published[1].

The most frequently used insoluble antigen is polyaminopolystyrene, which has been diazotized and coupled to protein antigens[2]. This derivative has the disadvantage that recovery of antibody is low, overall yields are low and appreciable amounts of non-specific protein are released with the antibody[1]. In addition, the coupling reaction is limited to substances which will couple to aryldiazonium compounds. To extend the scope and utility of this technique we developed the use of di*cyclo*hexylcarbodiimide as a coupling agent for chemically binding antigenic substances with basic amino groups, to insoluble varieties of carboxymethylcellulose[3]. Water-soluble diimides such as 1-*cyclo*hexyl-3-(2-morpholinyl-(4)-ethyl)-carbodiimide can also be used. Recovery of antibody complexed to the cellulose derivatives was in excess of 88 per cent. Since the original work was done, overall yields exceeding 90 per cent are now being consistently obtained from anti-BSA serum and anti-azophenylarsonate serum, by dissociating the antigen–antibody complex with 1 per cent saline acidified with hydrochloric acid to *p*H 2·3 for the former case, and to *p*H 2·0 for the latter. Human γ-globulins, bovine serum albumin, keyhole limpet hæmocyanin and other proteins can be coupled. Many enzymes couple and still retain activity. In addition, *p*-(*p*′-aminophenylazophenyl)-arsonic acid, ε-DNP-lysine and other organic amines reacted with carboxymethylcellulose and could be used to bind serum antibody. Coupling of the amines to carboxymethylcellulose was more effective at *p*H 3·6–4·0 than near neutral *p*H.

Carboxymethylcellulose coupled to antigens releases about 0·01 mg of non-specific protein per ml. serum/g carboxymethylcellulose. Another cellulose derivative commercially available ('Cellex *PAB*') has been bound to proteins by diazotization and coupling[4]. Any uncoupled diazo groups remaining were blocked by reaction with β-naphthol. While adequate for the isolation of antibody, it is a deep brown substance which is difficult to free of water-soluble material. The coupled 'Cellex *PAB*' retains about 0·1 mg of non-specific protein per ml. serum/g 'Cellex *PAB*'[3].

Coupling through azo links is useful and sometimes advantageous. We have now developed a simple, rapid method of synthesizing a new arylaminocellulose under mild conditions using a slight modification of the method we use for coupling proteins and haptens directly to carboxymethylcellulose (CMC)[3]. The product is a clean, white solid which can be diazotized, or used as it is with di*cyclo*hexylcarbodiimide for coupling to various organic and biological substances.

Benzidine, first suspended in water, was coupled to ten times its weight of carboxymethylcellulose by adding a tetrahydrofuran solution of di*cyclo*hexylcarbodiimide (DCC). The slurry was allowed to stand for 2 days at room temperature, after which the coupled material was washed exhaustively with acetone, dilute acid, base and finally water.

The number of benzidine groups coupled to the cellulose was determined by first reacting the benzidine derivative with a solution of benzenesulphonyl chloride in tetrahydrofuran (in the presence of triethylamine) to form sulphonamides with the free amino groups. After hydrolysing any anhydride that formed, the remaining free carboxyl groups were titrated with sodium hydroxide. Typical data were: 1·0 g carboxymethylcellulose of 0·78 m.equiv. free carboxyl groups per gram was reacted with 0·1 g of benzidine to form a derivative with 0·2 m.equiv. free carboxyl groups/g. By difference, the benzidine coupled amounted to 0·58 m.equiv./g or 0·1 g, signifying that all the benzidine reacted with the cellulose.

An immunoadsorbent was prepared by diazotizing the arylaminocellulose and coupling it to bovine serum albumin. Subsequent treatment with β-naphthol blocked any unreacted diazonium groups. 1 g of this material was poured into a 10-mm glass tube, with a glass wool plug, to make a column several centimetres long.

5 ml. of rabbit anti-bovine serum albumin was passed through the column followed by 1 per cent saline until no protein could be detected using a 1-mm flow-through cell in a spectrophotometer set at 220 mμ. Complexed material was eluted from the column with 1 per cent sodium chloride acidified to *p*H 2·3 with hydrochloric acid. All the specific antibody contained in the serum was picked up and afterwards released. The isolated antibody was not less than 80 per cent precipitable with specific antigen (Table 1).

Similar experiments were performed using a non-specific rabbit serum. In Table 2 the quantity of non-specific

Table 1. Isolation of Antibody from a 1-g Column of Carboxymethylcellulose Benzidine Derivative coupled to Bovine Serum Albumin

Serum added (ml.)	Total antibody passed through column (mg)	Total protein released at *p*H 2·3 (mg)	Precipitable protein (%)
5	10·50	11·25	82·1
5	6·30	6·78	80·5

Table 2. Comparison of Carboxymethylcellulose Benzidine Derivative and *p*-Aminobenzylcellulose Derivative for Release of Non-specific Protein at *p*H 2·3 from 1-g Columns

Column and antigen	Serum added (ml.)	Protein eluted Per ml.	Protein eluted Total (mg)
PAB–BSA	2	0·12	0·24
PAB–BSA	3	0·08	0·24
CMCB–BSA	5	0·18	0·90
CMCB–BSA	5	0·16	0·77

PAB, *p*-Aminobenzylcellulose.
CMCB, Carboxymethylcellulose coupled to benzidine.
BSA, Bovine serum albumin.

protein eluted from an immunoadsorbent made from a carboxymethylcellulose derivative with 0·52 m.equiv./g free amino groups is compared with that eluted from an immunoadsorbent made from a *p*-aminobenzyl derivative with 0·32 m.equiv./g free amino groups.

Under the conditions used, the retention and release of non-specific protein on these immunoadsorbents appear to be dependent on the number of β-naphthyl groups present. Carboxymethylcellulose, which is directly coupled to an antigen through an amide linkage and requires no blocking groups, retains no more than 0·011 mg for each ml. of serum passed through a 1-g column[3]. A *p*-aminobenzyl immunoadsorbent retains about 0·1 mg non-specific protein while the new arylaminocellulose, although still useful as an immunoadsorbent, has about twice the number of reactive groups as the *p*-aminobenzylcellulose and retains about twice the quantity of non-specific protein. By reducing the number of benzidine groups, non-specific protein adsorption should be correspondingly reduced. Such immunoadsorbents are useful because they contain proteins coupled at reactive sites other than those involved in the reaction with di*cyclo*hexylcarbodiimide.

We are now exploring the coupling of this new derivative to other proteins and organic molecules both by diazotization and by amide formation. By chemically coupling inhibitors or other complexing agents to the cellulose derivatives we expect that biologically active molecules and other substances can be concentrated and isolated.

This work was supported by the U.S. National Aeronautics and Space Administration contract *NASA*7–100.

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PATHOLOGY

Antigenic Properties of Human Tumours

This communication presents the preliminary findings of investigations on the antigenic properties of human tumours using heterologous antisera. Some attempts have been made to isolate and characterize tumour antigens.

The literature on this subject is voluminous, complex and even contradictory. There is some evidence for the presence of a new antigen or antigens in tumours, and in addition there is also evidence to indicate the loss of antigens.

The possible applications of immunology to the problems of clinical cancer are many and are intriguing. At present, however, they are mainly theoretical and more often purely speculative.

Because of the possible importance of any conclusions concerning the antigenic composition of tumours, it seemed that further investigations were necessary.

Human malignant tumours (predominantly carcinomas) and normal tissues were obtained mainly from surgical operations. A small proportion of the material was obtained from autopsies. The collection of corresponding normal tissues from the same patient was not always possible. Soluble extracts of the various tissues were prepared by a cryostat method[1] using phosphate-buffered saline, *p*H 7·6, as a solvent.

Antisera were prepared from a number of cancerous and normal tissues from different sites, one individual tissue being used to immunize one or more rabbits. The rabbits were injected with the various tissue homogenates and complete Freund's adjuvant. Immunization was continued after one month with tissue homogenates without adjuvant. Antisera were also prepared against protein fractions obtained by chromatographic methods. The antisera obtained were tested for relative potency and specificity.

The antisera were invariably contaminated with antibodies to serum proteins and blood cells. These antibodies were removed by repeated absorptions of the antisera with the corresponding antigens. Anti-tumour antisera were further absorbed with the corresponding normal tissues.

After repeated absorptions several antisera were obtained which reacted exclusively with tumours (Fig. 1). These absorbed antisera were used for the subsequent work.

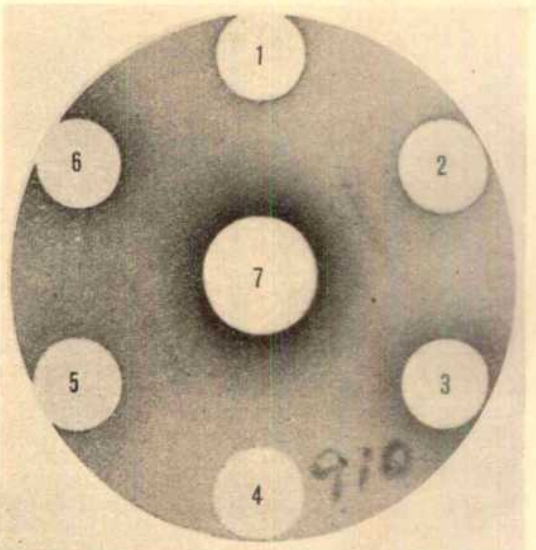


Fig. 1. Ouchterlony plate showing tumour specific reaction. 1, Normal tissue extract; 2 and 5, tumour extracts; 3 and 6, serum; 4, leucocyte extract; 7, anti-tumour antiserum

An antigenic analysis was made of 72 tumours and 31 normal tissue extracts by means of the Ouchterlony agar-gel double diffusion technique.

Tumour-specific reactions were observed with a number of the tumours when reacted with anti-tumour antisera. These positive reactions occurred regardless of whether the tumours were derived from different patients or from different sites. A higher proportion of positive reactions was observed with tumours from the same site as that of the immunizing tumour. Anti-tumour antisera did not react with the normal tissues.

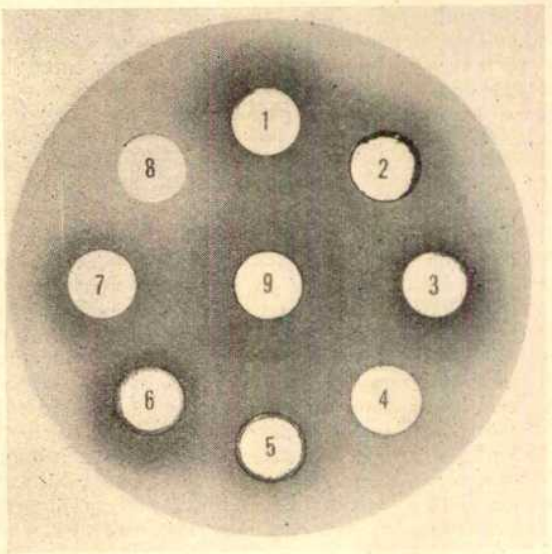


Fig. 2. Ouchterlony plate showing additional antigen in tumours when compared with autologous normal tissues. 1, 3, 5, 7, Different tumour extracts; 2, 4, 6, 8, corresponding normal tissue extracts; 9, anti-tumour antiserum

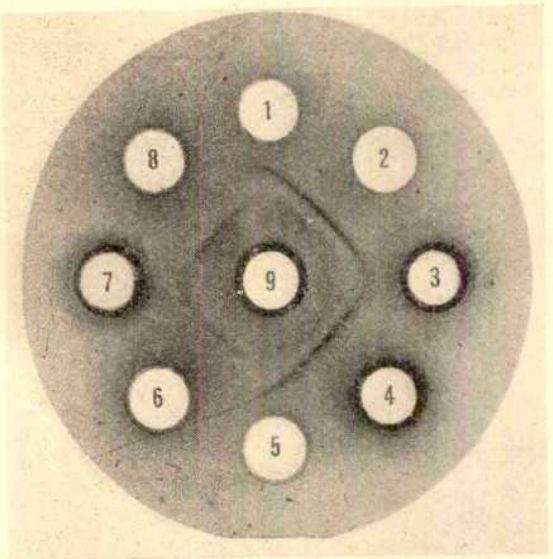


Fig. 3. Ouchterlony plate showing loss of antigens from tumours when compared with autologous normal tissues. 1, 3, 5, 7, Different tumour extracts; 2, 4, 6, 8, corresponding normal tissue extracts; 9, anti-normal tissue antiserum

Positive reactions were observed only with tumour extracts and no reactions were observed with the normal tissues from the same patient (Fig. 2). This confirms that the tumour-specific antigen is not due to iso-antigenic differences between the cells of genetically different individuals.

Loss of antigens was shown in at least a proportion of the tumours when these were reacted against anti-normal tissue antisera (Fig. 3).

No evidence was found for the presence of a soluble antigen present in HeLa cells when an extract was reacted with all the anti-tumour antisera. However, the result may not be conclusive as only HeLa cells from one source have been tested. It was not possible to detect, in extracts of fœtal tissues, antigens serologically identical with the tumour-specific antigen.

Attempts have been made to isolate and characterize these tumour antigens by chromatographic and electrophoretic techniques. The results of these experiments and of further extensions of the antigenic analysis of human tumours will be published in due course.

In summary, we have shown the presence of antigen or antigens in human malignant tumours which were not detectable in normal tissues, and, in addition, we have shown that there was some loss of normal tissue antigen from tumours.

Very recently, McKenna, Sanderson and Blakemore[2] have published results of a similar investigation which largely substantiate our own findings.

This work was supported by the Cowburn Research Fellowship, King's College Hospital Medical School (D. E. H. T.), and by grants from the British Empire Cancer Campaign for Research (M. W. and J. W.).

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Brain Tumours induced in Hamsters inoculated intracerebrally at Birth with Rous Sarcoma Virus

It has been previously shown that new-born hamsters are susceptible to the oncogenic effect of polyoma virus[1] introduced intracerebrally[2]. Likewise, *SV* 40 virus[3] which was shown to be oncogenic when injected subcutaneously into new-born hamsters[4] also proved capable of inducing papillary ependymomas after intracerebral inoculation into the same animals[5]. In recent years a variant of the chicken Rous sarcoma virus (RSV), the Schmidt-Ruppin strain[6], has been shown to be oncogenic in a variety of subcutaneously inoculated rodents, including hamsters[7]. The investigation described here was undertaken to determine the effects of intracerebral inoculation of this virus into new-born hamsters.

The Schmidt-Ruppin strain of virus was prepared by the technique of differential centrifugation[8] from wing's web sarcoma induced in 2–3 weeks old white Leghorn chickens. The virus was suspended in 0·05 M sodium citrate and stored in 0·5-ml. aliquots in sealed ampoules kept at −70°. A pool with a titre of $\log_{10}$ 6·58 plaque-forming units/ml. in chicken chorioallantoic membrane was used throughout the experiments. In order to overcome the toxicity of sodium citrate for the new-born hamster's brain and to further concentrate the virus, 0·5–1 ml. of the virus pool suspension was diluted to 10 ml. with cold phosphate buffer saline, *p*H 7·2 (PBS), and pelletized in a preparative Spinco centrifuge at 30,000*g* for 1 h, a procedure which is known to sediment more than 99·9 per cent of the suspended virus. The pellets were reconstituted in PBS and the titre of the final concentrations used in the inocula are shown in Table 1.

New-born Syrian hamsters (*Cricetus auratus*) were obtained from the Animal Production Unit of the National Institutes of Health, and inoculated when less than 24 h old with 0·02 ml. of viral suspension into the mid-portion of the right cerebral hemisphere.

After a period of time, varying with the titre of the inoculum (Table 1), the animals began to show somnolence and sluggishness followed in a few days by lateral posture of the head and paralysis first of the posterior limbs, then general paralysis leading to death.

At autopsy an increased volume of cerebrospinal fluid was noted in the RSV inoculated hamsters. The brain tissue appeared softer and more friable than normal. Microscopically it showed the presence of multiple, diffuse tumours located within the cerebral hemisphere bilaterally (Fig. 1), with only rare involvement of the meninges.

Table 1. NEW-BORN HAMSTERS INJECTED INTRACEREBRALLY WITH RSV SCHMIDT-RUPPIN STRAIN (POOL *SR* 26) FROM CHICKEN WING'S WEB SARCOMA

Exp. No.	Inoculum titre P.F.U.*/ 0·02 ml.	No. hamsters with neurological symptoms/No. weaned hamsters	Appearance of neurological symptoms (days: average)	Incidence of gliomas No.	%
1	$7·6 \times 10^6$	18/20	47	18	90
2	$3·024 \times 10^5$	14/14	18	14	100
3	$1·512 \times 10^5$	24/24	24	24	100
3	$1·512 \times 10^4$	15/15	36	15	100
3	$1·512 \times 10^3$	8/9	56	8	89
—	PBS†	0/12	—	0	—

* Plaques forming units in chicken chorioallantoic membrane.
† Phosphate buffer saline *p*H 7·2 used as diluent.

No extension to the ependymal lining of the ventricles was noted. These tumours consisted of two types of cells: (*a*) large round cells with basophilic cytoplasm and (*b*) bundled fusiform cells interlacing with delicate fibrillar strands. There was no collagen, and very little reticulum. Small amounts of glial fibrils were seen within the tumour. These findings favour a diagnosis of glioma. (We thank Dr. H. L. Stewart, chief of the Laboratory of Pathology of the National Cancer Institute, and Dr. K. Earle, chief of the Neuropathology Section of the Armed Forces Institute of Pathology, for a critical review of the histological sections.)

Further work on the histogenesis of these tumours is in progress. Controls inoculated with the suspending medium (PBS) without virus failed to show evidence of intracranial tumour or noteworthy pathological changes after 3 months of observation. The tumours induced by Schmidt-Ruppin RSV in hamsters differ from those induced in the same species by polyoma virus. The latter resemble human leptomeningeal sarcoma[9] and are mesenchymal type tumours arising from the arachnoid and from the adventitia of the meningeal and cerebral vasculature. They are located at the meningeal surfaces and only at a late stage infiltrate the deeper portions of the cerebrum. In these tumours, collagen and reticulum are easily demonstrated[2].

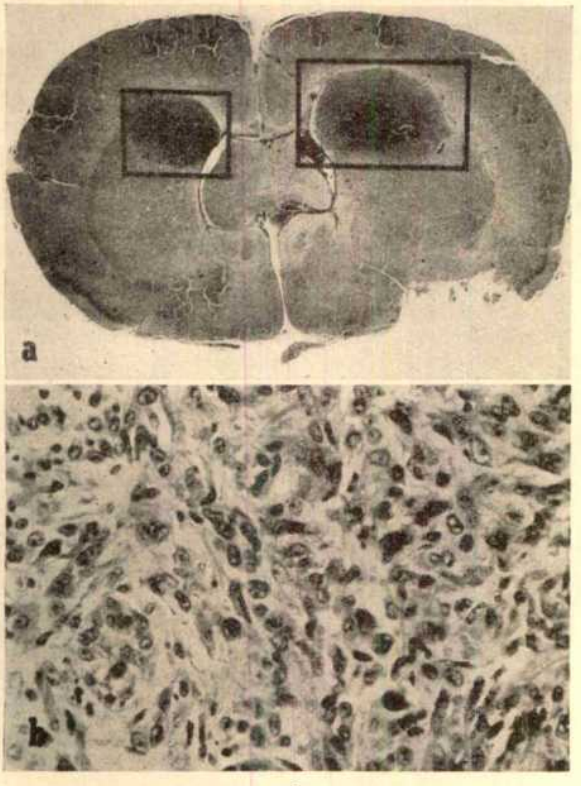


Fig. 1. Brain of hamster inoculated at birth with Schmidt-Ruppin RSV. (*a*) Bilateral tumours (squares) (phosphotungstic acid hæmatoxylin: × *c.* 20); (*b*) area of glioma cells (hæmatoxylin and eosin: × *c.* 390)

Unlike the tumours induced in hamsters with *SV* 40 virus[5], no involvement of the ependyma was found in the RSV-induced tumours. It is also noteworthy that the intracranial tumours induced in hamsters by the Schmidt-Ruppin strain of RSV differ from those induced in the chicken brain by the Bryan's strain of RSV. These latter are myxomatous lesions arising in the pial-arachnoid spaces[10–12].

Both mesenchymal type brain tumours and gliomas have been induced in mice with carcinogenic hydrocarbons[13]. It would appear, therefore, that viral oncogenesis may be more selective in that different types of brain tumours are induced in hamsters by different viruses, namely, leptomeningeal sarcomas with polyoma virus ependymomas with *SV* 40 and gliomas with RSV Schmidt-Ruppin strain.

The implications of these findings may prove of interest in the investigation of pathogenesis of human brain tumours.

Two main points pertaining to the biology of neoplasms induced by RSV in mammals remain to be clarified: (*a*) the incapability of recovering the virus from the tumours induced in mammals by the Schmidt-Ruppin strain; (*b*) the incapability of the Bryan's strain to induce such neoplasms.

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RADIOBIOLOGY

Prevention of Plutonium Deposition by Desferrioxamine-B

IN selecting chelating agents to test their ability to remove internally deposited metals, the magnitude of the stability constant of the complex is an obvious criterion. Schubert[1] rightly points out that a more practical guide is the ratio of the stability constant of the chelate, K_{ML}, to that for omnipresent calcium, K_{CaL}. This situation is illustrated in Table 1 for Fe (III) and Ca (II).

Table 1. STABILITY CONSTANTS $K_{ML} = \log_{10} \frac{[ML]}{[M]\,[L]}$

Where M = metal, L = chelating agent (ref. 2)

L	'Common' chemical name of L	K_{FeL}	K_{CaL}	K_{FeL}/K_{CaL}	Ref.
EDTA	Ethylenediaminetetraacetic acid	25·1	10·7	14·4	2
DTPA	Diethylenetriaminepentaacetic acid	28·6	10·1	18·5	2
DFAB	Desferrioxamine B	30·6	2·6	28·0	3

From these results one would predict the efficacy of decorporation of iron to run DFAB > DTPA > EDTA, and this is the case[4]. Similarly, Catsch assigns the superiority of DTPA over EDTA in the removal of certain elements to the greater ratio of the stability constants for DTPA with these elements and calcium[5]. Since DTPA is the

present agent of choice for the removal of certain internally deposited heavy metals, for example, plutonium[6], cerium[7], ruthenium[8], it was of interest to compare the effect of DFAB in this respect. Only the results for decorporation of plutonium will be reported here.

Eight-month-old female Sprague–Dawley rats were intravenously injected with 2·0 μc. $^{239}Pu^{4+}$ in 0·2 ml. of *p*H 5·0 citrate buffer. 1, 5 and 24 h later they were treated with 0·5 m.mol/kg of the agents (total treatment dose, 1·5 m.mol/kg). To prevent tetany, 0·4 m.mol of calcium as calcium gluconate–calcium glucoheptonate (Abbott Laboratories) was mixed with the DTPA just prior to injection. This was unnecessary with the DFAB solution, which was made up just before use and contained 0·1 m.mol of the methane sulphonate salt (CIBA compound No. 33112, 'Desferral') per 0·5 ml. Urine and fæces were collected daily from each group of ten rats. Five days after injection of plutonium the animals were killed and tissues analysed for plutonium by conventional methods.

To obtain homogeneity of group variances, resulting from the large differences between control and treated values, the results were transformed using the arcsine of the square root. The one-way analysis of variance technique was used to analyse the transformed data and Tukey's *T*-method of multiple comparison was used to compare the means.

The statistically summarized results of the non-transformed data are shown in Table 2. Two results of practical importance are apparent: (1) treatment with DFAB decreased bone deposition of plutonium to about one-half the value obtained with DTPA; (2) the two agents given together produced a partially additive effect. This latter observation was not expected, since usually two chelating agents merely compete with one another for the available metal, the sum of their actions being the same as the effect of the stronger agent by itself. The enhanced effect of DFAB is probably due to its different *in vivo* distribution and the consequent tapping of plutonium pools not available to DTPA. This is further suggested by the consistently lower bone deposition and higher fæcal output of plutonium in the DFAB-treated animals.

Table 2. Effects of Treatments on Plutonium Content of Tissues and Excreta Five Days after Administration

Treatment and route of administration	Per cent of ^{239}Pu dose: Liver $\bar{X} \pm S_{\bar{X}}$†	Kidneys $\bar{X} \pm S_{\bar{X}}$†	Femur $\bar{X} \pm S_{\bar{X}}$†	Accumulated excreta*: Urine	Fæces
DFAB, i.p.	1·3 ±0·1	2·1 ±0·2	0·14±0·01	62	20
DTPA, i.p.	0·58±0·07	0·24±0·01	0·28±0·02	67	17
DFAB, i.m.	1·3 ±0·1	1·5 ±0·1	0·16±0·01	66	23
DFAB, i.m. + DTPA, i.p.	0·39±0·04	0·54±0·04	0·13±0·01	73	21
No treatment	20·0 ±1	1·4 ±0·2	3·0 ±0·2	2·5	6·5

* Being pooled results, these data were not statistically analysed.

† Mean ± *S.E.*

Note: all treatments lowered the spleen content 5–7-fold below the control value of 0·35 per cent.

The poor removal of plutonium from the liver and the build-up in the kidneys when DFAB is used alone may be due to lability of the chelating agent[9]. The organic moiety of the Pu-DFAB complex may be destroyed, surrendering its plutonium to the tissue where DTPA, in the combined treatment, can pick it up and complete the removal.

While the measurement of the stability constants of the plutonium chelates of DFAB and DTPA are not yet completed, they are of similar magnitude. On the basis of the likely ratios of the stability constants of the two agents for plutonium and calcium, one would have expected DFAB to show even greater superiority than these results indicate. As this ratio becomes reasonably large, it seems likely that the relative ability of two agents to remove metal is more dependent on such factors as the tissue distribution, relative strength compared to tissue ligands, and stability and excretability of the sequestering complex. Still other factors are no doubt involved, as may be illustrated by the effect of DTPA in removing plutonium from the liver. DTPA is significantly more effective than other polyaminocarboxylic acids of comparable chelating ability and presumably similar distribution[10,11].

In view of its clinical use in the treatment of pathological iron deposition[12], DFAB might well be considered for human use in removal of plutonium. However, the combined use of two chelating agents should be approached with caution, since in our limited experience the DTPA–DFAB combination appeared to be more toxic than either agent alone.

I thank L. C. Gray for his assistance; R. L. Bushbom for the statistical treatment of the data; and Dr. H. Bickel and associates, and CIBA, Ltd., Basle, for donating the DFAB used in these experiments.

This work was carried out under contract *AT*(45–1)–1350 between the Atomic Energy Commission and the General Electric Co.

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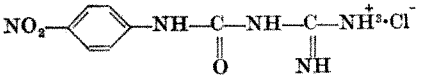
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BIOLOGY

A New Molluscicide 1-(*p*-Nitrophenyl)-2-amidineurea Hydrochloride (*T* 72)

Molluscicide Bayer 73 or 5,2-dichlor-4′-nitrosalicylanilide, better known as 'Bayluscid^R', has been shown to be a potent molluscicide[1]. The chemical similarity of *T* 72 to Bayer 73 (ref. 2) led us to investigate its possible molluscicide property. *T* 72 is known to be active against malaria[3–6] and against several intestinal infestations[7]. It has the following structural formula:



Our experiments were carried out with snails and fish. The snail *Melanopsis costata* (Ferrusac) was obtained from the estuary of the River Jordan; *Melanopsis praemorsa* (Linne) from the Baalbeck stream in Lebanon; *Radix peregra* (Müller) from Lake Yammoune in Lebanon; fish from the River Barada in Damascus.

A total of 135 specimens of *Melanopsis costata* were divided into nine groups and 160 *Melanopsis praemorsa* were divided into eight groups. They were placed in separate solutions of *T* 72 and Bayer 73 for 24 h and afterwards washed in tap water for a further 24 h. In accordance with Kärber's method, the *LC* 50 was estimated 48 h after the start of the experiments, but *Radix peregra* failed to survive in the 0·05 p.p.m. solution. The *LC* 50 values of the other species for *T* 72 and that of the fish for both preparations are shown in Table 1. The

Table 1. Toxic Concentrations of *T* 72 and Bayer 73

Preparation	Animal	Number of animals per group	*LC* 50 in p.p.m.
T 72	*M. costata*	15	22
T 72	*M. praemorsa*	20	17·8
T 72	Fish	11	78·7
Bayer 73	Fish	11	0·33

molluscicide concentrations of Bayer 73 established by us are in agreement with those recorded in the literature[1].

In order to determine whether the molluscicide action of *T* 72 is selective for snails we experimented with fish (88 for *T* 72 and 66 for Bayer 73) and found that they tolerated *T* 72 in molluscicide concentration. This is indicated in Table 1. Extreme motor activity seemed to be the main feature before death of those fish treated with Bayer 73. Concentrations of 0·5 p.p.m. and 0·6 p.p.m. killed the fish in 8 h and 1 h, respectively. Fish treated with *T* 72 remained prostrate but with re-inforced gill movements. A concentration of 90 p.p.m. killed four of the fish in 8 h and the remaining seven in the next 16 h. A concentration of 80 p.p.m. killed seven fish in 24 h. However, fish survived in a 70 p.p.m. solution over a period of 24 h. It should be noted that concentrations of 0·6 p.p.m. and 80 p.p.m. represented twice the *LC* 100 for snails as found in our experiments for Bayer 73 and *T* 72, respectively.

In view of the tolerance of *T* 72 by man and its low toxic effect in the rat under prolonged administration[3,4], water containing *T* 72 in molluscicidal concentration may be safely used for drinking purposes in restricted areas for a limited period. Moreover, *T* 72 can be viewed as a molluscicide belonging to a potentially new group of agents which, unlike Bayer 73, does not contain the salicylanilide structure. This observation does not support the idea that the salicylanilide structure is essential for molluscicide activity[1]. Since halogenation of *T* 72 might give rise to higher activity of the compound, we are proceeding with a study of several halogenated and aromatic derivatives.

We thank Dr. A. Zilch for help in identifying the snail species.

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Occurrence and Dispersal of *Verticillium* Conidia in Xylem Sap of the Hop (*Humulus lupulus* L.)

During investigations on the rate of colonization of wilt-sensitive and wilt-tolerant hop varieties by mild and virulent hop isolates of *Verticillium albo-atrum* Reinke and Berthold, conidia were found to be abundant in the xylem vessels of stems, and appeared to play an important part in the migration of the fungus in the hop plant.

The soil of potted plants was inoculated with *Verticillium*-infested hop-stem fragments, and at weekly intervals thereafter the stems of selected plants were cut about 5 cm above soil-level and a specimen tube, held in position by a cotton wool collar, was fixed over the cut end of each stump. The plants were then inverted so that sap expressed by root pressure during the next 4 h collected in the tubes. The number of conidia in the sap was estimated by spreading 0·5 ml. aliquots over the surface of nutrient agar plates and counting the colonies which developed. At the same time, transverse sections were cut from the stem, near the base and at various heights above the soil, and plated on to nutrient agar to isolate the fungus; similar sections were examined microscopically for the presence of hyphæ in the vessels.

Conidia were first detected in expressed sap three weeks after inoculation. The numbers in subsequent samples varied from 0 to 6,870/ml. sap and were unrelated to the time from inoculation. There might be abundant conidia in some aliquots of sap, and none in others, from the same plant. Stem-section isolations and observations showed that: (1) *Verticillium* was invariably present before mycelium could be observed microscopically in the vessels; (2) the fungus often occurred at considerable distances above soil level simultaneously with its first-recorded presence in the stem; (3) the distribution of the fungus was occasionally discontinuous; (4) the rate of spread of the fungus was greatly in excess (about ten times) of the maximum mycelial growth rate *in vivo*. Germinated and ungerminated conidia were observed in stained, centrifuged sap, but rarely in transverse sections of stems.

Estimations of vessel length by Greenidge's method[1] indicated that continuous open vessels extended three-quarters of the length of 4-week-old stems (that is, about 150 cm), and that by early summer open vessels were present virtually throughout the length of field-grown bines. Conidia injected directly into the xylem by a cone-frustrum method[2] or with a hypodermic needle were not, however, immediately transported throughout the stem. After 24 h *V. albo-atrum* was isolated only 20 cm above the injection point at about 100–150 cm, 7 days later, and after 2 weeks fungus was generally present in all parts of the stem except the finer extremities and leaf petioles. Colonization of the latter was slow and there was evidence that this might be associated with the absence of continuous open-vessel systems comparable to those in the stem.

The rapid dispersal of *V. albo-atrum* in the hop plant thus seems to be due to the transport of conidia in xylem sap; this movement is occasionally arrested and then followed by conidial germination and renewed sporulation. The dispersal system probably resembles that described for *Fusarium oxysporum* f. *cubense* in banana[3–6], although gel vascular occlusions may not occur in hop[7,8].

The marked time difference observed between wilting of hop plants after root inoculation and the rapid dispersal of the pathogen within mature xylem vessels is thought to reflect the time taken by the pathogen to colonize, by slow mycelial growth, the extravascular tissues of the root and also the closed xylem systems of the root tip[9] and leaf petiole.

Direct injection has demonstrated that the stems of wilt-tolerant hop varieties are slightly more resistant than those of wilt-sensitive ones and that virulent strains of *V. albo-atrum* are slightly more pathogenic to hop stems than mild ones, but these differences are insufficient to account for the contrast in behaviour of these hosts and pathogens following natural root infection. The root thus appears to be a determinative factor in the hop-wilt syndrome, as has previously been suggested by investigations of intervarietal graft-complexes of hop[10].

We thank Dr. A. G. P. Brown for his co-operation during part of this work.

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Asparagopsis in Europe

The spread of *Asparagopsis* in Europe has rightly attracted considerable attention, but the excitement with which each new record has been greeted has tended to obscure certain unanswered questions regarding two species at present accepted, *A. armata* Harv. and *A. taxiformis* (Delile) Trev.

Montagne[1] based the original treatment of the genus on *Fucus taxiformis* Delile[2], although changing the epithet to the illegitimate *delilei*; the combination *Asparagopsis taxiformis*, usually credited to Collins and Hervey[3], was, in fact, used by Trevisan[4] and afterwards overlooked. *A. taxiformis* has been reported widely from the tropical waters of the Pacific, Indian and Atlantic Oceans, the most northerly records in the latter being from Bermuda, the Canary Islands, and possibly the Azores[5], although the latter report has been questioned[6]. *A. taxiformis* has also been reported from the Mediterranean, where it is said to be restricted to the eastern region. Apart from some observations by Schiffner[7], which have been disputed, there appears to be only one record, from Algeria, of the occurrence of *A. taxiformis* as such in the western Mediterranean, and that is based on a drift specimen thought to have originated in the eastern region[8]. However, it should be noted that the type locality for *Falkenbergia hillebrandii*, the alternate phase in the life-history of *Asparagopsis taxiformis*, is the Island of Elba, and there are other early reports[9] of the occurrence of this entity in the western Mediterranean. *Asparagopsis armata* was first described from Australia during the last century and specimens referred to this species were first identified in Europe some forty years ago. Further reports of its occurrence in various localities in north-west Europe and North Africa have been made over the years, but there is still a marked disjunction in tropical waters between the European and Australian areas of distribution.

The first question to be answered concerns the relationship between *A. armata* and *A. taxiformis*. From the literature[6,10] it would appear that the only difference between the two algae is the occurrence of branches bearing spines in the former. The type material of *A. armata* is now preserved in the herbarium of Trinity College, Dublin, and, although the present location of Delile's type specimens is unknown[11], part at least of Delile's original collection of *A. taxiformis* has been found in the Lamouroux Herbarium during a visit to the Institut Botanique, Caen. Examination of these type materials and of other collections confirms that the presence or absence of spine-bearing branches is the only criterion by which the two entities can be distinguished. *A. taxiformis* and *A. armata* have been accepted as distinct species by all authors except Schiffner[7], who considered them merely as growth forms of a single species.

Schiffner's observations have been ignored or dismissed as the erroneous interpretation of badly prepared specimens[10], but some recent personal observations indicate that further investigation of the production of spine-bearing branches in this genus is necessary. During the past few years, several specimens of *Asparagopsis* in which the spine-bearing branches characteristic of *A. armata* were very reduced in size and number have been collected at various localities on the south and west coasts of Britain and Ireland, while in a single collection from the Isles of Scilly no trace of the spine-bearing branches could be found so that the fronds were otherwise indistinguishable from those referred to *A. taxiformis*. In connexion with this problem, it should be noted also that there are no apparent morphological differences between *Falkenbergia rufolanosa* and *F. hillebrandii*, the alternate phases in the life-histories of *A. armata* and *A. taxiformis* respectively.

The second question relates to the origin of the European material referred to *A. armata*. It has been accepted universally that the European material was initially of Australian origin but, in view of the doubts as to the relationship between *A. taxiformis* and *A. armata*, a reconsideration of the origin of the European material is warranted. Whether this can now be undertaken depends very largely on the extent to which the taxonomic problem can be elucidated.

The third question concerns the life-history of the entity in the British Isles. Tetrasporangia have never been detected in the *Falkenbergia* phase in Britain or Ireland, while plants of the *Asparagopsis* phase with carpospores appear to be diminishing in frequency[12]. The barbed branches of the *Asparagopsis* phase facilitate vegetative propagation and the floating 'balls' of the *Falkenbergia* phase, which occur so abundantly on occasions in British waters, have been observed to re-attach with ease[13]. The new attachment is relatively weak and the 'ball' is readily detached so that it may well be moved on many times during its life. These observations suggest that independent propagation of both the *Asparagopsis* and *Falkenbergia* phases is occurring and that the actual life-history of the entity in British waters may not be the functional sequence of sexual, carposporic and tetrasporic phases demonstrated in other areas[14]. It is probable that this independent vegetative propagation is a likely explanation for the small differences in geographical distribution of the two phases which have become increasingly marked during recent years and which have been attributed by various authors[15,16] to differences in ecological requirements.

These three questions demonstrate the deficiencies in our knowledge of *Asparagopsis*, which is by no means as complete as is often assumed. By directing attention to the crucial problem of the relationship between *A. taxiformis* and *A. armata* it is hoped that further investigations will provide the answers to this, and the related problems. In this connexion, reports of the occurrence of spineless specimens of *Asparagopsis* in Britain will be particularly valuable.

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Mechanism for Nitrogen-increased Shoot/Root Ratios

Aberg's review[1] cites Söding (*Die Wuchstofflehre*, Georg Thieme Verlag, Stuttgart, Germany, 1952) as suggesting that the generally recognized increased shoot/root ratios resulting from nitrogen fertilization are caused by nitrogen-increased growth hormone content which, in turn, inhibits root growth and promotes shoot growth in the intact plant. This suggestion appears reasonable and was supported by Bosemark[2], who compared the growth of roots in high nitrogen solution with that of roots growing in solutions containing growth hormones.

Strong experimental support for this hypothesis was obtained from two experiments conducted in this Labora-

tory to evaluate the influence of nitrogen and phosphorus fertilization on the extractable growth hormone content of soy-bean roots (*Glycine max* L.).

The first experiment consisted of the addition of minus phosphorus, minus nitrogen, minus nitrogen and phosphorus, and complete Hoagland's solution 2 to autoclaved, calcareous sand at regular intervals. Total plants were gathered at 11, 16 and 21 days after seedling emergence. The second investigation involved a factorial of three levels of nitrogen and three levels of phosphorus mixed throughout similar autoclaved calcareous sand. A tenth treatment consisting of twice the highest level of nitrogen and phosphorus in the factorial was also included. All other essential plant nutrients were supplied in optimum amounts. These plants were gathered 24 days after emergence.

The soybean roots were not nodulated. Root systems were not washed, and the loosely adhering sand particles were shaken free before the determination of fresh weight. The plant tissues were frozen immediately and stored at −29° C until extraction. Total roots per treatment (entire root system) were macerated with a mortar and pestle, and extracted with absolute methanol for 48–50 h at −12° C. The macerated roots and extract were washed and filtered under suction at room temperatures 22°–24° C. Filtered extracts were reduced in volume by vacuum evaporation at temperatures of less than 40° C. Aliquots of this extract were chromatogrammed by the descending technique using an isopropyl alcohol–water mixture (8 : 2) as the solvent. The developed chromatogram was air-dried and divided into 10 equal strips numbered from one to ten starting at the origin. Each strip was subjected to the oat coleoptile straight-growth test to evaluate its growth activity. Concurrent with the bioassays, longitudinal strips of the same chromatogram, or small aliquots of the extract under study, were applied to separate chromatograms and developed. These chromatograms were sprayed with a 2 per cent solution of *p*-dimethylaminobenzaldehyde in 1·2 N hydrochloric acid (Ehrlich reagent). Colour reactions with this reagent were used to attempt identification of substances in those strips of the chromatogram showing growth activity in the bioassay.

Growth activity in the bioassays was confined to chromatogram strip numbers 3, 4 and 5. Planimeter measurements of the area of Ehrlich-positive spots in strips 3, 4 and 5 were recorded as quantitative estimates of the relative amounts of growth-promoting substances on the chromatogram (R_F region of about 0·25–0·55 by colour reaction).

Tryptophan and an unidentified substance were found to be associated with the growth promotion in the oat coleoptile straight-growth test. Indolyl-3-acetic acid was not found in any of the root extracts. The unknown substance gave a yellow colour reaction with Ehrlich reagent, and occurred at slightly higher R_Fs than tryptophan on the chromatogram. It appeared to be differentially produced by soybean roots at higher nitrogen-levels in the root medium. This was supported by consistently greater growth activity in the bioassay at higher R_Fs on chromatograms of nitrogen-fertilized root extracts[3]. The regression equations and correlations reported are based on area measurements of chromatogrammed 100λ aliquots of the extract of the total root system per treatment. This area was divided by the total root weight to provide an estimate of the concentration of indicated growth hormone per gram fresh weight of root.

Fig. 1 shows the statistically significant inverse relationship between Ehrlich area (indicated growth hormone) per gram of root and the fresh weight of roots produced at each of the three harvests (exp. 1). The trend toward decreasing amounts of indicated growth substances per gram of root with increasing age was reduced by nitrogen fertilization[3].

The positive correlation between shoot–root ratios and the amount of indicated growth substances, where the

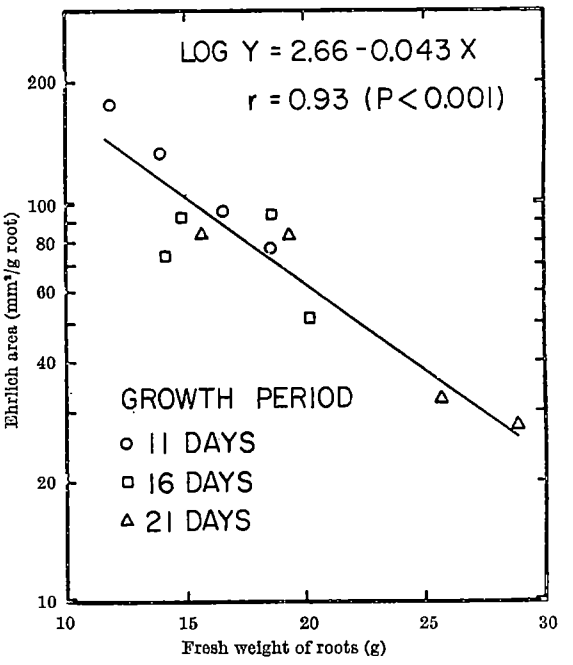


Fig. 1. An inverse relationship is found between the fresh weight of the roots and the logarithm of the Ehrlich area per gram of roots extracted

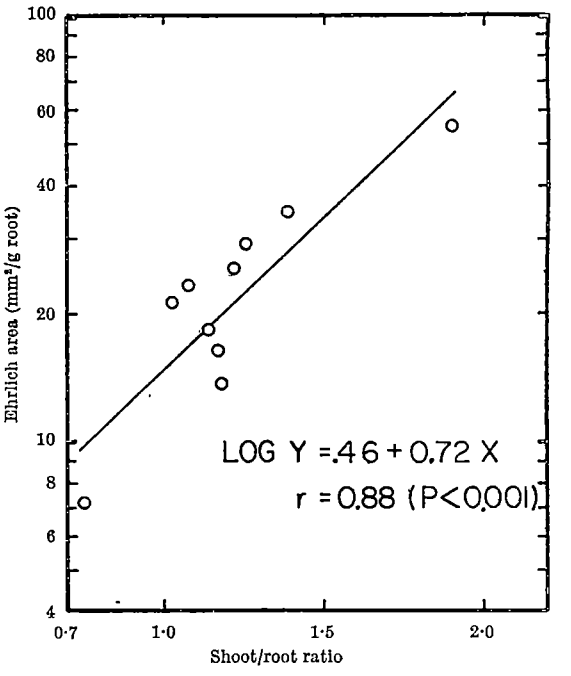


Fig. 2. The logarithm of the Ehrlich area increases with an increase in the shoot/root ratio of plants of the same chronological age. (Exp. 2)

roots were all the same chronological age, is illustrated in Fig. 2. The Ehrlich-positive area in chromatogram strips 3, 4 and 5 was also proportional to the level of nitrogen in the root medium as were the shoot/root ratios. The average Ehrlich-positive area per gram fresh weight of roots was 16 mm², 30 mm² and 55 mm² at the one, four and eight levels of nitrogen, respectively, while the average shoot–root ratios were 1·16, 1·32 and 1·90 at the one, four and eight levels of nitrogen, respectively.

Phosphorus had no consistent effect on shoot/root ratios, or indicated growth hormone content above deficiency levels[3].

A correlation coefficient of −0·72 ($P < 0·05$) between Ehrlich area per gram of root and total fresh weight of

roots per treatment was also obtained from the data of the second experiment. The regression equation for this experiment was log $Y = 1{\cdot}87 - 0{\cdot}013\,X$, where Y equals predicted mm² Ehrlich area per gram of root, and X equals the fresh weight of the roots (g).

The positive correlation between shoot/root ratios and indicated growth substance content, and negative correlation between indicated growth substance content and root growth (fresh weight), was considered to be direct evidence in support of Söding's suggestion that high levels of endogenous hormones in roots may inhibit root growth. The average root lengths in Exp. 2 were 51, 42 and 34 for the one, four and eight levels of nitrogen, respectively: thereby showing general agreement with Bosemark[2] that root length also was reduced by higher levels of nitrogen fertilization.

This work was partly supported by the U.S. Industrial Chemical Co., Inc.

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Determination of Cell Numbers in Dried Leaf Material

The method of maceration in chromic acid followed by counting, using a hæmocytometer, has been widely used in the estimation of cell numbers in fresh leaf material[1–3]. A new method has been developed by means of which cell numbers can be estimated for leaf material which has been oven-dried. The method has the following advantages: direct estimates of cell number can be obtained using dried material from growth-analysis investigations without the necessity of providing separate plants for sampling; this may be especially advantageous for work in glasshouse or constant environment rooms where space is often limited, average cell dry-weight is obtainable directly without the use of a separate sample, and dried material can be stored indefinitely without deterioration and macerated when opportunity arises. The procedure using dried material takes somewhat longer and the estimate of cell number obtained is sometimes slightly lower than that using comparable fresh leaves.

The method has been applied to leaves and leaf disks of French bean and *Canna edulis*, which have been oven-dried at 80° for 24 h and then weighed and stored. The first treatment consists of soaking the dried material in 50 per cent ethanol for 16–24 h. The alcohol is then drained off and the material soaked for a further 6–12 h in two changes of distilled water. The leaves or disks are then carefully blotted to remove the surface liquid and macerated overnight for 14 h in 5 per cent chromic acid (2 ml. per cm² was found to be sufficient for the materials investigated). The cells are then separated by tapping with a glass rod and the resulting suspension is passed rapidly through a fine pipette, made up to a suitable volume and counted using a hæmocytometer. Results comparing this method with that for fresh material are given in Table 1.

The new method gave estimates that were 1–17 per cent lower than the conventional method. As part of this difference could be due to genuine differences between similar leaves, a further comparison was made using disks punched from the same leaves and macerated either fresh or after drying. Here the number of cells per disk ($\times 10^{-6}$) was $2{\cdot}046 \pm 0{\cdot}175$ for fresh material and $1{\cdot}876 \pm 0{\cdot}138$ for dried disks. This difference of 9 per cent was statistically significant ($t = 2{\cdot}86$, $n = 22$). However, a lower value is not always obtained. A comparison for disks taken for the same leaves of *Canna edulis* gave the following values of cell number ($\times 10^{-6}$): for fresh disks $3{\cdot}962 \pm 0{\cdot}580$, and for dried disks $4{\cdot}008 \pm 0{\cdot}424$, the difference not being significant.

Table 1. Comparison of Cell Numbers in the First Pair of Leaves of French Bean Plants, One Leaf macerated after Drying, the other macerated Fresh

Plant	'Fresh' estimate ($\times 10^{-6}$)	'Dried' estimate ($\times 10^{-6}$)	Difference (%)
1	30·28	28·36	6·8
2	36·11	35·61	1·4
3	43·57	40·33	8·0
4	45·32	39·28	15·4
5	52·76	47·86	10·2
6	23·99	20·47	17·2
7	26·02	24·09	8·0

Where differences occur in the values obtained using the two methods it seems likely that they originate in the drastic disruption of the cell wall during the drying process, with the result that a certain number of cells are 'lost' since broken cells are not counted. In the case of *Canna*, disintegration does not appear to occur at all extensively, so that maceration after drying gives an accurate assessment of cell number. The differences can probably be disregarded for most purposes since variation in cell number in comparable leaves can be of the order of 15–25 per cent.

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ENTOMOLOGY

Spinning Apparatus of the Caterpillar of *Leucinodes orbonalis*

The spinning apparatus which has so far been investigated in Noctuidae, Saturnidae and Sphingidae becomes more specialized in the case of *Leucinodes orbonalis* (Pyralidae) with respect to its musculature and the structure of the silk press.

The silk glands are elongated tubular structures occupying a great part of the body cavity and lying below the alimentary canal. The accessory acinous glands described in noctuid and saturnid caterpillars[1] are absent in *Leucinodes orbonalis*. The ducts of these glands open at the base of the silk press, which has muscular walls and is completely enclosed within the hypopharyngeal-premental lobe. The hypopharynx is completely united with

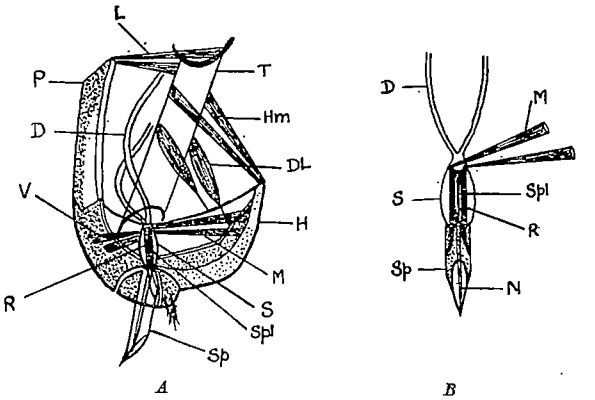


Fig. 1. *A*, Lateral view of the hypopharyngeal-premental lobe of *Leucinodes orbonalis*. *B*, Dorsal view of the silk press. *D*, duct of silk gland; *DL*, dorsal labial muscles; *H*, hypopharynx; *Hm*, hypopharyngeal muscles; *L*, ventral labial muscles; *M*, muscles of the sclerotic plate; *N*, terminal duct; *P*, prementum; *R*, sclerotic bar; *S*, silk press; *Sp*, spinneret; *Spl*, sclerotic plate; *T*, tentorial arm

the prementum, as has been reported in other silk-producing caterpillars[2], but the morphology of the spinneret indicates that it is a component part of the prementum. In the caterpillars of Noctuidae[3], Saturnidae and Sphingidae[4] the dorsal wall of the silk press is invaginated into the lumen and it contains a median sclerotic bar over which two or three pairs of dilator muscles are inserted. Histological investigations of the spinning apparatus of the caterpillar of *L. orbonalis* indicate that the dorsal wall of the press is not at all invaginated and the muscular layer is thicker. The dorsal wall is covered by a thin sclerotic plate formed by the fusion of two sclerotic bars which originate from within the side walls of the spinneret. This sclerotic plate remains opposed to the dorsal wall of the press and is operated by a pair of muscles which take origin from the hypopharyngeal-premental lobe and are inserted on the distal ends of the sclerotic plate. The sclerotic plate is thus movable and quite separate from the wall of the press. The contraction of the muscles withdraws the plate and the pressure so far exerted by it is released, resulting in the extension of the lumen of the press. At the same time the change in the position of the sclerotic plate makes the two bars straighten the spinneret slightly anteriorly, causing a temporary break in the flow of the fluid silk through the terminal duct of the silk press, which opens on the tip of the spinneret. A second pair of ventral muscles, which constitute the dilator of the lumen of the press, arises from the premental part of the hypopharyngeal-premental lobe and insert on the ventral wall of the press.

Up-and-down movements of the hypopharyngeal-premental lobe are brought about by three pairs of muscles. The hypopharyngeal muscles, which are the elevators, take origin from the upper region of the tentorial arms, and are inserted at the base of the hypopharyngeal part of the lobe. Their action is co-ordinated by the dorsal labial muscles, which arise from the middle of the tentorial arms and are inserted on the dorso-lateral margins of the premental part of the lobe. The ventral labial muscles are inserted at the base of the premental portion and originate from the region of the tentorial arms where they join the internal ridge of the frons and act as depressors. The sideways movement of the hypopharyngeal-premental lobe is effected by the elaborate arrangement of muscles of the head and prothorax.

I thank Dr. P. N. Saxena and Prof. S. Keshava for advice.

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Role of Insects as Vectors of Red Ring Disease

SEVERAL insect species associated with the coconut palm have been suggested as be vectors of red ring disease (Table 1).

Table 1

Order	Family	Species	References
Coleoptera	Curculionidae	*Rhynchophorus palmarum* (L).	1-8, 10-12
		Rhina barbirostris L.	3,6,7,11
		Homalinotus coriaceus Gyll.	3,6,7
	Scolytidae	*Xyleborus* sp.	11
Hymenoptera	Formicidae	(?)	2,11
Isoptera	Rhinotermitidae	*Coptotermes niger* Snyder	13
	Termitidae	*Nasutitermes ephratae* Holmg.	13

Cobb[4], Martyn[10], Fenwick[5], and Hagley[8] have stated that the causal organism, *Rhadinaphelenchus cocophilus* (Cobb), was recovered from the body surfaces of adult palm weevils, *R. palmarum*, and Hagley[9] has discussed the importance of these insects as vectors of the disease.

In recent studies conducted in the eastern and north-eastern regions of Trinidad adults of several other species collected from 188 diseased palms between 2 yr. and 7 yr. of age were examined by the methods previously described for *R. palmarum*[9]. *R. cocophilus* was recovered from four species of Coleoptera (Table 2).

Metamasius hemipterus, observed on 56·5 per cent of the palms examined, was most frequently taken under the external fibres surrounding the internodal stem region and in the upper leaf axils of the palm. At these sites the insects were normally not exposed to direct contact with diseased tissue. Although some individuals were recovered in the tunnels excavated by larvæ of *R. palmarum* in the internal diseased stem tissues, only 2·8 per cent of the total number examined were carrying nematodes.

R. barbirostris which was observed in localized areas was present on 10·7 per cent of the palms examined. The preferred sites of the insects were in the axils of the lower and older petioles and at the base of the stem. Adults were not observed in the internal stem tissues. Nematodes recovered from a single individual were all dead.

The histerid, *Hololepta* sp., was taken most frequently in palms showing severe palm weevil infestation. The tissues of these palms were in an advanced state of decay and 80 per cent of those examined did not contain living nematodes. The insect was recovered from 53·5 per cent of the palms examined and the nematodes recovered from six individuals were all dead.

Nematodes were also recovered from one individual of the dynastid, *Hemiphileurus* sp., which was found in the lower leaf axils of only four palms.

The small numbers of those insects which carried *R. cocophilus*, and the relatively low frequency with which

Table 2. THE RECOVERY OF *R. cocophilus* FROM INSECTS ASSOCIATED WITH DISEASED PALMS

Order	Family	Species	Total No. individuals recovered	No. carrying nematodes: Living	No. carrying nematodes: Dead	Av. No. living nematodes/insect
Coleoptera	Curculionidae	*Metamasius hemipterus* L.	465	8	5	12
		M. hemipterus hemipterus (L.)	22	—	—	
		M. obsoletus Gyll.	42	—	—	
		Rhina barbirostris L.	61	—	1	
		Homalinotus depressus L.	16	—	—	
		Parisoschenus sp.	406	—	—	
		Limnobaris sp.	123	—	—	
	Dynastidae	*Strategus aloeus* (L.)	8	—	—	
		Hemiphileurus sp.	4	—	1	
	Histeridae	*Hololepta* sp.	314	—	6	
	Scolytidae	*Xyleborus subaffinis* Egg.	396	—	—	
Hymenoptera	Formicidae	*Azteca* sp.	602	—	—	
		Odontomachus haematoda L. s.sp. *pubescens* For.	343	—	—	
		Wasmannia auropunctata Rog.	528	—	—	
		Anochetus emarginatus Fab.	175	—	—	
		Campanotus rectangularis Em.	71	—	—	
Isoptera	Termitidae	*Nasutitermes costalis* Holmg.	648	—	—	

they were observed, suggested that their role as vectors of red ring disease was of little significance.

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GENETICS

Genetic Variation of Alcohol Dehydrogenase in *Drosophila melanogaster*

A growing number of biochemical variations are at present being described in many organisms. When variations in proteins can be demonstrated to be under genetic control the interest and importance they hold are, to some degree, based on the background of genetic knowledge in the experimental organism. *Drosophila melanogaster* has recently provided knowledge of several inherited biochemical variations. The protein variations of Pantelouris and Duke[1] and the variations in the esterases[2,3], alkaline phosphatase[4,5], leucine aminopeptidase[6,7] and glucose-6-phosphate dehydrogenase[8] are among the latest to be discovered.

The basis for the observation of these variations is differential mobility, which is exhibited when different molecular forms of protein are subjected to the sieving action of starch-gel electrophoresis[9]. The method separates proteins according to both molecular size and charge; slight variations in either can be detected in the starch gel by a change in mobility. Quite often such variations can be demonstrated to be under genetic control. After electrophoresis various staining techniques[10,11] are used to localize enzyme activity in the gel.

When genetic electrophoretic differences are discovered between *Drosophila* individuals it is most common to find both parental proteins in the hybrids. In one case, however, in addition to the parental protein bands, a third band of intermediate mobility was found. The third or hybrid zone was found in the larval alkaline phosphatase enzyme[4,5] and represented one of the first hybrid enzyme molecules to be found in *Drosophila*. This communication reports the finding of a second hybrid molecule in the enzyme alcohol dehydrogenase.

Starch-gel electrophoresis was carried out using the discontinuous system of buffers by Poulik[12]. Single *Drosophila* individuals (larvæ, pupæ or adults) were homogenized separately in a small drop of distilled water. The resulting liquid was absorbed into a 4 mm × 6 mm piece of filter paper and inserted into a vertical slot in the starch gel. The samples were electrophoresed for about 2 h at a voltage gradient of 6–8 V/cm at room temperature. After electrophoresis the gels were sliced horizontally and stained for alcohol dehydrogenase activity in the following solution: 100 ml. *tris*hydroxymethylaminomethane-hydrochloride buffer, *p*H 8·5, 0·1 M 5 ml. 95 per cent ethyl alcohol; 25 mg diphosphopyridine nucleotide; 25 mg *p*-nitroblue tetrazolium; 7 drops 0·5 per cent methylene blue. Staining was complete in about 4 h and the gel was preserved in a water–methanol–acetic acid (5 : 5 : 1) solution. The *Drosophila* stocks used in this investigation were maintained on standard yeast corn-meal molasses culture medium.

Most of the *Drosophila* laboratory cultures which have been examined for alcohol dehydrogenase show a deeply staining (fast) band approximately midway between the start and the slow larval alkaline phosphatase variant. A second band, much fainter in staining intensity, migrates at nearly twice the rate of the main strong band. However, in one stock, Canton-*S*, the strong fast alcohol dehydrogenase was absent from the usual position and had instead a band (slow) migrating in the opposite direction, toward the cathode. A faint band was also present in this strain which coincides with the position of the fast strong alcohol dehydrogenase band (Fig. 1). All developmental stages, larvæ through adults, show the same alcohol dehydrogenase zones.

Alcohol dehydrogenase-slow flies of the Canton-*S* strain were mated with alcohol dehydrogenase-fast flies from some of the other stocks. The resulting hybrids possessed the parental alcohol dehydrogenase bands and, in addition, zones of intermediate mobility. Not only was there an intermediate or hybrid zone between the strong alcohol dehydrogenase zones but also between the faint bands (Figs. 1 and 2). A tentative explanation which appears satisfactory at the present preliminary stages is that the fast strong alcohol dehydrogenase band present in most stocks is due to homozygosity for one allele at an autosomal locus and that the slow alcohol dehydrogenase band is due to an alternative allele. The hybrid substance is probably the result of combination of unlike molecular sub-units in the heterozygote. Similar reasoning follows for the faint alcohol dehydrogenase bands. Whether or not it represents an independent system has not been determined.

The presence of sub-units in alcohol dehydrogenase has been indicated from other investigations. Alcohol dehydrogenase from yeast has been found to have a molecular weight of 151,000 and to contain four zinc atoms and bind four molecules DPN per molecule of enzyme. Liver alcohol dehydrogenase has two zinc atoms and binds two molecules of DPN with a molecular weight of 84,000. Treatment of the enzymes with zinc-complexing agents removes the zinc atoms and causes inactivation and dissociation into sub-units (cf. Malcolm and Webb[13]) of molecular weight 36,000.

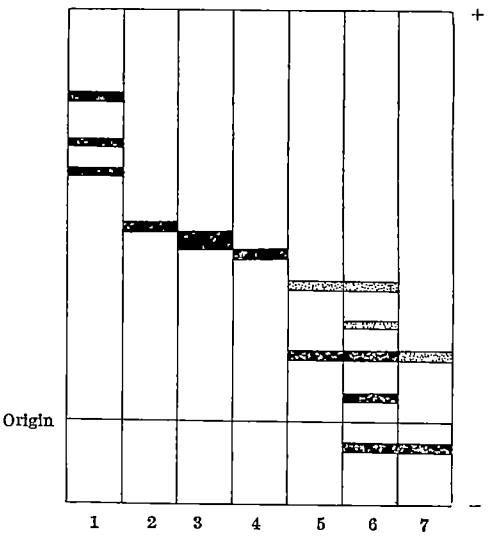


Fig. 1. Schematic comparison of protein patterns of larval alkaline phosphatase and alcohol dehydrogenase in starch-gel electrophoresis of single *Drosophila*. Sample 1 indicates the three main protein bands, samples 2–3 show the fast, heterozygous and slow alkaline phosphatase patterns and samples 5–7 show the fast, heterozygous and slow alcohol dehydrogenase patterns. The faint alcohol dehydrogenase bands are stippled



Fig. 2. Photograph of starch gel showing fast (*FF*), heterozygous (*FS*), and slow (*SS*) alcohol dehydrogenase

Although methanol will not serve as substrate, several other alcohols can be substituted for ethanol in the staining solution. Isopropanol is at least equally effective as a substrate as ethanol. Other alcohols produce less dense patterns.

Mixture experiments with fast and slow variants homogenized together failed to produce the hybrid band. A similar finding resulted from an identical experiment with fast and slow alkaline phosphatases[4,5].

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Hæmoglobin *H* Disease in Thailand: a Genetical Study

IN Thailand in 1952 Virginia Minnich and her colleagues first discovered inclusion bodies in the red cells of patients with thalassæmia-like syndrome[1]. This disease became known here as inclusion-body anæmia, but was later identified as thalassæmia-Hgb *H* disease, first reported by Rigas *et al.*[2]. Its genetical peculiarity was immediately recognized. In the majority of cases the abnormal pigment is not detected in either parent, or in the progeny. Thalassæmia stigmata are always present in one of the parents and in the patients themselves, and in some of the sibs and progeny. The associated thalassæmia is of the normal Hgb A_2 or alpha type.

Because of its horizontal distribution, it was suggested that the gene for Hgb *H* was recessive. Motulsky[3] in 1956 proposed that the apparently normal parent might harbour an *H* gene which could manifest only when present together with thalassæmia. In 1959 Jones *et al.*[4] identified the chemical nature of Hgb *H* as β_4. It was then suggested that there might be imbalances in the α-chain synthesis, leading to accumulation of the β chains which then polymerized to form β_4 or *H*. In the same year Ingram and Stretton[5] suggested that Hgb *H* may be formed in one of the following two ways: either, two mild α-thalassæmia occur together resulting in a reduction of the α-chain synthesis and polymerization of the excessive β chains, or, alternatively, there is a silent β-chain mutation causing excessive production of the β chains which, when present together with the α-thalassæmia gene, polymerize. The second hypothesis was favoured by Ingram and Stretton. Later it was found that in the patients with Hgb *H*, Hgbs γ_4 and δ_4 are also present[6–8]. It therefore appears more likely that reduction in the α-chain synthesis is solely responsible for the development of all three abnormal pigments.

In 1961 Koler and Rigas[9] carefully analysed the genetical data available up to then. They could find eleven sibships with one *H* parent, five of these sibships including one or more children with hæmoglobin *H*. Among 23 children of the *H* patients seven had hæmoglobin *H*. They argued against *H* being due to homozygosity of an α-thalassæmia locus. They explored the many possibilities, including a single mutation at the α locus, the β^H mutation with an α-thalassæmia gene, and the existence of an α-thalassæmia allele producing no demonstrable abnormality when present alone. They decided that evidence was not yet available to choose among these hypotheses.

In Thailand Hgb *H* disease is prevalent, over 140 cases having been investigated by us. The clinical and hæmatological data were recently presented at the Asian and Pacific Congress on Hæmatology in Jerusalem[10]. We have studied over 350 members of 42 unrelated families with this disease. The results are summarized in Tables 1–4.

With regard to the presence of α-thalassæmia gene, Hgb *E* trait (*A*+*E*) and α-thalassæmia-Hgb *E* will be treated as equivalent to normal and α-thalassæmia trait,

Table 1. PARENTS OF Hgb *H* PATIENTS

Normal	23	26	
A+*E*	3		
α-thalassæmia trait	32	35	0·2 > P > 0·1
α-thalassæmia-Hgb *E*	3		
Hgb *H*	4		
Total	65		

Table 2. MATING TYPES OF THE PARENTS OF 27 Hgb *H* SIBSHIPS

Normal × normal	0
Normal × α-thalassæmia trait	17
(*A*+*E*) × α-thalassæmia trait	2
Normal × α-thalassæmia-Hgb *E*	2
α-thalassæmia trait × α-thalassæmia trait	3
α-thalassæmia trait × α-thalassæmia-Hgb *E*	1
Hgb *H* × normal	1
Hgb *H* × α-thalassæmia trait	1
Total	27

Table 3. SEGREGATION RATIOS AMONG 154 SIBS IN 49 Hgb *H* SIBSHIPS

Normal	35
A+*E*	5
α-thalassæmia trait	39
α-thalassæmia-Hgb *E*	6
Hgb *H*	69
Total	154

Table 4. SEGREGATION RATIOS AMONG 62 OFFSPRINGS OF 19 Hgb *H* PATIENTS

Normal	16	
α-thalassæmia trait	38	
α-thalassæmia-Hgb *E*	1	
Hgb *H*	7	(in 4 sibships)
Total	62	

respectively. The recognition of the α-thalassæmia trait was based on the findings of the abnormal RBC morphology, decreased osmotic fragility, with normal serum iron and normal Hgb A_2 level. While it is fully realized that these criteria are not conclusive, up to now they are the only available ones.

The ratio of *H* individuals among sibs, after correcting for truncate selection, fits well with 1/4 (*a priori* expectation is 66·742 versus the observed 69). This, together with the frequent absence of Hgb *H* in the other generations, suggests that a single gene mutation is not the cause of *H* and indicates double-gene origin of the abnormal pigment, one from each parent.

Of the two abnormal genes harboured by the *H* person, one is generally accepted as an α-thalassæmia. There is much evidence against the idea that homozygosity of the α-thalassæmia locus is the cause of *H*—the persistent absence of thalassæmia stigmata in one of the parents, the lack of this disease among Negroes in spite of the prevalence of α-thalassæmia gene in this race[11,12], and the existence of a prototype for homozygosity at this locus, the hydrops fœtalis with Hgb Bart's[13,14]. Furthermore, if it were due to homozygosity of the α-thalassæmia locus, parent–child transmission might happen only when the *H* person married an individual with α-thalassæmia trait or with Hgb *H*. We have observed parent–child transmissions four times out of nineteen. This would mean that the frequency of α-thalassæmia trait in the population was 4/19 or 0·21. From this the frequency for Hgb *H* persons is expected to be approximately 0·011, which is much too high.

For convenience, let the *H* gene be taken to be the abnormal allele which the *H* person derives from the apparently normal parent. The *H* gene and the α-thalassæmia gene could be independent or allelic or linked.

With regard to them being independent, parent–child transmission must be very frequent and at least 1/4 of the offspring of *H* persons will have Hgb *H*. The observed 7 *H* out of 62 progeny is significantly less than 1/4 ($P < 0·02$). It would therefore appear that the *H* gene is either allelic or linked to the α-thalassæmia locus.

If the two genes are allelic, the *H* individual will never produce *H* progeny, unless he marries a person with an α-thalassæmia trait or with an *H* trait. That would mean that the combined incidence of α-thalassæmia trait and *H* trait is 4/19 or 0·21. The frequency of α-thalassæmia obtained from our survey here is 0·03. Therefore, that of *H* trait would be 0·18. From these an approximate expectation of the frequency of *H* persons is 0·00135. We do not yet know the incidence of *H* obtained directly from a survey investigation, because all our investigations were such that the patients were most likely not included. However, the incidence of *H* in the population is indirectly estimated. The patients with thalassæmia-Hgb *E* disease and those with Hgb *H* disease come to our attention for similar reasons. Lately, after a routine examination for RBC inclusion bodies and hæmoglobin electrophoresis on cellulose acetate strips, which is highly sensitive in detecting Hgb *H*, having been adopted, the number of patients with each of these diseases is similar. The gene frequency[15] for Hgb *E* is 0·069 and the frequency of β-thalassæmia trait from our unpublished study is 0·035. From these the expected frequency of thalassæmia-Hgb *E* is calculated to be 0·0012, which, as mentioned above, is also taken to be the approximate frequency of Hgb *H* disease. Mating between two *H* trait persons, which is frequent (0·0324), most likely does not result in offspring with the disease.

At present it is not possible to differentiate between allelomorphism and close linkage between the *H* and the α-thalassæmia genes.

In the offspring of *H*, if the *H* trait is not manifest, a phenotypic ratio of 1 normal : 1 α-thalassæmia trait is expected. But the ratio of 16 : 38 was observed. In the sibs of *H* the expected ratio is 2 normal : 1 α-thalassæmia trait, but 40 : 45 was observed. It would therefore appear that approximately half the *H* traits in the progeny and sibs of *H* persons do have hæmatological characteristics similar to the α-thalassæmia trait. Furthermore, it would appear that the manifestation of the *H* trait decreases with age.

In conclusion, the *H* gene, which is either allelic or closely linked to the α-thalassæmia locus and which, in the heterozygote state, appears to have, at least at younger ages, similar hæmatological findings to the α-thalassæmia trait, might well be another and milder form of α-thalassæmia.

Finally in view of the findings of Hgb *H* in the individual harbouring genes for α-thalassæmia and Hgb *Q*, which is an α-chain defect[16], in cases of leukæmia[17], in α-thalassæmia trait[12], heterogeneity of the genetic mechanism of its development is obvious, but all stem from various degrees of reduction of the α-chain synthesis.

We thank the Thai Medical Research Council and the China Medical Board, Inc., New York, for their support.

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PSYCHOLOGY

Arousal and Childhood Autism

The clinical features of the syndrome 'early infantile autism' were described by Kanner first in 1943 (ref. 1) and more recently in 1958 (ref. 2). The cardinal symptoms of this syndrome are: (*a*) an inability to form social relationships; (*b*) an 'obsessive insistence on the preservation of sameness'. The latter feature often has the corollary that catastrophic reactions are provoked by the introduction of novelty or the change of an established routine. A catastrophic reaction closely resembles infantile rage, consisting of screaming, weeping, threshing movements of arms and legs and sometimes self-directed aggression such as biting the limbs.

Investigation of a substantial number of children with Kanner's syndrome including systematic observation of their free-field activities has suggested a neurophysiological hypothesis to account for some aspects of their behaviour. The hypothesis is that in these children the non-specific activity of the reticular system is sustained at a chronically high and relatively inflexible level, or in other words, they are in a chronically high state of arousal.

This formulation would account for several features of their disorder.

(1) The resting electroencephalograms (EEGs) of these children are predominantly of two types: (i) records of very low voltage (< 25 μV) irregular activity irrespective of whether the eyes are open or closed, that is, 'flat' or 'desynchronized' EEGs; (ii) α-dominated records with eyes closed, similar to those found in adults, and flat EEGs with eyes open. Records which are consistently desynchronized or of higher frequency than is usually found in children of this age (2–6 years) may be evidence of an unusually high level of electrocortical activation or arousal in the waking state. Sleeping EEGs are generally within normal limits for the age-group.

(2) Two main characteristics of the syndrome are behavioural withdrawal and the continued performance of stereotyped behaviours such as rocking, grimacing, idiosyncratic hand movements. Investigations with animals have shown that both these behaviours are associated with states of high arousal[3,4].

(3) In a recent investigation[5], detailed observations were made on six children, aged 3–6 years, diagnosed as suffering from this syndrome. The waking EEG records made in the laboratory showed desynchronized activity. None of the children had neurological signs or a history of cerebral insult. Observations were made in a situation which, in other children, usually elicited exploratory behaviour[6]. These autistic children, however, showed marked stereotyped movements and very little exploratory behaviour. In fact, stereotypy was their predominant activity. It was found that stereotypy increased with increasing complexity of the environment and was least in evidence when the child was by himself in an unfurnished waiting-room. If stereotypy is a function of level of arousal then increased sensory bombardment (arising from increasing complexity) would result in more stereotyped behaviour. Conversely, this behaviour would be least likely when there was relative sensory deprivation. In fact we found that these children were least 'psychotic' in the empty room. (Adult schizophrenics have also been found to show less disturbed behaviour under conditions of sensory deprivation[7].) In these children stereotypies may be a device to prevent level of arousal exceeding critical limits, by blocking further novel sensory input.

(4) It has been possible to telemeter the EEGs of 2 autistic children while behavioural observations of their free-field activities were simultaneously being recorded. As before, stereotypies occurred least frequently in the unfurnished room, and in this situation the EEG was more synchronized and of higher voltage than in more complex environments. Stereotypies were more frequently accompanied by 'desynchronized' EEG activity than rhythmic activity.

(5) Nearly all autistic children seen by us have been suspected of deafness on referral, yet audiometry (where this has been possible) has seldom revealed any loss of hearing. They also manifest a higher threshold than normal children to painful stimuli. It has been shown that stimulation of the brain-stem reticular formation in cats produces blocking in sensory pathways the receptors of which are simultaneously stimulated[8,9]. It is possible that the chronically high level of arousal in these children similarly produces blocking of sensory pathways, thus accounting for their altered responsiveness to sound and pain.

(6) Autistic children strive to maintain 'sameness': as with complexity, novelty results in activation of the reticular system[10]; if this is already highly activated, either there will be an attempt on the part of the organism to prevent any further excitation by engaging in repetitive activities, or else the organism will succumb to excessive excitation when a catastrophic reaction will result.

It is perhaps relevant that the reticular system has been implicated in the schizophrenic disorders of adults[11,12], which may possibly be related to the present disorder; it has also been shown[13] that social withdrawal in many chronic schizophrenics is associated with increased physiological arousal. Furthermore, chlorpromazine, the action of which is to depress the activity of the reticular system[14], is effective in the treatment of many schizophrenic disorders of adults. Jasper[15] has stated more specifically, on the basis of Rothballer's work, that chlorpromazine has a differential effect on the thalamic and caudal portions of the reticular system, blocking the action of the tonic (caudal) system without affecting the rapid phasic system. If, as seems likely, chlorpromazine proves to be effective also in the treatment of these autistic children, it is possible that at least in some of them the ascending reticular activating system (ARAS) is more specifically involved in the disorder. It is premature, however, to suggest the nature or extent of this involvement, or its ætiology.

The work is supported by the Oxford Regional Hospital Board and the Nuffield Foundation.

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Lateralization and One-trial Inter-hemispheric Transfer of Avoidance Conditioning

MANY workers have demonstrated the use of spreading cortical depression to lateralize the effects of conditioning to one cerebral hemisphere. Russell and Ochs[1] have shown that food-reinforced bar-pressing lateralized to one hemisphere could be transferred to the other hemisphere with a single reinforced trial during bilateral cortical function. More recently Russell and Ochs[2] showed that a necessary condition for such one-trial inter-hemispheric transfer of a memory trace was the presence of reinforcement during bilateral function. If animals were allowed to perform the conditioned response in the absence of reinforcement no transfer was found.

Bureš and Burešová[3] have reported lateralization of simple avoidance conditioning to one cortical hemisphere. Animals were trained in a shuttle-box to a criterion of 9 out of 10 successful avoidance responses with one cortical hemisphere depressed. On the following day either 5 or 10 avoidance trials were given with both hemispheres functional. Thirty minutes later spreading depression was produced in the trained hemisphere and the animals trained to criterion. They found that if the animals were given 10 'interdepression' trials with both cortices functional, inter-hemispheric transfer of the memory trace to the untrained hemisphere was seen in terms of a significant

savings score. Further, five 'interdepression' trials were found to be ineffective in producing transfer.

The purpose of the experiment recorded here was to examine whether a single 'interdepression' trial could produce transfer of a lateralized avoidance response comparable to the one-trial transfer effects found for food-reinforced conditioning. Twelve rats were prepared with permanently indwelling cannulæ, one over each tempero-parietal region[4]. Close-fitting mandrins were used to keep the cannulæ patent. Spreading depression was produced by a 25 per cent solution of potassium chloride injected by a microdrive syringe. The volume of each injection was 0·008 c.c., which produced a depression of approximately 90 min duration. The effectiveness of each injection was tested before and after training by loss of the placing reflex in the contralateral forelimb of each animal.

Avoidance conditioning was obtained using a runway, 6 ft. long, with an electrifiable grid floor. Animals were placed in the start box and the door leading to the runway then raised. Simultaneously a buzzer (*CS*) was sounded followed 8 sec later by shock (*UCS*) in both the start box and the runway. On reaching the end of the runway, the rats gained access to the goal box, where they either escaped or avoided the shock. They were trained to a criterion of nine consecutive avoidance responses on each of the five days of training. A successful avoidance response was recorded when the animal reached the goal box before the onset of shock. Fifteen minutes before training, spreading depression was generated by injection in the left hemisphere of the animals on days 1, 2 and 3. On day 4 depression was produced in the right hemisphere. A single trial was given on day 5 with both hemispheres functional for half the animals. Ninety minutes later the right hemisphere was depressed and the rats run to criterion. The remaining animals on day 5 followed the same procedure with the omission of the 'interdepression' trial. The results may be seen in Fig. 1.

Acquisition of the avoidance response can be seen in terms of the successive decrements in trials to criterion over the first three days of training with the left hemisphere depressed. On day 4, when the right hemisphere was depressed, the performance was the same as day 1 for both groups. The effect of previous training was clearly lateralized to the hemisphere functional during the first three days of training. Student's '*t*' test showed no significant differences between days 1 and 4 for each group. Further, no differences were found between groups on any of the four days of training. This lateralization of learning, indicated by the absence of any savings for the untrained hemisphere on day 4, showed there was no spontaneous transfer of the memory trace between training sessions. A single 'interdepression' trial, however, given when both hemispheres were functional on day 5, did produce significant transfer effects on the experimental group compared to the controls (P 0·01). This significant drop in trials to criterion for the experimental group is also seen by comparing the scores for both groups on days 2 and 5. This is critical when it is remembered that day 5 was the second day of training with the right hemisphere depressed and day 2 was the second day of training with the left hemisphere depressed. If there was no transfer of the memory trace one would expect identical scores within groups for both days. Comparing the scores of day 2 with day 5, however, reveals significantly lower scores on day 5 for the experimental group (P 0·001), and no difference for the controls. It is interesting to note that, although one 'interdepression' trial produced positive transfer effects, it did not produce perfect transfer. This can be seen by a comparison of the scores for the experimental group of days 3 and 5, where the scores for day 3 are significantly smaller (P 0·001).

These results give generality to the experiment by Russell and Ochs[2] and differ somewhat from that of Bureš and Burešová[3]. Two points should be made here concerning this discrepancy: (1) Bureš did not use any conditioned stimulus in his avoidance procedure whereas in the present experiment shock was always preceded by a buzzer; (2) Bureš allowed only 30 min between 'interdepression' trials and the initiation of spreading depression whereas a 90-min interval was used in the present experiment. Ross[5], Chamberlain, Halick and Gerard[6], McGaugh, Westbrook and Thompson[7], and many others have pointed out that a period of time after learning is necessary for consolidation of the memory trace. Estimates of the length of this time vary; but it is quite possible that 30 min is not long enough for the trace to consolidate. If this is true then one would not expect to find positive one-trial transfer in such a case.

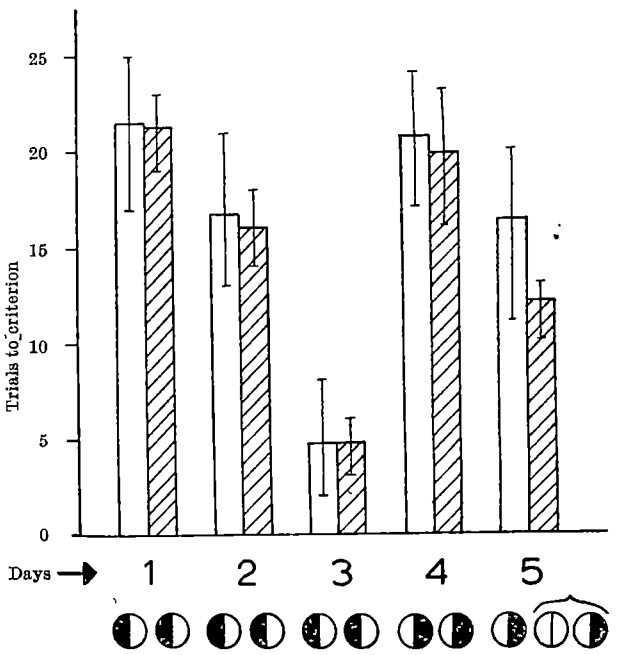


Fig. 1. Average number of trials to criterion are presented for both groups on each of five days of training. The clear histograms represent scores for the control group and shaded histograms the experimental group. The range of scores for each group is indicated by the vertical lines. The hemisphere depressed is indicated by the shading in circles below each histogram

Bureš, however, did find that 10 'interdepression' trials with a 30-min consolidation period were effective for transfer. This suggests the possibility of an interaction effect between the number of reinforcements and the length of the consolidation period. In other words, a single reinforcement might require a longer period to consolidate than would several successive reinforcements. It is clear that spreading depression as well as being a useful tool for lateralizing the effects of learning may further be used to shed light on the perseveration-consolidation theory of learning.

This work was supported by the Medical Research Council and the work was carried out at the Council's Unit for the Experimental Investigation of Behaviour, London.

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I. Steele Russell

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[1] Steele Russell, I., and Ochs, S., *Science*, **133**, 1077 (1961).
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[7] McGaugh, J. L., Westbrook, W. H., and Thompson, C. W., *J. Comp. and Physiol. Psychol.*, **55**, 710 (1962).

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, November 30

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (joint meeting with the I.E.R.E. Medical and Biological Group, at Savoy Place, London, W.C.2), at 2.30 p.m.—Discussion on "Lasers".

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 2.30 p.m.—Anniversary Meeting.

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Sir John Lockwood: "Higher Education". (Last of three Cantor Lectures on "A Comparative Study of World Trends in Education".)

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.15 p.m.—Dr. T. F. Gaskell and Captain G. S. Ritchie, D.S.C., R.N.: "Beneath the North Atlantic".

Tuesday, December 1

UNIVERSITY OF LONDON (at the Institute of Obstetrics and Gynaecology, Hammersmith Hospital, London, W.12), at 2 p.m.—Prof. P. Polani: "Human Chromosomes and Their Abnormalities".

UNIVERSITY OF LONDON (at Westfield College, Kidderpore Avenue, London, N.W.3), at 5.15 p.m.—Sir Edward Bullard, F.R.S.: "The Floor of the Ocean".*

INSTITUTION OF ELECTRICAL ENGINEERS, SCIENCE AND GENERAL DIVISION (joint meeting with the I. Mech. E. Automatic Control Group, at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. H. Nicholson: "Optimal Control of a Boiler".

UNIVERSITY OF LONDON (at Imperial College of Science and Technology, Prince Consort Road, London, S.W.7), at 5.30 p.m.—Dr. P. L. Willmore: "The Contribution of the Tape Recorder to Seismology".*

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. D. J. Bauer: "Antiviral Chemotherapy". (Eleventh of fourteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)*

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. Richard Wollheim: "On Drawing an Object" (Inaugural Lecture).*

INSTITUTION OF MECHANICAL ENGINEERS, MANIPULATIVE AND MECHANICAL HANDLING MACHINERY GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Bulk Unloading of Solids from Ships—Is It Time for New Methods?"

SOCIETY OF INSTRUMENT TECHNOLOGY (at Manson House, 26 Portland Place, London, W.1), at 6 p.m.—Mr. E. N. Martin and Mr. R. J. Weir: "Process Instrumentation up to 1984".

UNIVERSITY OF LONDON (in the Engineering Theatre, University College, Gower Street, London, W.C.1), at 6 p.m.—Prof. René Tavernier (Ghent): "Genesis and Classification of Brown Earth".*

PLASTICS INSTITUTE, LONDON SECTION (at the Wellcome Building, Euston Road, London, N.W.1), at 6.30 p.m.—Mr. B. S. Dyer and Mr. M. R. Day: "Reinforced Thermoplastics".

Wednesday, December 2

COLOUR GROUP (Great Britain) (in the Lamont Room of the National Book League, 7 Albemarle Street, London, W.1), at 3 p.m.—23rd Science Meeting.

SOCIETY FOR ANALYTICAL CHEMISTRY (at the Wellcome Building, Euston Road, London, N.W.1), at 3 p.m.—Meeting on "Solvent Extraction".

INSTITUTION OF MECHANICAL ENGINEERS, APPLIED MECHANICS GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 4 p.m.—Papers on "High-Rate Forming".

ROYAL STATISTICAL SOCIETY (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5 p.m.—Prof. J. W. Pratt: "Bayesian Interpretation of Standard Inference Statements".

FAUNA PRESERVATION SOCIETY (at the Offices of the Zoological Society of London, Regent's Park, London, N.W.1), at 5.30 p.m.—Dr. Hugh Lamprey (Principal of the College of Africa Wildlife Management): "The Work of the College".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. H. J. Sketch: "The U.K. 3 Satellite".

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, ELECTRO-ACOUSTICS GROUP (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Symposium of short papers on "Special Loudspeaker Applications".

SOCIETY OF ENVIRONMENTAL ENGINEERS, PACKAGING GROUP (in the Mechanical Engineering Department, Imperial College of Science and Technology, London, S.W.7), at 6 p.m.—Mr. R. A. C. Ives: "The Fragility of Simple Structures".

Thursday, December 3

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 10.30 a.m.—Discussion Meeting on "Some Aspects of the Stratosphere, Mesosphere and Lower Thermosphere" organized jointly by Prof. D. R. Bates, F.R.S., Prof. P. A. Sheppard, F.R.S., and Dr. R. C. Sutcliffe, F.R.S.

ROYAL INSTITUTION OF NAVAL ARCHITECTS (in the Weir Lecture Hall, 10 Upper Belgrave Street, London, S.W.1), at 4.45 p.m.—Dr. R. L. Townsin: "Boundary Layer Separation from Ship Models—a Note on Its Detection and Suppression with Some Examples"; Mr. R. E. Clements: "The Control of Flow Separation at the Stern of a Ship Model Using Vortex Generators".

INSTITUTION OF CIVIL ENGINEERS, TRANSPORTATION ENGINEERING GROUP at Great George Street, London, S.W.1), at 5.30 p.m.—Informal Discussion on "The Growth of Private Car Ownership and the Redevelopment of Public Transport" introduced by Mr. J. D. C. Churchill.

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. S. Shaldon: "Long-Term Hæmodialysis as a Substitute for Kidney Function". (Twelfth of fourteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)*

UNIVERSITY OF LONDON (at the Middlesex Hospital Medical School, Cleveland Street, London, W.1), at 5.30 p.m.—Prof. J. G. Mulnard (Université Libre de Bruxelles): "Experimental Approach to Mammalian Development".*

INSTITUTE OF REFRIGERATION (at the National College for Heating, Ventilating, Refrigeration and Fan Engineering, Southwark Bridge Road, London, S.E.1), at 6 p.m.—Mr. G. C. Hatcher and Mr. J. C. F. Payne: "The Application of Computers to Problems Associated with Refrigeration Engineering".

INSTITUTION OF MECHANICAL ENGINEERS, EDUCATION AND TRAINING GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "How to Encourage Schoolboys to Become Chartered Mechanical Engineers".

UNIVERSITY OF LONDON (in the Engineering Theatre, University College, Gower Street, London, W.C.1), at 6 p.m.—Prof. René Tavernier (Ghent): "The Soil Map of Europe".*

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (joint meeting with the Northampton College Chemical Society, at the Northampton College of Advanced Technology, St. John Street, London, E.C.1), at 7 p.m.—Dr. L. J. Bellamy: "Hydrogen Bonds".

Thursday, December 3—Friday, December 4

ROYAL PHOTOGRAPHIC SOCIETY (at the Institution of Electrical Engineers, Savoy Place, London, W.C.2)—Symposium on "Optimum Results from Colour Photography".

Friday, December 4

ROYAL AERONAUTICAL SOCIETY, ROTOCRAFT SECTION (at 4 Hamilton Place, London, W.1), at 2.15 p.m.—Mr. L. M. Hewin: "The General Requirements of the Three U.S. Armed Services for Helicopters and other VTOL Aircraft"; Major-Gen. N. Crookenden: "British Army Unit Light Helicopter Project".

SOCIETY OF CHEMICAL INDUSTRY, FINE CHEMICALS GROUP (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. D. J. Bauer: "Antiviral Chemotherapy".

TELEVISION SOCIETY (in the Conference Suite, I.T.A., 70 Brompton Road, London, S.W.3), at 7 p.m.—Mr. J. Weltman: "Television University".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Prof. C. C. Butler, F.R.S.: "High-Energy Nuclear Physics—Achievements and Future Developments".

Saturday, December 5

BRITISH INTERPLANETARY SOCIETY (at the Northampton College of Advanced Technology, St. John Street, London, E.C.1), from 10 a.m. to 12.30 p.m.—Meeting on "Astronautics Education".

ASSOCIATION OF CLINICAL BIOCHEMISTS, SOUTHERN REGION (at the Wright Fleming Institute, St. Mary's Hospital, Praed Street, London, W.2), at 10.30 a.m.—Meeting on "Forensic Toxicology".

BRITISH MYCOLOGICAL SOCIETY (in the Rooms of the Linnean Society of London, Burlington House, Piccadilly, London, W.1), at 2.15 p.m.—Annual General Meeting, followed by Prof. N. F. Robertson: "The Fungal Hypha" (Presidential Address).

LONDON COUNTY COUNCIL (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Dr. J. G. Sheals: "The British Museum (Natural History) Expedition to East Nepal".*

Monday, December 7

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 10 a.m. and 2.30 p.m.—Colloquium on "Programmed Test Equipment and Techniques".

BRITISH SOCIETY FOR THE PHILOSOPHY OF SCIENCE (in the Joint Staff Common Room, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Mr. G. C. Archibald: "Refutation or Comparison?".

INSTITUTION OF ELECTRICAL ENGINEERS, SCIENCE AND GENERAL DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Prof. J. C. West: "Non-Linear Circuit Theory".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 6 p.m.—"The Bridge" (Geographical Films).

UNIVERSITY OF LONDON (at Birkbeck College, Malet Street, London, W.C.1), at 6 p.m.—Prof. J. Th. Runnenburg (University of Amsterdam): "Some Practical Problems Solved with Simple Tricks from Probability Theory and Analysis".*

SOCIETY FOR ANALYTICAL CHEMISTRY, MICROCHEMICAL METHODS GROUP (at "The Feathers", Tudor Street, London, E.C.4), at 6.30 p.m.—Discussion Meeting on "The Characterization of Organic Compounds" opened by Dr. D. W. Mathieson and Mr. L. C. Cross.

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (joint meeting with the Paper and Textile Chemicals Group, at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Mr. I. F. Hendry and Mr. J. A. S. Newman: "New Developments in Papermaking".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

M.R.C. RESEARCH ASSISTANT IN THE DEPARTMENT OF PHARMACOLOGY for work on amine metabolism in neurological diseases, under the direction of Prof. H. Schnieden—The Registrar, The University, Manchester, 13 (December 5).

Assistant Lecturer in the Department of Medicine, University of Edinburgh—The Professor of Medicine, Department of Medicine, The Royal Infirmary, Edinburgh, 3 (December 7).

Assistant Lecturer in Zoology—The Registrar, The University, Manchester, 13 (December 10).

Lecturer (with an honours degree in geography and teaching experience in schools) in Geography and Education at the Institute of Education—The Registrar, The University, Sheffield (December 12).

Lecturer (man or woman graduate) in Geomorphology in the Department of Geography—The Secretary, Bedford College (University of London), Regent's Park, London, N.W.1 (December 13).

Senior Lecturer or Lecturer in Mechanical Engineering at the University of Natal, Durban—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, December 14).

Chair of Crop Husbandry and Horticulture at the University of Ghana—The Assistant Registrar, University of Ghana Overseas Office, 15 Gordon Square, London, W.C.1; or The Registrar, University of Ghana, P.O. Box 25, Legon, Accra, Ghana (December 15).

Chair of Geology at the University of Ghana—The Assistant Registrar, University of Ghana Overseas Office, 15 Gordon Square, London, W.C.1; or The Registrar, University of Ghana, P.O. Box 25, Legon, Accra, Ghana (December 15).

Research Fellow in Computer Science—The Secretary, University of London Institute of Computer Science, 44 Gordon Square, London, W.C.1 (December 15).

Lecturer or Assistant Lecturer in Statistics—The Secretary, The Queen's University, Belfast, Northern Ireland (December 17).

Lecturer (with a good honours degree in civil or mechanical engineering, professional and/or research experience, and preferably interest in strength of materials) in the Department of Engineering—The Secretary, The University, Aberdeen (December 19).

Lecturer or Assistant Lecturer (Probationary) (man or woman graduate) in Zoology—The Secretary, Bedford College (University of London), Regent's Park, London, N.W.1 (December 20).

Lecturer or Assistant Lecturer in Pure Mathematics; a Lecturer or Assistant Lecturer in Applied Mathematics; and a Lecturer or Assistant Lecturer in Statistics—The Registrar, The University, Leeds, 2 (December 31).

Lecturer (with special interests in the application of physical chemistry to pharmaceutical, medicinal or biochemical problems) in Physical Chemistry in the Department of Pharmacy—The Registrar, The University, Nottingham (December 31).

Senior Lecturer in History and Philosophy of Science at the University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, December 31).

Senior Lecturer in Mammalian Physiology in the Department of Biological Studies—The Principal, Lanchester College of Technology, Priory Street, Coventry (December 31).

Chair of Theoretical Physics at Queen Mary College—The Academic Registrar, University of London, Senate House, London, W.C.1 (January 1).

Research Assistant (with an honours degree in geology or zoology and preferably postgraduate experience in palæontology or micro-palæontology) in Palæontology, for duties which will involve the preparation, identification and cataloguing of carboniferous fossils—The Registrar and Secretary, University of Durham, Old Shire Hall, Durham (January 1).

Lecturer in Immunochemistry in the Department of Pathology—The Registrar, Monash University, P.O. Box 92, Clayton, Victoria, Australia; or The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (January 4).

Research Fellow in Environmental Archæology in the Department of Anthropology and Sociology, Research School of Pacific Studies, Institute of Advanced Studies, Australian National University—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 20).

Chair of Mathematics at the University of Otago, Dunedin, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1; or The Registrar, University of Otago (New Zealand and London, January 31).

Assistant Lecturer (with a good honours degree in biology or zoology) in Biology—The Registrar, University of Strathclyde, George Street, Glasgow, C.1.

Entomologist (preferably under 35, with an honours degree in agriculture, horticulture or an appropriate natural science, plus two years postgraduate training or experience) in Hong Kong, to undertake and supervise agricultural research work and to provide appropriate specialist advice and assistance in the field of practical agricultural entomology—The Appointments Officer, Room 301, Ministry of Overseas Development, Eland House, Stag Place, London, S.W.1, quoting Ref. RC213/81/02.

Graduate Research Assistant (preferably with training or previous research experience in exfoliative cytology) for work on cancer cell detection—The Dean, Guy's Hospital Medical School, London Bridge, S.E.1.

Horticulturist (preferably under 35, with a degree in agriculture, horticulture or an appropriate natural science, plus two years' postgraduate training or experience) in Hong Kong, to undertake and supervise agricultural research work, and to provide specialist advice and assistance in the field of horticulture—The Appointments Officer, Room 301, Ministry of Overseas Development, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 213/81/04.

Lecturer or Senior Lecturer (with a first- or second-class honours degree or equivalent, and preferably experience in teaching, industry or the Scientific Civil Service) in the Mathematics Branch—The Registrar, Royal Military College of Science, Shrivenham, Swindon, Wiltshire.

Master to teach Physics throughout the School, with a good share of VI Form work—The Headmaster, King's School, Worcester.

Research Assistants (2) (preferably with a degree in biochemistry or chemistry at a good honours standard, or graduate in a biological science, with suitable experience) in the Department of Applied Microbiology and Biology, to undertake studies related to the mechanisms of sporulation in filamentous fungi—The Registrar, University of Strathclyde, George Street, Glasgow, C.1.

Research Biophysicist in the Department of Pathology, for work concerned with the application and further development of an electron probe microanalyser in the investigation of trace element distributions in human diseases—Dr. A. J. Hale, Department of Pathology, St. Thomas's Hospital Medical School, London, S.E.1.

Technician in the Department of Biochemistry, to assist in research on the biochemical effects of radiation on normal and cancer tissues—The Secretary, The Medical College of St. Bartholomew's Hospital, West Smithfield, London, E.C.1.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Meteorological Office. Scientific Paper No. 20: The Interannual Variability of Monthly Mean Air Temperatures over the Northern Hemisphere. By J. M. Craddock. (Met. O. 755.) Pp. ii+10. (London: H.M. Stationery Office, 1964.) 2s. net. [610

Discovery or Invention? By Prof. I. D. Rattee. (Inaugural Lecture delivered in the University of Leeds on 20 January 1964.) Pp. 23. (Leeds: The University Press, 1964.) 2s. 6d. [610

Agricultural Research Council. Soil Survey of Great Britain—England and Wales. Bulletin No. 1: Soils of the Middle Teign Valley District of Devon. By B. Clayden. Pp. vii+111+map. (Harpenden: Rothamsted Experimental Station, 1964.) 25s. net. [610

Chelsea College of Science and Technology. Prospectus 1965–1966. Pp. 40+4 plates. (London: Chelsea College of Science and Technology, 1964.) [610

Other Countries

Institutt for Atomenergi, Kjeller, Norway. Kjeller Report No. 70: Measurements of HBWR II Core Parameters in the NORA Reactor. By K. Bryhn-Ingebrigtsen, N. L. Snidow, A. Myking and H. Smidt Olsen. Pp. v+12+14 figures. Kjeller Report No. 71: K–7 Transpo (One-Group Neutron Transport in a Heterogeneous Cylindrically Symmetric Reactor Cell). By R. J. J. Stamm'ler. Pp. v+27+8 figures. Survey of Activities 1963. Pp. 29. (Kjeller, Norway: Institut for Atomenergi, 1964.) [610

United States Department of Agriculture. Leaflet No. 530: Shade and Water for the Honey Bee Colony. By Charles D. Owens and S. E. McGregor. Pp. 8. (Washington, D.C.: Government Printing Office, 1964.) [610

Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Bulletin No. 98: Groundwater Resources of Fannystelle Area, Manitoba. By J. E. Charron. Pp. ix+21. 75 cents. Paper 64–10: Rocks and Minerals for the Collector—Bay of Fundy Area. By Ann P. Sabina. Pp. vii+96. 75 cents. Paper 64–25: Revised Stratigraphic Nomenclature for the Central Part of the Labrador Trough. By M. J. Frarey and S. Duffell. Pp. vi+13. 35 cents. Paper 64–40: Geological Survey of Canada—Radiocarbon Dates III. By W. Dyck and J. C. Fyles. Pp. ii+15. 35 cents. Paper 64–50: A Simple Method for Converting Measured X-ray Intensities into Mass Compositions. By G. R. Lachance. Pp. iii+8. 35 cents. (Ottawa: Queen's Printer, 1964.) [610

Indian Central Jute Committee. Annual Report of the Jute Agricultural Research Institute for the year 1962–63. Pp. 115. (Calcutta: Indian Central Jute Committee, 1964.) [610

Uganda. Annual Report of the Forest Department for the year ended 30th June, 1963. Pp. ix+79. (Entebbe: Government Printer, 1964.) Shs. 5/50. [610

United States Department of the Interior: Fish and Wildlife Service. Bureau of Sport Fisheries and Wildlife Research. Report No. 64: Some Chronic Effects of DDT on Cutthroat Trout. By Don Allison, Burton J. Kallman, Oliver B. Cope and Charles Van Valin. Pp. iv+30. (Washington, D.C.: Government Printing Office, 1964.) [610

Geological Survey of Uganda. Report No. 10: Explanation of the Geology of Sheets 19, 20, and 29 (Southern West Nile). By Dr. J. V. Hepworth. Pp. vi+128 (5 plates). Shs. 30. Index to Geological Mapping as at 31st December 1963. Pp. 1. (Entebbe: Government Printer, 1964.) [610

Conseil National de la Politique Scientifique. Inventaire du Potentiel Scientifique de la Belgique—Année 1961. Pp. 303. (Bruxelles: Conseil National de la Politique Scientifique, 1964.) [610

Smithsonian Contributions to Astrophysics, Vol. 8, No. 4: The Velocity of Faint Meteors. By Gerald S. Hawkins, Bertil-Anders Linblad and Richard B. Southworth. Pp. ii+133–139. (Washington, D.C.: Government Printing Office, 1964.) [610

Occasional Papers of the California Academy of Sciences. No. 47 (August 28, 1964): The Pinacate Region, Sonora, Mexico. By Ronald L. Ives. Pp. 43. (San Francisco, California: California Academy of Sciences, 1964.) [610

National Museum of Canada. Bulletin No. 202 (Anthropological Series, No. 69): Development of Basic Socio-Economic Units in Two Eskimo Communities. By Asen Balikci. Pp. x+114. (Ottawa: Queen's Printer, 1964.) 1.50 dollars. [610

New Zealand. Report of the Department of Scientific and Industrial Research for the year ended 31 March 1964. Pp. 87. (Wellington: Government Printer, 1964.) [610

The Year Book of the National Institute of Sciences of India, 1963. Pp. vi+241. (New Delhi: National Institute of Sciences of India, 1964.) Rs. 15. [610

Indian Council of Scientific and Industrial Research: Central Fuel Research Institute. Report of the Director for the year 1961–62. Pp. iv+127. (Jealgora, Dhanbad, Bihar: Central Fuel Research Institute, 1964.) [610

Bulletin of the American Museum of Natural History. Vol. 127, Article 4: Results of the Archbold Expeditions. No. 86: Summary of the Sixth Archbold Expedition to New Guinea (1959). By L. J. Brass. Pp. 145–216+plates 2–13. 2.50 dollars. Vol. 127, Article 5: The Relationships and Taxonomic Position of the Halfbeaks, Killifishes, Silversides, and Their Relatives. By Donn Eric Rosen. Pp. 217–268+plates 14 and 15. 1.50 dollars. (New York: American Museum of Natural History, 1964.) [610

Editorial and Publishing Offices of "NATURE"
MACMILLAN (JOURNALS) LIMITED
ST. MARTIN'S STREET, LONDON, W.C.2
Telephone Number: Whitehall 0081. Telegrams: Phusis London W.C.2

Annual subscription: Inland £13, Overseas £13 15s., payable in advance, postage paid to any part of the world.

Advertisements only should be addressed to
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Telephone Number: Holborn 4743

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MAN AND THE SECRET SPRINGS OF NATURE

SIR HAROLD HARTLEY'S Romanes Lecture, "Man and Nature", delivered at the University of Oxford on October 27, has all the sweep and vision that have marked recent reviews of the Carnegie Institution of Washington, with which, in style, it has something in common. Sir Harold's insight is all his own, though he is prompted by the observation of Lucretius with whose modernity he is confessedly amazed. It is indeed from a passage in George Santayana's essay on Lucretius that he derives his initial inspiration: "The man who discovers the secret springs of appearances opens to contemplation a second positive world, the workshop and busy depths of Nature, where a prodigious mechanism is continually supporting our life and making ready for it from afar by the most exquisite adjustments. The march of the mechanism, while it produces life and fosters it, yet as often makes it difficult and condemns it to extinction".

Sir Harold begins his lecture with a review of the way in which, in the half-century since Santayana wrote those words, enquiry into the 'secret springs' of Nature has revealed something of what was then a profound mystery. He quotes also Jowett's forecast almost a century ago, in his introduction to Plato's *Republic*: "The resources of the natural sciences are not half developed as yet; the soil of the Earth, instead of growing more barren, may become many times more fertile than hitherto; the uses of machinery greater, and also more minute than at present. New secrets of physiology may be revealed deeply affecting human nature in its innermost recesses. The standard of health may be raised and the lives of men prolonged by sanitary and medical knowledge . . . The ever-increasing power of locomotion may join the extremes of the earth".

Sir Harold moves easily over the realms in which Jowett's prophecies have been fulfilled and Santayana's perceptive interpretation justified during the past half-century and more. He has the deep understanding and perspective of the historian as well as the intellect of the scientist. The contributions of Pasteur and Ross are related with deep insight, as also are such more recent discoveries as Melvin Calvin's food-producing photosynthetic cycle, Sir Hans Krebs's metabolic cycle, and the discovery of the pattern of the coiled protein molecules in the nucleus of the cell and the physical basis of heredity. All this is set, not against forecasts of half a century or a century ago, but against the astonishing insight of Lucretius two thousand years ago.

Again and again Lucretius used words which fit our present-day knowledge. He recognized the cyclical character of Nature's processes, the life and death of living organisms and their return to the primordia to begin the cycle again, the alternation of the seasons, and of day and night. His reference to *natura gubernans* to keep successive changes in living organisms in proper balance is apposite to what we are just beginning to simulate with the aid of computers in our great chemical factories and oil refineries. Equally appropriate is the simile Lucretius uses to account for the rarity of the substances formed from the primordia, his reference to heredity "by which things can, as they grow, preserve their kind". Even of new views on the origins of life on this planet, Lucretius's words "buffeted by blows from limitless time, by trying movements and unions of every kind" fit the passage of organic compounds through the stage which Mr. N. W. Pirie calls 'biopoiesis', producing at last some primitive organism that could use radiation from the Sun to make food from water and return the oxygen to the air. Newton often quoted Lucretius, and their arguments touching the eternal nature of atoms are identical.

Sir Harold Hartley is not content merely to show how the torch of tradition is handed on through the ages, nor to outline this world of new particles and intricate cycles of living processes. We are beginning to see glimpses of the third stage through which Sir Cyril Hinshelwood, in a presidential address to the Royal Society, suggested that scientific theories may pass in the search for principles and in the conflict between imagination and the austere regard for truth—the new order which succeeds the early gross oversimplification, the distortion of the symmetry of the hypothetical systems and marring of neatness as recalcitrant facts increasingly rebel against uniformity. It was not necessary for Sir Harold in this lecture to refer to the corrective value of the historical perspective. The whole lecture is steeped in that sense, and the scientist at least has reason to be grateful to him for displaying so clearly the rashness of any neglect of the insight or interpretation or experience of the past.

But Sir Harold's own contribution is pregnant with meaning for to-day. In the latter part of his lecture he asks how this deeper understanding has influenced man's relations with Nature and he seeks to apply what lessons he draws to the pressing problems of to-day, particularly at the international level. First, he reminds us that it is misleading to think of a partnership with Nature because the common interest which partnership implies does not exist. We can learn from Nature and co-operate with her, but, although man has now largely mastered the constant threat from his environment under which he lived in the past, he has still to come to terms with the great power that his new understanding of Nature has put into his hands.

Sir Harold looks at the effect of these changes on man's philosophy and on his attitude to social and ethical concepts formulated in different circumstances, and it is in this context that he examines the question of food supply and population—by far the most urgent problem that the world is facing to-day. It is not sufficient to consider the many contributions that new techniques and the new understanding of the living process have made to food production, from improvements in yields and quality of crops, of breeds of animals, control of diseases and pests, and the improvement of soil fertility, for there is no finality. Nature does not take kindly to man's interference with her balance and man has ever to be on his guard and watchful of her reactions. The new science of ecology is dynamic, not static, and has much to teach.

Man has still much to learn, not only about food supply but equally also the use of his raw materials in general. Sir Harold is well aware of the new possibilities in the use of micro-organisms, but what he stresses above all is the constant need for forethought, the choice of means appropriate to the purpose to be served. This is of fundamental importance in designing aid to the developing nations, and its significance can scarcely be over-stressed. As Sir Harold observes, the average yields of crops and the

progressive improvement in those yields are much greater in the developed countries. It is not simply a matter of food surpluses on one hand and food deficiencies on the other; there are wide divergencies in the efficiency of the cultivator and in the resources he can command, as well as wide gaps between the primitive cultivator and the fund of knowledge long available to the developed countries. Progress in the developed countries is based on the fertility of the soil built up by generations of good farming practice, whereas in Africa and Asia the soil has often been depleted of nutrients by erosion and long years of exploitive agriculture. Emergent countries may have to start by making good long periods of neglect and depletion before many of the new techniques can yield results.

Apart from problems still unsolved as to how far existing knowledge regarding, for example, the ability of legumes to fix nitrogen is valid in the tropics, the innate conservatism of man poses social, political and technological problems which each country must solve for itself, with the appropriate means to which education, land tenure, irrigation and other technical factors contribute. Much more is at stake than production alone. More mechanization of agriculture will not provide the answer. In many countries the key factor is raising the efficiency of the individual cultivator and not the introduction of costly equipment which has proved its value under different conditions in the developed countries.

These considerations underline the importance of accurate surveys, such as Sir Harold Hartley has long urged, and which have now been made by the agencies of the United Nations. The problems are now defined, and what Sir Harold emphasizes in this Lecture is that the great challenge to the present generation, which involves the future of humanity and the stability of the world, is only to be met by some sacrifice of personal and national self-interest and by the recognition of the ultimate community of interest of the world in spite of fragmentation. Many difficulties have to be overcome and the greater freedom of world trade, the appropriate choice between various forms of aid, above all the devotion and inspiration of personal service, are not to be achieved by political dogma or formulæ. Each country must choose for itself the appropriate relation between man and machine, the wisest allocation of resources, without blindly following the economic, political, social, educational or technical patterns of other countries where the relation of population to resources is different.

Finally, Sir Harold sees the relation between man and Nature as something deeper than the relief of hunger. It is interesting to contrast this lecture with Lord Brain's presidential address to the British Association in August, in which Lord Brain covered some of the same ground as Sir Harold. The difference, however, is that Lord Brain emphasized how little we know of man himself, whereas Sir Harold emphasizes the vast potentialities of the knowledge we already have—but without dissenting from our need to know more about man himself. When Sir Harold insists that the deeper problem is to find a way of living that will give human satisfaction and hope, he is challenging us to search for that further knowledge of man's nature which will enable us to find the means to redress the imbalance between man and Nature.

That challenge is sharpened in Sir Harold's final words when, quoting a passage from Sir Geoffrey Vickers, he insists that man was not made for leisure, and that he will continue to accept the challenges of Nature as he has done in the past. Sir Harold did not elaborate the point. He was content to leave it in the context of the words of Sir Geoffrey which he quoted: "For each of us, as well as for society and posterity, the need to struggle is the chance to grow. The world is like a dark forge, lighted by the sparks which men strike as they beat the stuff of life into significance on the anvil of circumstance. The light is a function of the effort; we make it bright as day if we hammer hard enough; but we shall never dispense with the need to hammer, or live by the light of sparks struck yesterday".

That passage goes to the roots of Britain's own difficulties to-day, and Sir Harold's comment that man was not made for leisure indicates where its relevance lies. Preoccupation with leisure rather than effort is responsible for much that is amiss in Britain to-day in the whole social and political climate, as well as in her economic and industrial situation. Even advances such as the computer and automation make possible bring their own challenge and the need to come to fresh terms with Nature; challenges that are not simply technical or economic but social and moral as well. In this interdependent world it may well be that the challenge of leisure will be met in part as we respond to the challenge of the underdeveloped countries—framed so clearly in the problem of food and population.

IMAGE OF CHILE

THE scientific advice and financial assistance which the United States has been giving to the Central and South American countries is now well known. This has applied especially to the development of agriculture, but now such assistance is converging into scientific collaboration—much to the credit of the United States, having profound scientific knowledge and the policy to give advice and financial help, and also to the wisdom of the other American countries concerned.

The most recent evidence of this desirable scientific collaboration was presented by a conference on "Science and Development in Chile", held in Washington, D.C., under the joint auspices of the Embassy of the Republic of Chile and the U.S. National Academy of Sciences, during October 5–9.

The President of the United States sent the following message to members of the conference:

"In this third year of the Alliance for Progress, it is gratifying to see scientists of Chile and the United States sharing the fruits of their research in the Image of Chile Science and Development in Chile program, jointly sponsored by the Embassy of Chile and the National Academy of Sciences of the United States of America.

"The pressing need to apply the benefits of science and technology for the well-being of humanity makes it particularly appropriate that the scientists of our two countries work together in the spirit of hemispheric co-operation.

"To the distinguished scientists from Chile and to my fellow Americans who share our hopes and aspirations for the advancement of human welfare in all of the Americas, I bid a warm welcome and wish you every success."

Space will not allow a report of the symposium held, and the papers read, at the conference; but the closing remarks made by Mr. Dean Rusk, U.S. Secretary of State, and by the Ambassador of Chile to the United States are a source of inspiration to all countries, and therefore the substance of each speech is reported as follows.

SCIENCE AND DEVELOPMENT IN CHILE

By The Hon. DEAN RUSK
U.S. Secretary of State

When the United States pledged its commitment to the Alliance for Progress in 1961, it recognized the central part science and technology must play in the economic and social transformation envisaged under the Alliance. The experience of the first years of the Alliance has not lowered our assessment of the importance of science in this development process. But it has refined thinking on how science can most usefully spur economic growth.

In Latin America generally, scientific talent is limited in quantity—indeed only 2·3 per cent of the population is classified as professional or technical in any field. It has been necessary to establish priorities. This has led to extensive discussions between scientists and technologists, on one hand, and those responsible for allocating national resources on the other; and, as a rule, the result has been the sound conclusion that short-range scientific and technical projects, however interesting in and of themselves, are less urgent than research and teaching institutions capable of promoting science and technology. Much has been done, and is being done, in improving curricula, strengthening faculties, equipping laboratories with the most modern and sophisticated research devices, and providing interchanges between the United States and Latin American scientists.

There is general agreement, I believe, that most of the Latin American countries need to put more emphasis on improving their agriculture and on making their rural areas a more active market and stimulus for manufacturing. Indeed, this is true of most of the developing areas of the world. As I have recently pointed out, agricultural production in the developing areas, particularly in Asia and Latin America, is growing less rapidly than population —and if current trends continue, the point will not be too far off when world food stocks will simply not be enough to meet minimum needs in the deficit areas.

Although Chile has a rich agricultural potential, it has been a net importer of food for some years and now imports about 20 per cent, or 125 million dollars, of its food needs. Every dollar of increased food production has the potential of freeing a dollar of valuable foreign exchange to buy goods and services needed for Chilean development.

The Agricultural Development Project now being initiated in Chile is a good example of how applied science contributes to a total development strategy. This project seeks to increase farm production through research activity and improved rural education institutions. Closely correlated activities seek to stimulate food output and exports through changes in the agricultural credit and marketing systems and in overall national agricultural policy.

Another effective application of science to Chile's economic priorities is the Maule River Project, so reminiscent of the Tennessee Valley Authority development in the United States. By late 1967 a comprehensive regional development plan will be created for the Maule River Basin, to include an integrated and co-ordinated construction and financial schedule for individual projects. The multi-purpose water resource plan will include uses for agriculture, industry, power, recreation, flood control and conservation. Related projects will cover agrarian reform, transportation, marketing, education and training, housing, urban growth, community development and public health.

The Manpower and Educational Planning Project is a key to assuring that, in the future, human resources will make their optimum contribution to Chile's economic and social goals.

I believe these three projects, among the other technical assistance activities with which the United States is concerned in Chile, are excellent examples of the co-operative endeavour the States share under the Alliance. They are being implemented by Chilean institutions in collaboration with the Chile–California Programme and the U.S. A.I.D. Mission in Santiago, with funds provided both by the Government of Chile and A.I.D., and they are utilizing more fully than ever before the scientific capability of Chile and the United States in the service of the former's economic and social development under the Alliance for Progress.

Co-operation between Chile and the United States extends over a wide range of scientific subjects, including the sleep–wakefulness mechanisms. This has a somewhat special appeal to me. I have been told that science may make it possible for us to get on with less sleep at night. As I remarked on another occasion, I know some government officials who, of necessity, have already made considerable progress in that direction.

I turn now to the second main discipline of these seminars—earth sciences.

The same force which caused so much destruction in Chile during 1960 manifested itself in Alaska only last year. This awesome power of Nature disdains international boundaries. Investigations carried out in Chile on earthquake-resistant construction can have universal application. Development of techniques and instrumentation which can sense the early on-rush of earthquakes is as important in the eastern hemisphere as in the western.

New techniques to determine the location and extent of as yet unfound ore deposits are of great significance in the economic development of a country such as Chile, which has potentially such a great mineral wealth. I understand that investigations of this type are being actively pursued by the Institute of Geologic Research.

Investigations in physical oceanography offer many potential benefits, such as harnessing the tides and ocean currents for power. Exploitation of the plant and animal resources of the sea promises, I think, even more rewarding benefits. In a world where much of the population goes to bed at night hungry, where protein deficiency is widespread, the potential benefits from harvesting the sources in the sea are spectacular. The successful conclusion of experiments now under way in Chile producing fish protein concentrate on a pilot-plant scale could be of far-reaching benefit to mankind. We need more research in methods of influencing the biological cycle of the sea and in techniques leading to farming fish rather than hunting them. Such programmes might pose a challenge for scientists in the Fish Development Institute in Chile.

Progress in any of these scientific fields depends not so much on dollars as on manpower. The necessity for the development of trained manpower, including scientists, is an essential component of Chile's goals. It will continue to receive close attention by Chilean authorities and the U.S. A.I.D. Mission.

Because of the particular importance of science and engineering in economic development, a special committee of the Pan-American Union has been set up to investigate the science and engineering education programmes in Latin America. Investigations have already been carried out in several countries, and for Chile they have now been completed.

Let a meeting of scientists from Chile and the United States, gathered within the spirit of hemispheric co-operation, be interpreted by all as a certain sign that we stand together, ready to attack any and all to overcome any scientific or technological obstacles in the path of the economic and social progress of the peoples of the New World.

SCIENCE, FOREIGN RELATIONS AND GOVERNMENT IN DEVELOPING NATIONS

By HIS EXCELLENCY THE AMBASSADOR OF CHILE TO THE UNITED STATES

(Sergio Gutiérrez-Olivos)

Perhaps it may be surprising that a diplomatic mission should engage in a venture not normally associated with diplomatic duties and activities. This surprise would be justified if these symposia were purely and simply a scientific expression. As science and technology more and more saturate every fibre of our lives as individuals and communities, it becomes progressively more difficult for us to ignore the decisive part they play in all events in which we, as laymen, are daily called on to play a responsible part.

I am reminded of what the celebrated French General Lyautey said when he was assigned to an African garrison early in his brilliant military career. "What is your speciality, lieutenant?" his commander asked him. Lyautey replied: "I am a specialist, Sir, in general ideas". Permit me, then, to invoke my status as a non-scientist in developing some general ideas on the importance of the sciences in certain contemporary activities that are not *per se* scientific, such as foreign affairs and government.

A general belief exists in developing countries that only the foreign policies of the great powers are influenced by science and technology. There is some justification for this belief, because the inter-relationship of science, foreign policy and government in those powers is tangible and clear to the man in the street every time he opens his newspaper. Even though the developing nations do not face problems requiring foreign policy alternatives fixed at a given moment by the balance of space and/or nuclear progress; even though the day is still remote when such countries must resort to high theories of a mathematical content and to electronic computers to try to interpret and predict the course of international relations, there is ample evidence—as even a rapid survey reveals—that the inter-relationship of science, foreign policy and government has a concrete and decisive effect on the theory and practice of foreign relations of even minor powers.

The 'eclipse of distance' produced by advances in transportation and communication has, among other things, made the masses all over the world familiar with the way of life of the wealthier nations, and has fired the imagination and ambitions of vast sectors in such a manner that to-day the configuration of world politics is determined, at least in part, by tensions—national and international—that did not exist when each region was circumscribed by its local horizons and surrendered to its individual fate.

Progress toward development requires that Foreign Offices have access to—and that they examine, classify and file for ready use in case of need—a vast compendium of political, economic and scientific knowledge which grows by the minute. Unless they do so, the general lines of foreign policy, or a specific decision in a specific situation, can fall into serious and even irrevocable error.

Foreign policy to-day is made only in part in the cabinets of the Foreign Ministers. For the rest, it evolves at international meetings of varied political, economic, social—and, of course, scientific—hues, and frequently at meetings of a mixed type, such as that which produced the Antarctic Treaty. Let us remember that in the area of science and technology alone there were more than 300 international organizations in 1962, and that 50 of them were of an inter-governmental type.

Just as the conquest of underdevelopment calls for a planned strategy in utilizing internal resources, so does it call for a planned strategy in utilizing international resources, including those of a scientific nature.

The influence of science on the foreign policy of a developing country can assume a more subtle aspect, curiously akin to that of the major powers. In effect, their superior scientific development serves these powers to strengthen and increase their international prestige, attract domestic and foreign capital, and involve them in executing or taking part in technical assistance programmes elsewhere in the world.

With due and prudent regard for distances, these benefits and functions are not beyond the reach of certain developing nations. I have said that this 'Image of Chile' sought to show the United States public the state of certain specific areas of Chilean science and thereby promote the image of my country and the interest of the United States public in it. With all frankness, and without false pride or false modesty, I can say that Chile has made, and will continue to make, a valuable contribution to the development of other countries for which our technical and scientific resources are of interest.

Willy-nilly, the developing nations are being called on properly to understand and resolve, individually or collectively, certain questions of a very sophisticated scientific or technical nature: Will the right of 'innocent passage' apply or not to space? The assignment of frequencies for transmissions from satellites is already in sight. What kind of international law will govern 'occupation' of the Moon?

For Melba Phillips, "The truly central problem in economic development, and perhaps the most difficult of all, is education". I need not enlarge on the degree to which the answer to this problem depends on adopting a foreign policy geared to maximum utilization of available international resources and programmes, to attracting human talent from without, to a planned policy of immigration, to the recovery of scientists and technicians who have abandoned their own country for another, etc.

The process of regional integration is often thought of as being limited to economic and political considerations. It would not be amiss to emphasize that this process also has a high scientific and technological coefficient, which begins with the measurement of the physical and human potential of a region, continues along the difficult path of proper or improper utilization by each country of the opportunities that integration open to it, and so on.

Even though, as I have pointed out, the developing countries are removed from military decisions having an immediate and conclusive impact on world affairs, this does not mean that science and technology are irrelevant to the decisions they must make in their own interest or in their regional interest. Far from it. Which is the modern military hardware the country really needs and can efficiently operate? What is the optimum measure of 'self-protection' in relation to the optimum measure of development? What type of contribution can and must a country make to regional defence without prejudice to availabilities for internal security or local emergencies? To what extent can the scientific and technological education of the armed forces be a complementary factor in national development?

The so-called population explosion in Asia, Africa and Latin America poses difficult questions which are linked to moral and religious matters, but linked no less to the

advance of science. The dialogue on these problems involves the churches, most of them international in scope; scientific assemblies which are equally international in scope; and the conduct of each country, considered individually. It is now impossible to evade this dialogue, and the attitudes to be adopted, whatever they may be, must stem from a profound understanding of the economic, political, moral and scientific issues involved.

These are but some of the modern questions of a high scientific or technological content which developing nations must face. In short, within the framework of their responsibilities, within the framework of their obligations and opportunities, the developing nations must recognize an interdependence among science, technology and foreign policy that, on an appropriate scale, is comparable to that which is customarily attributed to the foreign policy of the great powers. Expressing it in another way, we have here something that was set forth by a task force of the United States Department of State, acting in accordance with certain recommendations of the Hoover Committee, as follows: "The Department is dealing on the one hand with foreign policy matters which have a great effect on United States scientific policy, and on the other hand with international scientific activities which have an impact on foreign policy".

James R. Killian, jun., recalls that Thomas Jefferson once observed that nothing had been heard from their Ambassador to Spain for more than two years, and that, if he did not hear from him in another year, he proposed to send him a letter. From one point of view, at least, those were enviable and happy times. The relationship between the national and the international to-day is immediate, imperative and inescapable.

Governments traditionally have recognized the interdependence between science and public administration in those agencies clearly concerned with such activities as agriculture, transportation and public health—to cite a few.

As I have noted, to-day science and technology are all-pervasive. Harrison Brown has told us that: "Just as the people in agrarian civilizations became dependent upon agriculture for their survival, so we to-day become dependent on our science and our technology". The time is past when it could be said that "the least government is the best government". That does not mean, of course, that the best government is necessarily the biggest. Far from it. But it should be recognized that the size of government tends to grow, even in countries that protest a doctrinaire distaste for this phenomenon.

This fact is reflected in what is happening to scientific research. According to the U.S. National Science Foundation, Federal funds for basic research constituted 45 per cent of the total national basic research expenditure from all sources during 1953–54. By 1957–58 this amount had grown to 51 per cent, and for 1960–61 it reached 60 per cent. This has been accompanied by a relative decline in the contributions from other sources. Industry's proportion fell from approximately 35 per cent in 1953–54 to 30 per cent in 1957–58, and 22 per cent in 1960–61. College and university contributions declined from roughly 18 per cent to 12 per cent over this period. Other non-profit institutions made up the remainder, and their contributions declined from not quite 10 per cent to approximately 6 per cent.

Is this invasion of government of a sphere that was almost immune to it a few short years ago a peculiar application of Parkinson's Law on bureaucracy? That might be true up to a point, but the real motivation is that scientific and technological progress has become a matter of prime national interest and, therefore, a matter of governmental responsibility.

We can verify this with readily accessible data. Profs. Harbison and Myers have established a correlation between the national income of certain countries and the development of their human resources. The results are surprising. If we compare the number of persons per 1,000 of population possessing at least 12 years of education with the *per capita* income, converted to dollars, we arrive at the following results:

	Persons per 1,000 of population with at least 12 years of education	*Per capita* income (U.S. dollars)
Nigeria	0·8	64
Egypt	10·7	116
Italy	31·4	594
U.S.S.R.	112·6	721
United States	288·0	2,800

The great task, then, that the governments of the developing countries now face—assuredly with the desirable, necessary and maximum co-operation of private institutions—is to devise a logical strategy of high-level manpower development and relate it to the broader objectives of economic growth. This is a difficult matter, which involves many purely political problems. In effect, natural pressure in the developing countries is exerted on broadening the base of primary and secondary education. The formation of a scientific and technological *élite* and, what is apt to be just as difficult, retaining this *élite* in the face of attractions offered by emigration to more developed countries, also requires a well-defined and vigorous national policy.

Finally, assuming that countries of intermediate development, as are some in Latin America at present, succeed in forming an intermediate *élite* of technicians and engineers, they still must face the problem of forming the contingent of truly original outstanding talent essential to the full development of their potential. Otherwise, their role in the world will be necessarily and indefinitely subordinated to the fresh and original progress of other areas of the world. It is true that the emergence of such talent answers no physical law—"the spirit blows where it will", say the Scriptures—but to some extent its appearance depends on a degree of probability linked to the dimensions of the educated sector of the population. Here is where we encounter anew a very necessary aspect, albeit somewhat ignored up to now, of the integration of the Latin American nations, which imperatively requires us to follow the example of Europe in basic modern research.

Thus, the development of science and technology is not merely a question of science and technology. In essence, it is a political question which affects all society. The United States has understood this, and that is why in recent years it has created a variety of scientific advisory agencies at all levels of Government, including the Presidency of the Republic and the Department of State.

We are not concerned alone with a matter which relates to the material progress of our communities, essential as that may be.

Lord Snow has intimated that professors and scientists should be entrusted with the world because they are a little better than other people. Robert M. Hutchins, from the Centre for the Study of Democratic Institutions, good-humouredly quarrels with this notion: "My view, based on long and painful observation", he says, "is that professors are somewhat worse than other people, and that scientists are somewhat worse than other professors".

I do not intend to take sides in this highly debatable issue. But let me express my honest conviction that the decision-making responsibility in a democratic government must repose in the hands of accountable political leaders (it does not matter if they are *also* scientists), accountable leaders responsible to the people.

In a world which is progressively more scientific and technological and in a society that seeks to preserve its democratic way of life, the diffusion of the pertinent basic knowledge among the greatest number is an essential in maintaining the national dialogue inherent in every democracy. Otherwise, weighty decisions relating to science and technology at best would slip from the control of the electorate and its representatives. A tiny group, which we assume to be competent and well intentioned,

would decide on fundamental questions at its own and exclusive discretion. By definition, this could constitute a very dangerous situation. "Democratic government cannot survive if the most important issues of public policy are inexplicable to citizens and their constitutional officers. Deliberation . . . is impossible if communication among experts and between them and citizens is not feasible."

Perhaps these observations on the inter-relationship of science, foreign policy and government, and its necessary impact on the democratic way of life that we are determined to defend and advance, might well be summed up in the words of the late President John F. Kennedy, when he spoke in October 1963 at the centennial of the founding of the U.S. National Academy of Sciences:

"I think", he said, "that never in the . . . history of science has the time been brighter, the need been greater, for the co-operation between those of us who work in government, and those of you who may work . . . in laboratories."

STRUCTURE OF GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE

STRUCTURAL SYMMETRY WITHIN THE MOLECULE

By Dr. H. C. WATSON and Dr. L. J. BANASZAK

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GLYCERALDEHYDE - 3 - phosphate dehydrogenase (GPDH) is an important enzyme in intermediary metabolism where its function is to catalyse the oxidation and subsequent phosphorylation of aldehyde substrates to the corresponding acyl phosphates[1].

$$R - CHO + NAD^+ + HPO_4^{2-} \rightleftharpoons R - CO - OPO_3^{2-} + NADH + H^+$$

This enzyme has now been prepared in pure crystalline form from yeast[2] and skeletal muscle[3] with molecular weights reported to vary from 118,000 to 150,000[1]. Recent chemical work has shown that enzymatically active GPDH is composed of four chemically similar if not identical sub-units of molecular weight about 35,000[4]. Each sub-unit binds up to one molecule of the coenzyme nicotinamide adenine dinucleotide (NAD^+)[5] and contains four free sulphydryl groups one of which is highly reactive and participates in the enzymatic reaction[6]. These chemical and physical properties suggest that this enzyme might be amenable to detailed crystallographic analysis.

So far crystals of pig, rabbit, lobster and yeast GPDH have been prepared, but only the lobster GPDH crystals have been found suitable for extensive X-ray diffraction work. Crystals of the enzyme inhibited with *para*-chloromercuric-benzoate (PCMB)[7] are isomorphous with the native enzyme, but crystals grown from a solution containing inhibited enzyme in the presence of excess PCMB are quite different. The molecular arrangement in this new crystal form requires that the molecules lie on dyad axes. The molecule therefore has two structurally identical halves each related to the other by a two-fold symmetry axis.

Separation and Purification of Lobster GPDH

The procedure adopted for the separation and purification of lobster GPDH is similar to that described by Allison and Kaplan[8]. The tails are taken from the live lobsters and frozen. The shells are removed from the tails and the muscle washed, minced and extracted with a solution of 0·001 M versene, 0·001 M β-mercaptoethanol. The soluble protein extract is separated from the mixture by centrifugation. Solid ammonium sulphate is added to the supernatant until the salt concentration reaches 50 per cent saturation. The precipitate is discarded and the supernatant saturated with ammonium sulphate. The precipitate containing GPDH is removed by filtration, dissolved and dialysed against 0·001 M versene, 0·001 M β-mercaptoethanol and recrystallized at least three times from 75 per cent saturated ammonium sulphate solution.

The activity of the enzyme is measured by observing the change in absorption at 340 mμ due to the conversion of NAD^+ to NADH in the presence of a substrate of mixed triosephosphate esters and arsenate.

This preparative procedure yields approximately one gram of pure crystalline GPDH for each kilogram of wet lobster muscle.

Crystals of the Native Enzyme

Crystals of GPDH grown at room temperature from a 3·0 M ammonium sulphate, 0·001 M versene, 0·001 mercaptoethanol solution (*p*H 5·5) are shown in Fig. 1. The crystals are optically biaxial and invariably grow with the {100} faces well developed.

X-ray diffraction photographs recorded using copper $K\alpha$ radiation show that the crystals are orthorhombic with space group $P2_12_12_1$. The $0kl$ and $hk0$ sections of

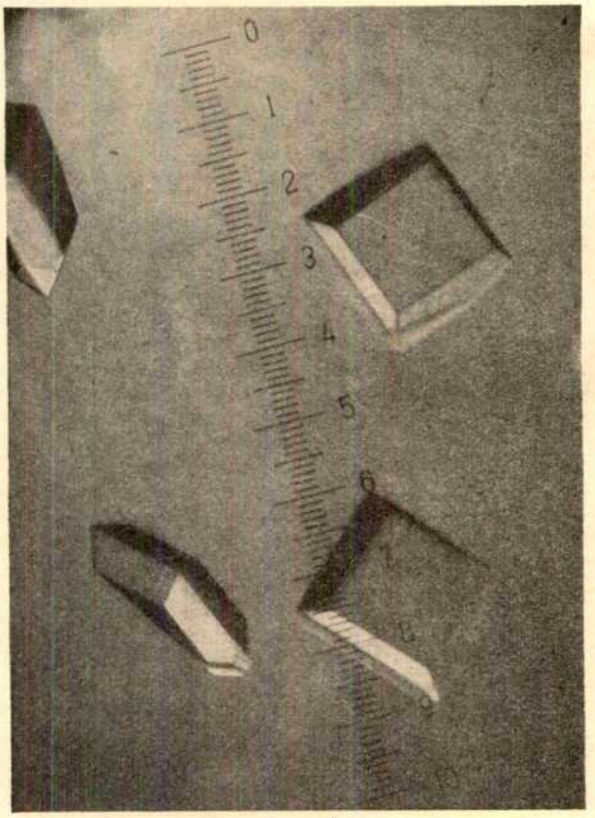


Fig. 1. Photomicrograph of crystals of native lobster GPDH. Each numbered scale division corresponds to 0·25 mm

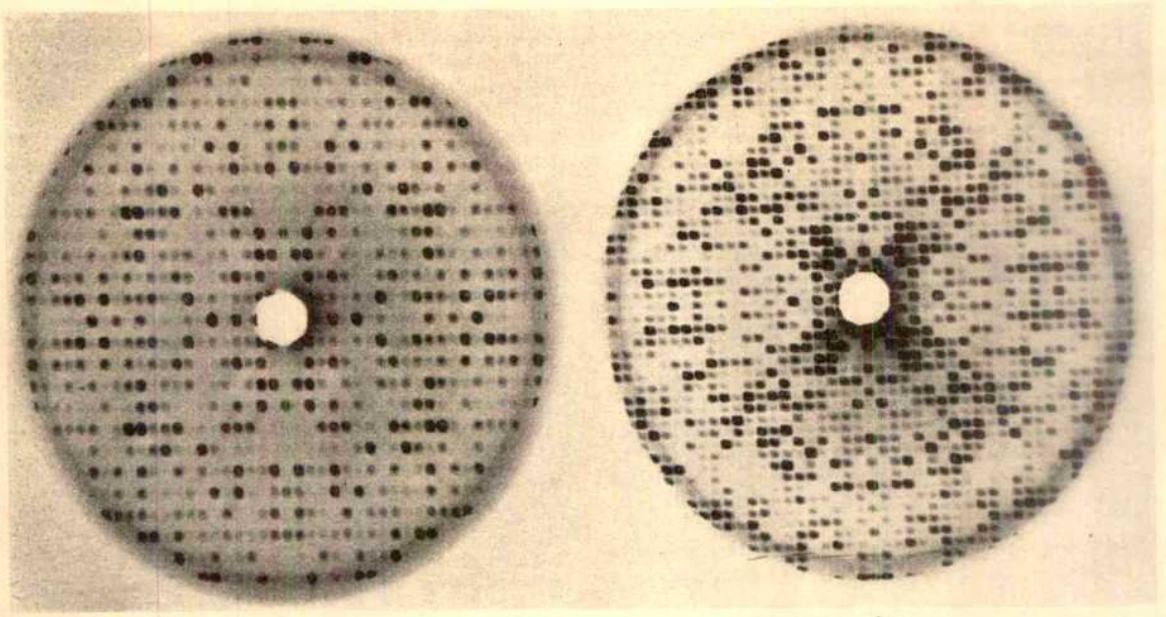


Fig. 2. Two X-ray diffraction photographs of native lobster GPDH. (*a*) the 0*kl* reciprocal lattice section; (*b*) the *hk*0 reciprocal lattice section. Both photographs are orientated with the b^* axis horizontal and contain reflexions to spacings which correspond to 6·3 Å resolution

the diffraction pattern shown in Fig. 2 indicate, at low resolution, the presence of a pseudo four-fold screw axis parallel to [001].

The unit cell dimensions are:

$a = 149 \pm 1$ $b = 140 \pm 1$ $c = 80{\cdot}5 \pm 0{\cdot}5$ Å

The volume of unit cell is: $1{\cdot}68 \times 10^6 \pm 5 \times 10^4$ Å^3

The diffraction pattern extends to spacings corresponding to a resolution of approximately 2 Å. The crystalline enzyme therefore has a defined structure extending to interatomic distances.

The density of the wet crystals as measured by flotation in a bromobenzene–xylene mixture is $1{\cdot}243 \pm 0{\cdot}002$ g/c.c., while the mother liquor is $1{\cdot}192 \pm 0{\cdot}001$ g/c.c. If a value of 0·75 c.c./g is assumed for the partial specific volume[9] and a molecular weight of 140,000[4] is used, the number of molecules in the unit cell is calculated to be 3·49. Since molecules in the space group $P2_12_12_1$ cannot be placed in special positions, the number of molecules in each unit cell must be a multiple of four. The crystals of native lobster GPDH therefore contain four molecules of approximately 140,000 molecular weight per unit cell.

The pseudo-space group $P4_12_12$ or $P4_32_12$ has eight non-equivalent positions[10] and therefore requires the four molecules in the unit cell to be placed on the space group's dyad axes. This restriction implies that, to within the limits of the pseudo symmetry, the molecules of GPDH have at least two structurally similar units and that these units are related by a two-fold symmetry axis.

Crystals of the PCMB-inhibited Enzyme

When four moles of PCMB per mole of protein are added to a versene, β-mercaptoethanol-free solution of lobster GPDH the enzymatic activity of the protein is reduced to less than one-hundredth of its former value. Crystals of the inhibited enzyme grow from a 3·0 M ammonium sulphate solution under conditions similar to those used for growing the native crystals. X-ray diffraction photographs show that these crystals are isomorphous with the enzymatically active GPDH crystals.

Crystals of lobster GPDH grown in the presence of thirty-two moles of PCMB per mole of protein are quite different from the native enzyme crystals. They are optically biaxial, rhombohedral plates with the {001} faces well developed. The crystals invariably appear with a dividing crack perpendicular to [001]. X-ray diffraction photographs show that these crystals are orthorhombic with space group $C222_1$.

The unit cell dimensions are:

$a = 94 \pm 1$ $b = 130 \pm 2$ $c = 137 \pm 2$Å

The volume of unit cell is: $1{\cdot}67 \times 10^6 \pm 6 \times 10^4$ Å^3

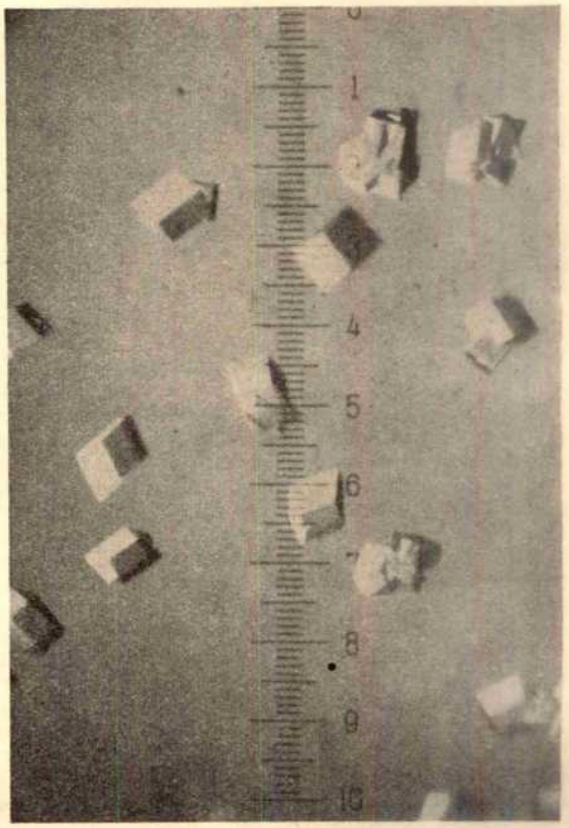


Fig. 3. Photomicrograph of crystals of the PCMB loaded GPDH. Each numbered scale division corresponds to 0·25 mm

Native GPDH crystals rapidly deteriorate when soaked in a large excess of PCMB, and small rhombohedral plate crystals are eventually formed.

The density of the wet rhombohedral plate crystals, as measured by flotation, is $1{\cdot}250 \pm 0{\cdot}003$ g/c.c. and the density of the mother liquor $1{\cdot}201 \pm 0{\cdot}002$ g/c.c. If values of 0·75 c.c./g and 140,000 are again used for the partial specific volume and molecular weight respectively, the number of molecules in each unit cell is found by calculation to be 3·50. Since the space group $C222_1$ has eight non-equivalent positions[10], the unit cell of the PCMB 'loaded' crystals must contain four molecules and these molecules must be placed on the space group's dyad axes.

Discussion

Chemical studies have shown that molecules of enzymatically active rabbit GPDH are composed of four similar if not identical sub-units[4]. Comparative investigations of rabbit, pig, yeast[11] and lobster[12] GPDH's indicate that these enzymes have similar sub-unit structures.

The crystallographical data presented in this communication indicate that the lobster GPDH molecule possesses at least one two-fold symmetry axis. The combined chemical and crystallographical results suggests that the lobster GPDH and, by inference, other glyceraldehyde-3-phosphate dehydrogenase molecules contain two pairs of chemically and structurally equivalent sub-units.

The sub-unit structure of GPDH is therefore analogous to that found for hæmoglobin[13] where two identical pairs of sub-units are related to one another by a two-fold symmetry axis. If, however, the sub-units of GPDH are identical, as the chemical evidence suggests[4,14], then the existence of the molecular dyad axis may represent only a partial expression of the full symmetry of a regular tetrahedral arrangement of sub-units.

Further crystallographical work on this enzyme is in progress. It is hoped that the active centre labelled PCMB derivative and an NAD^+ heavy atom analogue will be of use as isomorphous heavy atom derivatives.

We thank Dr. I. Harris for directing to our attention the GPDH enzyme and Dr. W. Allison for technical information concerning the preparation of the lobster enzyme. One of us (L. J. B.) thanks the U.S. National Institutes of Health for a post-doctoral fellowship.

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IMPERIAL CHEMICAL INDUSTRIES, LTD.

PETROCHEMICAL AND POLYMER LABORATORY

IT is now some two years since Imperial Chemical Industries, Ltd., announced the formation of a central research laboratory to supplement the research already being carried out by the manufacturing divisions of the Company, particularly the longer range research in the two fields of petrochemicals and polymers, both of which are obvious growth areas for the chemical industry. The establishment of the Laboratory is now well advanced and its pattern is therefore beginning to emerge. Before describing this pattern, however, it will be useful to fill in the background by summarizing the reasons for the decision to set up the Laboratory, and the plans which were made once that decision was taken.

The total research effort of the divisions of Imperial Chemical Industries, Ltd., is, of course, extremely substantial, and has already resulted in many inventions and developments. Moreover, each division carries out in the fields of interest to it a complete range of research from the long-term, such as might be carried out in any good university department, to the short-term investigations required for its day-to-day business. It may well be asked, therefore, why a central research laboratory was needed in addition to the massive forces already deployed. The answer is two-fold. First, as every research manager knows, when long- and short-term research are going on side by side in the same department, the long-range research always tends to be eroded by the pressures of shorter-term work or other urgent problems. Secondly, without a central research laboratory, there was no easy mechanism for Imperial Chemical Industries, Ltd., to start research in a completely new area outside the existing interests of any of the component divisions. These reasons led to the concept of the Petrochemical and Polymer Laboratory as a source of research effort comparatively free from short-term pressures, able to supplement divisional research effort at points of particular need, to look outside the existing interests of the Company, and also to tackle problems running across division boundaries.

The Petrochemical and Polymer Laboratory is not intended to be in any sense an academic institution; it is very much part of Imperial Chemical Industries, Ltd., and good communications between it and the rest of the Company are as important to it as good science and technology. This in fact is one of the reasons why the chosen site was Runcorn Heath, alongside the Research Department and Headquarters of the Mond Division, one of the biggest and most active of the Company (Fig. 1). Here the staff of the Laboratory has the advantage of daily contact with their colleagues in the Mond Division Research Department, and they are also within a hundred miles of five other divisions of Imperial Chemical Industries, Ltd.

Turning now to staff, the aim was to acquire about 100 graduates, about twice that number of junior supporting staff, and about 10 managers (plus administrative staff). Studies of the balance between disciplines indicated that the 100 research workers would be made up roughly of 55 chemists, 33 physicists, and 12 engineers; in fact the Laboratory is now more than half-way to this target, and may well be at nearly full strength by the end of this year. The staff is divided between five groups of varying sizes, known respectively as the New Chemistry, New Polymers, Materials Science, Molecular Structure, and Engineering Groups. These titles indicate clearly and correctly that a considerable proportion of the effort of the Laboratory will be spent on work other than chemical research. Much of this other work will be

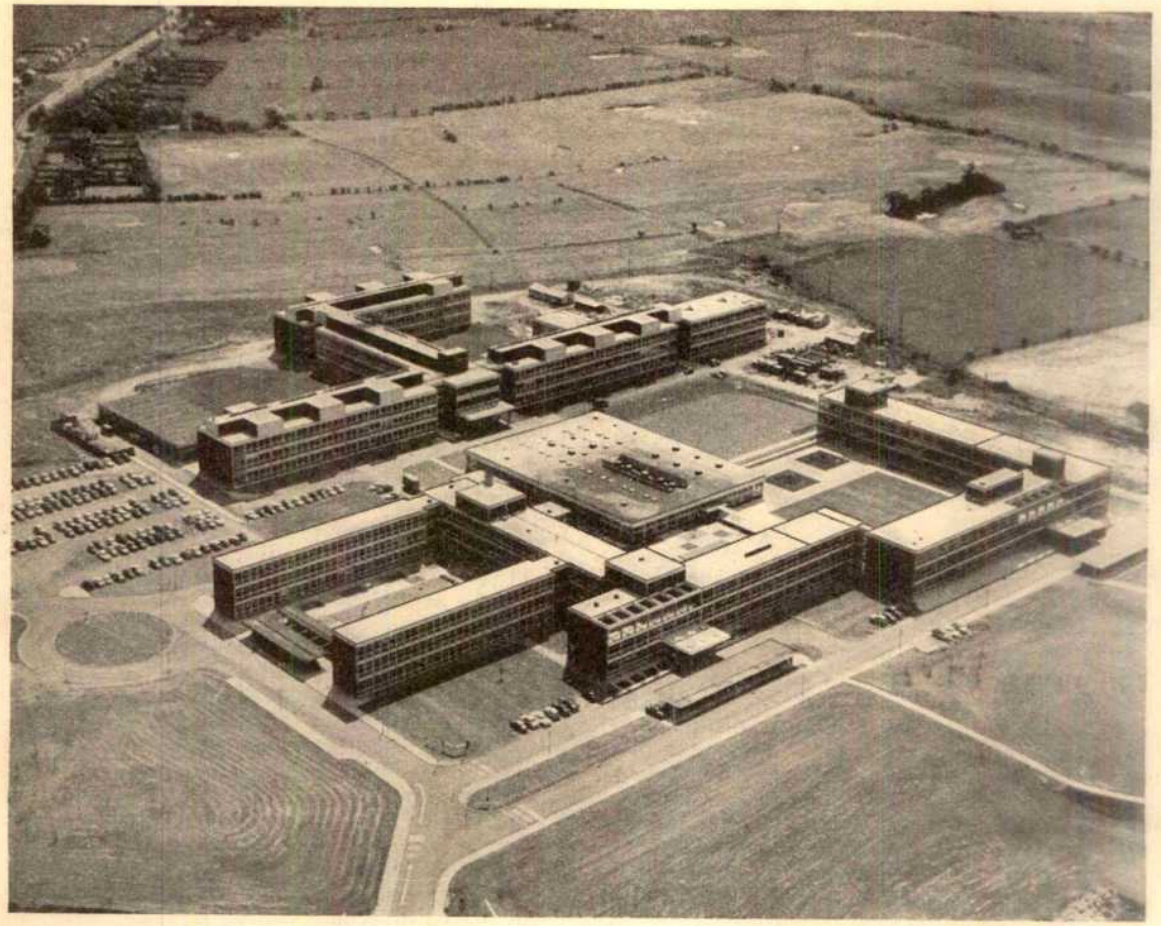


Fig. 1. Imperial Chemical Industries, Ltd., Runcorn Heath, with the research complex in the background

physical in nature, but physics applied in several very different fields. First there will be the use of physical techniques of all sorts for the investigation of molecular structure and for quantitative analysis (Molecular Structure Group); then there will be work on polymer physics and on the physical properties of solids in general (Materials Science Group); and the third field will be that of polymer application research, in other words of research into new methods of using or fabricating polymers, either new or existing ones (Engineering Group). Success in this field could be just as rewarding as a chemical invention; indeed, in the case of a polymer with valuable properties but incapable of being used by existing processes, the chemical invention might be sterile without the accompanying invention of a method of fabricating it. It is therefore considered vital for the chemists doing long-term speculative research to have alongside them people skilled on the application side, who will be constantly on the look-out for potentially valuable results, and who, from their knowledge of a wide range of technologies, could spot commercially promising developments at a very early stage. In fact, there should be a continuous interaction between these people and the chemists right from the initial choice of research topics, for while narrowly targeted research is not the Laboratory's function, equally it cannot afford to undertake all its research without ever considering possible end uses. The Laboratory must also ensure patent protection for its results by having its own patents section.

So far as equipment is concerned, one or two points are worth making. First, it is obvious now that infra-red spectrometers and gas liquid chromatography columns are as much standard tools for organic chemists as melting-point apparatuses used to be, and provision for such pieces of equipment has been made accordingly. Secondly, so far as physical equipment for the investigation of molecular structure is concerned, the Laboratory either has, or has access to on the site, a very complete range. It is, incidentally, the intention that the specialists working with these elaborate physical techniques such as nuclear magnetic resonance, electron-spin resonance, mass spectrometry, etc., will not only use their existing equipment to solve problems brought to them by the chemists, but will also carry on their own research into their particular fields with the view of extending the power and usefulness of those techniques.

The overall planning of the Runcorn Heath site was the responsibility of Frederick Gibberd and Partners; but the internal planning of the Mond Division and Petrochemical and Polymer Laboratories was based on experience within Imperial Chemical Industries, Ltd. The complete research complex consists of six buildings—three belonging to the Mond Division and three to the Petrochemical and Polymer Laboratory—all being linked at all levels to ensure close day-to-day contact between both the various disciplines and the two research organizations.

The three buildings of the Petrochemical and Polymer Laboratory consist of a chemistry block housing some 50 graduates, a physics block for 25, and an engineering or application block for 10–12 graduate research workers. The chemistry building, which was completed and occupied in early 1964, has three floors with a total floor area of some 30,000 sq. ft. A corridor runs the length of the building on each floor, the offices being on one side and laboratories on the other. These laboratories are of fairly normal design; each is intended for four graduates and their associated junior staff, having four large island benches with a number of side benches and generous provision of fume cupboards. There is a positive supply

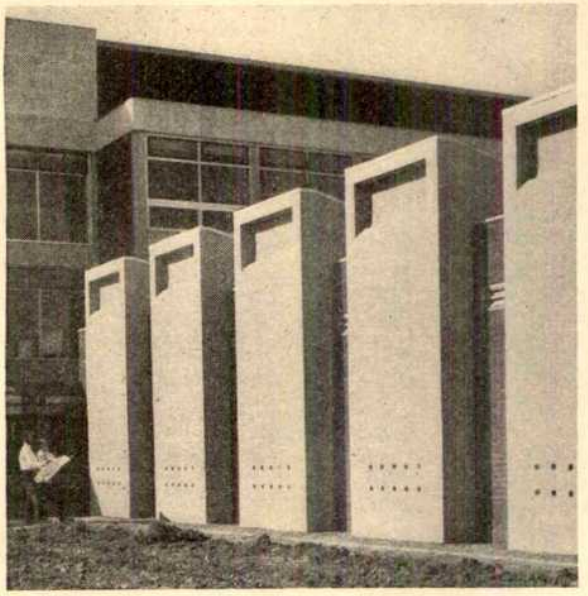


Fig. 2. Autoclave annexe showing reinforced concrete cubicles

of heated replacement air which is sufficient to allow the construction of further fume hoods. At one end of the chemistry building there is a 'tall apparatus area'. This is a 36-ft. high space occupied by a staircase with two landings, the floors of the landings being readily removable so that the full height of the building can be used if required.

The physics building, recently completed, is of the same height as the chemistry building but initially of only half its length. Since there is no longer the same need for plentiful fume cupboards and service provision, the internal layout is rather different, being designed to cater for the larger space and greater power requirements of the physicist. The unit laboratory, of which there are four per floor, is a 2-graduate unit some 38 ft. × 24 ft. overlooked by offices for the technical staff. There is no corridor as such, but a notional 5 ft. outside the offices is reserved for traffic.

The engineering building, also recently completed, is a single storey one basically designed to give a large interior space without obstruction and an 18-ft. high roof, in which can be placed or built any engineering apparatus which is necessary. This open space is overlooked by a double tier of offices at one end and is capable of extension by doubling its length. Outside the building, and along one of its sides, has been built the autoclave annexe. This contains eleven cubicles, six of steel and five of reinforced concrete, in which moderate pressure experiments can be carried out with maximum safety (Fig. 2). There is provision for remote control of the experiments and for the release of any pressure or fume generated in the cubicles. Nevertheless, the autoclave annexe is not intended for very hazardous or very high-pressure work; other more suitable sites are available, for example the Winnington Laboratories of the Mond Division some twelve miles away, where there is a wealth of experience of, and very good facilities for, work at really high pressures.

So far as the real purpose of establishing the Imperial Chemical Industries, Ltd., Petrochemical and Polymer Laboratory is concerned, it will of course be obvious from what has been said here that so far only the foundations have been laid. The laboratory is now entering on the period when the plans by which it has been shaped, both in terms of buildings and staff, will be really tested by the stern criterion of success or failure in inventing new products and processes for Imperial Chemical Industries, Ltd. Unfortunately, all those engaged in speculative research know only too well that no amount of planning can guarantee success; chance must play a part. However, one can at least attempt to reduce to a minimum the part played by chance in bringing about success, and this has been the aim throughout in planning and establishing the Petrochemical and Polymer Laboratory.

NEWS and VIEWS

The Advisory Council on Technology

The following have been appointed members of the Advisory Council on Technology under the chairmanship of Mr. Frank Cousins, Minister of Technology: Sir Leon Bagrit, chairman and managing director of Elliott-Automation, Ltd.; Mr. W. B. D. Brown, chairman of the Glacier Metal Co., Ltd.; Sir William Carron, president of the Amalgamated Engineering Union; Mr. C. F. Carter, vice-chancellor of the University of Lancaster; Dr. S. C. Curran, principal and vice-chancellor of the University of Strathclyde; Sir Arnold Hall, managing director and vice-chairman of the Hawker Siddeley Group and vice-chairman of Bristol Siddeley Engines; Mr. C. F. Kearton, chairman of Courtaulds, Ltd.; Prof. M. J. Lighthill, Royal Society research professor at the Imperial College of Science and Technology, and formerly director of the Royal Aircraft Establishment, Farnborough; Lord Nelson of Stafford, chairman and chief executive of the English Electric Co., Ltd.; and Mr. H. C. Tett, chairman of Esso Petroleum Co., Ltd. Prof. P. M. S. Blackett, professor of physics in the Imperial College of Science and Technology, is deputy chairman.

The 1964 Nobel Prize for Chemistry : Prof. Dorothy Crowfoot Hodgkin, F.R.S.

The work for which Prof. Dorothy Crowfoot Hodgkin was awarded the 1964 Nobel Prize for Chemistry consists of a remarkably coherent and well-planned series of investigations covering the whole field of organic structures of medical and biological importance. In 1932, when she started her work in Cambridge, all that X-rays could do was to confirm or correct formulæ arrived at by essentially organic chemical methods. Prof. Hodgkin's achievements lay in her transcending these limitations and making the X-ray method one of the major tools for the complete analysis down to accurate atomic positions. All the way through, her work was characterized by a consciously evolved strategy of analysis which showed itself both in the choice of the crystals to be examined and the crystallographic methods successively evolved to study them. She began with the sterols and combined extensive, comparative method with intensive, accurate structural investigations of selected crystals, notably those of calciferol and lumisterol. The structure of cholesterol iodide, which she carried out with Dr. C. H. Carlisle, was, in fact, the first complex organic structure without molecular symmetry to be analysed completely by X-rays. She was also at the very beginning of the X-ray analysis of proteins, and was the first to show that the insulin molecule, and, by extension, those of all proteins, possessed a rigid inner structure independent of the state of hydration.

On her return to Oxford she continued with a more definite bias towards molecules of physiological and medical importance. Her first great triumph was the elucidation with Bunn and others of the structure of penicillin, with 17 atoms in the molecule, in advance of

the chemical evidence. The next important molecule she attempted was to lead to her greatest triumph, the structure of vitamin B_{12}, the anti-pernicious anæmia factor. It was here that she was able to show how a knowledge of, and, better still, a feeling for, chemistry could be combined with the most refined physical analytical techniques. Her series of papers on this structure will always be a model of a tactical approach to a formidable problem. Her step-by-step advance, beginning with the recognition of the planar porphyrinoid ground around the cobalt atom, continued atom by atom until it included the whole closed loop, through the propanolamine and nucleotide group, which linked it ultimately with the cobalt atom itself. The completion of this work included the precise location of more than ninety atoms and is the present 'high-water mark' of crystallographic analysis. In establishing it, Prof. Hodgkin's work has set a new standard in X-ray analysis methods and has shown the way by which successively more difficult structures can be attacked.

The 1964 Nobel Prize for Physics

THE 1964 Nobel Prize for Physics is shared: one half has been awarded to Prof. Charles H. Townes, provost of the Massachusetts Institute of Technology, and the other half jointly to Prof. Nikolai G. Basov and Prof. Aleksandr M. Prokhorov, of the U.S.S.R. Academy of Sciences Lebedev Institute of Physics. The prize is awarded for work in the field of quantum electronics which led to the development of the micro-wave maser, and later to the laser.

Prof. Charles H. Townes

Prof. Charles H. Townes, who is forty-nine, obtained his bachelor's degrees at Furman University, South Carolina, and his M.A. at Duke University, North Carolina. He worked for his Ph.D. at the California Institute of Technology, and in 1939 joined the Bell Telephone Laboratories, where he remained until he was appointed professor of physics at Columbia University in 1947. Here he directed his attention to the problem of generating coherent radiation by using transitions between the energy-levels of molecules. The method, used in 1953 in the first maser, was based on the stimulated emission of radiation from excited ammonia molecules at a wave-length close to 1·25 cm. This maser was novel in two respects: its very low noise when used as an amplifier, and its high-frequency stability as an oscillator.

Prof. Nikolai G. Basov and Prof. Aleksandr M. Prokhorov

Profs. Nikolai G. Basov and Aleksandr M. Prokhorov developed independently an almost identical maser at the Lebedev Institute. Prof. Basov is a graduate of the Moscow Engineering and Physics Institute. He is forty-two and is at present deputy director of the Lebedev Institute. Prof. Prokhorov was born in Australia in 1916. After attending Leningrad State University he served in the Soviet Army during the Second World War and then joined the Lebedev Institute, where he became chief of the Oscillation Laboratory in 1954. He was awarded the Lenin Prize in 1959. Profs. Basov and Prokhorov are both corresponding members of the U.S.S.R. Academy of Sciences.

The operation of the first micro-wave masers evoked widespread interest in the application of the maser principle, and led to the development of the optical maser, or laser. This device was first discussed in detail in a paper published by Townes and Schawlow in 1958, and a little later Basov was among the first to suggest the use of stimulated emission in semiconductors as a means of generating coherent radiation.

Ministry of Aviation Chief Scientist : Dr. W. Cawood, C.B.E.

DR. W. CAWOOD has been appointed chief scientist of the Ministry of Aviation in succession to Sir Robert Cockburn, who has been appointed director of the Royal Aircraft Establishment, Farnborough (*Nature*, 204, 127; 1964). Dr. Cawood has been chief scientist of the Ministry of Defence (Army Department) since September 1960. He was born at Leeds in 1907 and educated at Archbishop Holgate's School, York, and the University of Leeds. During the period 1928–38, while engaged on research work, he produced many scientific papers on the physical properties of aerosols, the theory of coagulation, etc. In 1937 he was a member of the League of Nations International Atomic Weights Committee. He entered the Scientific Civil Service in 1938 and served for some eight years in the Headquarters Armament and Instrument Research and Development Branches of the Air Ministry and Ministry of Aircraft Production, becoming deputy director of armament research in 1945. He was promoted to chief scientific officer in April 1947, and served as deputy director of the Royal Aircraft Establishment until February 1953, when he was appointed principal director of scientific research (defence) of the Ministry of Supply. Some two years later he became principal director of scientific research (air) (later director-general of scientific research (air)); and in March 1958 he was appointed deputy controller of aircraft research and development. Two and a half years later he was transferred to the post of chief scientist of the War Office (now Ministry of Defence (Army Department)). His new appointment took effect as from November 30.

Head of Agricultural Bacteriology Division of the Ministry of Agriculture: Dr. J. G. Murray

DR. J. G. MURRAY has been appointed head of the Agricultural Bacteriology Division of the Ministry of Agriculture, and took up his new duties on November 1. Dr. Murray, who was born in Derrytrasna, Lurgan, was educated at Lurgan College and Greenmount Agricultral College, before entering Queen's University, Belfast, in 1934. After graduation, he joined the Ministry of Agriculture as an advisory officer, working mainly with the Milk Section. Since 1943 he has been a member of the research staff of the Ministry's Agricultural Bacteriology Division. He now also becomes head of the Agricultural Bacteriology Department at Queen's University. Dr. Murray is a member of the Council of the Society of Dairy Technology, a committee member of the Society of Applied Bacteriology and a member of two committees of the British Standards Institute. His particular research interest is the bacteriology of processed foods—especially milk and dairy products.

Meeting of Panel on WMO Development Fund

THE Panel of the Executive Committee of the World Meteorological Organization, established to approve projects for implementation in 1965 under the new WMO Development Fund, held a meeting at the Headquarters of WMO in Geneva, in November. The new Meteorological Development Fund of U.S. dollars 1,500,000 was established by the Fourth World Meteorological Congress in 1963 in order to enable significant and prompt assistance to be rendered to members during the period 1965–67. Its general purpose is that full advantage be derived from the opportunities which now present themselves for the improvement of man's basic understanding of the atmospheric processes. A plan for the operation of the new fund was approved by member governments earlier in the year, and assistance is to be made available in the following fields: (*a*) telecommunications facilities; (*b*) observational facilities (including those relating not only to conventional observations but also to APT and other satellite installations, automatic weather stations, ocean

weather ships and upper-air observations from merchant ships); (*c*) meteorological data processing, including electronic computers; (*d*) education and training; (*e*) any other activities related to the planning, establishment and operation of world, regional and national centres under the World Weather Watch not covered by the preceding items.

Wellcome Trust Grant for Research in Chemotherapy

THE Wellcome Trust is to make a grant of £30,000 to the Sir William Dunn School of Pathology at the University of Oxford. This will meet part of the cost of a new building and provide space for the work of Prof. E. P. Abraham on naturally occurring substances of biological and medical interest. In recent years Prof. Abraham's group has succeeded in isolating cephalosporin *C*, in determining its structure and in showing that a family of compounds of varying biological activity can be obtained from its nucleus. Members of this family promise to be of value in the treatment of penicillin-resistant staphylococcal infections and infections by bacteria from the intestinal tract. The grant will meet part of the cost of constructing an additional floor especially for the use of Prof. Abraham. The building will also house a new Medical Research Council Cellular Immunology Research Unit under the direction of Prof. J. L. Gowans, Henry Dale professor of the Royal Society.

The South African Institute for Medical Research

THE annual report of the South African Institute for Medical Research for the year 1962 records in impressive detail the very wide range of research being done by its staff all over South Africa (Johannesburg: South African Institute for Medical Research, 1964). Directed by Dr. J. H. S. Gear, the Institute not only conducts detailed research into many fields of medical investigation, but also provides a medical laboratory service for provincial hospitals in every part of South Africa and co-operates with such organizations as the World Health Organization and the U.S. Public Health Service. The report discusses this immense range of beneficent work under six main headings. The first of these is devoted to administration. The second and longest section deals with the work of the Research and Routine Division and covers almost every important field of medical research. The third section deals with the work of the Serum and Vaccine Division on diphtheria, typhoid, *Staphylococcus*, tetanus, gas gangrene, venoms and anti-venoms. The fourth section discusses the regional medical laboratory service, the fifth deals with research on pneumoconiosis, and the sixth the work of the Poliomyelitis Research Foundation. Like other publications of this kind, the report indicates, to those who read between the lines, not only the incalculable human suffering caused by disease, but also the devotion and industry of the many workers who are dedicating their lives to the alleviation and prevention of this suffering.

The Corrosion and Protection Association

A CORROSION and Protection Association has been established as an independent and comprehensive organization to embrace all aspects of corrosion and protection science and practice. It is intended that the Association shall serve the interests of all who are engaged in corrosion or protection work, and will not seek to rival existing groups, but rather to supplement them. It will especially strive to bring the latest advances before practising engineers, and its official journal will be *Corrosion Science*, which, in its new enlarged form, appearing monthly, will include sections devoted to research papers, present and future practice, and general information. Special terms for subscriptions to the journal will be available to members of the Corrosion and Protection Association. The inaugural meeting of the Association will be held at 2 p.m. on December 30 at the Sir John Cass College, Jewry Street, London, E.C.3. The business will be to adopt a constitution and to elect a Council and officers. Membership (£1 per annum) is open to all persons with scientific, technological, practical or commercial interests in corrosion and protection, and further information and forms of application for membership can be obtained from Dr. T. P. Hoar, Department of Metallurgy, Pembroke Street, Cambridge; Prof. T. K. Ross, Manchester College of Science and Technology; or Dr. L. L. Shreir, Sir John Cass College, Jewry Street, London, E.C.3.

Market Research

THE latest Library Association *Special Subject List* (No. 44) is *Market Research*, by D. C. Fanning (London: The Library Association, 1964. 5*s*.). A foreword by Viscount Chandos, president of the Market Research Society, shows how capital expenditure on industrial plant and building is generally decided by the results of market research: such factors as high rates of money at a given date are often over-stressed. Since a new plant frequently takes more than three years to bring into production, it is the size and nature of the market three years hence that are the primary considerations, and market research is the examination of these two subjects, projected three years ahead. The book list indicates directories and bibliographies as well as specific books on market research, motivation research, industrial market research, media research, techniques and methods, general text-books and periodicals. It will be useful to all concerned with market research in any of its many forms.

Wild-life Conservation

Oryx, the journal of the Fauna Preservation Society, continues to improve under its new editor. Conservation news from all parts of the world is well presented in Vol. VII, No. 5, August 1964, and a number of articles deal with special aspects of the subject. The most important is one by Dr. Fraser Darling on the ethical and technical problems of wild-life conservation, in which he emphasizes that animals and plants exist in an environment which is not merely physical or climatic but biotic and social. He gives a reasoned argument on the necessity for the management of wild-life in a world largely claimed by man for his own immediate needs, and shows the importance of controlling the numbers of wild animals in certain circumstances so that they shall not destroy their own environment. He does not, however, point out the obvious conclusion that man himself must control his numbers and bring himself into an ecological balance with his environment.

Clonal Selection Theory of Immunity

SIR MACFARLANE BURNET of the Walter and Eliza Hall Institute of Medical Research, Melbourne, writing in the *Australian Journal of Science* (vol. 27, July 1964), has described a Darwinian modification to the clonal selection theory of immunity. In the seven years since the clonal selection theory of immunity was first outlined a great deal of research has been concerned with testing its implications. The results can be summarized as being incompatible with the rather rigid form of the theory which postulates that all immunological patterns are normally present at the time of birth. On the other hand, there has been a striking swing away from the classical 'instructive' theories of antibody production to an acceptance of the central point of the clonal selection approach, that the pattern of antibody and of cell reactivity is determined wholly by genetic processes. The work of Gowans, in particular, has resulted in the establishment of the small lymphocyte as the commonest immunologically competent cell, and there appears now to be no controversy as to the importance of specific stimulation of immunologically

competent cells by antigen, or antigen derivatives, in inducing the change of small lymphocyte to pyrininophil stem cells, and the initiation of germinal centres or of plasma cell clones producing antibody. Present points of difficulty are related to the generation of the essentially unlimited range of immunological patterns by genetic (or somatic-genetic) processes; the range of potential immune reactivity of a lymphoid stem cell, and the possibility of transfor of genetic (immunological) information from one cell line to another. The essential modification of the original theory accepts the existence of frequent somatic mutation in all body cell lines at all periods of life and underlines the great significance of minor genetic change, possibly involving no more than the substitution of one amino-acid for another, in relation to the modern picture of γ-globulin structure. Equally we must accept as before the continuance of selective processes throughout life, both negative selection involving destruction or inhibition of physiologically immature cells reacting with antigen in relatively high concentration and positive selection for proliferation and antibody production following contact with antigen under other circumstances.

Woolgorong Stony Meteorite

NUMEROUS fragments of a stony meteorite were recovered from Woolgorong Station, north-east of Wullewa, in Western Australia, approximately 300 miles north of Perth, in 1961. This find almost certainly represents a possible fall noted in this locality in December 1960. A double report was heard at the time, but G. J. H. McCall and P. M. Jeffrey, writing in the *Journal of the Royal Society of Western Australia* (Vol. 47), consider that this does not necessarily imply a multiple fall, since phenomena of this kind are generally attributed to compression wave effects in the atmosphere. The meteorite is a veined and brecciated, grey hypersthene-olivine chondrite, Prior's class III, and shows evidence of pronounced recrystallization. Recovery of fragments from shallow burial in the soil, and from the surface, has allowed a confident reconstruction to be made. There is evidence that it had an aerodynamically stable form under conditions of atmospheric entry, since surface patterns and the distribution of thickened and thinned fusion-crust reveal a distinct orientation. Such orientation supports the idea that this was a single fall, complicated only by fragmentation at or near the point of impact.

Microscopic investigation has revealed considerable structural variation, some areas revealing well-formed, though recrystallized, chondrules. Others show almost complete recrystallization to a granular aggregate of polysomatic and larger fractured grains. In these areas of recrystallization the chondrular structure is only vaguely defined. Chemical tests, etch-tests, X-ray diffraction examinations and two full chemical analyses have been carried out, and the minerals determined include kamacite, iron sulphide (troilite ?), olivine (chrysolite-hyalosiderite), orthopyroxene, oligoclase, and possibly a calcium silicate (rankinite ?).

University News :

Aberdeen

THE following appointments to lectureships have been announced: Dr. W. Welsh (geology); Dr. M. N. Court (soil science).

Leeds

THE following appointments to lectureships have been announced: C. R. Abbott (pathology, with special reference to hæmatology); Dr. J. W. Taylor (gas engineering and general fuel science).

London

DR. JOHN ANDERSON, reader in medicine at King's College Hospital Medical School, has been appointed to the chair of medicine tenable at that school. Dr. P. N. Rowe, principal scientific officer in Chemical Engineering Division, Atomic Energy Research Establishment, Harwell, has been appointed to the Ramsay Memorial chair of chemical engineering tenable at University College from October 1. Dr. Kenneth Smith, of the Argonne Laboratory, has been appointed to the readership in mathematics tenable at Royal Holloway College.

Sussex

THE prospectus for 1964–65 of the University of Sussex gives particulars of the introduction of majors in philosophy in the School of African and Asian Studies; experimental psychology in the School of Biological Sciences; and social psychology in the School of Social Studies. Decisions regarding all three were taken too late for inclusion in the *Handbook of the Universities Central Council on Admissions*.

Eclipse of the Moon, December 19

THERE will be a total eclipse of the Moon on December 19, visible at Greenwich. The circumstances are:

Moon enters penumbra	Dec. 19d 00h 01m U.T.
Moon enters umbra	19d 00h 59m
Total eclipse begins	19d 02h 07m
Mid-eclipse	19d 02h 37m
Total eclipse ends	19d 03h 07m
Moon leaves umbra	19d 04h 15m
Moon leaves penumbra	19d 05h 14m
Magnitude of eclipse	1·18

Announcements

DR. E. J. DENTON, senior principal scientific officer at the Laboratory of the Marine Biological Association at Plymouth, has been appointed to a Royal Society research professorship at the University of Bristol. Dr. Denton, who took up his appointment as from October 1, 1964, will be attached to the University of Bristol but will continue to carry out most of his research work at the Plymouth Laboratory. Dr. Denton is distinguished for his contributions to the physiology of marine animals, in particular of their visual processes and maintenance of buoyancy.

THE Association of Commonwealth Universities has published, under the title *United Kingdom Postgraduate Awards, 1964–66*, information on such awards in a form suitable for quick reference. It does not include awards open to graduates of only one university, or those restricted to the academic staff of a single institution. A short list of awards tenable outside the United Kingdom by graduates of the United Kingdom is also included, and there are subject indexes to both lists, as well as an index to names.

A SYMPOSIUM on "Crustacea", under the auspices of the Marine Biological Association of India, will be held at Cochin during the second week of January. Further information can be obtained from the Convener of the symposium, Marine Biological Association of India, Marine Fisheries P.O., Mandapam Camp.

A SYMPOSIUM on "Morphogenesis and Differentiation in Relation to Cellular Fine Structure", arranged by the British Biophysical Society, will be held at the Imperial College of Science and Technology, London, during January 8–9. Further information can be obtained from Prof. R. E. Burge, Physics Department, Queen Elizabeth College, Campden Hill Road, London, W.8.

A JOINT meeting of the Biochemical Society and the Association of Clinical Biochemists will be held in Leeds during January 7–8. Colloquia on "Vitamin B_6 in Metabolism" and "Some Aspects of the Biochemistry of Protein and Peptide Hormones" will be held during the meeting. Further information can be obtained from the Meetings Secretary, Biochemical Society, 20 Park Crescent, London, W.1.

SCIENCE AND GOVERNMENT REORGANIZATION IN BRITAIN

A SENTENCE in The Queen's Speech at the opening of Parliament on November 3 stated that Britain's industries would be helped to gain the full benefits of advances in scientific research and applied technology. The Speech also announced that central and regional plans were being prepared to promote economic development, with special reference to the needs of the underemployed areas of the country. New arrangements (including the appointment of a Secretary of State for Wales, with a seat in the Cabinet, and a Minister of State, Welsh Office) would ensure that proper attention would be directed to the needs of Wales, and legislation would be introduced to provide for the appointment of a Highland Development Authority. Some light was thrown on these and other changes in the structure of the Government in the subsequent debate on the address to the Throne, although early in the debate Sir Alec Douglas-Home was critical of certain changes, particularly the fragmentation of the Treasury and some Ministries, such as the Ministry of Education and Science. He believed that to take the Ministry of Technology out of the latter Ministry was a real mistake.

Mr. R. Maxwell, in a maiden speech, however, welcomed the new Ministry of Technology, and also supported the proposal to review the *Concord* project. He referred to the backwardness of much of British industry in making use of science and technology as an aid to increased productivity, and suggested that one of the major obstacles was ineffective communication at various levels—between scientist and technologist and management, between industry and Government, between universities, colleges of technology and research associations. Like Lord Thomson of Fleet, Lord Melchett and Lord Boothby, in the corresponding debate in the House of Lords on November 5, he also emphasized the special importance of management. Lord Melchett, while agreeing as to the importance of efficient management and the structure of labour and management relations, questioned whether the establishment of a Department of Economic Affairs and a Ministry of Technology would do more than shift the emphasis and priorities within a system incapable of satisfactory rates of sustained growth. Nor did he think that the proposals for economic planning, the stimulation of science and technology, and the correction of assumed errors in past policies went to the roots of the matter. Mr. Maxwell, however, urged that the strength of our science depended on the initiative, imagination and intelligence of individual scientists and engineers, and thought that the Government's arrangements for organizing science and technology would meet the need.

Speaking in the debate on November 4, the Secretary of State for Economic Affairs, Mr. G. Brown, recognized the value of the work of the National Economic Development Council and said that it was the intention of the Department of Economic Affairs to take up the work of that Council, expand and develop it, and produce, in consultation with both sides of industry, through the Council, a plan for the next five years to guide the development of the economy and all the economic activities of the Government. Under the new policy the responsibility must be with the Government and in future this planning would be carried out in the Department of Economic Affairs, in close co-operation with the Treasury and other Departments concerned. Part of the staff of the Council's office had already joined his Department for this purpose, but there was a continuing need for an outside body. It was proposed to reconstitute the Council on much the same lines as it was based before with a membership of about twenty from Government, management and Trade Unions, with some independent members. Sir Robert Shone had been invited to continue as director-general. He thought that the regional councils would grow in importance and he was proposing that they should work in closer association with the Department of Economic Affairs and other Departments.

Mr. Brown then stated that the Government's regional economic plans would take their place within a coherent national framework, and effective means for consultation would be set up in the regions. The first major task was to fashion the national framework into which the detailed work of the regional investigations would fit, and this was one reason why the Government had started to modernize Whitehall. It was also intended that the regional plans should reflect regional needs, views and experience, and advisory regional councils would be set up with membership drawn from industry, local authorities, the universities, commerce and others concerned with strengthening and improving the fabric of regional life. These councils, to which the Government would look for considered and informed advice on regional problems, would participate in the examination and analysis which should precede sensible planning decisions and would work with regional planning boards consisting of representatives of the main social and economic departments. The chairmen of these boards would be appointed by the Secretary of State for Economic Affairs and each regional headquarters would have staff from that Department. The Secretary of State for Scotland would set up a Scottish planning council with similar functions and membership and the Scottish Development Group would be strengthened and constituted as a Scottish planning board. Similar arrangements would be made for Wales.

The resumed debate in the House of Commons on November 5 was concerned with technology. In opening the debate, Mr. Q. Hogg criticized particularly the decision to separate industrial and academic science as seen in the establishment of a Ministry of Technology and the Secretary of State for Education and Science. Mr. M. Stewart, who followed, pointed out that the previous Government had itself proposed to place the National Research Development Corporation with the Board of Trade, and went on to outline the new structure of the Department of Education and Science. This Department would include the Medical, the Agricultural and the new Science Research Councils. Some of the functions of the Department of Scientific and Industrial Research would be transferred to the new Science Research Council, which would also have responsibilities relating to the European Space Research Organization. A new National Environment Research Council would be established to bring together the activities of the Nature Conservancy, the Geological Survey and other groups of study including oceanography. There would also be the Advisory Council for Sciontific Policy, responsible, *inter alia*, for advising the Secretary of State on the allocation of resources between different fields of research.

The Ministry of Technology would assume the responsibility for the Atomic Energy Authority, the industrial side of the Department of Industrial and Scientific Research and the work now being done by the National Research Development Corporation. On the dissolution of the Department of Scientific and Industrial Research, Mr. Stewart pointed out that this was part of the previous Government's proposals, and although he did not refer to the Trend Report, as did Mr. E. Lubbock later in the debate, he did refer to the wide consultations between the late Government and all concerned with the proposed reorganization. He referred to the criticisms voiced by the Institution of Professional Civil Servants, though he did not point out as he might well have done, in view

of the extent to which the present proposals follow those of the Trend Report, that the demand for fresh consultation is largely specious. A government must take its own decisions once it is satisfied it has ascertained the facts and the views of those concerned. As to the status of members of the Institution under the new arrangements, he readily agreed as to the desirability of full consultation

In reply to specific questions, Mr. Stewart said that no decision had yet been taken regarding the National Physical Laboratory or as to whether there would be any change in the organization of the Atomic Energy Authority. He would not say yet whether the Committee on Scientific Manpower would continue as at present or whether separate committees would deal with technological and with scientific manpower. This was a matter which the Department of Education and Science and the Ministry of Technology would have to consider jointly. The Ministry of Technology would also be concerned with the development in the civilian field of the kind of research and development contract familiar in the field of defence. There would also be an Advisory Council on Technology with the Minister as chairman and Prof. P. M. S. Blackett as deputy chairman (see also p. 922 of this issue of *Nature*). Finally, there was a need to take a broader view of the educational process. It was necessary not only to provide a sufficient amount of skilled manpower but also to achieve in the population as a whole a general understanding of what a technological civilization meant.

This explanation of the Government's proposals was welcomed in a particularly well-informed speech by Mr. E. Lubbock, who asked what would be the role of the new Council of Engineering Institutions and whether they would be represented on the new Advisory Council: he thought it might well be a valuable source of advice. Otherwise there was little constructive comment in the debate, the main other topic being the future of the *Concord* project. This was raised by Mr. Lubbock and other speakers in relation to its effect on Britain's technology generally, and although it was originally raised by Mr. Q. Hogg, who also asked about Britain's support of the European Launcher Development Organization and the European Space Research Organization, Mr. Stewart left it to the Minister of Aviation, Mr. R. Jenkins, to reply in winding up the debate.

Sir Lionel Heald, who stressed the importance of the aircraft industry in Britain's technology, quoted Sir George Edwards's opinion that *Concord* represented the first full collaboration in such a venture by two great European powers and that it might be the first step to a world rationalization of the civil aviation effort. He was concerned as to the effect of discontinuing the *Concord* project on the other collaborative projects. Mr. N. Marten, like Mr. A. Neave and many others, was concerned about the future of the design teams and other workers in the industry if the project was discontinued. Mr. E. S. Bishop's concern that public expenditure on research and development should be well spent and not wasted was also generally shared.

Dr. J. Bray questioned whether discontinuance of the *Concord* project would, in fact, lead to a considerable loss of skilled manpower to the United States, and pointed out that the maximum number of workers who might be redundant by the end of 1965 was 6,000. Strong support for the project came from Mr. Angus Maude, who was convinced that supersonic transport was bound to come, but very reasonable answers were given to his specific questions by Mr. R. Jenkins in his reply on the debate. Mr. Jenkins reiterated that the only decision taken so far was to ask for an urgent review of the project, and he had personally explained to the French Minister of Transport in Paris Britain's doubts about the project. He had also had talks with the French Minister of Defence and the Minister of Science, who had French responsibility for the European Launcher Development Organization; a meeting had been arranged for January at the request of the French and Italian Governments, when the rising costs of this project and their allocation would be discussed.

As regards *Concord*, Mr. Jenkins said that neither Britain nor France had as yet spent or committed large sums, and up to the present time only about 3,000 people were involved, mostly in Bristol with about 400 in Coventry. The point had been reached where costs and the amount of labour involved would begin to rise fairly sharply. Besides these short-term reasons for desiring to review, there were long-term reasons. All should be satisfied that the counterbalancing technological and social considerations were commensurate with the costs. Whatever view was eventually taken of the *Concord* project, he was convinced that in the interests of both the French and the British aircraft industries, the two countries should work as closely as possible alongside each other. Collaboration so far had been happy and our doubts were entirely about the *Concord* project, not the collaboration. If the Government was convinced that the project was in the best interests of both countries it would wish it to go ahead, and even if there was an area of legitimate doubt a solution might still be found which would retain the possibility of developing a supersonic airliner which would be economic. But it could not be assumed that Britain's scarce resources permitted her to develop every existing possibility.

SCIENTIFIC RESEARCH IN SCHOOLS

THE president of the Royal Society, Sir Howard Florey, presided at a meeting in the Society's apartments at Burlington House, London, W.1, on September 30, to hear an account of some of the researches which have been carried out by practising teachers assisted by funds administered by the society's Research in Schools Committee.

The president, in his introductory remarks to an audience of more than 140 comprising school science teachers, their research advisers and officials of the Association for Science Education, said that the Council and Fellows take a great pride in the work done in schools, with the help of the committee, and it is crucial to Britain's benefit for this kind of work to be undertaken in schools. In addition, it gives teachers an insight into the frustration and disappointment which often go hand in hand with research.

After the president's remarks two papers were read, followed by a discussion and an interval for tea. Then two further papers were read and a further discussion was held. A brief summary of the four papers is given here.

Studies of Sympatric Evolution in the Butterfly, Maniola jurtina. By W. H. Dowdeswell, Winchester College.

The number of spots on the underside of the hind-wings of the meadow brown butterfly (*Maniola jurtina*) was a characteristic well known to be subject to the action of natural selection. Among the females, a typical South European spot-distribution with a high mode at 0 spots changed abruptly to a bimodal distribution (at 0 and 2 spots) in the vicinity of the Devon–Cornwall border where no physical barriers exist which might restrict the flight of the insect. There was evidence that the braconid *Apanteles tetricus* may be one of the agents tending to reduce spotting, while the bacterium *Pseudomonas fluorescens* may have the opposite effect. We might have a situation here comparable with that controlling sickle-cell anæmia in man.

The Lower Carboniferous Flora of the Tweed Basin. By A. G. Long, Berwickshire High School.

Primitive pteridosperm ovules from Berwickshire suggested that the integument evolved by fusion of separate sterile lobes around a megasporangium.

Two species of ovules occurred within cupules also composed of sterile cylindrical processes. Such cupules showed features comparable to angiosperm carpels and suggested that the pteridosperm cupule was the precursor of the angiosperm carpel.

Three *Lepidodendron* specimens showed the transition anatomy between stem and root without a stigmaria.

The nearest comparable flora was that of the Saalfeld beds in Central Europe.

The Stowe Radio Telescope. By J. M. Osborne, Westminster School, London.

A brief outline of the Stowe radio telescope was given. The original concept was described and the discussion with slides included the results obtained. Their significance in the field of amateur radio astronomy was mentioned.

The Szilard–Chalmers Reaction in Iodobenzene. By T. A. H. Peacocke, St. John's School, Leatherhead.

The original theory of the Szilard–Chalmers reaction explains the retention of iodine-128 in the parent molecule in terms of the recoil energy of the active atom. The recoil energy is about 200 eV and would result in the production of 5–7 free phenyl radicals. An alternative theory recently emphasized by C. N. Walton[1] considers the total energy release in terms of the electron excitation produced by the γ-rays emitted following the absorption of the neutron. This energy is greater than the recoil energy by several orders of magnitude and would produce many more free radicals than the 5–7 predicted by the original theory.

To test the rival theories iodobenzene has been irradiated with neutrons in the presence of scavenger iodine labelled with iodine-131, using the *Lido* reactor at the Atomic Energy Research Establishment. The accompanying γ-flux produces considerable radiolysis. To differentiate this from the effect of slow neutrons, some tubes were surrounded with cadmium. The observed retention of iodine-131 was measured in the absence of cadmium (R_1) and in the presence of cadmium (R_2). At neutron fluxes of the order of 10^8 n cm^{-2} sec^{-1}, R_2 is less than R_1; as the flux is increased the differences become smaller and at high neutron fluxes become negative. This could be explained in terms of the increased γ-flux inside the cadmium box as measured with the ferrous sulphate dosimeter. Approximate calculations indicate that 10^5 atoms of scavenger iodine are captured per Szilard event. This indicates the production of at least this number of free radicals, which would give strong support to the electronic excitation theory. The conclusions so far drawn must be tentative and further work is in progress.

Dr. F. P. Bowden, the chairman of the Committee for Scientific Research in Schools, in his closing remarks, said:

"This is the first occasion in the history of the society that scientific research carried out in schools has been presented in a formal discussion before it, which emphasizes the importance, at this moment of time, of science teaching in schools and it shows clearly the high level of ability with which some of our schools are manned on the scientific side. A wide increase of such research is without doubt one of our greatest needs in this country. Only a few days ago I was discussing with a research director of a major industry the implementation of his company's programme for the next five years. It was a highly imaginative programme and of great interest. Its successful prosecution, and that of similar companies, is vital to the economy of this country.

"It was clear that the programme could not be fully implemented because sufficient scientific and technical men were not available to carry out the work. There are many things we must do to cope with this situation and to breed more of them. It is clear that the cradle and source of them all is science in schools, and it is there that we must take urgent and effective action. This is one reason why we are grateful to the masters and the schools who are taking part in this scheme. There is no better way of showing schoolboys that science is an exciting activity than by doing creative work in it under their noses, and frequently with their participation. This is a fairly new scheme and it has developed quite rapidly—about 100 projects are in progress at the present time—but we would like to see it much more widely extended and we would be glad if you would tell your colleagues in other schools throughout the country about it.

"The other reason for our gratitude is a more usual one. One of the main functions and pleasures of the Royal Society is to hear of new scientific work and discoveries and to discuss them on their own merits. This has been an essential function of this meeting and it is very impressive to see the remarkably high scientific quality of the work which has been put before us. Of course, we have heard only a few papers; but it is typical of much of the work which is being carried out.

"We wish to express our thanks to the contributors and to the speakers for the very interesting afternoon they have given us, and to the president for presiding and taking part in the meeting. His presence here is a visible sign of the effective action which he, the Council and the officers of the society have taken throughout in supporting our committee and the scheme." R. H. Dyball

[1] Walton, C. N., *Radiochimica Acta*, 2, 108 (1964).

HYDROXYCINNAMIC ACIDS AND RELATED COMPOUNDS

THE Plant Phenolics Group recently changed its name to the Phytochemical Group with the object of broadening the scope of its discussions into the whole field of plant products; but for its first meeting under the new title, held in the Department of Chemistry of the University of Sheffield during September 21–23, it appropriately returned to the topic of plant phenols and in particular to hydroxycinnamic acids. Investigations of the structure and properties of natural products and in particular plant polyphenols have figured prominently in the researches carried out in Sheffield under a founder member of the group, Prof. R. D. Haworth, and these problems continue to interest his recently appointed successor, Prof. W. D. Ollis.

The meeting opened on September 22 under the chairmanship of Prof. Ollis, who welcomed the group to Sheffield. The first speaker, Dr. J. B. Harborne (John Innes Institute, Hertford), revealed the general background to the meeting in a paper entitled "The Occurrence, Distribution and Function of Hydroxycinnamic Acids in Plants", in which particular emphasis was laid on the taxonomic interest which these acids and their combined forms possess. They also play a central part in the biosynthesis of many of the more complex plant phenols, and in this context Dr. Harborne made brief mention of the problem of the complete absence in plant tissues of 3,4,5-trihydroxycinnamic acid suggesting that, for example, it may be replaced by gallic acid.

The four speakers who followed dealt with the problems associated with the determination of the structures of compounds recently discovered and found to contain one or more of the known hydroxycinnamic acids.

Prof. L. Birkofer (University of Cologne) unfolded the story behind the isolation, characterization and structure

determination of the acylated anthocyanins, pigments from *Petunia, Salvia, Raphanus* and *Matthiola* hybrids.

Dr. L. M. Scarpati (University of Rome) discussed in detail the problem of the isomers of chlorogenic acid, showing the caffeoyl ester group to be located at the 5 position of quinic acid in neochlorogenic acid and at the 4 position in 'band 510'. Isochlorogenic acid, a component of coffee beans and for a long time considered to be an isomer of chlorogenic acid, was revealed as a mixture of three di-*O*-caffeoyl quinic acids in which the 3,5 isomer predominates.

Dr. D. G. H. Daniels (Cereals Research Station, St. Albans) and Mr. M. O. Naumann (University of Sheffield) in two short communications announced the isolation and characterization of two new types of hydroxycinnamoyl esters, respectively, esters with a long-chain aliphatic ω diol found in oats and the 2-*O*-caffeoyl ester of the phenolic glucoside arbutin found in *Vaccinium vitis idaea*.

The session concluded under the chairmanship of Prof. J. W. Fairbairn and concerned the metabolism of hydroxycinnamic acids in higher plants. Miss J. J. Corner (Low Temperature Research Station, Cambridge) read a paper in which the distribution and formation of the *O*-glucosides and glucose esters of the hydroxycinnamic acids were critically evaluated. Dr. M. H. Zenk (University of Munich) discussed the experimental evidence underlying the important $C_6C_3 \rightarrow C_6C_1$ pathway for the formation of plant phenols and proposed a biochemical mechanism for this reaction.

The metabolism of the hydroxycinnamic acids was again considered in the final session on the morning of September 23 when Prof. R. D. Haworth took the chair. Prof. R. A. Thomson (University of Aberdeen) and Prof. E. E. Conn (University of California) discussed the question of the biosynthesis of coumarin from respectively the chemical and biochemical points of view. The pathway of coumarin metabolism was elegantly traced by Prof. Conn, and Prof. Thomson directed attention from a purely chemical point of view to the important step of *ortho*-hydroxylation, indicating the possible ways in which this transformation might be achieved.

Prof. W. D. Ollis (University of Sheffield) followed with a review of the biogenetic relationships among C_{15} compounds derived from cinnamyl precursors; in particular he detailed the possible ways in which the dalbergiones and 4-arylcoumarins might be formed and propounded a theory of oxidative biogenesis to explain the production of the associated benzophenones.

The final paper in the meeting was read by Dr. M. Zucker (Agricultural Experimental Station, Connecticut) and concerned an investigation of the biogenesis of chlorogenic acid showing that this is believed to involve a transformation of phenylalanine → cinnamic acid → 3-*O*-cinnamoylquinic acid → 3-*O*-p-coumaroylquinic acid → chlorogenic acid.

The meeting was well attended, and the lively formal and informal discussions which followed many of the lectures indicated the interest in phytochemical problems which it is the Group's policy to discuss.

POP-OUTS ON CONCRETE SURFACES

THE term 'pop-out' is technically applied to a particular type of disruption or disintegration of concrete surfaces, in serious cases only shortly after casting, with rather special significance. The subject has recently been discussed in a paper by G. M. Idorn (Concrete Research Laboratory, Karlstrup, Denmark) entitled "A Concrete Jetty with Frost Damage" (*Magazine of Concrete Research*, Cement and Concrete Association, 16, No. 47; 1964).

Mr. Idorn finds that pop-outs on concrete surfaces usually result from exposure to freezing and thawing, involving swelling pressure from ice in certain types of coarse aggregate occurring in Danish gravels. "Pure and silicified limestones and opaline and chalcedonic porous cherts in Danish aggregates are liable to give such pop-outs. These rocks account for about 20–40 per cent in ordinary gravel aggregates (considerably less in the sands). . . . There is always the possibility that a pop-out has developed because of the swelling pressure from alkali-silica gel in a porous rock particle when it contains or consists of opaline or chalcedonic silica. . . . The development of alkali-silica gel in a reactive rock particle can also bring about alteration in the interior pore-structure, so that any inherent tendency to be affected by freezing and thawing will increase." This raises again, but in a somewhat different form, the much-debated problem of alkali-reactive aggregate constituents as contributors to disruption of concrete, for example, the mineral chlorophaeite in some dolerites (*Nature*, 196, 943; 1962).

Whereas chlorophaeite and certain volcanic rock-types as pebbles have been suspect in causing damage to concrete by virtue of their chemical vulnerability and without the assistance of frost, it is now clear that the incidence of frost may considerably enhance the rate at which disruption of the concrete concerned can happen. Pop-outs produce unsightly 'pock-marks', rather like burst blisters, on concrete surfaces where these physico-chemical mechanisms are at work; they lead to extensive scaling. Four general types are recognized by Mr. Idorn: (*a*) capillary water in porous pebbles freezing to generate pressure on covering mortar; (*b*) alkali-aggregate reaction in porous cherts, etc., causing fracturing along a 'reaction rim'; (*c*) in rocks of very low permeability and negligible capillary water, ice may form on the surface of the pebble, destroying the bond, causing covering mortar or laitance to fall off; (*d*) growth of microscopic ice lenses in the thin layer of mortar or laitance over the pebble, a variation of type (*c*).

Pop-outs may not, however, fit all cases of surface deterioration of concrete, especially in circumstances where the potential causes of disintegration and exfoliation of mortar, as outlined above, are not in evidence. Mr. Idorn describes his inspection of a jetty in a Baltic harbour where a thick mass-concrete superstructure has suffered severe deterioration due to exfoliation of the mortar, resulting in pools of water where this disruption has occurred. In this case the aggregates used are said to be of excellent quality, ". . . strong, impermeable and chemically inert, and . . . no reason to question the quality of the cement". The concrete had been exposed to severe frost action involving numerous cycles of freezing and thawing during several winters. Of the type of deterioration, he says: "The aggregate pebbles show no sign of disruption themselves, but severe spalling and exfoliation have taken place on the horizontal faces. . . . There are many of the exposed coarser-aggregate pebbles forming pop-outs like the types (*c*) and (*d*) . . ." (above). It is emphasized that there is no sign of chemical reactivity in the aggregates used in this concrete, nor is chemical reaction due to sea-water likely, the salinity of the Baltic being quite low.

These researches and this particular case of the Baltic jetty are revealing, because they clearly demonstrate the mechanical effects of frost attack on concrete surfaces, whether accelerated by pop-outs or otherwise. The need for further investigations of similar damage, particularly in other countries where this has occurred, is implicit in this most informative essay.

NEW SEA-WALL AT ABERDEEN

THE subject of coastal protection is always with us but is seldom nationally publicized until a catastrophe occurs, when a combination of storm and excessively high tides causes the sea to overwhelm existing defences, flooding land behind them, resulting in grave damage to both life and property, to say nothing of the sea-walls and groynes concerned.

Recent work on a section of the coastline between the north pier of Aberdeen harbour and the mouth of the River Don is a case in point. In this example, lack of toe piling coupled with erosion of foreshore during the winter of 1961–62 determined the complete collapse of some 100 ft. of the then existent sea-wall and caused severe damage to the front of the apron over several hundred feet. According to *Concrete Quarterly* (No. 61, April–June 1964; Cement and Concrete Association, London), "The new work now nearing completion consists of two parts. The first and minor portion consists in rebuilding and strengthening portions of the existing wall extending some 2,500 ft. north from the beach bathing station. . . . A completely new wall has been built over the remaining 5,000 ft. or so of sea-front from the existing wall to the Don. A flexible facing of precast concrete blocks, with a post-tensioned precast coping, has been employed".

The construction of flexible revetments by mortaring stone or masonry blocks with a flexible material is a technique which has been known for centuries, ". . . a revetment on the banks of the River Tigris in ancient Assyria, built in 1300 B.C. of limestone blocks and brickwork mortared with bitumen and bituminous mixtures, is still in use to-day, and in good condition, more than 3,000 years later". This new sea-wall at Aberdeen uses much the same formula, only precast concrete blocks instead of limestone, etc., with bituminous jointing material, on a slope of 1 : 2. At the foot the wall is supported by steel sheet-piling topped with an *in situ* concrete capping beam. "Precast ribs at 29-ft. 5-in. centres span between the footing at the bottom and the *in situ* concrete foundation to the precast concrete coping at the top, and give lateral rigidity to the blockwork." It is recorded that some 42,000 precast blocks were involved in this project; these were vacuum-cast on the Miller system; this permits de-moulding to be done within about 4 min; also the use of steel moulds enabled thousands of components to be cast from the same moulds.

Construction of new groynes was an integral part of the scheme, not only to arrest northward drift of littoral sand, but also to help protection of the shore; these groynes are of greenheart timber. Finally, to make doubly sure, and to counteract erosion and stop scour at the northern end of the impermeable wall, there is a terminal section of gabions—permeable wire cages filled with stone—used as a transition section between the wall itself and the existing beach. The illustrations accompanying this article in *Concrete Quarterly* adequately convey the essential constructional details of this new sea-wall, equally a sense of the solidarity of this recent addition to Britain's coastal protection, a matter of great credit to all the engineers concerned.

CHROMOSOMES AND HUMAN MALFORMATION

IN the space of a few years, the enquiry into human variability has been raised from a somewhat speculative stage to the level of exact observation, said Prof. J. A. Böök in a lecture* entitled "Some Mechanisms of Chromosome Variations and their Relation to Human Malformations". The exact observations referred to chromosomal investigations, which have been made possible in recent years through improved techniques of culturing human cells *in vitro*.

Since 1959, when it was first demonstrated that certain chromosomal abnormalities were associated with specific congenital malformations[1,2], a large number of instances have been reported. These cytological abnormalities, Prof. Böök pointed out, were not new cytological phenomena, but demonstrated that man, like other animals and plants, was occasionally subject to abnormalities in the structure of the hereditary system.

The occurrence of an extra chromosome (trisomy), arising from normal parents by chance meiotic or mitotic non-disjunction, was the commonest chromosomal abnormality. In most instances the origin of the abnormal karyotype could be correlated with above-average maternal age—thus revealing the increased risk of chromosomal accidents by ageing. In addition to the three well-established trisomic states [13–15 syndrome, 17–18 syndrome and Downs syndrome (trisomy 21)], the possible differentiation of a fourth syndrome was outlined—involving trisomy for a small acrocentric chromosome—presumably No. 22. From five presumptive cases of this 22 trisomy, the defects in common included microcephaly, deafness, large, low-set malformed ears and pre-auricular papillomata.

The presence of a complete extra set of chromosomes (triploidy) still constitutes a rare abnormality and, apart from the sporadic occurrence in spontaneously aborted fœtuses[3–6], only three instances are known in living individuals[7–9]. The first instance was reported in 1961 (ref. 7) from Prof. Böök's laboratory and occurred in a male infant aged 14 months. In common with the two subsequent cases, the infant was a chromosome mosaic containing both diploid and triploid cells, the latter having the suggested origin from a second fusion with a retained polar nucleus. The most recent observations on this child after an intervening period of three years have shown that the proportion of triploid cells in the skin tissues has markedly decreased from an original figure of 84 per cent to a value of 10 per cent; the decrease being credited (from observations on tissue cultures) to abundant mitotic irregularities in the nature of bridges, lagging chromosomes and tripolar spindles. It should, however, be pointed out that no mitotic instability has been observed in any of the other instances of human triploidy, and it remains to be seen whether the triploid cells in the two other living cases are selectively eliminated. It is further noteworthy that, in the case reported by Ellis *et al.*[8], the child was considerably older (6 years) and yet

* The Galton Lecture delivered before the Eugenics Society in London on May 20, 1964.

had 50 per cent of its skin cells triploid. If the triploid cells in this case are being eliminated, their elimination is occurring at a much slower rate.

Translocations, said Prof. Böök, were probably quite common, but were only microscopically recognizable when relatively large unequal chromosomal segments had been involved in the interchange. The translocations may be transmitted through several generations of phenotypically normal individuals and their presence may only come to light from recurrent malformations, which in some cases may affect 50 per cent of the offspring. In addition to the now well-established examples of translocation between the acrocentric chromosomes (centric fusions), a maternal karyotype was described with a presumptive translocation of the short arms of one of the large chromosomes with a sub-terminal centromere (No. 4–5), to the short arm of one of the small acrocentrics. Multiple congenital malformation with an early death occurred in the progeny when the karyotype displayed effective trisomy for the short arm involved in the translocation.

A similar type of interchange was then considered as the most likely basis for a number of individuals having mental retardation, broad face with flat nose, micro- and retrognathism, hypertelorism, bilateral epicanthus, impaired hearing, retardation of growth and a high-pitched wailing cry. The karyotypes associated with these malformations all showed a deficiency or monosomy for the short arm of a chromosome in the 4–5 group.

Finally, the importance of meiotic observations was discussed in relation to the confirmation of deductions based on somatic chromosome complements. This aspect was illustrated with a male karyotype in which two of the large acrocentrics were represented by a large metacentric element and which, somatically, could have represented a translocation between either homologous or non-homologous chromosomes. The existence of trivalent associations at meiotic metaphase clearly demonstrated an origin from a non-homologous interchange.

In this survey, Prof. Böök excluded all abnormalities involving the sex chromosomes, and limited his text to observations made principally in his own laboratory. His lecture, therefore, clearly demonstrated that with his colleagues he has made many significant contributions in the field of human cytogenetics. J. R. Ellis

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THE ANTRAL HORMONE GASTRIN

Structure of Gastrin

EXISTENCE of the antral hormone, gastrin, was suggested first in 1905, when Edkins[1] showed that crude extracts of mucosa from the antral region of the stomach stimulated gastric secretion in anæsthetized cats. Eventually Edkins's observations were confirmed; it was shown that gastrin was distinct from histamine and probably proteinaceous in nature[2]. Only very recently, however, has the successful isolation of pure gastrin enabled precise physiological and chemical characterization. In 1964, Gregory and Tracy[3] described the isolation from hog antral mucosa of two closely similar polypeptide hormones (gastrins I and II), both of which evoked a remarkable range of physiological responses from alimentary tract structures. In particular, both hormones were very powerful stimulants of gastric acid secretion when injected subcutaneously into conscious dogs[3]. Gastrin II has also been shown to stimulate gastric secretion in human subjects[4]. On a molar basis, it is some 500 times more effective than histamine, and has clear advantages over histamine as a secretory stimulant in assessing gastric secretory function[4].

Through the kindness of Prof. R. A. Gregory, we have been able to carry out chemical studies on both hormones. Our results described here show that gastrin II has the heptadecapeptide amide structure:

$$\begin{array}{l} \overline{\text{L}}\text{Glu.Gly.Pro.Try.Met.Glu.Glu.Glu.Glu.Glu.Ala.Tyr.} \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad \text{Gly.Try.Met.Asp.Phe.NH}_2 \end{array}$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad\ \ \text{SO}_3\text{H}$$

and that gastrin I differs from gastrin II only in lacking the tyrosyl sulphate ester group.

Amino-acid composition. Acidic hydrolysates (6 N HCl/105°/18 h *in vacuo*) were analysed for amino-acid and ammonia content by the automated ion-exchange method of Spackman, Stein and Moore[5] (Beckman amino-acid analyser model 120*B*). Similar results were obtained with both hormones, and were consistent with the foregoing structure. An unexpectedly high recovery of tryptophan (60 per cent) was obtained in the acidic hydrolysates. This is attributed to the high purity of the isolated gastrin. In contrast, tryptophan-containing peptides which had been separated by chromatography or electrophoresis on paper yielded no tryptophan after acidic hydrolysis. Alkaline hydrolysates (N $Ba(OH)_2$/105°/18 h) revealed the same amino-acid composition, except that gastrin II yielded no tyrosine. Chromatography of a basic hydrolysate of gastrin II on 'Dowex-50' cation-exchange resin and elution with water afforded a strongly acidic amino-acid (anionic at *p*H 2·1), which was identified as tyrosine-*O*-sulphate by comparison with a synthetic sample[12]. Enzymatic evidence has been obtained for the L-configuration of all the constituent amino-acids, except those of the *N*-terminal tripeptide sequence.

End-group analysis. No free terminal amino or carboxyl groups could be detected in either hormone by standard methods. Gastrin II failed to react with Sanger's reagent (1-fluoro-2 : 4-dinitrobenzene), and gastrin I yielded only a colourless derivative of *O*-dinitrophenyl-tyrosine. Neither hormone gave a coloration with ninhydrin. No terminal acetyl, or other simple acyl groups, could be detected by the hydrazinolysis method of Phillips[6]. At this stage it was considered likely that the peptide chain was terminated at its amino-end by a pyroglutamyl residue, and supporting evidence for this was obtained during the course of amino-acid sequence studies.

Carboxypeptidase was without significant action on either gastrin I or gastrin II, and hydrazinolysis failed to liberate a C-terminal amino-acid from gastrin II. Enzymatic degradation (*infra*) afterwards showed that the C-terminus of the peptide chain was blocked by the single amide residue.

Amino-acid sequence of gastrin II. Gastrin II was degraded by treatment with cyanogen bromide (a reagent reportedly[7] specific for cleavage at methionine residues), with chymotrypsin (high specificity for cleavage at aromatic residues), and with enzymes of low specificity, papain and subtilisin. Partial hydrolysis of gastrin II by

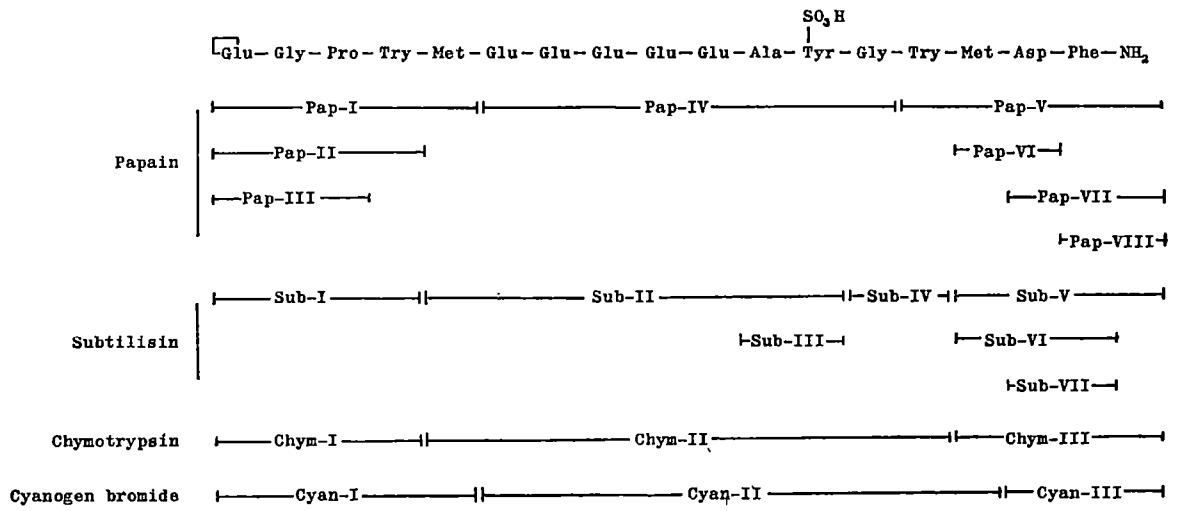


Fig. 1. Peptides obtained by degradation of gastrin II

aqueous acid was also investigated briefly. The most useful results were obtained from the papain and subtilisin digests; the resulting relatively complex mixtures of peptides were fractionated by a combination of paper electrophoretic and chromatographic methods. Most of the isolated peptides were obtained in amounts sufficient for quantitative amino-acid analysis after acidic hydrolysis. Tryptophan was determined after basic hydrolysis or further enzymatic or chemical degradation, or its presence was inferred from a positive Ehrlich test on the intact peptide.

(1) *Papain degradation.* Digestion of gastrin II was carried out at *p*H 7·05 and 37° for 22 h, using papain previously activated with potassium cyanide (enzyme : substrate ratio, 1 : 20). The eight peptides designated Pap-I to Pap-VIII in Fig. 1 were isolated. Pap-VIII was identified as phenylalanine amide by comparison with a synthetic sample. It must, therefore, have been derived from the C-terminus of the peptide chain. The C-terminal sequence, Try.Met.Asp.Phe.NH_2, followed unambiguously from the compositions of the peptides, Pap-V, Pap-VI and Pap-VII. The three peptides, Pap-I, Pap-II and Pap-III, formed a ninhydrin-negative series, and their behaviour on electrophoresis (monoanionic at *p*H 6·1, neutral at *p*H 2·1) confirmed the absence of free amino-groups, and was consistent with the presence of terminal pyroglutamyl residues. They must, therefore, have been derived from the N-terminus of gastrin II. The partial sequence, Glu(Gly,Pro)Try.Met, followed from their compositions. Further digestion of the pentapeptide, Pap-I, with carboxypeptidase liberated methionine and tryptophan, with no trace of the other constituent amino-acids. Furthermore, the residual peptide isolated after carboxypeptidase treatment of Pap-I contained equimolecular ratios of glutamic acid, glycine, and proline. In view of the known inability of carboxypeptidase to hydrolyse past proline residues[8], the sequence of Pap-I, and of the N-terminus of gastrin II, could confidently be expanded to Glu.Gly.Pro.Try.Met.

The central peptide, Pap-IV, was shown to have N-terminal glutamic acid by reaction with fluorodinitrobenzene. Four cycles of the Edman degradation (followed both by identification of the liberated phenylthiohydantoins and by quantitative analysis of the residual peptide after each stage) showed that glutamic acid occupied the first four positions in the peptide chain. The C-terminal amino-acid was identified as glycine (carboxypeptidase). The partial sequence Glu.Glu.Glu.Glu(Glu,Ala,Tyr)Gly was thus established for Pap-IV. The complete hydrolysis of Pap-IV by leucine aminopeptidase showed that all five glutamyl residues were linked by their α-carboxyl groups, and that all the constituent amino-acids were of the L-configuration.

(2) *Subtilisin degradation.* Digestion of gastrin II was carried out at *p*H 8·01 and 37° for 20 h, using a commercial subtilisin preparation ('Nagarse'), enzyme : substrate ratio, 1 : 16. Isolation of peptides Sub-V, Sub-VI and Sub-VII confirmed the C-terminal sequence already deduced. Furthermore, the complete hydrolysis of Sub-VI by leucine amino-peptidase established the α-linkage of the aspartyl residue in this C-terminal sequence. The central peptide, Sub-II, and the dipeptide, Sub-IV, provided overlaps with the papain peptides which confirmed the arrangement of N-terminal, central, and C-terminal sequences suggested above. The central peptide, Sub-II, had N-terminal methionine (Edman degradation), confirming the alignment of Pap-I and Pap-IV. The C-terminal amino-acid was tyrosine (hydrazinolysis), which enabled the sequence of Pap-IV to be extended to:

Glu.Glu.Glu.Glu(Glu,Ala)Tyr.Gly.

The dipeptide, Sub-III (anionic at *p*H 2·1, that is, contained the tyrosine as its *O*-sulphate), was shown to have the alanine N-terminal (Edman degradation), completing the above central sequence. The dipeptide, Sub-IV, was identified as glycyltryptophan by both N-terminal (Edman degradation) and C-terminal (hydrazinolysis) analysis, confirming the alignment of Pap-IV and Pap-V, and completing the entire amino-acid sequence of gastrin II.

(3) *Other degradations.* Cleavage of gastrin II by chymotrypsin and by cyanogen bromide was examined in less detail, but the compositions of the resulting peptide fragment were in all cases consistent with the amino-acid sequence deduced from the papain and subtilisin degradations. Gastrin II was not a good substrate for chymotrypsin, and conditions could not be found for specific cleavage at all the aromatic residues. Hydrolysis at the tryptophan residue (position 4) was rapid, and the N-terminal tetrapeptide, Chym-I, was readily isolated. Cleavage at the second tryptophan residue (position 14) was much slower. The C-terminal tripeptide, Chym-III, was isolated in pure form, but the central peptide, Chym-II, was contaminated with the longer peptide comprising Chym-II + Chym-III. No evidence was obtained for cleavage at the tyrosine-*O*-sulphate residue[9], and the electrophoretic behaviour of Chym-III suggested that the phenylalanine–amide bond was also intact.

The normal conditions[7] for the selective fission of peptides at methionine residues by cyanogen bromide had to be modified because of the insolubility of gastrin II in

dilute hydrochloric acid. However, a moderately satisfactory cleavage was obtained using aqueous trifluoroacetic acid as solvent, and the expected peptides, Cyan-I, II and III, were isolated in low yield. Cyan-I and Cyan-II were presumed to contain C-terminal homoserine lactone in place of the original methionine residues, in accordance with the accepted mode of cleavage by cyanogen bromide. The ninhydrin-negative peptide, Cyan-I, was neutral on electrophoresis at both *p*H 2·1 and 6·1, confirming the absence of terminal carboxyl and amino groups, and of the second carboxyl group of the glutamyl residue. This, again, is in accord with the pyroglutamyl formation. Further support for this structural feature was obtained by the isolation of pyroglutamic acid itself from partial acidic hydrolysates of gastrin II.

Amino-acid sequence of gastrin I. Many of the foregoing degradations were afterwards repeated on gastrin I. All the results were in agreement with the identical amino-acid sequences of the two hormones, except that gastrin I lacked the phenolic sulphate ester group. For example, the central peptides, Pap-IV, derived respectively from gastrins I and II, reflected the same differences in electrophoretic behaviour as were found[3] with gastrins I and II themselves (gastrin II is more acidic than gastrin I), whereas peptides derived from the N- and C-termini of the two hormones appeared to be identical. Furthermore, the central peptide, Pap-IV, from gastrin I liberated all the constituent amino-acids, including tyrosine, on digestion with leucine aminopeptidase, whereas the corresponding gastrin II peptide yielded predominantly tyrosine-*O*-sulphate. The two peptides differed also in their reaction with carboxypeptidase. Pap-IV derived from gastrin I liberated both glycine and tyrosine on digestion with carboxypeptidase at neutral *p*H. However, the gastrin II peptide liberated only glycine, and the residual peptide terminating in tyrosine-*O*-sulphate was inert to further hydrolysis. This behaviour is reminiscent of the inhibitory effect of C-terminal cysteic acid residues on the action of carboxypeptidase[6].

Further proof of the structural relationship between the two hormones has been obtained by their interconversion. Treatment of gastrin II with a mixture of trifluoroacetic and 3 N hydrochloric acids (3 : 1) at room temperature for 70 min results in 70 per cent conversion to gastrin I. Resynthesis of gastrin II from gastrin I has been achieved by treatment of an alkaline solution (*p*H 10) of the latter with pyridine–sulphur trioxide complex, a reagent previously used for the sulphation of the phenolic hydroxyl group of tyrosine[10].

The amino-acid sequences revealed by the foregoing degradations contain several unusual features worthy of comment. The presence of tyrosine-*O*-sulphate in a peptide hormone does not seem to have been encountered before, although the amino-acid has been identified in fibrinogen peptides[11]. The free amino-acid also occurs in urine[12]. The complete absence of basic amino-acids and the high concentration of acidic amino-acids in both gastrins is also noteworthy, especially the presence of five glutamyl residues in a contiguous sequence. The terminal pyroglutamyl residue is not without precedent in natural peptides[13], although it is possible that *in vivo* the hormones possess a glutaminyl residue in this position. On the other hand, the N-terminal pyroglutamyl residue and the C-terminal amide may have the *in vivo* function of protecting gastrin from destruction by amino- and carboxy-peptidases. It is interesting to note, however, that, with the exception of the C-terminal amide function, none of the structural features mentioned above is absolutely essential for biological activity[14].

We thank Prof. R. A. Gregory and Dr. H. J. Tracy for natural gastrin. We thank Imperial Chemical Industries, Pharmaceuticals Division, for a gift of the automatic amino-acid analyser, and for financial support, and Mr. D. Wheeler for assistance. We also thank Dr. D. Boulter and his associates of the Department of Botany, University of Liverpool, for preliminary amino-acid analyses obtained with a 'Technicon' autoanalyser.

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Synthesis of Gastrin

Synthesis of gastrin was, in our view, essential both to confirm the sequence deduced from degradative investigations[1] and to remove the possibility that a small constituent, not giving a colour with ninhydrin, had escaped our notice. In the event, synthesis of gastrin I[2] gave the clue to the structural difference between gastrins I and II, effectively revealing the presence of sulphate in the latter. As gastrin II has been prepared from gastrin I[1], this synthesis covers both hormones.

The final step in synthesis was removal of *t*-butyl groups, masking the γ-carboxyl groups of the five glutamyl residues, by treatment of the penta-*t*-butyl ester of gastrin I (Fig. 1, line 6) with 98 per cent trifluoroacetic acid at 20° for 1 h. The product was purified by chromatography on 'Sephadex *G*25' in 0·04 M ammonium bicarbonate, and gradient elution from aminoethylcellulose[3]. In this chromatography, in high-voltage electrophoresis (pyridinium acetate *p*H 6·1), and in thin-layer chromatography, the purified product was indistinguishable from gastrin I, and it gave full biological responses when injected subcutaneously into a conscious dog. Although the identity of the synthetic and natural products is not in serious doubt, we intend to compare enzymatic degradations of them in order to obtain even more conclusive evidence by 'finger-printing'.

The penta-*t*-butyl ester was assembled (Fig. 1)[4] from three components comprising residues 1–5, 6–13 and 14–17 of the sequence. The central (6–13) penta-*t*-butyl monomethyl ester (line 3) was prepared from alanyltyrosylglycine methyl ester by elongation in 10 steps, of which only the last two are shown. This polyester was coupled with the (1–5) azide (line 3) in dimethylformamide solution at 0° during three days; in some experiments Curtius degradation of the azide, shown in a low methionine content of the product, was troublesome[5]. Selective saponification of the extended polyester, suspended in 75 per cent aqueous dioxan, by 4 equivalents of sodium hydroxide during 1·5 h at 20°, yielded an acid (line 5), comprising residues 1–13, which was coupled with the (14–17) tetrapeptide amide (line 5) via the mixed anhydride with diphenyl phosphoric acid[6]. The yield from this coupling was only about 10 per cent, but this can

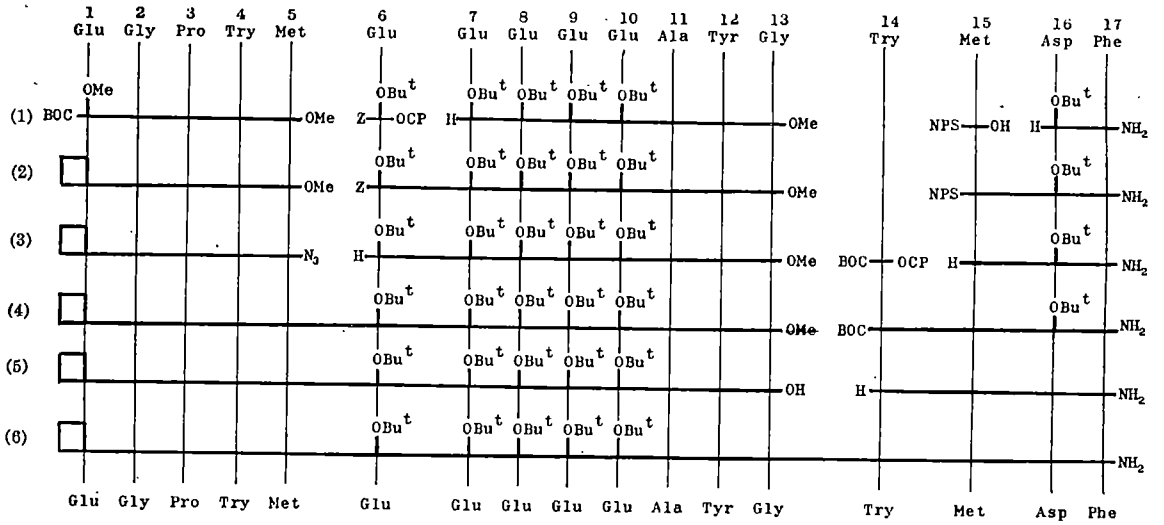


Fig. 1. BOC, $(CH_3)_3C.O.CO$; Z, $C_6H_5.CH_2.O.CO$; But, $C(CH_3)_3$; NPS, o-$NO_2.C_6H_4.S$; OCP, $O.C_6H_2Cl_3(2,4,5)$; Glu, pyroglutamyl

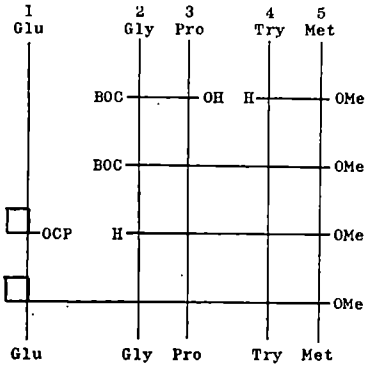


Fig. 2

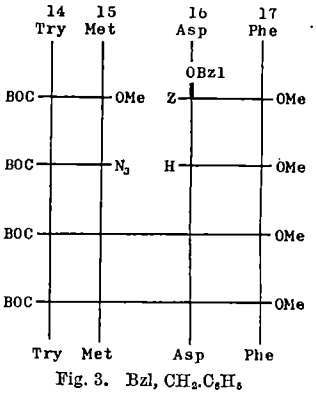


Fig. 3. Bzl, $CH_2.C_6H_5$

probably be improved by variation of technique. Another possible source of improvement is our recent finding that the butyloxycarbonyl group can be preferentially removed from the fully protected (14–17) amide (line 4); the resultant ester-amide can doubtless be coupled by other means.

The (1–5) and (14–17) components actually used in the first successful synthesis were prepared as indicated in Fig. 1 (ref. 4), but the following alternative methods are more convenient. Thus, the (14–17) tetrapeptide amide (Fig. 1, line 5) is made more efficiently (Fig. 3) by an azide coupling of two dipeptides, ammonolysis at the tetrapeptide stage, and removal of the single protecting group[7]. Again, tryptophylmethionine methyl ester, prepared from the butyloxycarbonyl derivative used in the foregoing preparation, can be condensed with butyloxycarbonylglycylproline by di*cyclo*hexylcarbodi-imide at 0° (Fig. 2). Then, after removal of the butyloxycarbonyl group, reaction with the 2,4,5-trichlorophenyl ester of pyroglutamic acid in dimethylformamide at 20° for 60 h yields the (1–5) pentapeptide ester, identical with that prepared by the alternative route (Fig. 1, line 2).

A second synthesis of gastrin I has also been achieved by means of the 2,4,5-trichlorophenyl ester of pyroglutamic acid, probably the most convenient reagent for introducing a pyroglutamyl residue. The final stage is reaction between this active ester and the free (2–17) peptide amide together with 7 equivalents of triethylamine in dimethylformamide during five days at 37°. This hexadecapeptide was constructed from the (2–5) butyloxycarbonyl-tetrapeptide ester (Fig. 2) through an azide coupling with the (6–13) pentabutyl monomethyl ester (Fig. 1, line 3) and subsequent attachment of the (14–17) tetrapeptide amide (Fig. 1, line 5).

Throughout this work the L-forms of amino-acids were used and the syntheses were designed to preserve all the optical activity. At each stage the peptides gave satisfactory amino-acid analyses (Beckman 120*B*). Details of this work, of investigations of alternative syntheses, and of syntheses of smaller peptides related to portions of gastrin will be published elsewhere.

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[6] Cosmatos, A., Photaki, I., and Zervas, L., *Chem. Ber.*, 94, 2644 (1961).
[7] Synthesis of (14–17) tetrapeptides by these and other methods is covered by pending patent applications in the name of Imperial Chemical Industries, Ltd.

Physiological Properties of a Series of Synthetic Peptides structurally related to Gastrin I

In our description earlier this year of the isolation and properties of gastrins I and II, we showed that both these peptides displayed, in apparently identical form and degree, a wide range of actions in the alimentary tract[1]. At one time or another in the past, all these actions had been recognized to be properties of more or less crude extracts of antral mucosa, and it had been widely supposed that they were attributable to the presence in such extracts of a variety of substances besides the hormone gastrin, which was believed only to stimulate gastric acid secretion.

The actions of the pure natural peptides (gastrins I and II) may be summarized as follows:

(1) *Stimulation of gastric acid secretion.* Subcutaneous, or slow intravenous, injections in appropriate dosage cause a prompt and protracted secretion of highly acidic gastric juice containing little pepsin.

(2) *Inhibition of gastric acid secretion.* In conscious dogs provided with denervated fundic pouches, small rapid intravenous injections inhibit a steady background response maintained by repeated small subcutaneous injections, or slow intravenous infusions, of histamine or of gastrin I or II.

(3) *Stimulation of pepsin secretion.* In the type of experiment described under heading (2), a large output of pepsin is observed in the juice which is secreted as the inhibition wears off. A combination of inhibition of acid (and volume) secretion and stimulation of pepsin secretion is also demonstrable in denervated pouch dogs following large single subcutaneous injections.

(4) *Stimulation of pancreatic secretion.* In conscious or anæsthetized dogs, intravenous injections cause a prompt increase in the volume-flow and enzyme output of pancreatic juice.

(5) *Stimulation of gastric tone and motility.* In conscious dogs provided with denervated fundic pouches, small rapid intravenous injections cause a prompt and powerful spasm of the gastric musculature, usually followed by some increase of motility at a lower level of tone before the normal rhythm is resumed.

(6) *Effects on small intestinal tone and motility.* In conscious dogs provided with isolated jejunal loops, small rapid intravenous injections cause a prompt sustained spasm or rapid series of vigorous contractions, which quickly gives place to more prolonged inhibition, chiefly of tone but often of motility as well.

(7) *Biliary tract and blood-pressure.* In anæsthetized dogs, neither of the gastrins causes contraction of the gall-bladder (cholecystokinin-like activity), nor does either significantly influence the rate of flow of hepatic bile. In similar circumstances neither gastrin has other than a small and inconstant depressant effect on the blood-pressure.

Towards the end of the work described in the previous paper, which culminated in the total synthesis of gastrins I and II, we were enabled through the kindness of our colleagues to investigate the physiological actions of a series of synthetic peptide derivatives comprising, or closely related to, various portions of the gastrin molecule. Most of these peptides were produced during the programme of synthesis, but some were made at our request. We have made a preliminary examination of these peptides for the presence of the types of activity enumerated here for gastrins I and II (with the exception of actions on the biliary tract). In these experiments the same methods, and for the most part the same group of surgically-prepared conscious dogs, were used as described in detail by us[1].

The prime objective of this investigation was to ascertain, as a starting point for the preparation of analogues, which types of activity were shown by each peptide; it was hoped that some localization of function in different

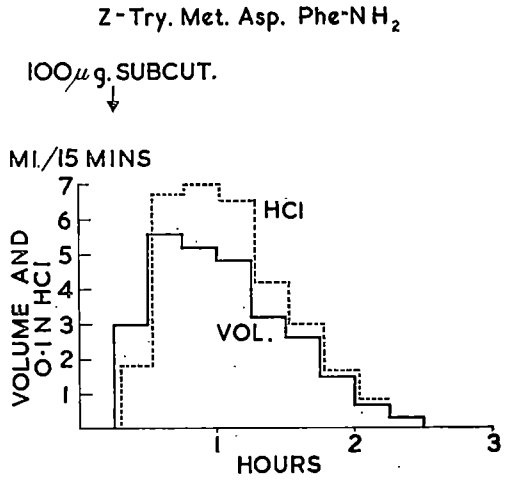


Fig. 1. Response of a conscious dog provided with a denervated fundic pouch to a single subcutaneous injection at arrow of 100 μg of Z.Try. Met.Asp.Phe—NH_2 dissolved in 1 ml. saline

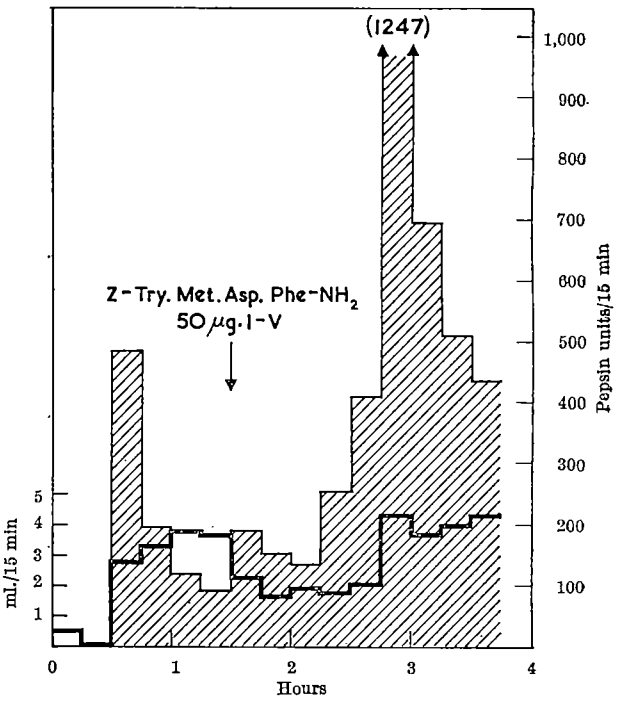


Fig. 2. Volume-flow (heavy line) and pepsin output (shaded areas) of gastric juice from a denervated fundic pouch in a conscious dog. Histamine acid phosphate 0·15 mg was injected every 15 min throughout the experiment. At arrow, 50 μg of Z.Try.Met.Asp.Phe—NH_2 dissolved in 1 ml. saline was injected rapidly intravenously

regions of the total molecule might thereby be revealed. At this stage it seemed of little value to attempt any formal comparison of potency in respect of any type of action. However, the amount of each peptide injected, at least in initial trials, was 50–100 μg; and, since ample data were already available for the actions of gastrins I and II when used in such amounts, it was possible to draw some tentative conclusions as to potencies. Comparisons were facilitated by using the same dogs for similar types of test where possible; and these dogs had also been used for previous studies with the gastrins.

In experiments on the stimulation of gastric acid secretion by subcutaneous injections, a wide range and pattern of dosage was used in order to exclude the possibility that a negative result was in reality due to the production of inhibition by too large an initial dose (see under heading (3)). In testing for inhibition of gastric acid secretion and stimulation of pepsin secretion by intra-

Table 1

	Peptide	Physiological actions								
		HCl secretion		Gastric motility		Intestinal motility		Pancreatic secretion		Pepsin secretion
		Stim.	Inhib.	Stim.	Inhib.	Stim.	Inhib.	Vol.	Enzyme	Stim.
	$\overline{\text{Glu.Gly.Pro.Try.Met.(Glu)}_5\text{.Ala.Tyr.Gly.Try.Met.Asp.Phe.NH}_2}$	+	+	+	0	+	+	+	+	+
(1)	Z.Asp.Phe.NH_2	0	0	0	0	0	0	0	0	0
(2)	Z.Met.Asp.Phe.NH_2	0	0	0	+	0	0	0	0	0
(3)	Z.Try.Met.Asp.Phe.NH_2	+	+	+	+	+	+	+	+	+
(4)	Z.Gly.Try.Met.Asp.Phe.NH_2	+	+	+	?+	+	+	+	+	+
(5)	Z.$(Glu)_5$.Ala.Tyr.Gly.Try.Met.Asp.Phe.NH_2	+	+	+	+	+	+	+	+	+
(6)	$\overline{\text{Glu.Gly.Pro.Try.Met.(Glu)}_5\text{.Ala.Tyr.Gly.Try.Met.Asp.Phe.NH}_2}$	+	+	+	0	+	+	+	+	+
(7)	$\overline{\text{Glu.Gly.Pro.Try.Met.(Glu)}_2\text{.Ala.Tyr.Gly.OMe}}$	0	0*	0	0	0	0	0	0	+
(8)	$\overline{\text{Glu.Gly.Pro.Try.Met.OMe}}$	0	0*	0	?+	0	?+	0	0	+
(9)	Z.$(Glu)_4$.Ala.Tyr.Gly.OMe	0	0	?+	0	0	0	0	0	?+
(10)	Z.Gly.Try.Met.Asp.Phe.OMe	0	0*	0	0	0	0	+	+	?+
(11)	TFA-NH_3^+.Try.Met.Asp.Phe.OMe	0	0*	0	0	0	0	+	+	+
(12)	TFA-NH_3^+.Try.Met.Asp.Phe.NH_2	+	+	+	+	+	+	+	+	+

* Background histamine response augmented. ?+ Effect very small or doubtful.

venous injection against a background response (headings (2) and (3)), repeated subcutaneous injections of histamine were used as the stimulus.

In Table 1 are set out the constitutions of the 12 peptides to be described on this occasion, compared with that of gastrin I, and the types of activity found in respect of each of them. The results are expressed in the following form: + indicates a decisively positive result, and 0 a negative result; ?+ denotes that the effect observed was very small or of doubtful significance. The effects on gastrointestinal tone and motility were more variable in occurrence and pattern than the other actions, due, no doubt, to spontaneous variations in sensitivity and rhythmical activity of the animal preparations on different occasions, and the component of these responses most difficult to evaluate was that of inhibition following stimulation in the gastric musculature. A further problem in these experiments arose from the observation that, during a given experiment, earlier injections of the same or different peptides influenced later responses (generally decreasing them) although recovery from the earlier responses had apparently ensued. Where, as sometimes happened, a negative result was obtained on one occasion, and a decisively positive one on another, the result is expressed in Table 1 as positive (+).

The following conclusions can be drawn from the present findings:

(i) The entire range of physiological activities displayed by natural gastrin I are possessed by the C-terminal tetrapeptide sequence Try.Met.Asp.Phe—NH_2 (peptides 3 and 12). Whether the N-terminal residue was protected by a benzyloxycarbonyl group (No. 3) or not (No. 12) appeared to have no significant influence on activity. There was some indication that both peptides showed an inhibitory action on gastric tone and motility following stimulation, after intravenous injection; this action has not been consistently observed with natural gastrin. The power to stimulate acid secretion on subcutaneous injection appeared to be about one-fifth that of gastrin on a simple weight basis, and the ability to inhibit gastric acid secretion when injected intravenously seemed to be of about the same relative potency.

(ii) As the peptide chain was progressively lengthened towards the N-terminus (peptides 4, 5 and 6) the general potency of the molecule increased; No. 6, which differed from gastrin only in having two instead of five glutamyl

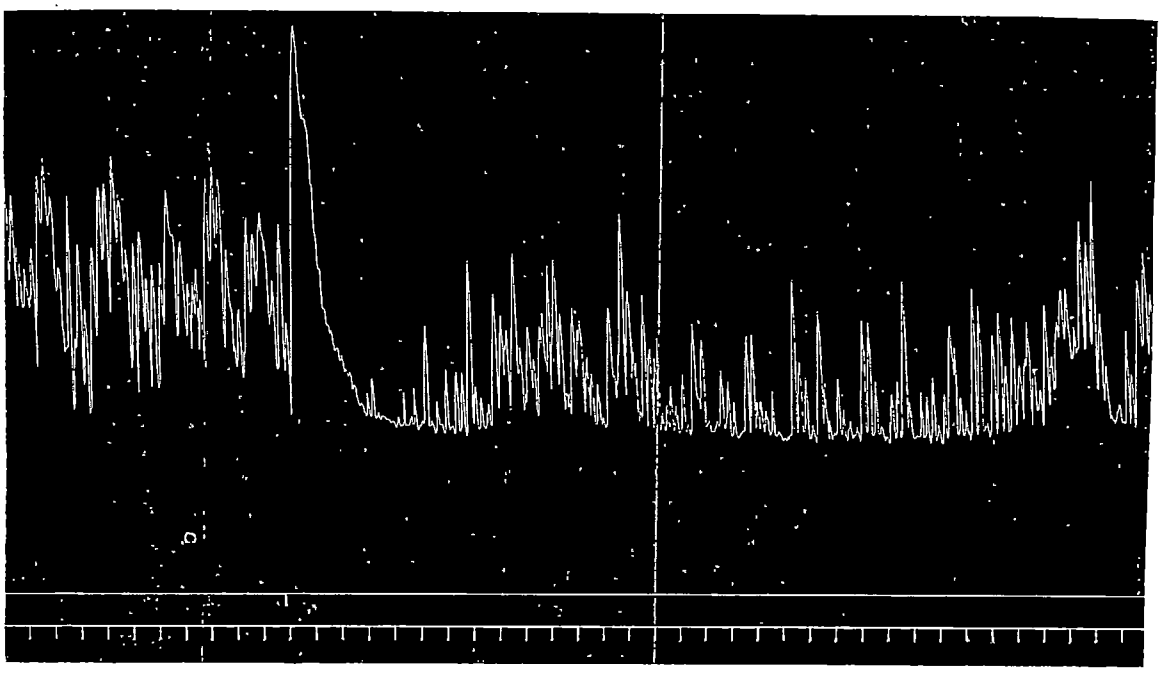


Fig. 3. Record of tone and motility in the denervated fundic pouch of a conscious dog. At signal, 50 μg TFA-NH_3^+.Try.Met.Asp.Phe—NH_2 dissolved in 1 ml. saline was injected rapidly intravenously. Time-signal 10 sec

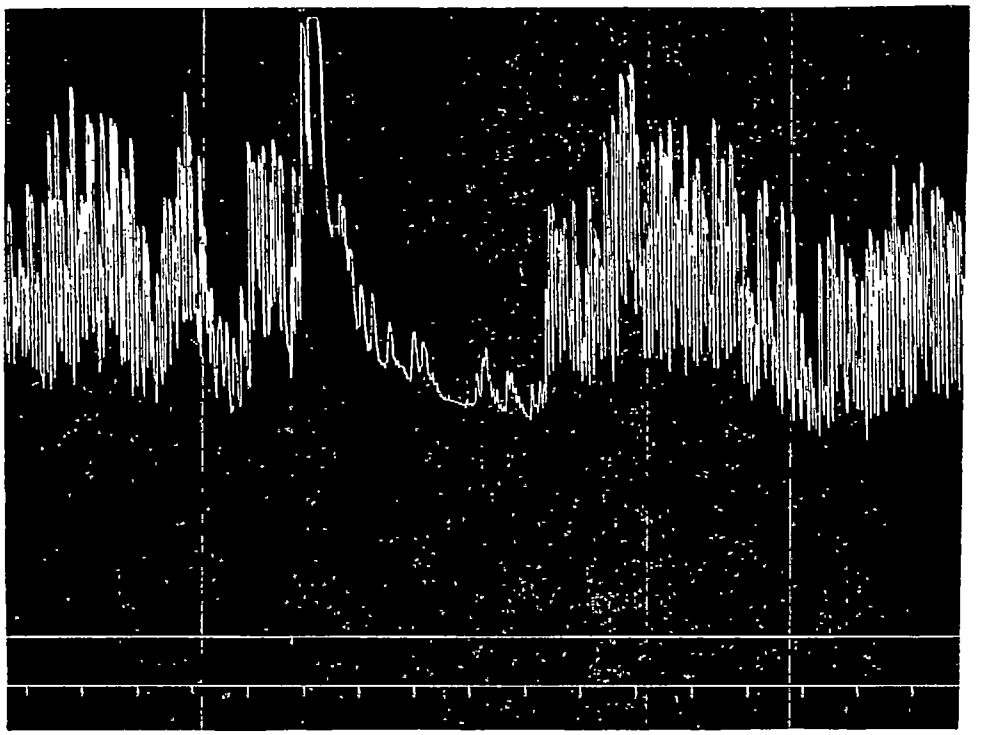


Fig. 4. Record of tone and motility in an isolated jejunal loop (Thiry fistula) of a conscious dog. At signal, 50 μg of TFA-NH_3^+.Try.Met.Asp.Phe—NH_2 dissolved in 1 ml. saline was injected rapidly intravenously. Time-signal 10 sec

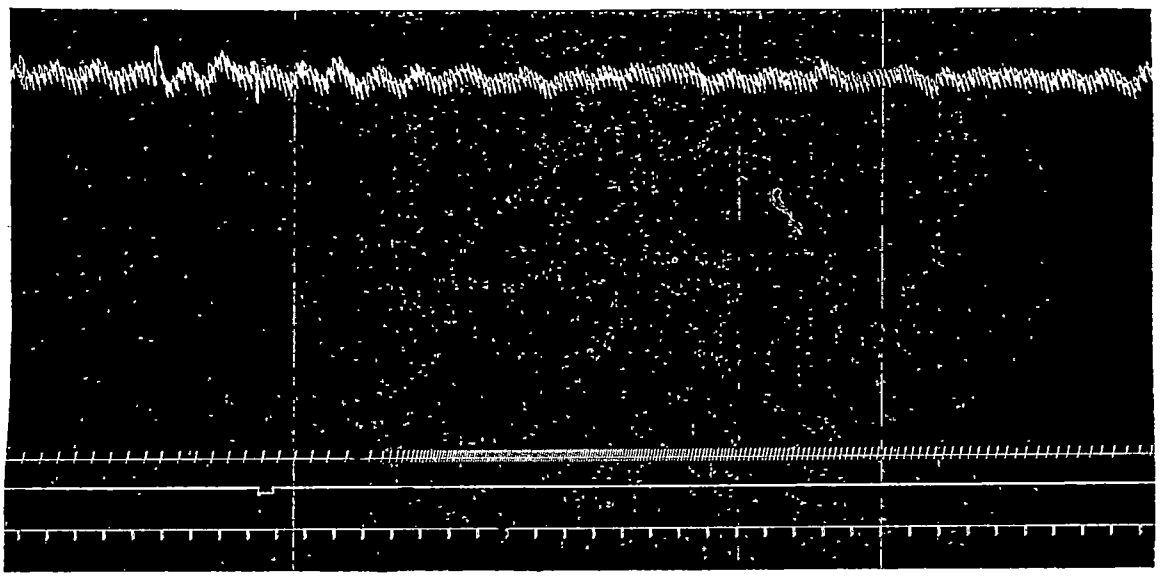


Fig. 5. Record of arterial blood-pressure and drops of pancreatic juice in a chloralosed dog. A background flow was maintained throughout the experiment by slow intravenous infusion of a commercial pancreozymin-free secretin preparation. At signal, 50 μg of Z.Try.Met.Asp.Phe—NH_2 dissolved in 1 ml. saline was injected rapidly intravenously. Time-signal 10 sec

residues in the body of the molecule, could not be distinguished from gastrin itself in any way.

(iii) The C-terminal di- and tri-peptide derivatives (Nos. 1 and 2) were apparently inactive, except for some indication of inhibition of gastric tone and motility by No. 2.

(iv) The N-terminal sequences Nos. 7 and 8 and a central sequence (No. 9) showed no significant power to stimulate gastric acid secretion when injected subcutaneously in single or repeated doses, nor did they stimulate pancreatic secretion or influence gastrointestinal motility; but two of them (Nos. 7 and 8), when injected intravenously against a background histamine response, augmented this, and there was a small or moderate increase in pepsin output.

(v) The C-terminal pentapeptide and tetrapeptide lacking the amide group (Nos. 10 and 11) could not be shown to stimulate gastric secretion when injected subcutaneously. When injected against a background histamine response, they augmented this and caused a slight or small increase in pepsin output, as had peptides 7 and 8. They had no action on gastrointestinal tone and motility, but still retained the power to stimulate pancreatic volume-flow and enzyme secretion. These actions of No. 11 are in notable contrast to those of No. 12, for the two compounds differ only in that the masking amide group is replaced by

an ester group; the same is true of peptides 4 and 10. Some examples of the closely similar actions of peptides 3 and 12 are shown in Figs. 1–5; these may be compared with Figs. 1, 6, 7, 8 and 9 in Part II of ref. 1 which displays the corresponding effects produced in similar experimental conditions by natural gastrin.

It is remarkable that, of the 17 residues of gastrin, only the C-terminal tetrapeptide sequence is required for the entire range of physiological activities displayed by the total molecule, and that these actions should be so radically changed by the absence from this sequence (or from the corresponding pentapeptide, compare peptides 4 and 10) of the amide group which masks the C-terminal residue. Naturally, at this stage a number of questions concerning the properties of these peptide derivatives remain unanswered; but the investigation continues, and a fuller account will be given elsewhere. It seems clear, however, that the simple tetrapeptide sequence, Try.Met.Asp.Phe—NH_2, with its well-developed and distinctive actions on the gastric and pancreatic gland-cells and on the gastric and intestinal musculature offers promise for the study of structure-function relationships by the synthesis of analogues and exploration of their properties along lines similar to those described here.

This work was supported by grant *AM*–04786 from the U.S. National Institutes of Health.

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Physiological Laboratory,
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DEGRADATION OF TYPE XIV *Pneumococcus* POLYSACCHARIDE BY INDUCED ENZYMES

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Methylation investigations[1] had shown that type XIV *Pneumococcus* polysaccharide contains on average, in every 6 moles of sugar residues, 2 moles of D-galactopyranose non-reducing end-groups, 1 mole of D-galactopyranose linked 1 : 3, 1 mole of D-glucopyranose linked 1 : 4 and 2 moles of *N*-acetyl D-glucosamine linked 1 : 4 : 6. The technique of sequential enzyme induction[2,3] has been used to elucidate further the fine structure of *S.* XIV.

S. XIV (0·1 mg/ml.) was incubated shaken at 34°/24 h with a dense suspension of succinate-adapted cells of *Klebsiella aerogenes* N.C.I.B. 9479 in sterile succinate medium adjusted to *p*H 6·8 with potassium hydroxide[4]. D-Galactopyranose, characterized as the β-penta-acetate and assayed[5,6], was recovered (0·5 mole, calculated on the 6 moles of sugar residues of the polysaccharide cited above) from the dialysable fraction of the cell-free culture filtrate. The partially degraded *S.* XIV, when used for a further induction at 34°/24 h, gave a further amount of D-galactose (0·55 mole) and a trisaccharide (0·16 mole)[5–8].

Two further inductions resulted in the liberation of additional amounts of trisaccharide (0·32, 0·22 moles, respectively). The induced enzyme present in a 48-h cell-free culture filtrate was a specific β-D-galactopyranosidase, hydrolysing lactose and *o*-nitrophenyl-β-D-galactopyranoside but without action on melibiose, cellobiose, maltose or *N,N'*-diacetylchitobiose. When *S.* XIV was re-incubated with the isolated induced β-D-galactosidase, galactose and the trisaccharide were again detected.

The neutral reducing trisaccharide was characterized as *O*-β-D-glucopyranosyl (1 → 4)-*O*-(2-acetamido 2-deoxy)-β-D-glucopyranosyl (1 → 3)-D-galactopyranose. Analysis after acid hydrolysis showed that it contained equimolar amounts of glucose, galactose and *N*-acetyl glucosamine, and did not react with the Morgan–Elson[9] reagents. It was not oxidized by either galactose[6] or glucose[7] oxidases, but both these enzymes reacted with the corresponding sugars in the hydrolysate. Following sodium borohydride reduction, acid hydrolysis revealed glucose and glucosamine, while, after consecutive periodate oxidation, sodium borohydride reduction and acid hydrolysis, glucosamine only was detected. Mild acid hydrolysis of the trisaccharide yielded *inter alia* two disaccharides, characterized respectively as 4-*O*-β-D-glucosyl 2-acetamido 2-deoxy D-glucose and 3-*O*-(2-amino-2-deoxy)-β-D-glucosyl D-galactose.

The release of the trisaccharide in the medium was followed by analysing aliquots of the dialysable fractions for total hexoses[5], galactose[6], glucose[7], hexosamines[8] and calculating the trisaccharide content from these figures. The trisaccharide was a substrate for β-glucosidase, releasing D-glucose (characterized by its specific oxidation by glucose oxidase[7]) and a disaccharide characterized as 3-*O*-(2-acetamido 2-deoxy) β-D-glucosyl D-galactose. Acid hydrolysis of periodate oxidized *S.* XIV had yielded the same disaccharide after acetylation[11]. After mild acid hydrolysis of *S.* XIV, and acetylation of the amino sugars[12], five disaccharides had been isolated: lactose, 4-*O*-β-D-glucosyl 2-acetamido 2-deoxy D-glucose, 3-*O*-β-(2-acetamido 2-deoxy) D-glucosyl D-galactose, 3-*O*-D-galactosyl D-galactose, 3-*O*-β-D-glucosyl D-galactose; also a trisaccharide, *O*-β-D-galactosyl-(1–4)-*O*-β-D-glucosyl (1–4) 2-acetamido 2-deoxy D-glucose.

When the trisaccharide was used as an inducer with *K. aerogenes* cells, a β-glucosidase was induced, for which cellobiose was a substrate, but not maltose, lactose, melibiose or *N,N'*-diacetyl chitobiose. A disaccharide, characterized as 3-*O*-β-(2-acetamido 2-deoxy) D-glucosyl D-galactose, appeared in the medium; the rate of formation was readily followed by increasing response to the Morgan–Elson reagents[10] while the total hexosamine content[9] of the medium remained unchanged. The presence of D-glucose in the medium was detected by glucose oxidase[7], although this sugar was readily fermented[3]. The disaccharide was not oxidized by galactose oxidase. When used as an inducer with *K. aerogenes* cells, a β-D-glucosaminidase was induced, for which chitin and *N,N'*-diacetyl chitobiose were substrates but not the other disaccharides previously cited. The progress of enzymatic hydrolysis of the disaccharide was followed by the appearance in the culture medium of D-galactose, specifically oxidized by galactose oxidase. The disaccharide was hydrolysed by a specific β-D-glucosaminidase to D-galactose and *N*-acetyl D-glucosamine, characterized by its positive reaction with the Morgan–Elson reagents[9].

The presence in the trisaccharide of two β-glycosidic linkages was thus established by the anomeric specificity of the induced enzymes formed when the isolated trisaccharide and disaccharide acted as inducers; these findings were confirmed by the action of enzymes of known specificity. The galactose non-reducing end-groups initially removed from *S.* XIV were also β-glycosidically linked.

The first four units of *S.* XIV were thus: β-D-Galp 1–4 β D-Gp 1–4 β D-GpNAc 1–3 D-Galp. *S.* XIV was a substrate for β-D-galactosidase, galactose oxidase, β-glucos-

aminidase and a constitutive α-D-galactosidase, isolated from *Streptococcus bovis* Bailey strain 1 (ref. 13), which cleaved melibiose but not lactose.

After repeated inductions using *K. aerogenes* cells, a non-dialysable residue was obtained. This contained no glucose, had $[\alpha]_D^+$ 27° (*c* 0·12 in N NaOH) and contained 0·97 moles *N*-acetyl glucosamine per mole galactose. This residue precipitated with anti-XIV serum and cross-reacted with human anti-*B* serum. A disaccharide, characterized as *O*-galactosyl 2-amino 2-deoxy glucose, was recovered from the products of mild acid hydrolysis of the residue. Periodate oxidation of the residue, followed by acid hydrolysis, revealed glucosamine but not galactose. The residue was no longer a substrate for β-galactosidase. It was, however, a substrate for α-D-galactosidase of *S. bovis*[13], which cleaved D-galactose, characterized in the dialysable fraction by its specific reaction with galactose oxidase. The residue was itself a substrate for galactose oxidase. It was also a substrate for β-glucosaminidase (induced by chitin oligosaccharides in *K. aerogenes* cells; or bee venom). Either of these enzymes, when incubated with the residue, removed a disaccharide characterized as 6-*O*-galactosyl 2-acetamido-2-deoxy D-glucose.

The presence in the non-dialysable residue of α-linked galactose non-reducing end-groups was indicated by their removal by α-galactosidase; the cross-reaction with anti-*B* serum supported this view, since blood group *B* substance contains α-linked D-galactose units in the antigenic determinant groups[14]. The failure of the non-dialysable residue to induce a β-glucosaminidase supports this view, while the isolation of a galactosyl-*N*-acetyl glucosamine from the action of a specific β-glucosaminidase suggests the presence of β-linked *N*-acetyl glucosamine units in the residue.

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THALIDOMIDE AND INDUCED AMPHIBIAN METAMORPHOSIS

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ATTEMPTS to determine the cause of the teratogenic action of thalidomide have emphasized the need for more experimental procedures for producing the embryotoxic effects of drugs[1]. The use of mammalian fœtuses[2] for this purpose suffers from two drawbacks: (*a*) complications due to maternal responses to the drug; (*b*) the difficulty of accurate timing of the initiation of embryonic morphogenic processes. In some respects, therefore, the developing chick embryo[3] represents a better experimental system than the mammalian fœtus[2]. In our laboratory, we have been investigating the biochemistry of the precocious induction by thyroid hormones of metamorphosis in the giant American bullfrog (*Rana catesbeiana*) during the long (2–3 years) pre-metamorphic period. Biochemically and morphologically, accelerated metamorphosis induced by these hormones is qualitatively indistinguishable from the slow, normal process[4]. The ease with which effects of various substances on the developing frog embryo could be investigated is obvious. It is also possible to correlate chronologically and accurately any effect to the time of onset of morphogenic changes, such as limb growth, skeletal modifications, tail resorption, etc., following induction of metamorphosis. These considerations prompted a test of the effect of thalidomide during precociously induced metamorphosis in bullfrog tadpoles. The results described here show that thalidomide is quite non-toxic to pre-metamorphic frog embryos but acquires a strong but transient toxicity during early metamorphosis.

Rana catesbeiana tadpoles (6–8 cm long), purchased from Lemberger Co., Oshkosh (Wis.), were maintained at 20°–24° in aerated tap water on a diet of boiled spinach. Thalidomide (α-*N*-phthalimidoglutarimide) and related substances were dissolved in methyl cellosolve or alkaline 1 : 2-propanediol; triiodothyronine was dissolved in 10 per cent 1 : 2-propanediol at *p*H 8·8. The usual experimental procedures consisted of placing groups of 6–10 tadpoles in 2–4 l. of water containing the substance to be tested, or the solvent blank, for periods of 1–6 weeks before metamorphosis was induced by adding 3–15 μg of 3,5,3′-triiodo-L-thyronine/l. medium. In some experiments the teratogenic agent was injected or added at various time-intervals after the thyroid hormone was administered. The water was changed at regular intervals throughout the course of the experiments. The onset and rate of metamorphosis were recorded by determining the ratios of tail-length : total body-length, and hind-limb length : total body-length.

As shown in Table 1, thalidomide had no harmful effects on pre-metamorphic bullfrog tadpoles maintained in water containing doses as high as 200 mg/l. and for periods up to 2·5 months. There were also no signs of toxicity if 1–2 mg of thalidomide were injected intraperitoneally. However, immersion of the tadpoles in low concentrations of 10–20 mg thalidomide per litre proved to be lethal, in all animals, within 4–8 days after induction of metamorphosis. The time of death following the induction of metamorphosis depended largely on the dose of thalidomide and the hormone. In every case, thalidomide had also completely inhibited the onset of metamorphosis as judged by a variety of indexes such as tail and intestinal resorption, eruption or growth of hind or front limbs, appearance of adult hæmoglobin in blood, etc. The early metamorphic changes in control animals were detected between 4 and 8 days after induction.

Fig. 1 illustrates the time-course of these effects as seen in a typical experiment. In this experiment, administration of triiodothyronine provoked the usual accelerated pattern of induced metamorphosis after a lag period of 3–4 days. Those embryos which were treated with thalidomide alone retained normal body dimensions throughout the course of the experiment. Independently of the duration of pre-treatment with thalidomide, an attempt to induce metamorphosis with triiodothyronine caused the death of all animals on the fifth day after the hormone, with no signs of an initiation of metamorphosis as seen at post-mortem. The extreme sensitivity to this teratogenic agent developed by tadpoles was only manifest for the first four days after induction of metamorphosis, as seen from experiments in which thalidomide was given after triiodothyronine administration (Fig. 2). If given on the same day as the hormone or within 3 days thalidomide inhibited metamorphosis as well as being lethal. It did not prevent the progress of differentiation when metamorphosis had

Table 1. Effect of Thalidomide on Triiodothyronine (T_3)-induced Metamorphosis in *Rana catesbeiana* Tadpoles

Thalidomide dose (mg/l.)	Pretreatment duration before T_3	Dose of T_3 (μg/l.)	Survival 7 d. after T_3	Metamorphosis onset (5 d. after T_3)
0	0	0	12/12	–
200	2 weeks	0	8/8	–
200	2 weeks	10	0/8	–
0	0	10	8/8	+
60	10 weeks	0	8/8	–
60	10 weeks	10	0/8	–
20	1 week	0	10/10	–
20	1 week	10	0/10	–
20	1 week	3	2/10	–
0	0	3	7/8	+

Tadpoles were maintained in water containing different amounts of thalidomide for varying periods of time before and after the induction of metamorphosis with triiodothyronine.

Table 2. Effect of Thalidomide, 'Doriden' (Glutethimide) and L-Glutamine on Induced Metamorphosis

Drug	Dose (mg/l.)	T_3	Survival 7 d. after T_3	Onset of metamorphosis on fifth day after T_3
None	0	–	11/12	–
None	0	+	8/10	+
Thalidomide	20	–	12/12	–
Thalidomide	20	+	1/10	–
'Doriden'	20	–	2/12	–
'Doriden'	10	–	9/10	–
'Doriden'	10	+	8/10	+
L-Glutamine	80	–	6/6	–
L-Glutamine	80	+	6/6	+

Tadpoles were maintained for 12 days in water containing the material under test before induction of metamorphosis with 6 μg/l. of T_3.

been induced 4 or more days prior to its administration and also had diminishing lethal effects with increasing time-intervals between the hormone and thalidomide.

A few substances related to thalidomide were tested in order to determine the specificity of lethal effects of this substance in amphibian embryos with respect to its teratogenicity in mammalian species. Of particular interest is 'Doriden' (glutethimide), a sedative which has been widely used in clinical practice but with no known teratogenic properties[1,5]. Both 'Doriden' and thalidomide have the glutarimide ring in common. Unlike thalidomide, 'Doriden' was highly toxic to non-induced tadpoles, with an approximate LD_{50} at 20–25 mg/l. When used at concentrations below 10 mg/l., 'Doriden' failed to prevent the onset of metamorphosis and the mortality frequency was the same in induced and non-induced tadpoles. Glutamine also had no inhibitory effects on the early stages of induced developmental changes. This brief comparison does not conclusively establish the specificity of thalidomide action, as other unrelated substances may inhibit metamorphosis in frogs[6].

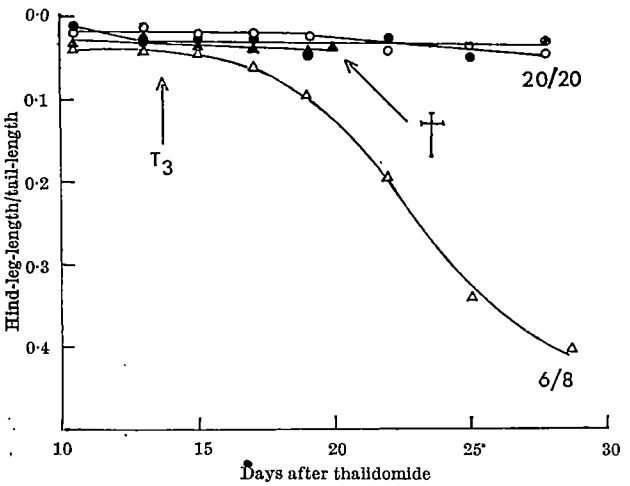


Fig. 1. Effect of pretreatment of *Rana catesbeiana* tadpoles with thalidomide (30 mg/l.) on the induction of metamorphosis by triiodothyronine (10 μg/l.). Treatment with thalidomide was begun on day 0 and continued thereafter. Triiodothyronine was given at the time indicated by the vertical arrow and treatment continued thereafter. ○, controls (no treatment), ●, thalidomide alone; △, triiodothyronine alone; ▲, thalidomide pretreatment and triiodothyronine. The ratio of hind leg to tail-lengths is here used as the index of metamorphosis. Figures at the end of the curves indicate the proportion of the animals surviving at the end of the experiment. † indicates the time (pointed out by the arrow) at which all the animals were dead. T_3, triiodothyronine

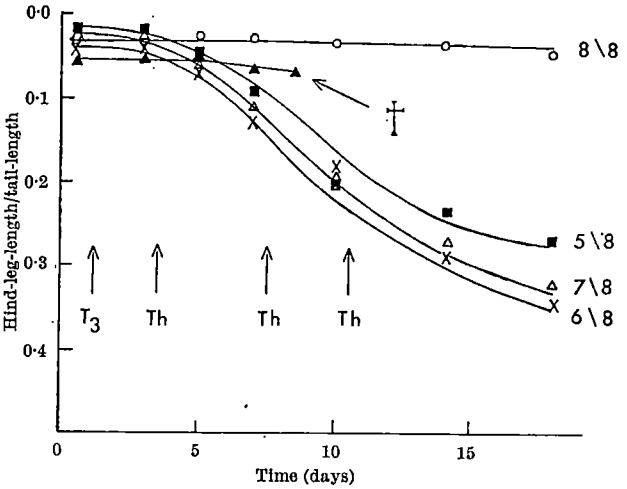


Fig. 2. Transient toxicity of thalidomide as seen after the administration of the drug (30 mg/l.) at increasing time-intervals (indicated by vertical arrows) after metamorphosis was induced by triiodothyronine (6 μg/l.). ○, controls (no treatment); triiodothyronine administered to all other groups. △, no thalidomide; ▲, ■, and *X*, thalidomide treatment begun 2·2, 6·2 and 9·2 days, respectively, after the induction of metamorphosis with triiodothyronine. Th, thalidomide. Other details as in Fig. 1

Judging from the nature of teratogenic effects of thalidomide in mammalian embryos[1,2], I expected at first only a partial inhibitory effect of thalidomide on some specific developmental processes during amphibian metamorphosis, such as a disturbance in limb formation, but not in tail or intestine resorption. The failure to observe such specific effects makes it impossible to say whether identical mechanisms are involved in the embryotoxic effects we have observed in the frog as that in the production of phocomelia in man and other mammals. It is, however, interesting that in both cases the action of thalidomide is only manifest when it is present at stages of differentiation coinciding with limb development and other skeletal changes. It is also noteworthy that during the 72 h following induction of metamorphosis, when thalidomide exerts its lethal action, the extensive modifications in the rate and nature of nuclear and cytoplasmic RNAs synthesized in the embryo are at their peak level[7]. The possibility of testing drugs directly on the isolated embryo and the experimental induction of developmental changes which can be accurately timed justifies further examination of amphibian metamorphosis as an additional system for investigating the embryotoxic effect of substances like thalidomide. If the transient, induced sensitivity of the tadpoles at the onset of metamorphosis and the teratogenicity of thalidomide in mammalian embryos represent different manifestations of the same phenomenon, then the amphibian system of the type described here can be potentially useful as a screening procedure for predicting harmful effects of drugs on embryonic development.

I thank Mr. A. J. Bell for his assistance. I also thank the Distillers Co., Ltd., for a gift of thalidomide, and Dr. D. F. Elliott, of Ciba Research Laboratories, Horsham, for the sample of 'Doriden'.

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EARLY LYMPHATIC REGENERATION IN THYMECTOMIZED RADIATION CHIMERAS

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THOUGH thymectomy of adult animals has no detectable effect on their immune reactivity, there is now ample evidence that the presence of the thymus is required to ensure normal restoration of immune reactivity in sublethally irradiated adult mice[1,2] as well as in lethally irradiated mice surviving with an isologous bone marrow graft[3]. The mechanism by which the thymus, whether left *in situ*, grafted subcutaneously or enclosed in a diffusion chamber, effects such morphological and functional recovery of the irradiated lymphatic system has not been fully elucidated.

The apparent effectiveness of thymus grafts implanted in diffusion chambers no doubt favours a humoral factor as the active principle. Investigations of cell migration, however, have indicated that the thymus (*in situ* or grafted subcutaneously) provides a suitable environment for lymphoid cell proliferation and maturation both in the developing young animal[4] and in the recovering, irradiated adult animal[5].

It has been repeatedly emphasized that the two hypotheses, namely: (1) a thymus passage of lymphoid precursor cells followed by 'peripheralization', or (2) a humoral stimulation of lymphoid proliferation and maturation elsewhere in the lymphatic tissues, need not be mutually exclusive and may, in fact, both be operative.

Chromosome markers have been used during investigations concerned with the involvement of the thymus in the migration of injected donor cells in isologous radiation chimeras. With this method Ford and Micklem[5] have demonstrated that the thymic cell population is derived from the injected bone marrow, even when lymph node cells had been injected simultaneously. The permanent population of the chimera's lymph nodes was also derived from the donor bone marrow, not from the injected lymph node cells. This undoubtedly suggested a selective attraction of the thymus for lymphoid precursors (contained in the donor bone marrow) followed by seeding of supposedly 'instructed' cells to the periphery. However, the earliest identification by Ford and Micklem of the thymus cell population was made six weeks after irradiation and bone marrow treatment, so that the initial fate of the injected cells was, in fact, not observed.

It seemed interesting to examine the fate of the injected donor marrow both in normal and thymectomized chimeras during the early period after irradiation. Since thymectomized chimeras do not regain normal immune reactivity, the proliferative pattern of injected lymphoid precursor cells might be expected to be different from that seen in non-thymectomized controls.

In the present study the isologous donor marrow was labelled with tritiated thymidine. Localization and proliferation of hæmopoietic cells labelled with tritium can be readily followed autoradiographically after injection into lethally irradiated animals[6]. Unlike in chromosome investigations, donor cells which are not dividing in the host can be recognized while the fact that the histological pattern of the surrounding host tissues remains intact is also advantageous.

Female (*CBA* × *C57BL*)F_1 mice were thymectomized or sham-thymectomized when 6–8 weeks old. Four weeks thereafter they were lethally irradiated (912 r., 250 kV, half-value layer 2·15 mm copper) and 3 h later given 25 × 10^6 tritium-labelled viable isologous bone marrow cells intravenously (viability determined by dye exclusion). To obtain a maximal number of labelled 'precursor' cells the donor marrow was obtained from isologous F_1 chimeras (one week after irradiation and bone marrow injection). Preliminary tests had shown that such 'regenerating' donor marrow produced an identical pattern of initial hæmopoietic and lymphatic regeneration in irradiated hosts, as did 'normal' donor marrow.

The donor mice were labelled *in vivo* by two intravenous injections of 25 μc. tritiated thymidine (Schwartz Bio-Research, Inc., N.Y., spec. act. 1·9 c./mM), with an interval of 10 h; they were killed 1 h after the last injection and a bone marrow suspension in Tyrode's solution was obtained from the femurs. Autoradiographs of the donor marrow (Kodak '*AR* 10' stripping film, six weeks' exposure, staining with hæmatoxylin and eosin) showed labelling of about 40 per cent of all cells, with an average of about 20 grains per nucleus.

The danger of damage to the proliferative potential of the labelled cells has been kept to a minimum by labelling the donors with about 2 μc. tritium/g body-wt.; this amount of tritium is accepted as innocuous to the proliferative potential of the animal's bone marrow[7]. In view of previous work along similar lines, the likelihood of host cell labelling and autoradiographic detection of 're-utilized' thymidine is extremely small in the present experimental design.

The recipients were killed at 3 h and at 1, 2, 4, 6 and 8 days following irradiation and injection of the labelled bone marrow, one thymectomized and one sham-thymectomized mouse at each interval. Autoradiography was done on sections of bone marrow, spleen, mesenteric lymph nodes, thymus (in the sham-operated mice), lung, liver and intestines. Initial settling and proliferation of labelled donor cells were scored as reported previously[6]. Though a precise morphological differentiation of the various cell types is difficult in tissue sections covered with stripping film and stained afterwards, it was usually possible to determine whether agglomerations of labelled cells were of the myeloid, erythroid or lymphoid series.

Contrary to what one might expect in view of the marked difference of immune reactivity at a later stage, the thymectomized and the sham-thymectomized mice showed identical localization, proliferation and initial follicle formation by labelled mononuclear cells.

Fig. 1 gives a schematic representation of the findings for both groups of chimeras. A few hours after the injection of the bone marrow suspension the labelled cells were still randomly distributed. Far from seeking the thymus (in the sham-operated controls), the labelled cells generally seemed to avoid that organ already at this early stage. The few cells that could be located in the thymus were rather highly labelled large cells reminiscent of reticulum cells.

During the next few days (1–2 days) labelled cells had virtually disappeared from all except the lymphatic and hæmopoietic tissues. At that time, large numbers of them could be seen in the spleen and lymph nodes, which were still highly atrophic due to radiation damage. Agglomerations of cells of the erythroid or myeloid series, containing up to 30 labelled cells and occasional labelled mitoses, indicated the onset of hæmopoietic regeneration in spleen and lymph nodes which eventually led to dilution and disappearance of the label in the erythroid and myeloid

* Member of the Biology Division of Euratom.

cell lines. This no doubt explains the scarcity of labelled cells in the bone marrow sections by the time the donor cells started repopulating the marrow cavity (day 4–6).

Agglomerations of labelled mononuclear lymphoid cells were rarely seen during the first two days in any organ. Between the 4th and the 6th day, however, the spleen and lymph nodes slowly regained their pre-irradiation histological pattern; areas containing labelled and unlabelled mononuclear cells were now visible round the central arterioles (the sites of the lymphatic follicles before irradiation), while extensive hæmopoiesis was evident in the red pulp causing an enlargement of the spleen. Labelled mononuclear cells were found exclusively in these peri-arteriolar areas, often in large numbers and arranged somewhat concentrically. A similar concentration and presumably proliferation of mononuclear cells were seen in the sub-cortical areas of the still atrophic lymph nodes. Again, labelled mononuclear cells, obviously lymphoid in nature, were seen exclusively in these areas of early follicle formation, usually in groups of 10–30 cells (Fig. 2). Most of the labelled cells had clearly proliferated (dilution of the label); others may have divided very slowly or not at all as judged from the rather high grain count on some nuclei on day 8.

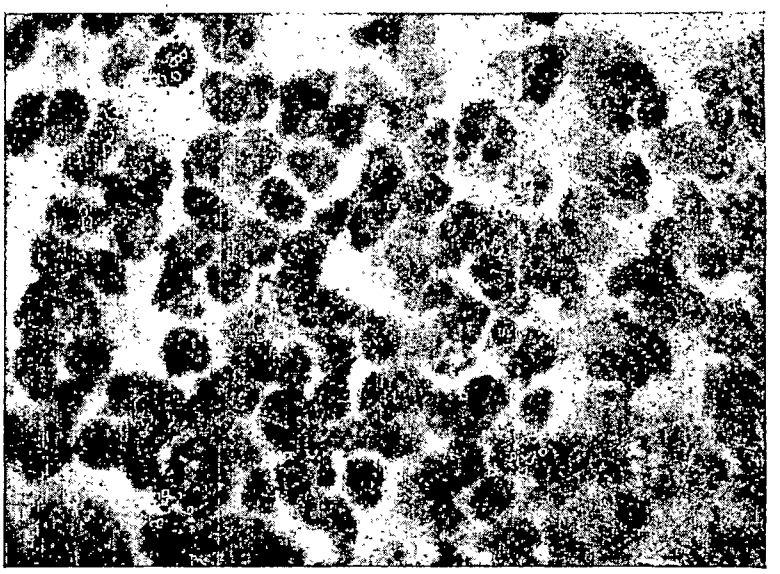


Fig. 2. Group of labelled mononuclear donor cells in lymph node of thymectomized chimera on the sixth day. Cells with a relatively high number of grains may have divided once or not at all after their transplantation

The thymus in the sham-thymectomized controls remained atrophic during the entire first week. The small number of labelled cells observed therein from the very beginning could still be spotted in the thymus sections on the 6th and 8th day. Contrary to what was seen in spleen and lymph nodes, proliferation of labelled cells could not be observed in the thymus. A return of the lymphatic cortex of the thymus was first seen on day 8, but these lymphocytes were not labelled.

Though not yielding a detectable difference in the pattern of lymphoid regeneration in thymectomized and non-thymectomized chimeras, the experiments described here nevertheless allow the following conclusions: The identical distribution and proliferation of apparently all cell lines in the two kinds of chimeras suggest that, whatever the influence of the thymus on restoration of the lymphatic system in chimeras may be, it is not discernible during the first 8 days of recovery, at least not morphologically. The sham-thymectomized controls seemed to confirm this impression since there was no traffic of labelled cells in the thymus during that period. The few large labelled donor cells, found in the thymus at any time, never showed a tendency to proliferate during the period when obvious proliferation of presumably immature labelled lymphoid cells was occurring in the spleen and lymph nodes. When lymphoid proliferation was finally resumed in the thymus cortex on day 8, these lymphoid cells were not labelled. They were, therefore, not derived from the few obviously inert labelled donor cells continuously present in the thymus. Since a chimera's thymocytes will be of the donor type, as has been repeatedly proved, it seems plausible that they were seeded from the 'primitive' follicles in spleen and lymph nodes, where proliferation of labelled mononuclear cells had been going on since the 4th day; this had led to a loss of the label in most of these cells on day 8. An alternative hypothesis would assume a separate kind of 'stem cell' for thymocytes in the donor marrow which had never incorporated any label. Afterwards, these 'thymocyte-precursors' would have localized preferentially and unrecognized in the recipient's thymus. Such an alternative is, however, rather unlikely.

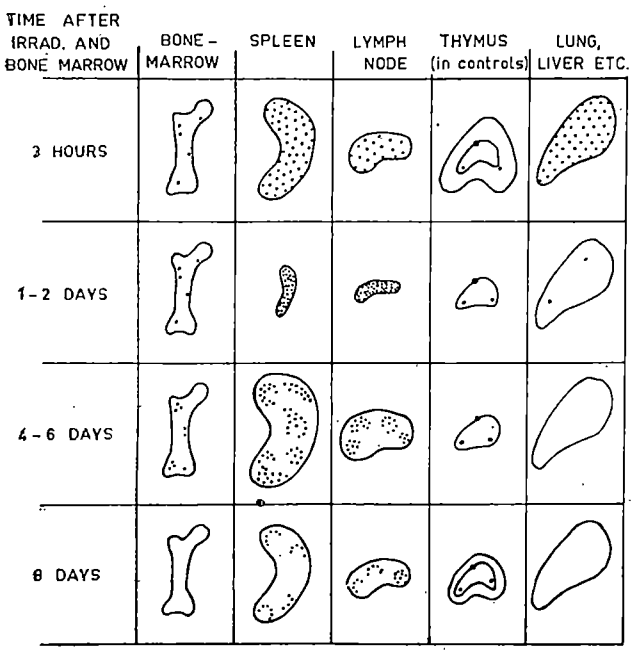


Fig. 1. Distribution and relative number of tritium-labelled donor cells in thymectomized as well as in non-thymectomized isologous chimeras

It seems, therefore, that a 'thymus passage' of lymphoid precursor cells in the non-thymectomized animals is unlikely during the first eight days, while a humoral influence, if already existent, has no effect on the morphology of initial lymphoid regeneration. It is postulated that if a thymus passage of lymphoid cells for the acquisition of immunological competence indeed takes place, it starts after a latent period of at least one week when mononuclear cells, probably derived from primitive follicles in spleen and lymph nodes, start to repopulate the thymus cortex.

We thank Dr. D. W. van Bekkum for advice.

This work was performed under contract with Euratom (European Atomic Energy Community), 51–53 rue Belliard, Brussels, Belgium.

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EFFECT OF SERUM FROM NORMAL AND DIABETIC SUBJECTS ON INCORPORATION OF GLUCOSE 1-CARBON-14 INTO INDIVIDUAL FATTY ACIDS OF RAT ADIPOSE TISSUE

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WE have compared the effect of serum from control and untreated insulin independent diabetic subjects on incorporation of glucose 1-^{14}C into fatty acids by rat epididymal adipose tissue.

In the first group of experiments, serum from six controls and five untreated hyperglycæmic insulin-independent diabetics, with fasting blood sugar less than 150 mg per cent, was obtained in the fasting state, 20 and 120 min after ingestion of 100 g of glucose. Glucose concentration in serum was adjusted to 300 mg per cent in all investigations. Two ml. of serum containing 5 μc. of 1-^{14}C glucose were incubated with fragments of epididymal fat pad from 200–225-g Wistar rats[1]. Serum from controls and diabetics was incubated with adipose tissue from the same group of rats. After incubation for 3 h, the tissue and serum were extracted[2], fatty acids were saponified, and the carbon-14 in one aliquot was determined in a liquid scintillation spectrometer. Another aliquot was methyl esterified[3]. The methyl esters were then separated on a 20 per cent diethylene glycol succinate column. Each peak and the areas between the peaks were trapped separately by a method developed in this laboratory[4] and carbon-14 in individual fatty acids was determined.

Serum from the five hyperglycæmic diabetics stimulated less incorporation of glucose 1-^{14}C into fatty acids than did serum from six controls. Because of the small number of samples, differences were only significant when the effects of serum obtained 20 min after glucose were compared. However, differences were almost significant ($P < 0{\cdot}10$) when fasting, and 120 min post-glucose sera were compared. Further, there was no overlap between individual values in the two groups. It therefore appears that the net effect of serum from mildly hyperglycæmic, ketosis-resistant diabetics on incorporation of glucose 1-^{14}C into fatty acids is less than normal (Fig. 1).

Serum from controls and hyperglycæmic diabetics had similar effects on the distribution of carbon-14 among the individual fatty acids with one noteworthy exception—percentage of fatty acid carbon-14 in oleic acid was significantly less when adipose tissue was incubated with fasting, and post-glucose serum from the hyperglycæmic diabetics.

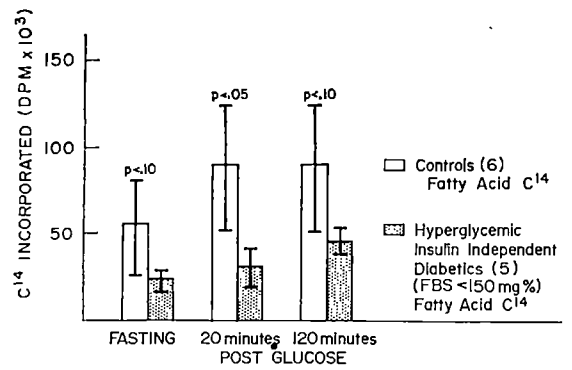


Fig. 1

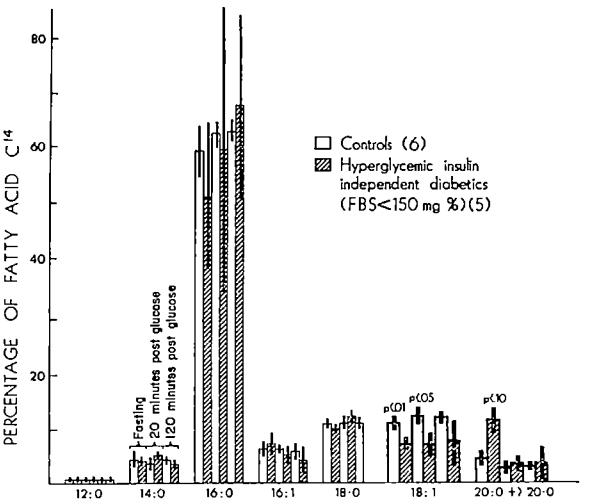


Fig. 2

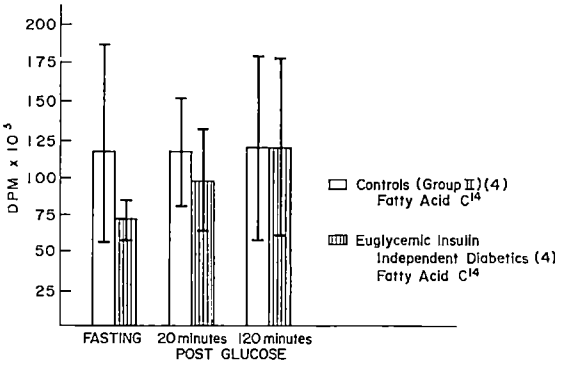


Fig. 3

In contrast, no significant difference was noted between the effect of fasting, 20 or 120 min post-glucose serum obtained from four untreated diabetics with normal fasting blood sugar and four other controls examined at the same time. Nor was a difference in incorporation of carbon-14 into individual fatty acids found (Figs. 3 and 4).

Incorporation of carbon-14 into fatty acids was much greater in the second group of experiments in both controls and diabetics than in the first group. It appears that the sensitivity of adipose tissue to stimulation of incorporation of glucose 1-^{14}C into fatty acids by serum varies with different groups of rats. Therefore, control and diabetic sera should be tested against adipose tissue from the same group of rats.

With the increase in incorporation of carbon-14 into fatty acids, there was a striking increase in percentage of carbon-14 in oleic acid. When total disintegrations per minute in fatty acids were plotted against percentage of carbon-14 in oleic acid, there was a striking positive cor-

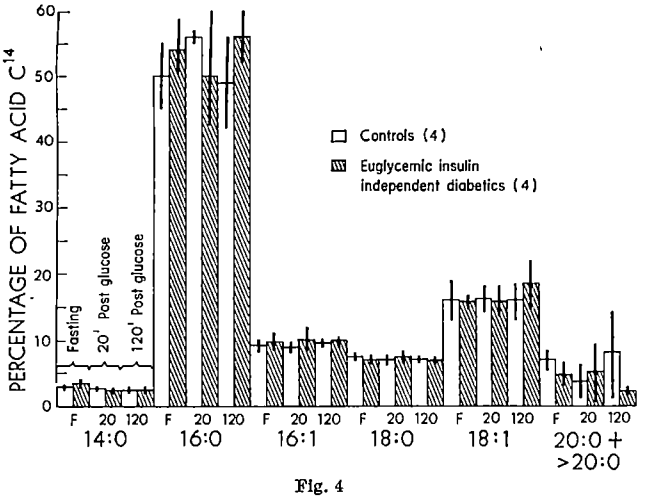


Fig. 4

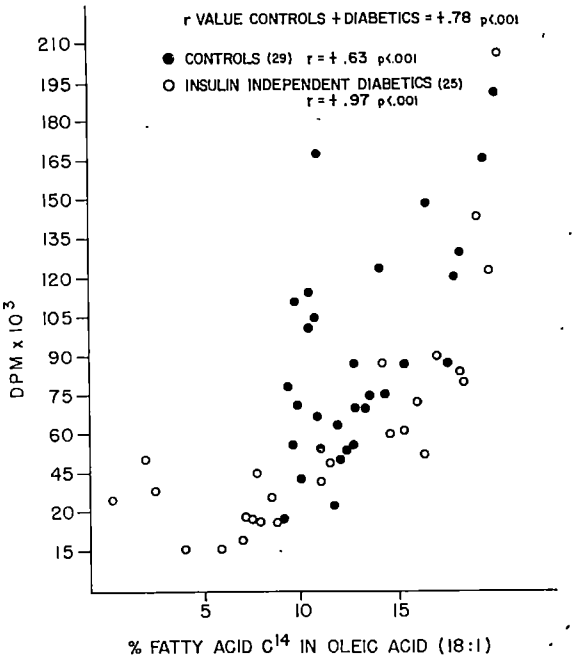


Fig. 5

relation. This was particularly remarkable when fat pads were incubated with serum from diabetics (Fig. 5).

It therefore appears that the net effect of serum from normal and diabetic subjects on stimulation of formation of fatty acids by epididymal adipose tissue is related in some way to stimulation of formation of one particular fatty acid, oleic acid. It is of interest that compounds which may be oxidized by mitochondria, such as ketones, or may be involved in electron transport (quinones), or may serve as hydrogen donors (ascorbic acid), almost selectively stimulate formation of oleic acid in preparations of human blood cells[5]. This fatty acid appears to be made in a mitochondrial system separate from that for long chain, saturated fatty acids[6]. Degradation of oleic acid formed by blood cells shows a distribution of carbon-14 compatible with formation of this fatty acid by a chain lengthening pathway, presumably mitochondrial[7]. Perhaps the disproportionate stimulation of oleate formation in these various situations is a reflexion of increased oxidation by mitochondria. Formation of oleate from stearate appears to require TPNH[8]. It is conceivable that formation of oleate in the mitochondria could serve to re-oxidize the reduced TPNH formed at the isocitric dehydrogenase step. Formation of this fatty acid might then be expected to increase whenever mitochondrial oxidations proceed at a rate which exceeds the capacity of transhydrogenase or of the TPNH cytochrome *c* reductase systems to re-oxidize TPNH.

Benjamin and Gellhorn have shown that formation of oleate from stearate is greatly decreased in liver and adipose tissue from alloxan diabetic rats. This defect was corrected by administration of insulin *in vivo*, but not *in vitro*, and was apparently unrelated to total fatty acid radioactivity[9].

The present work demonstrates that stimulation, by serum, of incorporation of glucose 1-^{14}C into adipose tissue fatty acids, presumably an effect of insulin in the serum, is closely related to stimulation of oleic acid formation. Whether this indicates a specific effect of insulin on formation of oleic acid or is due primarily to increased substrate utilization by mitochondria is unknown.

We thank Mr. John Juntunen for assistance. The work was supported by grants from the National Institutes of Health (*AM*-5024-08; *AM*-07089-02; and *AM*-05891-2).

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DUAL ONTOGENY OF THE MALE *Coccophagus gurneyi* COMP. (HYMENOPTERA: APHELINIDAE): A PHENOTYPIC PHENOMENON

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THE ontogeny of the male *Coccophagus gurneyi* Comp. exhibits two courses, ectoparasitic and endoparasitic, with correlated differences in form and behaviour. The course followed by a particular individual is determined by the environment of the deposited egg. Larval morphology indicates ectoparasitism to be the primitive course.

A prerequisite for the dual ontogeny of the male in the aphelinid *Coccophagus gurneyi* Comp., a species which reproduces parasitically on certain mealybug species in the genus *Pseudococcus* (Homoptera: Coccidae), is the divergent ontogeny of the sexes, this being female development as a primary parasite and male development as a secondary parasite of the host mealybug.

The *C. gurneyi* male, like the males of many other aphelinid species, is a general parasite of coccid-inhabiting primary parasites. However, it differs from the males of

other known species of similar habit in possessing the extraordinary capacity of being able to develop along two different paths that are initiated by the two distinctive sites of the deposited egg.

This male dual ontogeny consists of direct and indirect secondary parasitism of mealybugs that serve as hosts of the species, the male *C. gurneyi* developing endoparasitically on a parasitic full-fed larval female when the relation is direct, and ectoparasitically when the relation is indirect. (When secondary parasitism is direct, the parental female attacks only the parasitized host of the primary, or the primary itself; when indirect, the parental female attacks the host of the primary, whether or not that host contains a parasite[1].)

The fact that the larvæ of *Coccophagus gurneyi* are able to develop both externally and internally on several species of mealybug-inhabiting parasites was first noted in 1928 (ref. 2). That this capacity was limited to male larvæ was not known until 1936 (ref. 3).

The phenotypic aspects of the direct and indirect secondary parasitism exhibited by the male *C. gurneyi* remained obscure until their elucidation in 1963 by the late Giulio Zinna. His observations (unpublished)* showed that the indirect secondary relation initiates ectoparasitic development and is established when the haploid egg is deposited in the viscera of a mealybug; the direct secondary relation which initiates endoparasitic development is established when the haploid egg is deposited in the viscera of a parasitic larva inhabiting the mealybug.

The reproduction of the *C. gurneyi* female as a primary parasite of the citrophilus mealybug, *Pseudococcus fragilis* Brain, and of the long-tailed mealybug, *P. adonidum* (L.), has been known since 1928 when the species was imported into California from Australia. Despite such primary reproduction the *C. gurneyi* female does not discriminate between parasitized and unparasitized mealybugs, and prefers to oviposit as a secondary rather than as a primary parasite, when attacking hosts already inhabited by a parasitic larva.

Compere and Smith[2] observed that when citrophilus mealybugs inhabited by the larvæ of the encyrtid *Tetracnemus pretiosa* Timb. are exposed to attack by an excessive number of *C. gurneyi*, the bodies of such larvæ are frequently distended by the numerous *C. gurneyi* eggs deposited therein.

The combination of the obligatory hyperparasitism which characterizes the male of *C. gurneyi* and the availability in the field of primary parasites such as *T. pretiosa* —by assuring the continuity of the male population and the early mating of the *C. gurneyi* female—may be responsible for the citrophilus mealybug population in California being continuously at very low densities[4].

Although *T. pretiosa*, in the absence of *C. gurneyi*, is capable of controlling citrophilus mealybug populations[2], the decimating effect of the hyperparasitic habit of *C. gurneyi* on the *T. pretiosa* population apparently precludes the latter being the numerically dominant species.

Cendaña[5] was the first to note that the progeny of unmated *C. gurneyi* females could not complete their development in hosts not inhabited by parasitic larvæ. However, because he failed to experiment with hosts that were inhabited, he erroneously concluded that males were produced only by mated females. That hyperparasitic development was restricted to the male and was dually ontogenetic was then unsuspected, the essential character of the differential development of the sexes in many Aphelinidae being unknown[6].

Compere and Smith[2] also noted that when a *C. gurneyi* female attacked a host which was not inhabited by a parasitic larva, her eggs generally were deposited free in the host's body cavity and only occasionally in the visceral



Fig. 1. Photograph of *Coccophagus gurneyi* eggs in tap water after removal from the body fluids of a larval *C. gurneyi* female. The single egg showing a fully developed embryo is haploid; the other four are presumably diploid and non-viable because they were deposited in an unfavourable environment

organs. This seemed confirmed by the fact that many supernumerary eggs were phagocytized, a process that occurs in the host's body fluids. Later, Cendaña[5] corroborated this observation. He concluded that the supernumerary eggs deposited by 'mated' *C. gurneyi* females were subject to 'true' phagocytosis as described by Timberlake[7]. Bess[8] reported that female-producing eggs when in *P. fragilis* were more likely to be phagocytized than when in *P. adonidum*, and that when in other species most, if not all, the female-producing (diploid) eggs were readily phagocytized.

When both haploid and diploid eggs are deposited within the body fluids of a larval primary parasite (Fig. 1), only the haploid eggs develop successfully, the diploid eggs aborting without having been phagocytized. It is noteworthy that rarely, if ever, are the male-producing (haploid) eggs subject to phagocytosis whether in the body-fluids of a coccid or of a hymenopterous larva.

Zinna's 1963 observations, however, showed that, when an unparasitized host mealybug is subjected only to a single oviposition by either a mated or unmated *C. gurneyi*, the egg almost invariably is deposited in the sub-œsophageal ganglion. The characteristic stance taken by the female when thus ovipositing is that of standing on the thoracic dorsum of the host facing anteriorly. Previously, Cendaña[5] had noted that the female preferred this oviposition site, but failed to realize its significance.

Unfertilized eggs, when deposited in the sub-œsophageal ganglion of a mealybug, develop, but the development of fertilized eggs if placed in such a situation is questionable.

The facultative ability of the *C. gurneyi* male to develop either ectoparasitically or endoparasitically on the full-fed larva of a primary parasite is not dependent on the species of host mealybug, as erroneously assumed by Flanders[3].

On July 27, 1963, a full-fed female larva of *C. gurneyi* removed from the body of a citrophilus mealybug bore on its surface a yellow, highly sclerotized first-instar male larva (Fig. 2), apparently feeding. The host female larva was then dissected in tap-water and found to contain four translucent second-instar males each partially encased by



Fig. 2. The first instar male of *Coccophagus gurneyi* when ectoparasitic. Photographed after hatching and before feeding. Note the incomplete segmental bands on the thorax and the six anterior abdominal segments that permit body expansion when food is ingested

* Dr. Zinna's observations were made while working under a NATO grant at the Biological Control Laboratory, University of California, Riverside, in 1963. He was then on leave from his duties as assistant director, National Center of Biological Control, Portici, Naples, Italy.



Fig. 3. The endoparasitic second instar male of *Coccophagus gurneyi* before (top) and after (bottom) hatching. Note head capsule, arrow, and exuviæ of the first instar. Oxygen enters the tracheal system by osmosis, not through the spiracles as when ectoparasitic

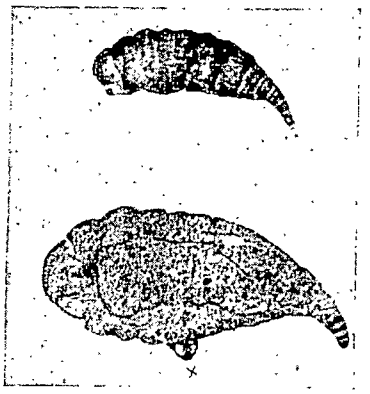


Fig. 4. Ectoparasitic first-instar male of *Coccophagus gurneyi* (top) in condition to moult, and the ectoparasitic second-instar male (bottom) after moulting. Second instar with body somewhat compressed to expose gas-filled tracheal loop. Note the head capsule (*X*) and 'caudal' segments of the first instar

the exuvia of the first instar and the chorion of the egg (Fig. 3).

When ectoparasitic, the larva casts its first-instar exuvia after hatching (Fig. 4). When endoparasitic, the first larval ecdysis occurs before hatching (a phenomenon first observed by Zinna). The second instar in either case possesses a gas-filled tracheal loop with seven pairs of spiracular branches. Only two of these pairs are connected to spiracles. When the second instar is endoparasitic the proximal portion of each spiracle-connected branch lacks gas (oxygen?).

The presence of non-functional spiracles in second-instar males indicates that with this species indirect secondary parasitization evolved prior to direct secondary parasitization.

The third instar male, whether endoparasitic or ectoparasitic, has seven pairs of open spiracles. Prior to emerging from the primary parasite, the endoparasitic male is encased by a sheath of cocooning material as are many endoparasitic chalcidoids[9]. This sheath may be necessary to protect the seven pairs of open spiracles from the host's body fluids. The sheath, which occurs as a very thin transparent film adhering tightly to the male's body, may have been formed, in part, during the second instar. When the male is ectoparasitic this sheath is lacking.

In one 'direct secondary' experiment in which specimens of citrus mealybug, *Planococcus citri* (Risso), were parasitized by *Leptomastix dactylopii* (July 5) and later (July 7–8) exposed for 24 h to unmated females of *C. gurneyi*, a *Leptomastix* female that had mummified its host mealybug, and then pupated, yielded a third instar *C. gurneyi* male (July 16).

In an 'indirect secondary' experiment in which *P. citri* was first parasitized by unmated *C. gurneyi* (July 26) and then by *L. dactylopii* (August 8), the emergence of a male *C. gurneyi* began August 27. The length of the male's life-cycle was prolonged by the interval between the two exposures to parasitization.

Cendaña[5] removed from a parasitized mealybug an unhatched live male larva 85 days after it had been deposited as an egg. This larva had apparently moulted once, but had retained the moult skin in position.

Table 1. Sequence of Developmental Phenomena characterizing the Dual Ontogeny of the *Coccophagus gurneyi* Male

Indirect	Direct
Differential host relations	
Haploid egg	
Deposited in viscera of mealybugs	Deposited in viscera of primary parasite of mealybugs
Minimum period of embryonic development 3–4 days	
Respiratory system of unhatched larva with two pairs of spiracles	
Growth	
Discontinuous	**Continuous**
All instars feed ectoparasitically	Only last stage of third instar feeds ectoparasitically
Hatching of egg dependent on consumption of host viscera by the primary host	Hatching of egg dependent on first larval ecdysis
First instar	**First instar**
Body segments become indurated forming yellow distinct bands. Pear-shaped when engorged	Body segments do not become markedly indurated and yellow. 'Hymenopteriform' when engorged
Respiratory system	
Second larval instar	
Two pairs of functional spiracles	Two pairs of non-functional spiracles
Third larval instar	
Seven pairs of spiracles	
Protected by larva-secreted sheath	Not protected by larva-secreted sheath

The facultative dual ontogeny of the *C. gurneyi* male (Table 1), therefore, is dependent on whether or not the mealybug host is inhabited by a primary parasite at the moment that the unfertilized (haploid) egg is deposited. If such a larva is present, its viscera receives the haploid egg; if absent, the mealybug's viscera receives the egg.

In the first case, male development is more or less continuous, whereas in the second case it is discontinuous, the hatching of the male larva being contingent upon the mealybug being inhabited later by a full-fed larva of a primary parasite.

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ORIGIN OF METEORITES

By B. J. LEVIN

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IN the report to the eleventh U.S.S.R. Conference on Meteorites (held in Moscow during May 1964) I proposed that the most highly differentiated and recrystallized types of meteorites, namely, irons, stony irons and achondrites, represent a product of a phase separation in chondritic material which occurs by diffusion in the solid state. This process must have been a continuation of the transformation of carbonaceous chondrites into ordinary

chondrites, which, according to Mason[1], was due to dehydration, reduction and recrystallization in the solid state. (Mason designates the formation of chondrules as spherulitic crystallization. However, the primitive chondrules are not completely crystalline but contain glass. Moreover, the investigations of microstructures of carbonaceous chondrites[2,3] had shown that the formation of 'high-temperature' minerals begins with the formation of minute globules (microchondræ). Probably the process of chondrule formation (at least its initial stage) is similar to the separation of glass into two metastable liquids which was recently examined in the laboratory during the course of developing a glass-ceramic[4].)

The formation of Widmanstätten structures in octahedrites occurred at 350°–500° C over a period of 10^7–10^8 years. The diffusion coefficients are very temperature-dependent and at greater temperatures much larger changes must have occurred during the same interval of time. But the central parts of larger asteroids were about 10^9 years at temperatures of 800°–1,000° C (ref. 5).

A greater solubility of smaller grains due to surface energy (surface tension) follows in the agglomeration of nickel–iron into larger inclusions and redissolution of small ones. The first step of this process occurred during the transformation of carbonaceous chondrites into ordinary chondrites. In the central parts of parent bodies, where the thermal changes proceeded much beyond the formation of crystalline chondrites, the agglomeration of nickel–iron into larger and larger bodies had to produce 'inclusions' of the size of largest iron meteorites found on the Earth. To achieve this in 10^9 years the diffusion coefficient must be of the order of 10^{-3}–10^{-10} cm²/sec. Such values could be attained at 8,000°–1,000° C.

At fragmentation of parent bodies large iron inclusions became separated from surrounding silicates because of differences in densities and elastic properties. These surrounding silicates, which were also strongly altered by diffusion processes, were almost free of nickel–iron. Achondrites must represent samples of them. As was noticed by Prior (1918), many achondrites are similar to the silicate parts of some stony irons.

The stony irons are probably samples in which the separation of metal and silicates was stopped at some intermediate stage by cooling. They do not come from some branch but from the main path of formation of irons. When silicates became locked inside metal the more diffusible ones escaped while the less diffusible became trapped. This was probably the formation process of pallasites. The predominance of olivine in them is possibly due to its high melting temperature. At temperatures more than 500° below the melting point it probably has a low solubility in nickel–iron and/or small diffusion coefficient. The pallasites with rounded or angular olivine-grains must have originated at different temperatures, but in such an interval of time that the temperature-dependences of the 'force of crystallization' and of the surface-tension coincided.

The pallasites, the great nodules of troilite and graphite in metal, are usually interpreted as formed in the melt. But the rate of gravitational differentiation in such a melt is very fast even when gravity is low (Anders, E., personal communication).

On the other hand, the tendency towards phase separation existing above the melting point in many cases continues to exist at lower temperature as well. But in the solid state enormous intervals of time are needed to permit this phase-separation tendency to manifest itself. The meteorites, or rather the meteorite parent bodies, had life-histories which just fitted the necessary conditions.

If such large-scale diffusion occurred in meteorite parent bodies, then chemical fractionation by diffusion must have occurred on a large scale. The fractionation of trace elements in meteorites possibly can be explained in this way, including a varying behaviour of some elements which can be due to differences in environment and temperature.

I trust that this re-interpretation of observed features of meteorites will confirm that diffusion in the solid state was a decisive process in meteorite parent bodies, no part of which was ever molten.

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MICROWAVE FARADAY ROTATION IN FERRITE POWDERS

By Dr. PREM SWARUP and NARENDRA KUMAR
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A SYSTEMATIC investigation has been made of non-reciprocal microwave rotation through ferrite powder, magnetized below saturation, as a function of magnetizing field and packing density at 9 kmc/s at room temperature. Philips 'Ferroxcube IV' fine powder was pressed into standard cylindrical pellets (2 cm long and 1·95 cm in diameter) of varying packing density. The experimental arrangement consisted of a 2 k 25 klystron, a standard x-band microwave bench (consisting of isolator, attenuator, wave-meter and tuner) feeding a circular wave-guide in TE_{11} mode, which in turn was terminated in a matched 1N23 detector through a swivel-head joint calibrated in degrees. A solenoid slipped over the circular wave-guide provided the variable longitudinal magnetization. The ferrite sample filled the circular wave-guide completely.

Assuming lossless permeability tensor, the specific rotation of the plane of polarization of a plane wave is given by[1]:

$$\frac{\theta}{l} = \frac{w\sqrt{\varepsilon}}{2c}\left[\left(1+\frac{w_m}{w+w_0}\right)^{1/2} - \left(1+\frac{w_m}{w_0-w}\right)^{1/2}\right] \quad (1)$$

where θ is the rotation of the plane of polarization in radians; l, length of the ferrite rod (2 cm); ε, dielectric constant of ferrite (about 13); c, velocity of light; w, operating angular frequency ($2\pi \times 9$ kmc/s); w_0, resonance frequency given by Kittel's formula; w_m, resonance frequency corresponding to saturation magnetization ($\gamma 4\pi M_0$); γ, magnetogyric ratio ($1{\cdot}76 \times 10^7$ radians/sec/œrsted); M_0, saturation magnetic moment per unit volume.

In the case of unsaturated ferrite operating at a frequency much below resonance, the relation (1) can be simplified to:

$$\frac{\theta}{l} = \frac{\sqrt{\varepsilon}\, w_m}{2c}. \quad (2)$$

ε and w_m are both functions of the packing density while w_m depends on the applied magnetic field also. The dielectric constant of the powdered sample is related to the packing density by the relation[2]:

$$\frac{\varepsilon - 1}{\varepsilon + 2} = C_1\rho \quad (3)$$

where ρ is the packing density and C_1 is a constant. This gives the value of dielectric constant of the sample as:

$$\varepsilon = \frac{1 + 2C_1\rho}{1 - C_1\rho} \quad (4)$$

To evaluate w_m in case of unsaturated ferrite powder, M_0 is to be replaced by the unsaturated value M, as such:

$$w_m = \gamma 4\pi M = \gamma 4\pi H_{\text{eff}} X \quad (5)$$

where γ, magnetogyric ratio; X, static low field susceptibility; H_{eff}, effective magnetic field in the sample after taking account of the demagnetization factor N_z. The expression for H_{eff} is given by:

$$H_{\text{eff}} = \frac{H_a}{1 + 4\pi X N_z} \quad (6)$$

where H_a is the applied field. The susceptibility also depends on the packing density. The permeability varies with the density in a fashion analogous to the permittivity and is given by:

$$\frac{\mu - 1}{\mu + 2} = C_2\rho \quad (7)$$

where C_2 is a constant. Since $\mu = 1 + 4\pi X$, X is given by the expression:

$$\frac{4\pi X}{3 + 4\pi X} = C_2\rho \quad (8)$$

substituting equations (8) and (6) in equation (5), the expression for w_m is given by:

$$w_m = \frac{\gamma H_a 3C_2\rho}{1 + C_2\rho(3N_z - 1)} \quad (9)$$

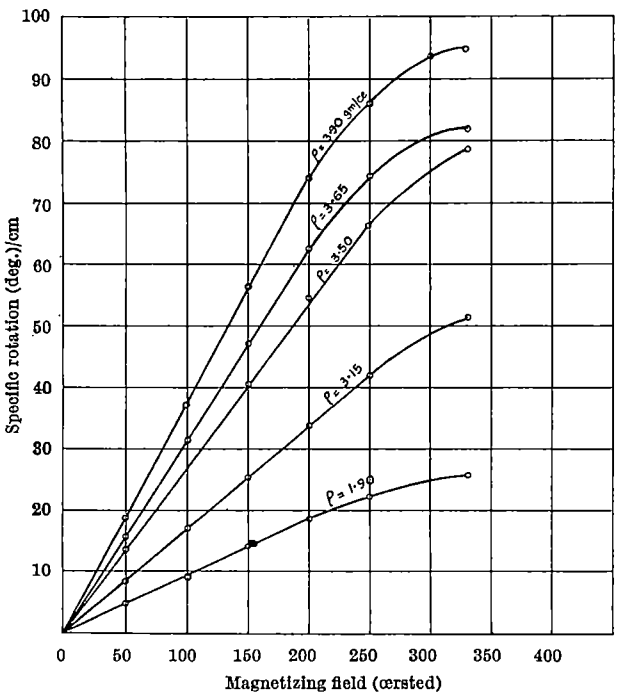


Fig. 1. Specific rotation in ferrite powder samples of varying density plotted against the applied magnetizing field at 9,060 Mc/s

substituting the expression for ε and w_m in the expression (2), the specific Faraday rotation is found to be given by:

$$\frac{\theta}{l} = \frac{(3\gamma)}{(2c)} \times \frac{\sqrt{(1 + 2C_1\rho)}}{\sqrt{(1 - C_1\rho)}} \times \left[\frac{C_2\rho}{1 + C_2\rho(3N_z - 1)}\right] H_a \quad (10)$$

It is seen from relation (10) that: (*a*) the specific rotation is linearly dependent on the applied field; (*b*) the specific rotation is highly superlinearly dependent on the packing density.

Finely powdered ferrite was pressed under various pressures into cylindrical samples of packing densities varying from 1·9 g/c.c. to 4 g/c.c. The specific rotation was measured in each case in the axial magnetic field up to 330 œrsteds. The experimental results showing the specific rotation versus magnetic field are plotted in Fig. 1. It is observed that in all the cases the specific rotation is linearly proportional to the applied field in the unsaturated region. In order to verify the nature of variation of the specific rotation with packing density, the equation (10) was used for theoretical computation. The various constants were determined experimentally. The constants are as follows: ρ, 1·9–3·9 g/c.c.; c_1, 0·20; c_2, 0·23 (c.g.s., unrationalized system of units was used); N_z, 0·12.

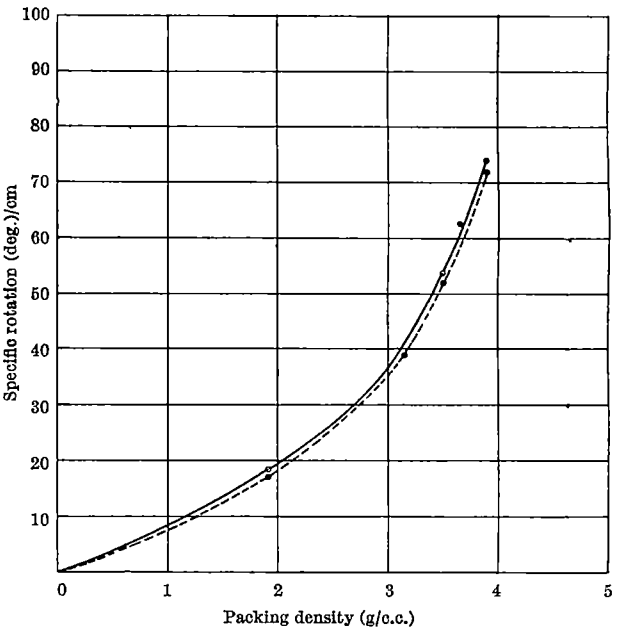


Fig. 2. Specific rotation in ferrite powder samples at an applied magnetizing field of 200 œrsteds plotted against the density at 9,060 Mc/s. ——, experimental; — — —, theoretical

The demagnetization factor N_z was estimated by a magnetostatic method using the constancy of the tangential component of the magnetic field across the cylindrical surface of the sample immersed in axial magnetic field. The theoretical graph and the experimentally observed data on the variation of the specific rotation with packing density are plotted in Fig. 2. It is observed that the nature of the specific rotation variation with the packing density agrees excellently with the theoretical curve, though the numerical agreement is not to be over-emphasized.

We thank Lieut.-Colonel S. Mishra for providing facilities for work and advice and Commodore M. K. Lele, dean of the Institute, for permission to publish the results.

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Supplement to NATURE

No. 4962 DECEMBER 5, 1964 Vol. 204

FROM A DROP IN THE OCEAN?

The Chemical Origin of Life

By Alexander I. Oparin. (A Monograph in American Lectures in Living Chemistry.) Pp. xxvii+124. (Springfield, Ill.: Charles C. Thomas, 1964.) 6.75 dollars.

OPARIN has already made outstanding contributions to the most fundamental problem of biology, and *The Chemical Origin of Life* summarizes the present state of his thinking. Apart from being profound scientifically, it is scholarly in range and style, and it has the touch of poetic inspiration which belongs to all the best science.

Oparin observes that "the origin of life is an inalienable part of the general orderly process of development of the Universe in which each successive stage is inseparably bound up with the one before and can only be understood in the light of a study of that predecessor". Consistently with this view he devotes his first chapter to a broad description of astronomical and geological dating, the evolution of different stellar types, the origin of elements, and the formation of the Earth. About 2×10^9 years ago it probably possessed an atmosphere containing water vapour, hydrogen sulphide, ammonia and hydrocarbons, but no significant amount of oxygen. The atmosphere as we know it to-day is perhaps about half this age, and came into being by the action of various forms of life.

The second chapter is devoted to the way in which organic compounds were built up on the primeval Earth under conditions when the ultra-violet radiation was much more intense than now (in the absence of an ozone screen) and when there were no micro-organisms to devour the compounds which might be formed. The main reason, as Oparin remarks, why life cannot now arise *de novo* under natural conditions is that it has already arisen. To form an idea of the course of events, therefore, it is no good looking for evidence left over from the past, but we must rely on reconstruction experiments in the laboratory. These, in fact, show that compounds like amino-acids can be formed by the action of silent electrical discharges on mixtures of methane, ammonia, hydrogen and water vapour; thence in various ways of which analogues are known, optical asymmetry could have arisen. Elaboration and polymerization of the primary organic compounds can also be envisaged, and this now includes the possibility of non-enzymatic synthesis of polynucleotides. The various kinds of model experiment are discussed in some detail.

At the beginning of Chapter 3 we have reached by purely abiogenic means a 'nutrient broth' containing organic compounds similar to those which build up living matter to-day. How do self-reproducing organisms arise in this organic chemical free-for-all? Oparin considers that a key step in the evolution is the formation of 'coacervate droplets' from solutions containing polymerized substances. Polymers do, in fact, separate out from aqueous media in droplets well demarcated from their environment. This boundary formation is a first essential in the evolution of individual organisms. Numerous observations and experiments on coacervate droplets of various kinds are described and in some of these systems significant biochemical reactions can be made to take place efficiently. Coupled biochemical reaction in the droplets can also be believed in, and we arrive at the stage of what Oparin calls 'protobionts'. These could increase their mass at the expense of their environment. They can incorporate extra components, catalysts and so on. Presently an element of replication is introduced when polynucleotides engage in the now well-known game of 'base pairing'. The ordering of amino-acids then becomes coupled with this. "A mechanism of this sort could, of course, only have arisen in the course of the prolonged evolution of living systems. However, we now have fully grounds enough for believing that even at far earlier stages of the development of organic matter, the inclusion of polynucleotides in coacervate droplets or protobionts had a certain effect on the polymerization of the amino-acids in those systems". It must have needed some luck even in 10^9 years. But Oparin's case, even if there are passages of superficiality, is well argued and, on the whole, well documented.

Chapter 4 covers a wide range of biochemical facts in a discussion of the course which the further evolution of the first living organisms may have taken, a central thesis being that the complex mechanisms known to-day must have been elaborated by a process of gradual addition and improvement.

The final section is devoted to a brief examination of geological evidence relating to the chronology of the whole story.

The book is beautifully written, and if we do not feel that all the mysteries are solved the questions are presented and much evidence is marshalled in a way which sheds real illumination. C. N. HINSHELWOOD

PRIMATE EVOLUTION

Evolutionary and Genetic Biology of Primates

Edited by John Buettner-Janusch. Volume 1. Pp. xiv+327. 86*s*. Volume 2. Pp. xii+330. 89*s*. 6*d*. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1963 and 1964.)

A NEW generation of students has moved into the field of primate research—not only new, but also a much more numerous one than we have ever known before. It makes its presence felt in ways far more novel than just the perennial debate about the relevance to human evolution of some fossil fragment which usually becomes known through the heat of partisan advocacy rather than as a result of dispassionate anatomical study. We have symposia on the behaviour, anatomy and physiology of the primates. We have new journals devoted to primate studies. Regional primate centres spring up in the United States at a cost of millions of dollars a year. Some enthusiasts are even trying to describe all this field of interest by the term 'primatology', although here we hope unsuccessfully, since this is scarcely a happy step in a far too rapidly fragmenting world of science.

The two volumes edited by Dr. J. Buettner-Janusch, and entitled *Evolutionary and Genetic Biology of Primates*, are the latest sign of this renaissance of interest in the primates. They are a valuable addition to the library of our knowledge of the mammalian order to which man belongs.

The opening chapter of Volume 1, written by the editor, describes the order and discusses its classification. It is followed by a critical and scholarly reappraisal of fossil primates deriving from Tertiary deposits, and from which we learn that more fossil genera are known in the order of Primates than in all but six of the thirty-three orders of mammal recognized by Gaylord Simpson.

Chapter 3 deals with functional and structural phylogenetic aspects of the primate nervous system, including the visual apparatus. The fourth considers the skin of primates in general, and the fifth the sweat glands of the Lorisidae. The chapter which follows provides an up-to-date account of what is known of nerve endings in primate skin. The final chapter of Volume 1 is a first-rate summing up of present-day knowledge of the chromosomes of primates.

Volume 2 opens with an account of the taxonomic implications of the susceptibility of primates to different viruses. This is followed by an illuminating chapter on primate serum proteins. Chapter 3 deals with primate hæmoglobins. The three final chapters deal in turn with the reproductive physiology and behaviour of the Lemuroidea, the use of the hand in lower primates, and with what are called the 'behavioural displays' of the order.

So rich a fare does not lend itself to review. All that I can do is to light arbitrarily on a few striking facts, for example, that man sweats more and more readily than any other animal, and that his sweat glands are more responsive to different stimuli than those of any other creatures; that "the primate can be characterized by the innervation of its skin just as correctly as by its bipedal gate [*sic*] and its dental formula"; that the pattern of cutaneous nerve endings is the same in all Primates; and "that a ball of non-myelinated neurofibrils has been found in the hairless skin of all Primates studied to date". It is interesting to learn that chromosome numbers in primates vary from $2n = 34$ to $2n = 72$, and that the fact that the only common denominator is 2 rules out the possibility of polyploidy having been a mechanism of chromosome evolution in Primates. It is equally interesting to read that "the karyotypes of many groups of primates studied show a remarkable correlation between low chromosome numbers and low numbers of acrocentric chromosomes and between high chromosome numbers and high numbers of acrocentrics". This suggests that centric fusion has played an important part in the chromosome evolution of the Primates.

We learn, too, that there is a correlation between the spectrum of susceptibility of primate cells to a given virus on one hand, and chromosome counts on the other, and that the anthropoid apes are prey to all human virus diseases that have been tested so far, while Old World monkeys are susceptible to many viruses which affect neither New World monkeys nor the lemuroids, which in turn are both susceptible to some other range of the spectrum of human viruses.

From the bibliographies at the end of each chapter it would seem that few, if any, of the authors who contribute to these two volumes began working in their particular fields of primate study more than ten years ago. Some, indeed, reveal themselves as post-doctoral research students. This makes for a refreshing treatment of the subjects which are discussed. But it also diffuses a certain *naïveté*. For, however up-to-date the chapters, the scope of the two volumes is neither as comprehensive, nor is their focus as novel, as the reader is encouraged to believe. Unless one stretches the meaning of the term beyond recognition, the editor's view that his two volumes constitute a "representative" sample of contemporary research on the primates can scarcely be accepted. All but one of the contributors are young American writers. Some important fields of primate study that are being cultivated in the United Kingdom are not even mentioned by name, and presumably work in other countries has also failed to gain recognition. Equally, the present collection of essays is certainly not the first attempt to bring to bear the results of physiological and other functional investigations on the evolutionary history of the Primates. A volume in which I attempted to do just this more than thirty years ago has, no doubt with justice, and certainly without regret on my part, passed entirely into the limbo; it is not even referred to by name in the present volumes. A certain lack of restraint and discernment also intrudes here and there. It is odd, for example, to read that Landsteiner's discoveries about the specificity of antibodies were "empirical as a phenomenon and vague as a concept". When Landsteiner's generalizations saw the light of day some sixty years ago they represented something far newer in the world of science than anything which has yet been provided by the present set of authors. It is almost as though one of to-day's Ph.D. students in physics were to describe J. J. Thompson's contributions to our understanding of the atom as incomplete and lacking in precision. The quality of scientific novelty and imagination with which Landsteiner's concepts still endure is, alas, hardly characteristic of most of the fact-garnering which goes on to-day in the particular branch of zoology with which Dr. Buettener-Janusch has concerned himself.

But, nevertheless, there is something inspiring about the enthusiasm of the authors who have contributed to his two volumes. Let us hope that the sense by which some of them are animated, that there is some mystery about primate evolution which is bound to be unfolded as new facts become available, will one day be realized.

SOLLY ZUCKERMAN

BIOLOGICAL BASIS OF PSYCHOSOCIAL EVOLUTION

Human Biology

An Introduction to Human Evolution, Variation and Growth. By G. A. Harrison, J. S. Weiner, J. M. Tanner and N. A. Barnicot. Pp. xvi + 536. (Oxford: Clarendon Press; London: Oxford University Press, 1964.) 42*s*. net.

THE proper understanding of human biology is becoming increasingly necessary as a background for all our efforts at practical, social, economic and cultural development. This book is important as being, so far as I am aware, the first to treat it as a subject of study in its own right, as part of a general university curriculum.

It is a valuable attempt at establishing what Prof. Medawar in his foreword calls "a new conception of the natural history of mankind". If it fails fully to achieve this highly desirable result, it is because it has not been adequately planned so as to present human biology in an integrated way, as the study of the unique organism called man, and therefore with its own important difference from general biology.

Let me begin, however, by emphasizing the large amount of valuable facts and ideas, largely based on the most modern disciplines and techniques, which it makes available on human genetics and evolution, growth and development, physiology and biochemistry, pathology and demography, variation and ecological adaptation.

Dr. Tanner's section on "Human Growth and Constitution" is admirable, bringing out the progressive acceleration of pre-adolescent development and its striking individual variation, the concept of physical developmental age and its relation to mental development, the classification of physique and its relation to disease and behaviour. (The lack of planning has resulted in Dr. Barnicot also including a treatment of human physique and its variation in his section.)

My only criticism of Dr. Tanner is that he makes no reference to the facts and implications of differential (allometric) growth-gradients (as opposed to maturity gradients), in spite of the exhaustive treatment of the subject by Rensch in his fine book, *Evolution Above the Species Level*, where he points out that both in development and evolution the description of characters has had to give place to a study of gradients and their effects. No mention is made of Rensch's remarkable demonstration that in many groups (including some invertebrates) increased absolute brain-size is correlated with improved

behavioural performance, and that this is due to the positive allometry (and therefore relatively as well as absolutely greater size) of the higher, more recently evolved regions of the brain, though this is of special relevance to man's evolution and present variation.

Harrison's section gives an excellent summary of human genetics which will be especially valuable to medical men, psychiatrists, psychologists, social scientists and historians, especially in view of the numerous 'anti-geneticists' still unfortunately to be found in their ranks. The long-overdue inclusion of E. B. Ford's rational nomenclature for the blood-groups in a general text-book is welcome.

None of the authors draws the important distinction between survival selection and reproductive selection, though it is especially relevant in man, where reproductive selection has become relatively far the more important. The results of both are lumped together under the fashionable but misleading term 'Darwinian fitness' (it was Spencer who influenced Darwin to use the unfortunate phrase 'survival of the fittest' in later editions of the *Origin*; and Darwin glimpsed the difference by distinguishing sexual from natural selection).

I hope that in the new edition, which will assuredly soon be called for, they will point out that man shows the highest degree of genetic variation known (Roger Williams's work is not cited), and that this makes possible the desirable 'varied excellence' and division of cultural labour that are basic for social structure, education, and possible eugenic improvement.

I hope, furthermore, that they will devote more space to the subject of morphism, the convenient term for Ford's balanced genetic polymorphism. Apart from colour-blindness and PTC taste-blindness, they do not treat the remarkable human sensory morphisms, such as smell-blindness, to hydrocyanic acid and the scent of freesias, taste-blindness to benzoate of soda, brucine and two separate components of mannose, and myopia; nor do they discuss the fascinating suggestion, in Darlington and Mather's *The Elements of Genetics*, of a correlation between O blood-group frequency and ease of pronouncing fricative dental sounds like *th*: this could readily be scientifically investigated, but the challenge has not yet been taken up.

Yet morphisms are an outstanding challenge to the biologist, since they all involve special genetic mechanisms, selective balance, morph-ratio clines, interesting geographical distribution, and evolutionary persistence (thus PTC taste-morphism certainly exists in chimpanzees, and probably in rats). And sensory morphisms are of special interest to the human biologist since they can most readily be detected and investigated in man, and their balance of selective advantage and disadvantage (which the authors have well brought out in regard to the non-sensory sickling hæmoglobin morphism) can be explored by experiment in animals as well as by comparative analysis in man.

As regards blood-group morphisms, it will be important to discover why in most ethnic groups the morph-ratios appear to remain constant over long periods, whereas in, for example, sickling the morph-ratio changes rapidly in new ecological (malaria-free) environments.

Any genetically determined characters the incidence of which is, say, more than 0·25 per cent must be in morphic balance. This will prove of great importance for psychiatry when the undoubted genetic basis for various mental diseases with high incidence, such as schizophrenia, has been properly ascertained.

Harrison and Weiner's section on "Human Evolution" and Weiner's on "Human Ecology" are full of valuable up-to-date information, notably on hominid evolution in the Pleistocene, on the adaptation of ethnic groups to different habitats, on tolerance and acclimatization to heat, cold and climatic variables in general, and on the history of population-control in the pre-technological world. On this last point, however, Weiner fails to point out that population-increase has now entered on a wholly new 'explosive' phase, which urgently demands large-scale research and study by biologists, social scientists, economists, politicians, publicists, and religious leaders, with immediate practical follow-up, if we are to avoid disaster and lay the basis for a population policy for the human species.

My only other criticism is that Harrison and Weiner's excellent account of the evolutionary history of the primates is almost too detailed for a book of this type, while they fail to mention the basic fact that large evolutionary advances occur by the rare emergence of new dominant types of organization with new and improved patterns of general organization. On the other hand, their account of the evolution of human behaviour is too brief, and makes no mention of its biological basis in modern ethology—even the word is absent from the index, as well as names like Lorenz and Tinbergen.

There remains one major defect in the book as a whole. In spite of Prof. Medawar's stress in his foreword on the distinctive features of human biology, among which he includes "love, showing off, real or sham aggression", and especially man's distinctive methods of communication and behaviour and consequent "non-genetical system of heredity", there is no adequate treatment of what I would call its basic premiss—the uniqueness of man as an organism, and the unique consequences flowing from that fact.

Man is unique in virtue of a combination of characters—erect posture, the highest known variability, front-to-front copulation, manipulative hands, size of cerebral cortex, tool-making, neoteny and a much-prolonged period of immaturity and educability, true (symbolic) speech and other communication systems, and the capacity for conceptual thought, creative art and purposeful planning. These in combination give him a highly effective second hereditary system in the shape of cumulative tradition. In the new resultant psychosocial phase, evolution is primarily manifested by cultural instead of genetic transformation. Hence the anagenetic improvement of cultures, their cladogenetic (confusingly spelt kladogenetic in this book) diversification, and their occasional stasigenetic overstabilization, together with the novel integrative process of cultural diffusion. Hence also the recent preponderance of reproductive over survival selection, to which I have already referred. These processes all rest on purely biological foundations, but are unique properties of evolution in its human phase, and should be fully dealt with as such.

Clearly a book on human biology should not attempt to provide potted summaries of social anthropology and religion, political and social structure, economics and ethics, history and the arts. But if it is to deserve its title, it must give a general account of the unique characteristics of man and of the main principles and features of his psychosocial evolutionary transformations. Let us hope that a second edition of the present work will include such an account: it is badly needed. Meanwhile, let us be grateful to the four authors for their valuable contributions to this new and rapidly growing field of study.

JULIAN HUXLEY

CHIMERAS AND CLASSIFICATION

Classification and Human Evolution

Edited by Sherwood L. Washburn. Pp. viii+371. (London: Methuen and Co., Ltd., 1964.) 63*s*. net.

THIS useful volume *Classification and Human Evolution* might have been more appropriately entitled "Human Evolution and Classification", for it is evident that the symposium on which it is based was initially stimulated by some of the remarkable discoveries of fossil primates (and particularly of fossil hominids) reported in recent years.

When fossil remains of creatures are found showing a mixture of anatomical characters that in modern types have been taken as distinctive of different taxonomic categories, their taxonomic position inevitably becomes a matter for debate. More than this, they also arouse renewed interest in the validity of accepted systematics as applied to zoological groupings in general. There is of course nothing novel in taxonomic reappraisals of this kind; they have frequently been required in the past following palæontological studies of hitherto unknown types of fossil vertebrates. But the discovery of fossil hominids and other higher Primates has now directed the attention of palæo-anthropologists to evolutionary principles already well recognized by their zoological colleagues, such as mosaic evolution, the gradational expression of micro-mutations, the importance of the selective effects of changing ecological conditions, the need to base definitions of major taxonomic groups on evolutionary trends rather than on the terminal results of evolution, and so forth.

During the past few years a number of symposia and colloquia have been published as the direct result of discoveries of extinct representatives of the higher Primates, and *Classification and Human Evolution* is one of them. The contents of such collections of papers and essays are bound to be of unequal value simply because they are the products of different authorships. They occasionally tend to repetitiveness for the reason that the same authors dwell on the same themes in successive publications, and they are sometimes characterized by a certain *naïveté* of approach from contributors who are not sufficiently acquainted with well-established principles of evolutionary processes and zoological taxonomics. Not that these are necessarily faults; for one thing, repetition and re-emphasis may be of considerable importance in order to gain acceptance of conclusions resulting from new discoveries. Naturally, it has always been recognized that definitions of taxonomic categories would become more difficult and more arbitrary as gaps in the evolutionary record become filled by intermediate links. But taxonomic definitions and distinctions have to be made simply as a matter of practical convenience, even if they are arbitrary. Whether *Oreopithecus* is a catarrhine monkey that has paralleled the Pongidae in many of its anatomical characters, or whether it is a pongid retaining some catarrhine characters, or whether (as has been suggested) it is an aberrant Pliocene offshoot of the Hominidae—all these are possibilities to be considered, and it is no doubt wise of Dr. W. L. Straus in his discussion of this problem to temporize by placing it in a family of its own, Oreopithecidae. On the other hand, the question whether *Australopithecus* is to be properly placed in the family Pongidae or in the Hominidae has now passed beyond the realm of serious controversy, for this early Pleistocene genus is well recognized to be a primitive hominid. Such problems as these are only to be decided by a close scrutiny of the combinations of morphological features that they present, and it is interesting to note that much more consideration is now being given to their functional and behavioural significance.

The chapter by G. A. Harrison and J. S. Weiner on "Some Considerations in the Formulation of Theories of Human Physiology" is of importance in this regard, as also are the chapters by S. L. Washburn on "Behaviour and Human Evolution", and by J. Napier on "The Locomotor Functions of Hominids". It may be that undue anxiety about the niceties of definitions is sometimes to be detected; for example, the suggested distinction between 'tool-modifying' and 'tool-making' seems to be a distinction without any obvious difference (in any event, in reference to simple bone or stone implements), and the impossibility of making a sharp distinction between brachiators and non-brachiators, which of course was to be anticipated, has now become clear from the anatomical characters of the Miocene ape *Proconsul*. The fact is that most Primates (even *Homo sapiens*) might be called brachiators in the sense that they can, or do on occasion, use their arms in swinging from one branch to another, but it is still a matter of convenience to limit this term to those Primates that use this mode of progression to the exclusion of leaping or running among the branches of trees by the propulsive force of their hind-limbs. Whether it is wise to invent the term 'semi-brachiator' for those monkeys that are somewhat more inclined to use their arms in arboreal locomotion is perhaps questionable, for this may lead to still further attempts at defining intermediate functional grades—'semi-demi-brachiators' and so forth.

The ancillary evidence for taxonomic relationships of Primates based on serology, the molecular constitution of proteins, the patterns of chromosomes, and the principles of genetics are dealt with in this book by authorities on these different subjects, with the interesting result that their conclusions serve in large measure to confirm the taxonomy previously based almost entirely on morphological criteria. It is true some writers have gone so far as to claim that the gorilla and chimpanzee should be included with *Homo* in the one family Hominidae, but it seems most unlikely that such a drastic redrafting of the classification of the higher Primates will be acceptable. After all, the distinctive characters of man, morphological, functional and psychological, are so obtrusive as to demand a far-reaching divergence from the modern anthropoid apes, even if they do not demand a divergence so far back in geological time as one or two extremists in the opposite direction would like to think.

WILFRID LE GROS CLARK

ANTIQUITY OF MAN

The Problem of Man's Antiquity

An Historical Survey. By Kenneth Page Oakley. Pp. 85–155. *Bulletin of the British Museum (Natural History), Geology*, 9, No. 5. (London: British Museum (Natural History), 1964.) 28*s*.

The Prehistory of East Africa

By Sonia Cole. Pp. 382. (London: Weidenfeld and Nicolson, Ltd., 1964.) 55*s*. net.

The Evolution of Man

By G. H. R. von Koenigswald. Pp. 148. (Ann Arbor Science Library: Ann Arbor, University of Michigan Press, 1962.) 5 dollars.

MOST people would agree that complete, impersonal objectivity in pioneer research is an impossibility. Even if there is no prejudice, the intellectual and imaginative equipment of an original scientist will affect the nature of his results. Accepting this as inevitable and not necessarily damaging, it still comes as a shock to discover how often preconceived ideas have affected the investigation of human origins.

There is, of course, nothing like a fake for exposing such weaknesses among the experts. For example, to look back over the bold claims and subtle anatomical distinctions made by some of our greatest authorities concerning the recent human skull and modern ape's jaw which together composed 'Piltdown Man', rouses either joy or pain according to one's feeling for scientists. As these books show, there is no reason to suppose that tendencies to error have grown very much less.

Dr. Kenneth Oakley's agreeable survey, *The Problem of Man's Antiquity*, can be seen as a history of changing attitudes and prejudices in human palæontology. Inevitably it begins with the greatest of them all: the refusal during the first two-thirds of the nineteenth century to recognize the immense antiquity of the human fossils and artefacts that were then being brought to light. While much of the resistance was due to religious opinion

on the mode of man's genesis, scientific conservatism also played its part. As Sir Charles Lyell commented, new finds of ancient man provided "Unwelcome intelligence opposed to the prepossessions of the scientific as well as the unscientific public". Dr. Oakley's material illuminating this great dispute includes a lively satirical drawing of the Paris Academy debating the affair of the Moulin-Quignon jaw (a fraud, as it turned out). Touches such as this, together with the sensitive approach and writing, make *The Problem of Man's Antiquity* an excellent example of the value of the history of science as a bridge between the 'Two Cultures'.

After the triumph of the evolutionary point of view, Dr. Oakley shows how the vast oceans of Palæolithic time that had been opened to exploration were gradually charted. Cultures were discovered, classified, and began to be given their chronological place relative to one another and to their geological context. This last-mentioned process is still very far from complete, while the situation has been revolutionized during the past decade by methods of absolute dating through radioactivity and chemical analysis. Dr. Oakley ends his survey with a suggestion for systematizing chronological terms. He proposes a double series of symbols (*R* 1–4 and *A* 1–4) for quick reference to the different forms of relative or stratigraphical dating and absolute or chronological dating. Although some of us would like to resist any specialist devices that raise unnecessary barriers against the uninitiated, there is no doubt that this system would be of convenience to professionals, and seems likely to be widely adopted.

The only thing to be regretted in this survey is that it stops too soon. One would very much like to have Dr. Oakley's latest judgements on the great series of fossil finds made in Africa during the past forty years—and particularly on the fossil men of East Africa that form the core of Mrs. Cole's book.

The Prehistory of East Africa is one of the best regional prehistories ever written. Indeed, it is more than than that, for although the bulk of the material comes from Tanganyika, Kenya and adjacent regions, the discussion of human origins and the earlier fossil hominids reaches out to involve the whole of the Pleistocene world.

Any book of this title is bound to have Dr. Louis Leakey as at least an unofficial hero. The work which he and his family have done in East Africa, and the brilliance of its results, should make one of the greatest heroic epics of science. Most important of all have been their discoveries in the natural section through Pleistocene time at Olduvai Gorge in Tanganyika. Here the Leakeys have not only found Early and Middle Pleistocene fossil hominids that are of outstanding significance, but have also been able to relate them to the evolution of the hand-axe cultures.

Where Dr. Leakey is a hero there will be argument and partisanship. For, even more than most men of original genius, he exemplifies the theory that the mental equipment of such men will shape and colour their interpretations. In the first place his impetuous enthusiasm has made him—as Mrs. Cole would put it—a "splitter" rather than a "lumper". That is to say, as new fossils have been extracted from their ancient matrices by Leakey or his wife or son, and he has recognized their exciting points of difference, he has tended immediately to proclaim each as representing a new genus—when later it has sometimes seemed wiser to recognize only a specific or sub-specific status.

In the second place, if this is not too disrespectful a way to put it, he has always played a hunch that our ancestral line of pre-*sapiens* hominids split off from the rest earlier rather than later. That is why he was, in a sense, working against himself when he found the decidedly pithecoid man, whom he named *Zinjanthropus*, apparently associated with the pebble tool culture at Olduvai; and why, when he found what he held to be a larger-brained, more progressive type at a lower level, he claimed it as "a quite distinct form of early hominid", and suggested that it was this man and not the unwelcome *Zinjanthropus* who had made the pebble tools. Many authorities would prefer to see *Zinjanthropus* himself as a racial variety of *Australopithecus robustus*, and the 'pre-Zinj' breed also among the australopithecines—although possibly looking towards the progressive type, *Telanthropus*.

Mrs. Cole deals with these crucial issues with admirable detachment. She also allows more room for manœuvre in evolutionary argument by tentatively accepting the recent potassium–argon datings, which would give *Zinjanthropus* an age of at least a million years, and approximately double the hitherto accepted length of the Pleistocene period.

Although the importance of East Africa in the work on human origins focuses interest on this part of her book, Mrs. Cole deals equally well with other subjects and later periods. There is a full discussion of climatic phases, of the whole sequence of stone-using cultures, of the beginnings of cultivation, of pottery making and the introduction of iron. There is a chapter on rock paintings and engravings and another on the equally difficult problems concerning modern African races and their history.

Mrs. Cole covers a wide field of knowledge with the precision to be expected of serious scientific exposition, and with exceptional clarity and style. But what makes her book even more outstanding is her courage in making it at the same time a human document. She enables us to see the settings of excavations, the conditions under which people worked, the telling personal details of the great moments of discovery—all are well described and fully integrated with the scientific narrative. *The Prehistory of East Africa* deserves a very special prize.

Prof. von Koenigswald's little book seems to show a bias cruder and far less acceptable than Dr. Leakey's zestful championing of his 'prepossessions'. The author occupies the chair of palæontology in the State University of Utrecht, and has himself obtained important pithecanthropine fossils in Java. It is impossible not to feel that it is this background that has caused him to declare that probably "Asia was the original centre of the hominids"—so opposing the African claims to primacy which seem every year to be more strongly based. He also sets the whole range of African australopithecines outside the direct line of our human ancestry, and claims that they, too, may have originated in Asia. He gives no consideration to the possibility of a much longer duration for the Pleistocene Age, nor, although his book was first published a year after their discovery, does he mention either 'pre-Zinj' or the Chellean man from Olduvai. (There are, indeed, signs that part of his text was written before any australopithecines were known from East Africa.)

Moreover, Prof. von Koenigswald's special knowledge of dentition makes him give what might be judged excessive weight to this limited part of the evidence, while at the same time loading both text and illustrations with more facts about teeth than are likely to be welcome to the general public. *The Evolution of Man* contains plenty of useful factual material, much of it set out in tabular form, but it can scarcely be recommended as a well-balanced handbook.

JACQUETTA HAWKES

PIONEER OF MODERN ANATOMY

Andreas Vesalius of Brussels, 1514–1564

By Prof. C. D. O'Malley. Pp. xv+480+64 plates. (Berkeley and Los Angeles: University of California Press; London: Cambridge University Press, 1964.) 10 dollars, 80*s*. net.

NO full-scale life of Vesalius has previously been written except that of Moritz Roth[1]. Since Roth's biography a wealth of Vesalian literature has appeared, including Harvey Cushing's *Biobibliography*, published

in 1943 and edited by John Fulton, and Charles Singer's studies. Furthermore, much new information has come to light in the intervening seventy years. Prof. C. D. O'Malley, the pre-eminent authority on Vesalius, has been engaged on this definitive biography for many years, and all medical historians will welcome the fruit of his labours in this well-produced volume.

Galen's anatomy was based on dissection of animals, and when applied to man was often confusing and erroneous. For centuries his teaching was held to be incontrovertible and above criticism. Yet, as Prof. O'Malley shows in his first chapter on pre-Vesalian anatomy, certain enlightened men like Berengario of Bologna and Leonardo da Vinci were dissecting human bodies and making their own observations which did not always accord with Galenic teaching.

Andreas Vesalius, son of an apothecary to the Emperor Charles V, was born in Brussels on December 31, 1514. He received a classical education at the University of Louvain; and in 1533 studied medicine at the University of Paris, where he worked with Guenther of Andernach and Sylvius and learned Galen's anatomy. Returning to Louvain for his M.B. degree, he carried out further dissections and discovered the corpus luteum in a female body. In 1537 he published his *Paraphrasis* (probably his M.B. thesis) which was a translation of the writings of Rhazes with a comparison of Arabian therapy with that of the Greeks. In the same year the University of Padua conferred the degree of M.D. on Vesalius and appointed him professor of surgery and anatomy.

At Padua, Vesalius became distinguished as an anatomist and teacher who taught anatomy not from books but from scientific observation. In 1538–39 he published three brief works, the *Tabulae anatomicae*, a revised edition of Guenther's *Institutiones anatomicae* and the *Venesection letter*. They still embodied Galenic teaching. From 1539 until 1540 Vesalius and John Caius lived together in a house in Padua engaged on Galenic texts. Vesalius contributed to the Giunta edition of Galen's *Opera omnia*, 1541–43. Ultimately, the friendship of these two great men was broken off, because Caius adhered to Galen's anatomy in which Vesalius had already found flaws.

In 1543 *De Fabrica* (with its *Epitome* for the use of students), the anatomical work which replaced Galenic tradition by scientific observation, was published at Basle and made Vesalius famous for all time. It evoked admiration, praise, controversy and bitterness from the Galenists, including in 1551 a virulent attack from Vesalius's old teacher, Sylvius. Guenther, on the other hand, was encouraging and friendly. In 1543 Vesalius resigned his chair to become physician to the Emperor Charles V. He accompanied the emperor in his many campaigns and after his abdication to Spain, becoming later physician to King Philip II. Vesalius's reputation was now international, for after King Henry II of France sustained a fatal head injury in a tournament, he was summoned to Paris where he met Ambroise Paré in consultation at the king's bedside.

By 1563 Vesalius was anxious to regain his chair at Padua, which he was offered on the death of Fallopius. He obtained leave from King Philip to make a pilgrimage to Jerusalem. Prof. O'Malley shows that there is little foundation for the legend that this journey was made because Vesalius had done a necropsy on a Spanish grandee whose heart was still beating. The pilgrimage was performed; but on the return journey to Padua Vesalius became ill and died in 1564 on the Island of Zante.

It may be noted that the *De Fabrica* soon came to the notice of English anatomists and surgeons, for as early as 1545 Thomas Geminus brought out an abridgement entitled *Compendiosa totius Anatomie delineatio* with copper-plate engravings copied from Calcar; it was dedicated to Henry VIII. In 1553 Geminus published an English translation of his compendium, made by Nicholas Udall, the author of the first English comedy, *Ralph Roister Doister*. This was dedicated to Edward VI. The third edition in 1559 was dedicated to Queen Elizabeth I.

In this book Prof. O'Malley has blended knowledge of medical history, of anatomy, of physiology, scholarly appraisement, original investigations and researches and literary charm together, and the result is a biographical masterpiece. There are also notes and references to the copious Vesalian literature.

Vesalius in the *De Fabrica* produced a great work of original research. As Charles Singer was wont to say, "he is the ideal subject for the investigation of the beginnings of science". It is therefore important to trace all the processes in his life—mental, social, personal, political—by or through which Vesalius, starting as a classical scholar, became a scientific inquirer. This task, one of supreme difficulty, has been accomplished by Prof. O'Malley. He has done a great service to history and to comprehension of the evolution of science.

[1] Roth, Moritz, *Andreas Vesalius Bruxellensis*. Pp. 500+viii+30 plates. (Berlin, G. Reimer, 1892.)

ZOO MAMMALS

The Management of Wild Mammals in Captivity

By Lee S. Crandall. Pp. xv+761+11 plates. (Chicago and London: University of Chicago Press, 1964.) 13.50 dollars; 97*s*.

WILD beast shows have been a popular entertainment for several thousand years and are now as popular as ever and more numerous than at any previous time. A minority of people object, for moral or sentimental reasons, to the keeping of wild animals in captivity, but the vast majority never consider these matters; those who do pause to think appreciate the educational and recreational value of living zoological collections. Wild animals are now exhibited in all parts of the world; zoos are of almost universal appeal, as is shown by the rapid increase in their number everywhere and the crowds of people who flock to them. The influence of television in spreading this cult has been considerable, and the increasing public awareness that the human population explosion is threatening the continued existence of many of the larger and more spectacular animals has given added interest and concern.

It is peculiar that, until well on in the present century, the management of wild animals in captivity was largely a matter of rule-of-thumb, lore, and the 'green fingers' of those gifted with a 'way with animals'. An immense amount of lore had been accumulated by zoo men, much of it by oral tradition, less by the written word, but until the twentieth century zoologists were so pre-occupied with the remains of dead animals in the museum and laboratory that few had directed serious attention to the many problems confronting those in charge of menageries. When zoology began paying court to physiology as well as morphology, and the expanding experimental approach demanded ever-increasing numbers of experimental animals, the scientific methods devised for the management of the laboratory animal-house were soon adopted and adapted by the better zoos. As a result the health, longevity and breeding success of captive wild animals have improved enormously during the past twenty-five years; many animals formerly regarded as impossible to breed, or even to maintain alive, in captivity are now the pride of their keepers. More recently, the rapidly progressing discipline of ethology is adding valuable knowledge to the art of animal keeping, and is showing how the mental well-being of captive wild animals is as important as their bodily health.

The zoo-keeper, however, has a double problem, for he is concerned not only with the good management of his

animals but also with their exhibition to the public—a group of animals in perfect health living in conditions closely simulating those of the natural environment, where they remain cosily concealed all day, may be eminently satisfactory to the research zoologist, but a complete failure to a public interested only in looking at unfamiliar strange creatures. Zoos, therefore, must compromise, and always remember that their first purpose is to show animals to their public in a manner as æsthetically pleasing as possible while maintaining them in full health and vigour in reasonably congenial surroundings. Zoos, too, have much to teach the public about its attitude towards menagerie animals; there still lingers much of the old-fashioned 'stir him up with a long pole and make him roar' spirit, and an almost universal anthropomorphic approach. Education is gradually overcoming these primitive reactions and leading to a dawning appreciation of the true interest and value of the world's fauna to mankind.

Lee S. Crandall worked for fifty years in the New York Zoological Park, the final fifteen or so as general curator. He is pre-eminently one of those fortunate people gifted with 'green fingers', and to his natural gifts he has added a life-time devoted to the scientific study of animals, especially those in zoological collections. He is *persona grata* with his colleagues throughout the world and has thereby reaped a rich harvest from the experiences of his friends. Now he has set down all he knows at first hand, and all that he has learnt from his confreres, about the management of wild mammals in captivity—and what a splendid book it makes!

Wild Mammals in Captivity is an exhaustive treatise, and there can be little of importance relating to the subject that has been overlooked, in spite of the author's modest disclaimer. It goes systematically through the orders of living mammals, omitting only the Cetacea, dealing with all the species that have ever been kept in zoos and mentioning some others that have not. Descriptions are given of all the main groups and of many individual species, with a summary of their distribution and habitats, followed by particulars of the natural diets and those found acceptable in captivity. Details are given of the enclosures and housing that have been provided in different menageries, and records of longevity and breeding successes are added. More valuable even than these are the personal experiences of the author with an enormous number of different species, set down clearly and with the occasional touch of humour that makes them fascinating reading. The book is very well documented and well indexed, and the typography and format are excellent. It is embellished with some splendid photographic illustrations; that of the gibbons in the New York Zoological Park drinking by dipping water from the lake below them while riding the branch-tips of an overhanging tree is a beautiful composition, apart from its zoological value.

Crandall's book will be the zoo-keeper's bible for many a year, and all zoologists interested in mammals, whether or not they are directly concerned with zoos, will feel a debt of gratitude to him for the immense labour he has devoted to the making of this outstanding and comprehensive work.

L. Harrison Matthews

INSECT PHYSIOLOGY WITHOUT TEARS?

The Life of Insects

By Prof. V. B. Wigglesworth. (The Weidenfeld and Nicolson Natural History.) Pp. xii+360+36 plates. (London: Weidenfeld and Nicolson, 1964.) 55*s*. net.

THIS is the first zoological contribution of the new Weidenfeld and Nicolson Natural History, which is planned as a series of volumes by authors who are all well-known scientific authorities, each of whom will deal with some specific group of organisms*. The effect of this policy is predictable. No one will be surprised that this book by Sir Vincent Wigglesworth, about the life of insects, is very largely concerned with insect physiology. He makes use of his unrivalled personal knowledge in this field to illuminate and give meaning to the wonders of insect life. The book is written in a clear and lively style and Lady Wigglesworth has provided many new line drawings, most of which are excellent and likely to find wider use. The half-tone and colour plates are very good, with the exception of parts of Plates 6 and 7, where reds have been printed as brown and white a strange blue shade.

The seventeen chapters cover a wide range of topics such as movement, diet, eggs, luminosity, vision, offence and defence, population problems and insect societies, and the book ends with a 30-page illustrated catalogue of the major insect orders. The discussion of physiological mechanisms in insects is often illuminated by simple analogies with familiar processes. Thus, the hardened insect cuticle is compared with a starched shirt-front and the method by which insects survive extreme cold is compared with our use of antifreeze in cars. However, in the chapter on movement, the mechanism of insect flight is likened to that of a 'flying machine'. This charming Edwardian term conjures up mental pictures of Blériot and the machines flown by his contemporaries. Will the younger generation think only of the supersonic jet or will they be sufficiently sophisticated to wonder which of the many different possible mechanisms of powered flight is really intended? What a pity the specific analogy with certain designs of helicopter was not pressed more closely!

The chapter on growth and metamorphosis is particularly stimulating, because this is one of the author's special interests. The treatment tends to be dogmatic rather than critical, so that the vexed question of whether there is a true 'prepupal' stadium within the puparium of flies is not discussed, but avoided both in the text and in the illustration of the metamorphosis of the house-fly.

The amateur entomologist should not be deterred from collecting and studying the Arctiid moths by the alarmist report that "In some insects such as the tiger moths (Arctiidae) these spines become contaminated at times with the venom from the body and then their effects can be severe". This sweeping statement is, in fact, based on very limited positive evidence. The rarity of reports of any reaction to these common gaudily coloured moths suggests there may be variable human susceptibility to them.

The attempt to popularize results which have been published in the scientific literature has its pitfalls, and Sir Vincent has occasionally fallen into the trap by supplying common names for insects incorrectly. Thus the fig insects are not gall-wasps, and *Nysson* is not a bee but a sphecoid wasp. Regrettably there are also many mistakes in the scientific names both of insects and plants—sometimes species are given names which have not been in current use for almost a century; there are many simple spelling mistakes of the names of common insects (the generic name of the orange-tip butterfly is rendered in three different ways) and there are some misidentifications of insects which are illustrated. An author finds it almost impossible to avoid errors of this kind, so the editors must in future take the necessary steps if this series is to be authoritative.

One regrets these minor faults in a book which nevertheless deserves to reach a wide public. The sixth form school-boy and the undergraduate will get a very good introduction to insect physiology. The amateur entomologist, with his specialist knowledge of one particular group, and the general naturalist, who have been speculating about the ways insects work, will begin to realize that professional entomologists are now finding

*[See also p. 958 of this issue of *Nature*.]

answers to many of their questions. University teachers should take account of the book, which brings an up-to-date factual account of insect physiology within the reach of all undergraduates. It will allow teaching to concentrate more on the processes of research, on how we plan our observations and measurements and how we interpret them to reach the conclusions so persuasively and simply expounded in this book. G. C. Varley

THE FUNCTIONAL UNIT OF LIFE

Allgemeine Cytologie:

Eine Einführung in die Funktionelle Morphologie der Zelle. Von Prof. Ekkehard Grundmann. Pp. viii+423. (Stuttgart: Georg Thieme Verlag, 1964.) 59.70 D.M.

TO-DAY the gulfs in our ignorance are widening because the quantity of information forthcoming is so great. The more we learn the more we realize how little we really know. Even in his own field of study the scientist must restrict his attentions to a relatively small area.

True progress in science, however, depends on integrated knowledge, which can be provided by reviewing the relevant information in a comprehensive manner. The part played by those responsible for 'synthesizing' scientific information becomes more and more important, but their task also becomes more difficult due to the increasing amount and complexity of the information they have to handle. This is probably the reason why, nowadays, monographs are usually written by several authors, each having a restricted interest and knowledge of the subject under review. As a consequence, these 'monographs' tend to lack uniformity and leave many gaps in our knowledge.

Allgemeine Cytologie is the work of one author who is well known in the field of cell morphology, pathology and cytochemistry. The sub-title indicates the scope of the book; it is intended as an introduction to the functional morphology of the cell. According to Prof. E. Grundmann, the cell is the 'ultimate' organized unit of life and therefore knowledge of it is essential to all who are studying or are interested in biological sciences and medicine. Commencing with the discovery of the cell, the review is all-embracing, and it would seem that every structure and particle which have ever been seen, named or described by cytologists, pathologists, geneticists, cytochemists or electron microscopists, are given space in the book. This gigantic task involved the use of about 3,000 references. There is no doubt that the book is a valuable source of information on everything which concerns the cell.

After a brief historical review, the reader is introduced to the architecture of the cell; 100 pages are devoted to the nucleus, a similar number to the cytoplasm and even more to the various processes of cell division. In presenting his information the author follows a definite pattern: first he deals with the morphology, then with the chemical structure or composition of the particular cell constituent, and finally he discusses its function and relationship to other units of cellular metabolism. Prof. Grundmann tries hard to be objective, and as a consequence the text is full of references. It is not a rare occurrence to come across sentences broken at two or three places with the names of several authors. The reader might question the value of the references to descriptive works of the nineteenth century which occur throughout the book. What is the advantage, apart from a purely historical interest, of knowing who first saw and named the astrosphere, sphäroplasma, holschisis, kinoplasma, paragenoplastin, archoplasma, or phragmatoplast? The author's attempt to ascribe roles to them is frequently based on either circumstantial evidence or only assumption. Considering the vast field of information which is summarized in the book, it is not surprising to find, occasionally, a certain lack of critical judgement. The fine structure of the chromosome and the modern concept of DNA replication are discussed in seven pages, while twice as much space is given to 'amitosis', a special type of nuclear division, the occurrence of which is restricted to particular cells. On the other hand, the author deserves praise for the short chapters (Rückblick) in which topics previously described are summarized, the important findings are pointed out and attention is directed to the gaps in our knowledge concerning this particular subject.

Technically, *Allgemeine Cytologie* is well produced, the illustrations are clear and the diagrams are informative. The references are in small print and occupy 50 pages in double columns. There is a comprehensive subject index which makes access to information an easy task.

Next year it will be the three hundredth anniversary of the discovery of the cell. In *Micrographia* (1665) Robert Hooke published two pictures showing "minute bodies" in cork; to him they resembled little empty compartments with firm boundaries. During the ensuing 300 years similar "empty compartments" have been observed to contain the most astonishing array of structures, each with a purpose in cellular function, now well summarized by Prof. Grundmann. His monograph is a fitting tribute to the forthcoming tricentenary. P. C. Koller

CELL BIOLOGY

The Cell: Biochemistry, Physiology, Morphology

Edited by Jean Brachet and Alfred E. Mirsky. Vol. 6: Supplementary Volume. Pp. xiv+564. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1964.) 128*s*. 6*d*.

THIS is the sixth volume of a large and important treatise on cell biology which has been published over the past five years. Its six articles are concerned with topics which were either absent from the earlier volumes or were only touched on briefly.

The first two articles are on the Protozoa. K. G. Grell discusses their nuclei, and W. Trager their cytoplasm. Both authors give a certain amount of space to protozoan physiology, but their main emphasis is on structure, dominated in Trager's article by the recent work with the electron microscope. There is no doubt that a knowledge of Protozoa ought to be more widespread among cell biologists. They show many interesting variations on the common pattern of higher cells and they should be as helpful as invertebrate physiology has been in the development of mammalian physiology. To take two examples, Trager points out that the kinetoplast may provide important clues about the development of mitochondria and the nature of independent organelles, and the abnormal nuclear divisions of many Protozoa (Grell has striking photographs of these in *Aulacantha*) may help us to relate the amitotic division of bacteria to true mitosis.

J. R. Raper and K. Esser give an excellent account of the fungi. In the comparatively short space of a hundred pages, they deal clearly and comprehensively with the genetics as well as the morphology and the complex patterns of life-cycles and sexuality. Fungi, like Protozoa, are a group which should not be left only to the specialists. Although *Neurospora* and yeast have been worked on widely, there are many other fascinating organisms among the fungi. It may well be, as the authors suggest, that they are going to provide the best material for solving the central problem of differentiation.

Another good and comprehensive article (the longest in the book) is that by S. F. Jackson on connective tissue cells. She discusses this subject at all levels, from molecules

to whole cells, and acts as an admirable guide through a complex literature. R. Cordier gives a shorter general survey of the structure of sensory cells in the Metazoa, mostly at the level of the light microscope. The subject, though not the account, is somewhat unsatisfactory at the present time because of the difficulties of relating form to function. Cordier concludes "... that morphological research on sensory cells, even at the submicroscopic level, does not furnish a safe and reliable base on which the satisfactory understanding of their function and activity could be founded", and contrasts this with recent progress on secreting cells.

Finally there is an article on the form and function of the plant cell by B. R. Voeller, with electron micrographs by Ledbetter and Porter. The author does not have space to do full justice to the subject, particularly on the physiological side, and this is indeed a criticism which might be made of the treatment of the botanical material throughout *The Cell*.

Since this may be the last volume, it is worth making a few remarks about the whole series. It is, without doubt, an impressive treatise of high standard which should be in all biological libraries. Equally, it is unlikely to be on the personal shelves of many biologists because it is too expensive and, for students, too detailed. It is made up of detailed review articles and it should therefore be compared with a review journal such as *The International Review of Cytology*. It has the advantage of an editorial policy that can ensure a more or less complete coverage of a field. But a journal has the even greater advantage that it can keep up to date by repeating reviews in a rapidly moving field. As an example, the two reviews on nucleo-cytoplasmic relations in the first volumes of *The Cell* already show signs of ageing. For these reasons, I would predict that the days of the review treatise on this scale are passing.

J. M. MITCHISON

BIOLOGY OF RUSSIAN INLAND WATERS

Fauna and Flora of the Rivers, Lakes and Reservoirs of the U.S.S.R.

By V. I. Zhadin and S. V. Gerd. Translated by A. Mercado. Edited by R. Finesilver. Pp. 626. (Jerusalem: Israel Program for Scientific Translations, 1963.) 128*s*.

NEARLY one-sixth of the land surface of the world lies within the U.S.S.R. and many of the world's largest lakes, river systems and reservoirs are among her numerous water bodies. Considerable resources are devoted to hydrobiological research, much of which is little known in the Western world. Despite the language difficulty, the original Russian version of this book has been an indispensable source of information for hydrobiologists, so an English translation is most welcome.

Better accounts of the general principles of fresh-water biology can be found elsewhere, and these sections have an elementary and old-fashioned approach with some loose writing. Nevertheless, the emphasis put on unfamiliar aspects stimulates thought, and the sections on topics which have been particularly examined in the U.S.S.R., such as rivers and reservoirs, are full of interest. Investigations of the evolution of lake populations and the distribution of species receive special attention and several Russian systems for classifying water bodies are described.

There is a wealth of information on the location, morphology, characteristics and populations of all the larger water bodies (except the Caspian and Azov Seas) as well as the better-investigated smaller water bodies. Leading Russian hydrobiologists and their laboratories are introduced. Special attention is directed to the management of reservoirs and other water bodies, to exploit their biological resources, for which the Russians have many new and unusual ideas.

There is a curious statement on p. 35 (apparently correctly translated) about bacteria being "primary producers", which may be justifiable, but not from the context. The unfamiliar use of "dystrophy" for the condition of severe oxygen deficit under a snow and ice cover should be noted.

The print shows through the pages more than one would expect from an expensive book, and the photographs (which were poor in the original) are not well reproduced. The line drawings, however, are excellent. Many more references to original work would have greatly increased the value of the book. Only authors, water bodies and species are indexed. This deficiency is accentuated by the frequent use of unusual and superfluous technical words and only partly offset by the detailed contents list and the repetitive text. The translation appears to be free from serious error, but there are a few curiosities and some words and phrases which are American or Russian rather than English.

D. F. WESTLAKE

FIGHTING FOREST FIRES

Soviet Progress in Forest Fire Control

Edited by N. P. Kurbatskii. Authorized translation from the Russian. Pp. iii+38. (New York: Consultants Bureau, 1964.) 10 dollars.

RUSSIA has a history of forest fires, but only in comparatively recent times has any attempt been made to estimate the areas involved. These statistics indicate that enormous losses of timber occur as a result of the fires, many of which seem to be located in Siberia. There is no indication in *Soviet Progress in Forest Fire Control* of the causes of the fires. There is one mention of forests being burnt along the Siberian railroad from Tomsk to Krasnoyarsk, a distance of some 300 miles, but the reader is left to make his own deduction of the cause. However, it is pointed out that "during the years of Soviet power the flammability of forests decreased substantially". To substantiate this statement, statistics are given for two 4-year periods, 1906–10 and 1940–44, when the area of forest fires was respectively 269·2 and 124·8 thousand hectares!

The first report in this book is on the comparative fire resistance of different tree species in the Taiga zone, near Tomsk. Statistics are used to demonstrate the differences, but really no new facts emerge. No one would argue with the summing up that, "the thicker the bark of trees, the deeper the root system, the higher the crown, the lower the resin content and content of volatile oils, the less the danger of fire injuries".

The second article is on the effect of relief on forest fires in Western Sayan (west of Lake Baikal). One-third of the forests of the U.S.S.R. are in the mountains, where they are important not only on account of the timber they yield but also for their protective and water regulating roles. The author considers the problem of the relief on the conditions of the outbreak, development and after-effects of forest fires in mountainous areas, but, in trying to suggest control measures, he appears to rely on water courses to act as natural barriers. He states that a network of fire-fighting barriers will be practical only in forests that are being exploited. In other words, as is well known, a good communication system does help in fire-fighting.

The principles of extinguishing forest fires are discussed in a third article, and some of these go into great mathematical detail concerning rates of absorption and heat liberation. Indeed, the author is at great pains to describe how water and fire-resistant chemicals function, but at the end of the article the reader is left wondering to what

use this information has been put in *Soviet Progress in Forest Fire Control.*

The last report is an analysis of the calorific value of moss, fern, grass, conifer needles, branchlets and resin.

This publication is somewhat disappointing; this may be due in part to the expectation gained from the title that the reader is going to learn a lot about forest fire control. He does not, which is a pity.

C. J. TAYLOR

THE WORLD OF PLANTS

The Life of Plants

By E. J. H. Corner. (The Weidenfeld and Nicolson Natural History.) Pp. xii+315+41 plates. (London: Weidenfeld and Nicolson, 1964.) 55*s.* net.

BOTANISTS often wonder why their subject, critically concerned as it is with the food and therefore the primary welfare of the whole human race, has remained so long the Cinderella of the biological sciences. A cynic might suggest that the neglect springs from a deep-seated disinclination in the human mind to grapple with essentials, but there is another and simpler reason. When botany and zoology emerged from their purely descriptive phases the latter was able to advance rapidly because zoologists, being themselves examples of their own subjects, could interpret a good deal about them in terms of personal experience—an advantage that was denied to the botanists. For this reason there has always been a certain difficulty in coming to grips with the study of plants, which has in consequence tended to become either chemistry or physics or, alternatively, taxonomy. Only in recent years has an awareness begun to grow that an enquiry into the principles on which the life of plants is ordered may reveal truths quite as important as those derived from the study of animals. *The Life of Plants* is a valuable amplification of this still small voice and deserves a warm welcome.

The author has a mind of considerable originality and powers of penetration, and he has made good use of both to write an introduction to the world of plants which is novel without being eccentric. Furthermore, the book is very well produced, and notable because nearly all the many illustrations are either new, in the sense that they have not appeared in a book of this kind before, or have been drawn or re-drawn with skill and care, though it must be said, more in sorrow than in anger, that Fig. 96, with its Mercator's projection, is the exception which proves the rule.

The book has fifteen chapters of which the first six provide a remarkable study of the algae, mainly from the point of view of their significance as the modern representatives of the aquatic forerunners of the present sub-aerial land-flora, and it is pleasant to notice here a long-overdue tribute to the neglected writings of A. H. Church. The next six chapters are, in effect, almost entirely concerned with seed-plants, though many others are included, and these amount to what has been sadly lacking in the past, namely a broad general exposition of the flowering plants by one with a really wide interest in, and experience of, tropical vegetation, and this part of the book is certainly likely to draw most attention and applause. Mr. Corner would have been more than human had he refrained from riding one or two hobby-horses, but even these exercises are spirited, and excellent for the sluggish botanical liver. The last three chapters deal with Mr. Corner's second (or is it first ?) love, the fungi, and they are as interesting as the rest.

To do full justice to this unusual book is a hard task and perhaps the most sober verdict that can be brought in with regard to it is that no one can possibly read it without being a better botanist and without knowing more about plants afterwards than he was or did before. To advanced students, and especially to the best of them, it will be a delight, and something to be savoured when all else begins to pall.

Apart from its factual content the book does, however, raise two issues of considerable interest and wider context. The first, and lesser, is how far it is possible to breathe new life into familiar and perhaps dry facts by clothing them in new words. Mr. Corner's expressive style of writing is one of the pleasures of his books, but at times it seems to take him dangerously near incoherence and there is a risk here and there that the less-sophisticated reader may fail to understand, or may even misunderstand, his meaning. The book is unlikely to prove easy reading to anyone without a sound grounding in botany.

The second point is more serious. The central theme of the book is, as it must be in all biological writings which are neither formal nor systematic, that of organic evolution. A writer dealing with this subject can easily slip into a "slough of despond" from which escape is difficult, and it is a tribute to Mr. Corner that he avoids this peril. Nevertheless one must feel some regret that so acute a thinker has not, apparently, been able to free himself more completely from the indoctrination of an outworn Darwinism, or to take more account of the change in attitude towards this in recent years.

R. GOOD

A NEW AGRICULTURAL ATLAS

An Agricultural Atlas of England and Wales

By Dr. J. T. Coppock. Pp. 255 (205 maps). (London: Faber and Faber, 1964.) 63*s.* net.

THIS new *Agricultural Atlas of England and Wales* contains, in addition to a large number of maps, a substantial text providing historical and explanatory comment. The author is to be congratulated on its comprehensive nature, because only by reference to such a detailed study can the considerable diversity of agricultural activity in these areas be appreciated.

After a brief statement of the aims of the *Atlas* and a description of the methods used in its preparation, the first chapter presents an interesting discussion of the factors, other than the more obvious physical and economic ones, which can affect enterprise choice and combination in farming. The second and third chapters provide a well-illustrated physical and economic background for the distribution of crops and livestock. There are then three chapters dealing in some detail with the distribution of tillage crops, grassland and horticulture, and a chapter covering the distribution of the various classes of farm livestock. In these four chapters, which comprise the major portion of the *Atlas*, the problems of describing crop and livestock distribution graphically have been given full consideration. By using more than one map for the individual tillage crops and types of grassland, the author has attempted to illustrate not only their distribution as a proportional area but also their relative importance. In order to map the regional importance of different classes of livestock, a weighting system based on relative feed requirements has been used. This converts the various classes of livestock into a common unit, the "livestock unit". The resulting difficulties of map interpretation can, however, be overcome by reference to the accompanying text. The distribution of separate classes of livestock is shown both in relation to the distribution of other livestock and in relation to land use. An interesting feature of the chapters on tillage crops and livestock is the inclusion of maps indicating the most common associations of crops and of livestock, and how these associations are distributed.

The remainder of the book is devoted to a chapter dealing with combinations of enterprises in farming, and

a chapter discussing the future possibilities for mapping more fully the agricultural information which has been, and is, accumulating in such quantity throughout England and Wales.

There are three appendixes and a select bibliography. The first appendix provides additional information about sources and the construction of individual maps. The second, contributed by A. Sentance, of Birkbeck College, London, is a technical description of the processing of data for the *Atlas* by computer. The third contains details of the various crop, livestock and enterprise combinations, and some selected statistics. The bibliography is restricted to publications of direct relevance to the contents of the *Atlas*, and is helpfully classified by reference to the various chapters. J. L. BEVERIDGE

MILESTONES IN VIROLOGY

Selected Papers on Virology

Edited by Nicholas Hahon. Pp. xv+363. (Englewood Cliffs, New Jersey, and London: Prentice-Hall, Inc., 1964.) 60*s*.

AN alternative title to this book might be "The Birth of a Science"; virology, that ugly word of doubtful parentage, is now a dynamic biological discipline.

For his compilation, Dr. Hahon has selected forty papers, spanning nearly two centuries, beginning with the paper by Edward Jenner on cowpox in 1789, and ending with the recent work of Trentin, Yabe and Taylor on the quest for human cancer viruses published in 1962.

It is difficult to make a representative selection of only forty papers out of such an immense field; but the author has carried out his invidious task with care and perspicacity. Whatever his choice, it is inevitable that some criticism of it would be made. One feels that Iwanowski deserves more than casual mention, for it was he who first proved the existence of the 'ultramicroscopic filterable viruses', to use a phrase now long obsolete, by showing that the agent causing tobacco mosaic was still infectious after passage of a Pasteur–Chamberland filter candle. F. C. Bawden and N. W. Pirie also deserve a niche in this hall of fame, since it was they who showed that the virus of tobacco mosaic, isolated by Stanley in 1935, was a nucleo-protein.

The time has long gone by when it could be stated that "plant viruses are of an entirely different nature from that of the animal viruses". We should recognize now that there are not four different kinds of infectious agents, each in its watertight compartments of bacterial, plant, insect and animal hosts. The science of virology comprises the study of viruses, no matter from what type of host they are derived. Once freed from the host cell, the similarity between some viruses of completely different origin is remarkable, and who would be bold enough to say at a glance whether a small near-spherical virus, when viewed in the electron microscope, was that of poliomyelitis or turnip yellow mosaic ?

The boundaries which separated these four types of viruses are indeed becoming indistinct and doubtful, and the versatility of viruses and their ability to adapt to different hosts are increasingly alarming. Certain plant viruses can multiply in their insect vectors; the virus of swine influenza, which is thought to be a relic of the great influenza pandemic of 1918–19, can multiply in a parasitic lung worm as well as in the pig, and a plant virus causing 'wound tumours' in legumes is thought to have a serological relationship with one of the reoviruses pathogenic to man.

The final paper in this book deals with an absorbing aspect of virology, the viral causation of tumours and the possible relationship of viruses with human cancer. It shows that a virus from man, one of the adenoviruses, has oncogenic properties and produces tumours when injected into new-born hamsters. Again, who would have predicted that the Rous sarcoma virus, originally only transmissible between certain strains of chickens, is now apparently capable of producing tumours in Primates ?

By compiling this book, Dr. Hahon has rendered a great service to virology. He has brought within easy reach many early papers, often difficult to obtain, and, in the increasing *tempo* of virus research, has emphasized the discoveries of major importance. K. M. SMITH

DRUGS AGAINST VIRUSES

The Background to Chemotherapy of Virus Diseases

By Prof. C. H. Stuart-Harris and Dr. Lois Dickinson. (A Monograph in the Bannerstone Division of American Lectures in Living Chemistry.) Pp. xi+175. (Springfield, Ill.: Charles C. Thomas, 1964.) 10.50 dollars.

THERE is a feeling in the air that after many years of frustrated hopes we may be about to get somewhere in the field of chemotherapy for viruses. We have learnt so much more recently about the chemistry of viruses and how they multiply that it is now possible to approach the subject in a logical way: no need any more to take bottles off the shelf in alphabetical order and test them in a hit-or-miss manner.

This little book is by the professor of medicine in the University of Sheffield and a senior worker at the Research Department of Boots Pure Drug Co., Ltd., in Nottingham. Between the two, they well know the difficulties of the subject both from the clinical and laboratory angles. The background of the subject is presented in the first three chapters on the chemical composition and multiplication of viruses, their pathology and on the phenomena of virus interference and interferon. The matter is briefly presented in an interesting and up-to-date manner, but does not contain anything much that is not covered in similar accounts in recent text-books and reviews. Chapter 4 gets down to the subject proper, describing recent experimental work in the field, with results obtained in tissue culture, in infected eggs and in laboratory animals. Formulæ of the more important groups of compounds are given and the rationale of their use is discussed. Chapter 5 contains a particularly useful account of the criteria necessary to establish that a drug is of value in a human infection. I myself feel that rather more prominence might have been given to the very encouraging results of using a thiosemicarbazone compound for prophylaxis of variola in Madras. In Chapter 6 the authors deal with the rickettsiæ and the psittacosis–lymphogranuloma–trachoma (P–L–T) group. They write that "the taxonomic position of the rickettsiæ and, to an even greater extent, of the P–L–T group of organisms is still controversial". This is scarcely true any longer: it is almost universally agreed that these are bacteria rather than viruses. The authors contrast them with "the true viruses", but occasionally talk of them as if they really were viruses. Their inclusion in this book is understandable, since it is only against them that chemotherapy has proved its worth: knowledge about this may prove of value in describing a 'background'; it at least shows that we need not despair of tackling an obligate intracellular parasite. A final chapter deals with antibacterial chemotherapy, the action of steroids, and the use of serum as additional aids in the fight against viruses.

Despite our hopes for the future, present achievements in the field of virus chemotherapy are admittedly very meagre. This book simply tells of present difficulties and will serve, one hopes, to consolidate a jumping-off ground for future advance. But for less than 120 pages of straight text, a price of 10.50 dollars is indeed fierce.

C. H. ANDREWES

CHEMOTHERAPY

Advances in Chemotherapy

Vol. 1. Edited by Abraham Goldin and F. Hawking. Pp. xi+579. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1964.) 125*s*.

THE number of 'Advances in . . .', 'Reviews of . . .' and, in general, scientific books which carry the names of editors and not those of authors on their front leaf is increasing steadily and overwhelmingly like cars on the road. This simile is not so far-fetched as it sounds, because the capacity of libraries (and the brains of their users) is limited as much as is the space for traffic. One is therefore inclined to utter a 'Gertrude Stein-like' sigh when looking at another publication: "A Review is a review is a review is a review . . .", or "Advances are advances are advances are advances . . .".

My plea to publishers and would-be editors alike is: Please think again before committing yourself to another tome or series of tomes, unless they fill a real need. Having said all that and believing strongly in this warning against the rapid increase of modern scientific publications, it must be stated with the same force of conviction that *Advances in Chemotherapy* does fill a gap, and is well designed, and that the first volume is very promising.

The names of the editors, one a leading cancer expert, the other a well-known team leader of research into the treatment of infectious diseases, are a guarantee that the choice of new articles by (so it is hoped) new workers in chemotherapy will be a good one. This is indicated in their preface to the first volume which contains the basic outline of the principles which will be applied to future progress reports. There follows a homily on the historical side: nothing new, but well summarized in eight pages.

The editors state in their introduction that articles on clinical chemotherapy, as such, will not ordinarily be included, except in certain cases. This is a pity, because it reveals the enormous rift between experimental and clinical science which instead of getting narrower is widening all the time. Luckily, the editors have thrown their restrictions out of their editorial window and allowed Dr. Zubrod to discuss his ideas about preclinical studies and the quantitative treatment of clinical trials with reference, as he says, to the complex and difficult problem of diseases of unknown ætiology such as the acute leukæmias. A chapter follows on the chemotherapy of protozoal diseases with special reference to trypanosomal ones, and an excellent and brief monograph on viral diseases by R. L. Thompson. Hope is expressed for a frequent review of this topic activated recently by the clinical results with isatin thiosemicarbazone derivatives and the upsurge of interest in the tumour virus field. As an example of the revival of alkaloids as therapeutic agents the survey of the *Vinca rosea* group of natural plant products illustrates that chemotherapeutic remedies of natural origin are not confined solely to products of moulds and micro-organisms. It appears that the *Vinca* alkaloids, which are chemically complex polycyclic indole derivatives, have not only anti-leukæmic and anti-tumour properties but also show, possibly, some effects on viruses. A very extensive and excellent review by Foley and Epstein treats critically a very important aspect of cancer chemotherapy. In several instances the difficulty of interpreting results in animals by clinical terms is discussed in the book. Is it therefore right to go one step further back and use cell culture in its various forms for examination of anti-tumour remedies? It would seem that the authors have made a good case in favour of cellular systems to complement findings in whole animals, but they have gone to greater length than the immediate application of a technique to screening procedures. They thoroughly discuss the properties of cell lines, their nutritional and biochemical status and their possible morphological and mutational changes during prolonged culture. One of their sub-chapters which might carry the greatest promise for future work in relation to drug evaluation deals with organ culture.

The last three contributions describe the present state of research into the role of immuno-reactions during anti-parasitic chemotherapy by Goble, an extensive treatise on drug synergism in cancer chemotherapy by Venditti and Goldin and a stimulating chapter on newer ideas on enzyme inhibition by N. O. Kaplan and M. Friedkin. The existence of a host–drug–parasite complex prompts Goble to review, and most competently so, the interplay of antigen–antibodies not only with infected hosts but also with drug-treated hosts. This is most useful, as the old tag, 'Let Nature take its course', has gained some credence in that more attention is being directed to the part played by the defence mechanisms available to the host even during and after drug therapy.

The question of improving chemotherapeutic effects by applying truly synergistic mixtures or by enhancing the specificity and selectivity of a drug is particularly important in cancer chemotherapy, where the tumour in many instances does not behave like a parasite. It is therefore valuable to be given assessments of nearly all the present-day potential approaches by two experts of the National Cancer Institute, Bethesda. Mixing biochemical, biological and clinical arguments, they arrive at interim conclusions which represent an excellent stepping stone for further experiments and controlled trials.

The contribution from Brandeis University brings up to date the problems of antimetabolites by adding to the classical ones with their slight modification of normal substrates, the unrelated ones which, so it is claimed, achieve their effect by 'allosteric inhibition'—an expression proposed by Monod and Jacob. This widens enormously the possibilities of preparing regulators which will interfere not with peripheral but with central cellular mechanisms.

The volume is well edited and follows the best traditions of Academic Press, but regrettably the subject index is skimpy. If reviews or advances have to be produced, a high-quality index is as important as the high level of contributions.

F. Bergel

ASPECTS OF CHEMOTHERAPY

Antimicrobial Agents and Chemotherapy—1963

Proceedings of the Third Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, D.C., October 18–30, 1963. Edited by J. C. Sylvester. Pp. xiv+806. (Ann Arbor, Michigan: American Society for Microbiology: distributed in Europe and London by H. K. Lewis and Co., Ltd., 1964.) 12 dollars.

ANTIMICROBIAL Agents and Chemotherapy—1963 consists of 139 papers, which between them touch on many aspects of chemotherapy. Some sections deal with new antibiotics which are receiving clinical trial, some with the further evaluation and limitations of antibiotics the place of which in medicine is already established, and others with more general aspects of the prevention and management of refractory or dangerous infections. Much of their contents is concerned with bacterial infections in man or laboratory animals; but one article discusses the use of sulphonamides, different antibiotics and nitrofurans for the treatment of bacteriæmias in fish—a problem of some importance to the 770 commercial fish hatcheries in the United States; another is concerned with the value of antibiotics, particularly hygromycin *B*, as anthelmintics in veterinary medicine; several deal with antifungal substances, or the treatment of fungal infections; and a few describe antibiotics which have antitumour activity.

The pattern of the articles on the laboratory and clinical evaluation of antibiotics gives some indication of the nature of the clinical problems in bacterial chemo-

therapy which now have particular importance. Thus, a good deal of space is given to gentamycin, a family of antibiotics produced by species of *Micromonospora*, which appears to be related chemically to neomycin and kanamycin. This product aroused interest because it showed high activity against many Gram-negative pathogens, including *Pseudomonas aeruginosa*, which is resistant to most of the antibiotics in common use. But, although it may have a place in the chemotherapy of urinary tract infections, toxicity appears to limit the usefulness of gentamycin for the treatment of bacteriæmias.

A further section contains articles on the use of one of the cephalosporins—cephalothin—and some of the new penicillins for the treatment of a variety of infections, including infections of the urinary tract and bacteriæmias caused by penicillinase-producing staphylococci and by Gram-negative bacilli. These substances are made attractive by their rapid bactericidal action and low toxicity, and it seems that the cephalosporins show the interesting property of provoking no reaction in patients hypersensitive to the penicillins. Together, they widen considerably the possibilities of safe and effective therapy in bacterial disease. But some organisms, such as species of *Pseudomonas*, have so far remained outside their scope, and the existence of different β-lactamases with penicillinase or cephalosporinase activity, or both, still imposes limitations on their range of usefulness.

Despite the revolution in bacterial chemotherapy brought about by antibiotics, the continued importance of measures designed to diminish the incidence of infection in hospitals can scarcely be over-emphasized. The authors of one paper estimate that 31,000 deaths from bacteriæmia by Gram-negative rods might have been prevented in American hospitals in 1962 if safer methods of catheterization had been used. Other papers deal with cross-infection and infection after surgery.

In addition to many articles concerned with the use of antibiotics there are several papers on the chemistry of antibiotics, including two on unusual peptides, one paper on the role of acetate in biosynthesis and others which deal with the mode of action of penicillin, streptomycin, erythromycin and polyene antibiotics respectively. One of the latter, by J. T. Park, leads to the interesting conclusion that penicillin inhibits enzymes concerned with mucopeptide synthesis only under conditions of cell growth and that this is the real reason why penicillin only kills growing cells.

This book contains a number of articles of high quality and its range of topics is likely to make it of wider interest than some of its predecessors. E. P. ABRAHAM

PROBLEMS OF NUTRITION

Proceedings of the Sixth International Congress of Nutrition

Edinburgh, 9th to 15th August 1963. General Editors: C. F. Mills and R. Passmore. Pp. xv+683. (Edinburgh and London: E. and S. Livingstone, Ltd., 1964.) 105*s*. net.

THE sixth International Congress of Nutrition, held in Edinburgh during August 9–15, 1963, centred on a series of symposia on the following subjects: nutrition and health; nutrition and early growth; dietary factors in the genesis of cardiovascular disease in man; nutrition in developing populations; animal production under adverse climatic conditions; nutritional and functional aspects of trace element metabolism; protein–calorie deficiency and its amelioration; absorption of nutrients; endocrine function and reproduction in relation to nutrition; balance experiments and isotopes in nutritional investigations. The papers delivered at these symposia, and also those delivered at a special plenary session on "Nutrition as the Servant of Man", are reproduced in full in the *Proceedings*. The volume also contains a message from H.R.H. the Duke of Edinburgh, an address by the president of the Congress, D. P. Cuthbertson, special plenary lectures on "Nutrition and Numbers" and "Nutritional Factors in Gallstone Formation" by J. G. Harrar and H. Dam, respectively, and abstracts of some 450 papers read at sessional meetings. About 1,300 names are given in a list of members; the Congress was thus not exceptionally large as international scientific congresses go nowadays.

In the wide field of subjects covered, there was some emphasis on nutrition in the developing countries, though the majority of members came from the United Kingdom and the United States, with continental European countries less fully represented. The small number of members from Asia, Africa and Latin America made useful contributions. An optimistic note was struck in many of the papers dealing with protein–calorie deficiency disease, reflecting the progress made in investigating and attacking this world-wide problem during recent years. There was, however, recognition of the vital importance of the 'population explosion' and its effects on world food requirements. Advances in biochemical and physiological research related to nutrition were the theme of some of the symposia, for example, those concerned with growth, trace elements, absorption, endocrines and the use of isotopes in nutritional investigations. The symposium on diet and cardiovascular disease produced nothing very new. At the special plenary session on "Nutrition as the Servant of Man", held at the end of the Congress, papers were delivered on "Food as a Function of History", "Nutritional Problems of Tanganyika", "Nutrition and World Health", "Application of Nutritional Research to Practical Problems of Nutrition", "The Challenge of the Pre-school Child", and "The Pre-school Protection Programme". These titles illustrate some of the broad issues which particularly interest workers in nutrition and associated fields to-day.

The volume is excellently printed and the editors are to be congratulated on having prepared it for publication in so short a space of time. It does not deal with the social side of the Congress, which was well organized; it can be taken for granted that participants in any scientific congress held in Edinburgh will enjoy themselves. The purpose of this Congress as a whole was well stated in the Duke of Edinburgh's message: "Knowledge in the field of nutrition has grown enormously during the past few years, and it has become more than ever imperative for those engaged in providing and applying this new knowledge to come together periodically and to survey the position". W. R. AYKROYD

LAMARCK AS A GEOLOGIST

Hydrogeology

By J. B. Lamarck. Translated by Prof. Albert V. Carozzi. Pp. vi+152. (Urbana, Ill.: University of Illinois Press, 1964.) 4.75 dollars.

JEAN Baptiste Lamarck (1744–1829), the founder of invertebrate palæontology and the begetter of a theory of evolution, indirectly contributed much to the advancement of the science of geology. His systematic study of fossil shells in the Paris Basin helped to establish the science of palæontological stratigraphy, while his theory of evolution passing through the hands of Lyell and Charles Darwin provided geology with a method and a doctrine. In contrast to these important contributions to science, we have the work, *Hydrogéologie*, which was published in 1802 at his own expense. The book is something of a curiosity, because, while it raises new and most fundamental problems in geology, at the same time it produces what would be considered nowadays the most fantastic solutions. Lamarck's contemporaries derided it, later generations forgot it, and historians of geology, with the

notable exception of Archibald Geikie, have ignored it. However, the book has many merits from the points of view both of the problems it raises and the attempts it makes to solve them. The English translation of *Hydrogéologie* by A. V. Carozzi along with his valuable annotations and critical notes represent an important contribution to the history of science. This critical apparatus comprises a large part of this edition. It not only makes Lamarck intelligible to the modern scientist but also puts his work into proper perspective.

Four fundamental problems are discussed by Lamarck: denudation of land by running water and formation of valleys and mountains, origin of continents and oceans, displacement of continents and oceans, and organogenic formation of rocks. In his discussion on the degradation of land, denudation and deposition of sediments in seas and oceans, Lamarck, although aware of the existence of highly inclined strata, assumed that the continents were covered by horizontal strata forming plateaux and carved out by rivers. From this he deduced that all mountains, except those of volcanic origin, were formed from watershed ridges of the plateau. Lamarck was obviously influenced in his conclusions by his immediate surroundings—the Paris Basin. Hutton, on the other hand, living in Scotland had a great advantage over Lamarck due to the diversity of the surrounding countryside. Oceans and seas occupy hollows, while continents are bulges in the Earth's crust. According to Lamarck the hollows are scoured by tidal ebb and flow, while continents represent elevated sea bottoms, a conclusion proved by the abundance on the continents of strata containing marine fossils. Again, forces due to the Moon produce westward drift of oceanic waters, which explains their westward drift over the continents.

While in the first chapter we gain a glimpse of geomorphology, in the second we meet geophysics, or, more precisely, isostasy—where Lamarck discusses the 'centre of the globe' not coinciding with the 'centre of gravity' of the Earth, in reference to the asymmetrical disposition of land masses and oceans. The idea that continents and oceans show a constant displacement contained the embryo of an idea which led to the science of palæogeography and palæostratigraphy. While Hutton, in his famous treatise, laid great emphasis on magmatic processes and said little about organisms, Lamarck as a biologist (he invented this term) grossly over-estimated the influence of organisms as rock builders. He correctly stated that limestones were formed by the remains of calcareous organisms, but then he also stated that clay was formed by plants, obviously mistaking plant ash for clay. Bad as it sounds, this statement is followed by a lengthy argument to prove that in Nature there is a tendency for compounds, such as minerals, to decompose into their constituent elements, while the formation of compounds is due entirely to living organisms, the only pure element being silica. To cross the t's and dot the i's, Lamarck, at the end of his book, provided an "Analytical Index" and here are parts of two entries: "Granite. It is not a primitive material because its components or their derivatives originate from living organisms which therefore must have existed previously . . ." and "Silica or vitreous earth. It is the only existing elementary earthy substance, and consequently the only one which is not of compound nature . . .".

It is to some extent a pity that a genius like Lamarck should have produced such a speculative book in which genius and ridicule appear to be closely intermixed. Obviously this can be put down to his neglect of geological field studies and also to his ignorance of contemporary literature, especially, for example, Hutton's, whose original paper was published in 1788 and his book in 1795, and whose theory is mentioned by J. C. Delamétherie in his *Théorie de la Terre* published in Paris in 1795. However, as the saying goes, it is easy to be wise after the event. Lamarck, in his little book *Hydrogéologie*, raised many of the fundamental problems which concern geologists to-day: geomorphology and the evolution of the land surface, migration of land and sea, and problems of geophysics and palæogeography, gradualism versus catastrophism, actualism versus diluvianism, and the organic origin of certain rocks. Finally, it should be pointed out that Lamarck's fundamental philosophical concept of the world as evolving in a plenitude of time is similar to the fundamental concept of Hutton.

Hutton: "Here is a compound system of things, forming together one whole living world; a world maintaining an almost endless diversity of plants and animals, by the disposition of its various parts, and by the circulation of its different kind of matter."

Lamarck: "On our planet, all objects are subject to continual and inevitable changes which arise from the essential order of things. These changes take place at a variable rate according to the nature, condition, or situation of the objects involved, but are nevertheless accomplished within a certain period of time."

Hutton: "But though, in generalizing the operations of nature, we have arrived at those great events, which, at first sight, may fill the mind with wonder and with doubt, we are not to suppose, that there is any violent exertion of power, such as is required in order to produce a great event in little time; in nature, we find no deficiency in respect of time, not any limitation with regard to power. But time is not made to flow in vain; nor does there ever appear the exertion of superfluous power, or the manifestation of design, not calculated in wisdom to effect some general end."

Lamarck: "Time is insignificant and never a difficulty for Nature. It is always at her disposal and represents an unlimited power with which she accomplishes her greatest and smallest tasks. The modifications to which all things in this world are subjected concern not only their shape and nature, but also their mass and even their location."

S. I. TOMKEIEFF

RELATIONSHIP BETWEEN SPECTRA AND CHEMICAL STRUCTURE

Introduction to Infra-red and Raman Spectroscopy

By Norman B. Colthup, Lawrence H. Daly and Stephen E. Wiberley. Pp. xii+511. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1964.) 86*s*.

Spectra Structure Correlation

By John P. Phillips. Pp. ix+172. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1964.) 48*s*.

SPECTROSCOPIC measurements are nowadays so much a part of everyday chemistry that there is an obvious need for introductory texts which explain the principles involved and make clear the areas of useful application. *Introduction to Infra-red and Raman Spectroscopy* and *Spectra Structure Correlation* are only two of a substantial number of books of this kind that has appeared in recent months, and that of Colthup, Daly and Wiberley, for example, covers much the same sort of ground as Rao's *Chemical Application of Infra-red Spectroscopy*, issued recently by the same publisher. Several other texts are known to be under way, and the chemist is likely to be presented with a plethora of introductory material from which he may find it hard to choose that which best fills his needs.

There is, however, a good deal of scope for variation in treatment and in emphasis. A chemist wishing to use these methods needs to know at least a little about vibration/rotation spectra and of the effects of molecular symmetry on band shapes in the vapour phase. He must

understand enough about symmetry properties and group theory to be able to apply the basic selection rules, and it is at least highly desirable (and in many branches of chemistry, essential) that he is familiar with the concepts of normal co-ordinate treatments and the applications of matrix methods to the detailed analysis of the spectra of small molecules. Add to this the necessity for some instruction in instrumentation, and experimentation, the enormous volume of empirical (and therefore largely incompressible) data on group frequencies, and the much smaller volume of work which contributes to their understanding, and one can see that a good deal of careful selection and compression is required if one is to produce a book and not a many-volume encyclopædia. Indeed, most of these topics (all of which are dealt with in the Colthup text) already have several advanced texts of their own.

In the Colthup book the emphasis is almost equally divided between group frequency data and the more fundamental and instrumental aspects. The first chapters on vibration/rotation spectra and on experimentation are adequately done but are in many ways essentially similar to the monographs which have already appeared in many compendia on physical methods in organic chemistry. It is difficult to see how it could be otherwise with the limited space available, but the facts are clearly stated. The third chapter on the classification of molecules is a particularly interesting one, dealing as it does with symmetry properties, point groups and the application of group theory. This is the area which the average chemist with no spectroscopic training finds most difficult to understand and where there is a real shortage of sufficiently elementary texts to enable him to get to grips with the subject. This chapter and those on the theoretical analysis of molecules and the calculation of thermodynamic functions are well done and attempt to develop the subject in a way that can be followed by those who have no previous knowledge of group theory or matrix algebra. The illustrations showing symmetry elements are particularly good, but the compression required to cover so much ground in so short a space will take its toll of many an organic chemist, who will find he needs to re-read these chapters very slowly and perhaps many times before he has assimilated their contents. Nevertheless, he will be wise to do so, as he will find it hard to locate short elementary treatments of these topics.

The substantial chapters on various aspects of group frequency also suffer somewhat from compression. The chapter on the origins of group frequencies is well done and an astonishing amount of factual data is packed into the 150 pages dealing with group frequencies of specific groups. These chapters are well worth while and probably represent the best that can be done in the limited space available, although with some of the minor groups the compression leads to the text merely restating in words the frequency ranges which are already available in the tables or charts. Perhaps the most significant weakness is the absence of sufficient guidance on the relative reliabilities of the individual correlations. The section on the trustworthy OH stretching frequencies is followed by that on the untrustworthy OH bending modes and there is little indication that the stretching bands are to be preferred. It is true that anyone who has read the earlier chapters with sufficient care could infer this for himself, but organic chemists are not always systematic readers. This point is important because in almost any journal nowadays one can find papers showing how very much more the inexperienced man can read into a spectrum than can his more experienced counterpart.

Despite these limitations this is an excellent text and can be strongly recommended. It fulfils its difficult introductory task extremely well.

The text by Phillips is also an introductory text, but of a different kind and using a much broader brush. Within the limits of 172 pages it reviews spectral structure correlations from the far ultra-violet to the far infra-red. With so wide a scope, something has to go, and in this case it is the instrumental and theoretical side. The author rightly states that theoretical discussions of the broad spectrum here considered, even at the minimum level, would leave no room for the text. This is therefore a handbook and survey of data, tersely and concisely presented. Measurements from several spectral regions often provide a degree of certainty which cannot be obtained from any single region alone, and the collection together of the various correlations is therefore long overdue. The job has been well done, within the limitations of size.

The utility of the book will depend very much on the background of the reader. For those who have some reasonable grounding in spectroscopic theory this will provide a handy, concise manual of correlation data. It will, no doubt, be welcomed also by many who have no such background, but they should be more wary of using it, as the pitfalls of using spectral correlations without some understanding of their origins are many and deep. None the less there are other texts available which will provide the necessary background of theory, and it must be said that this is a useful volume which fulfils well the aims of the author, and it will certainly be widely used in the laboratory. The fact that it is relatively inexpensive is also something deserving of comment and praise.

L. J. BELLAMY

DEVELOPING FIELDS IN POLYMER SCIENCE

The Chemistry of Cationic Polymerization

Edited by P. H. Plesch. Pp. xv + 728. (Oxford, London, New York and Paris: Pergamon Press, 1963.) 200*s*.

Newer Methods of Polymer Characterization

Edited by Bacon Ke. (Polymer Reviews, Vol. 6.) Pp. xvi + 722. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, 1964.) 185*s*.

Configurational Statistics of Polymeric Chains

By M. V. Volkenstein. Translated from the Russian edition by Serge N. Timasheff and M. J. Timasheff. (High Polymers: a Series of Monographs on the Chemistry, Physics and Technology of High Polymeric Substances. Vol. 17.) Pp. x + 562. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, 1963.) 150*s*.

MANY fields of polymer science are to-day in a state of rapid development in which, although classical treatments still provide a broad basis for interpretation, new concepts, refinements and information are being added at an increasing rate. Stimuli given by the work of Ziegler, Natta and many others have intensified work on polymerization and led to the discovery of new catalysts and methods and to new theories of mechanisms. The production of stereoregular polymers and of block and graft copolymers, the search for polymers of high thermal stability and other recent developments have resulted in the production of many complex and often highly sophisticated polymers which require very detailed knowledge in order to understand their structures and interpret their properties. New methods of examination and characterization of polymers, often involving advanced physical techniques and instrumentation, have been and are being developed. New approaches to problems of polymer conformation, configuration and shape have become necessary.

Information regarding such present-day developments is usually scattered in journals, and detailed information, because of limitations of space, is often absent. Comprehensive accounts and critical reviews, at the research-level, are therefore of great value to the polymer scientist. The

rate of development in some fields has become so great that it is often difficult for a single author to produce such an account or review. The alternative, a collection of contributions from experts on different aspects of the field, requires careful editing in order to ensure that the right level is maintained and serious overlapping does not occur. In either type of book it is desirable that recent work should be seen against a background of earlier investigations and theory, and it is essential that references be as up to date as possible. The three books under review illustrate, to varying extents, these points and some of those in the preceding paragraph.

That edited by Dr. Plesch provides an account of addition polymerizations in which the chain carrier is a cation. Although emphasis in recent studies of polymerization has perhaps been on stereospecific and radical polymerizations, cationic polymerization has not been neglected and the publication of this book is timely. Contributions from eighteen well-known experts lead the reader from the relevant fundamentals of organic chemistry to detailed consideration of the cationic polymerization of a wide range of compounds. The first chapter deals with the existence, formation, stability and structure of carbonium ions and is logically followed by the consideration of organic reactions related to cationic polymerization. The mechanisms of radical, cationic and anionic polymerization are then compared, so that cationic polymerization is seen in relation to a wider context. Successive contributions then deal with the cationic polymerization of olefines, aromatic compounds, polyenes and non-conjugated diolefines, vinyl ethers, cyclic and other oxygen compounds, nitrogen compounds and sulphur compounds, the last-mentioned including radical and anionic mechanisms as well as cationic. Other contributions deal with cationic reactions of polymers, cationic graft polymerization and cationic polymerization induced by high-energy radiation. A final chapter contributed by the editor, although short, gives valuable information on experimental techniques.

Omissions, such as separate accounts of heterogeneous reactions and complex catalytic systems derived from metal halides, are pointed out by the editor and are not serious. The level of the contributions is uniform and is that of a research monograph. Inevitable overlapping seems in most cases to clarify rather than confuse. Earlier work is critically considered, and the literature is well covered to the end of 1960. In addition, many important investigations published in 1961 and 1962 are cited in appendixes and editor's notes. The latter, distributed liberally throughout the book, also provide many stimulating comments. Dr. Plesch is co-author of one of the contributions and author of two others. These, the level of the book as a whole and his editor's comments, reflect his enthusiasm and influence in the production of a critical, authoritative and well-balanced review of cationic polymerization.

Newer Methods of Polymer Characterization also consists of separate contributions from different experts, each dealing with a method or methods at present in use, but which has or have not generally been previously described in detail. Each generally proceeds from a brief theoretical interpretation to applications of the method and a discussion of its scope and potentialities. Apparatus and techniques are only described in cases where such information is not readily available. As is perhaps to be expected, emphasis on different aspects varies. Applications are emphasized in a chapter on investigations of microtacticity which critically considers solid-state and solution methods. Another discusses applications of high-resolution nuclear magnetic resonance to configurational and structural problems. A chapter on optical methods of characterization describes different methods of attacking the problem. Five other chapters, dealing with polarization and deuteration in infra-red spectra, optical rotatory dispersion, fluorescence techniques, small-angle X-ray investigations and electron diffraction, may also be classed as optical methods. Two chapters, by the editor, deal with differential thermal analysis and a new method involving differential thermometric measurements during mechanical deformation. The remaining chapters deal with solution or related properties. Two of them, one on column fractionation and largely restricted to elution chromatography of polyolefines and the other on elasto-osmometry, apply a method to a particular problem. Another, on osmometry and light-scattering at elevated temperatures, deals with new techniques in well-established methods. The remaining contributions are concerned with density-gradient centrifugation, flow birefringence and monolayers of polymers.

The influence of the editor is also apparent in this book. In addition to his two contributions he has maintained a reasonably uniform level and generally avoided overlapping with other review publications. Each chapter provides references up to the end of 1962, and in some cases references to work in 1963 are also given. The book as a whole is a valuable and reasonably concise review of some newer methods of characterization and their use and potentialities in polymer research.

Configurational Statistics of Polymeric Chains is by a single author. Knowledge of chain configurations and their statistical distribution is essential to an understanding of the molecular properties of polymers and their behaviour in bulk and in solution. Interpretation of chain configurations in terms of hindered rotations about single bonds in the chain provides a basis for the use of mathematical treatments of models for polymer chains. This approach, extensively used by the author and his collaborators, is developed in detail. As in *The Chemistry of Cationic Polymerization*, the first chapters provide a background, giving a succinct account of relevant polymer theory and detailed consideration of internal rotation in small molecules and rotational isomerism. The concepts developed are then applied to polymeric chains. Different models used to represent such chains are critically discussed. The properties of crystalline polymers and the stereo-chemistry of polymer chains are topics in a chapter which includes theories of melting and chain configurations in crystals. A wide range of polymers, including natural ones, is considered. Real polymer chains are contrasted with model chains and the effects of various factors on chain dimensions are assessed. A chapter on optical anisotropy of polymer chains includes accounts of anisotropic polarizability, photo-elasticity in polymers and the Kerr effect in polymer solutions. A final chapter deals with the stretching of polymeric chains and networks and includes the thermodynamics of rubber elasticity and the network theory of high elasticity.

Treatment is inevitably often mathematical and the methods are frequently advanced. The scope of the book is wide including the consideration of many properties of polymers related to chain configuration and even embracing such aspects as the genetic code in polynucleotides. It was originally published in Russian in 1959 and references to the original literature up to 1958 are full and detailed. As Prof. P. J. Flory states in a foreword, the comprehensive coverage of earlier work lends perspective to newer developments. The rapid progress in this field has inevitably made some sections out of date although this is offset to some extent by Prof. Volkenstein indicating, in the preface, some of the developments made since the book was written. In spite of some omissions of recent work, this book, containing much valuable material not otherwise readily accessible, will be of great value to all research workers concerned with problems related to the configurations of polymeric chains.

Each of the three books represents an authoritative and critical account of a field of polymer science which is in process of rapid development. The level in each case is that of a research monograph. Each book will stimulate further research and increase the already high rate of

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edited by **R. E. Burgess**

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edited by **Wilhelm Foerst**

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(R688) January 1965, about 250 pp., 80s.

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edited by **Thomas B. Drew, John W. Hoopes, Jr.,** and **Theodore Vermeulen**

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(A324) Volume 5, January 1965, about 325 pp., 100s.

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development of the field or fields concerned. In view of the amount of information in each, and its importance, their prices are not excessive. W. R. MOORE

MASS SPECTRA OF ORGANIC COMPOUNDS

Interpretation of Mass Spectra of Organic Compounds

By Dr. Herbert Budzikiewicz, Prof. Carl Djerassi and Dr. Dudley H. Williams. Pp. xiii+271. (San Francisco: Holden-Day, Inc., 1964.) 8.75 dollars.

ALL laboratories of reasonably advanced organic chemistry now have equipment for the determination of ultra-violet and infra-red spectra and many have nuclear magnetic resonance spectrometers, the cost of the machine and its subsequent maintenance being, of course, the limiting factor. Comparatively few laboratories in Britain have modern mass spectrometers, although the number of laboratories so equipped will certainly increase as fast as funds permit. The mass spectrometer has many uses, but its prime use to the organic chemist is the information regarding chemical structure which it so readily and effectively supplies: this use was first appreciated and applied on any considerable scale by petroleum chemists, but the appreciation is now widely increasing.

It is noteworthy that books on modern mass spectrometry, cited by the authors of *Interpretation of Mass Spectra of Organic Compounds*, number only seven, the first being Dr. J. H. Beynon's *Mass Spectrometry and its Applications to Organic Chemistry*, which appeared in 1960 and which the authors of the present book justly describe as a classic. Prof. Djerassi and his colleagues consider that the many facets of mass spectrometry dealt with in these books, such as instrumentation, sample handling, free radicals and ionization potentials, analytical applications, etc., need not be repeated in the present volume, which deals almost exclusively with the determination of organic structure, and with the spectral fragmentation of organic compounds, which is, of course, an intimate part of the structure elucidation. Aliphatic hydrocarbons are not included, because they are dealt with adequately in the earlier books and are not of general interest to 'structural' organic chemists.

The book considers first the simple and common functional groups, such as the carbonyl, hydroxyl and amino functions, and discusses the fragmentation processes initiated by them on electron impact. The generalizations so obtained are then illustrated by their application to the tropane alkaloids, which have three such groups attached to a small alicyclic framework. Other classes of comparatively simple organic molecules are then considered. A second volume will later deal with more complex polycyclic compounds, particularly those of natural occurrence.

It is also noteworthy that much of the material in the present volume has been published very recently or is still unpublished, and the book contains therefore the latest relevant results obtained in Prof. Djerassi's very vigorous school of research at Stanford.

In a preliminary "Note to the Reader", the authors have attempted "as precise a definition as possible of the different bond fissions and transfer reactions", and they make some refreshingly direct statements. "Standard organic chemical concepts . . . are so readily applicable to the interpretation of mass spectrometric fragmentation processes that a certain sloppiness in symbolism has crept into most of the recent literature." Then after a common example of mechanism, they add that this representation "implies the shift of a proton, which very probably does not bear any resemblance to reality". This 'sloppiness in symbolism' (being, of course, the outward expression of an inner sloppiness of thought) is common in many branches of organic chemistry, and it is heartening to know that the authors will have none of it.

This volume can be strongly recommended to chemists as a clear yet thorough introduction to this branch of mass spectrometry. F. G. MANN

GAS KINETICS

Experimental Methods in Gas Reactions

Second edition. By Sir Harry Melville and B. G. Gowenlock. Pp. viii+464. (London: Macmillan and Co., Ltd.; New York: St. Martin's Press, Inc., 1964.) 84*s*.

THE original edition of this book achieved a unique position among research workers in Britain as a source of information on practical experimental methods in gas kinetics that was often difficult to find. Much of its success was undoubtedly due to the exceptional experimental ability of its two authors, whose personality and outlook pervade the whole text.

To revise such a personal work requires considerable courage even when done by the original authors, as the impact of the experimental scientist is usually one of the first casualties in the war against time. The second edition has, however, been revised by a newcomer, admittedly under the guidance of Sir Harry Melville, but unfortunately he has left the manostat and explosion vessel a fair way behind. Perhaps inevitably the book loses something of its flavour, and the somewhat untidy bittiness of its approach, which seemed an essential part of the character of the old volume, now, accentuated by the process of selection and addition necessary to include contemporary material, tempts one to suggest that it might have been better to start afresh.

As the preface to the second edition points out, "Farkas and Melville" has been out of print since 1946 and many of the younger generation of experimentalists may well be not very familiar with it. (I was a little disconcerted to find that in our own library both copies have now been relegated to the reserve stock dungeons.) For this reason perhaps it is only fair to comment on the present edition on its own merits.

Following a useful introductory chapter on the kinetic theory of gases, a wide variety of practical apparatus and methods for the measurement and control of pressure and temperature are considered. The simple feed-back circuit for the constant temperature operation of Pirani gauges on p. 90 seems a happy intrusion into the vacuum field from high-speed anemometer practice and might well be extended to provide fast response katharometers for gas chromatography. The acoustic pump mentioned on p. 53 deserves more attention than it has received. There are some areas where the treatment given is scarcely adequate to do anything useful, such as the very brief discussion of thermistors, but a good range of references is provided. Occasionally, important omissions are obvious as, for example, in the gas control section where the use of critical flow orifices for flow control (for small flows watch bearings are very suitable) is not mentioned.

The chapter on preparation of gases and volatile compounds is excellent and contains a wealth of material which it would be quite laborious to find from other sources. However, in the next chapter, in spite of the authors' comment in the preface, the emphasis on the older methods of gas analysis at the expense of gas chromatography seems incongruous in the light of the fact that the latter has displaced almost every other method. Although a useful survey of most aspects of this important method is given, the treatment is so compressed that it can be misleading. For example, the flame thermocouple detector, which even R. P. W. Scott would recognize as obsolescent, is given undue emphasis, while the difficulties of achieving high sensitivity for permanent gases using ionization methods are not mentioned.

The two final chapters deal with photochemical techniques and experimental methods for investigating gas reactions. The former includes a good description of Norrish's flash photolysis apparatus, but undoubtedly the latter is the most valuable in the book. High-pressure reaction apparatus, static and flow methods, explosive reactions and heterogeneous catalytic systems are all covered well from a practical point of view and will amply repay reading before starting an experimental programme in these fields.

The general presentation of the volume is very similar to the original edition, but the collection of references to the end of each chapter is a significant improvement. A useful bibliography of more specialized texts is included for each chapter as an appendix. D. H. Desty

SOME REFRACTORY METALS

The Science and Technology of Tungsten, Tantalum, Molybdenum, Niobium and Their Alloys

Edited by N. E. Promisel. (Based on an AGARD Conference on Refractory Metals held at Oslo University Centre, Oslo-Blindern, Norway, 23–26 June 1963. AGARDograph No. 82.) Pp. xiii+588. (London and New York: Pergamon Press, 1964.) 140*s*. net.

THE Science and Technology of Tungsten . . . , edited by N. E. Promisel, an acknowledged authority on refractory metals, is a comprehensive version of papers and discussion presented at the conference organized by the Advisory Group on Aeronautical Research and Development on "Refractory Metals" in Oslo during June 1963.

The book is divided into seven sections. The first consists of three short introductory papers which set out concisely the present achievements and long-term objectives of refractory metals technology. Section 2 deals with the physical metallurgy of alloys and, of the four papers in this group, two are devoted to the important aspects of interstitial solutes and their influence on physical and mechanical properties. The emphasis is placed on theoretical rather than on practical considerations. Section 3, entitled "Properties and Engineering Application", follows naturally, and all five papers contain valuable data in respect of the interpretation of physical and thermochemical properties in terms of engineering performance and fabrication. Of special interest is a paper by C. A. Verbraak on the effects of high-energy rate-forming on properties.

Section 4, "Deterioration and Protection", is the longest in the book (140 pages), and it is encouraging to note that a real attempt to cope with the fundamental aspects of oxidation and protection is evident in all four papers and two reports which form this group. A great deal of *ad hoc* experimental work has been a feature of past contributions in this field, and the uniformly clear presentation of facts and interpretation of results is a refreshing novelty. Oxidation phenomena and gas/metal interactions are discussed respectively by Kofstad and by Speiser and St. Pierre. The paper by Di Stefano and Hoffman on corrosion in refractory metal–alkali metal systems underlines the growing importance of this subject for heat-exchange in advanced nuclear reactors. The last papers deal specifically with protective coatings and present a comprehensive up-to-date account of the substantial progress made in this field during the past few years.

The important aspects of analysis and testing are covered in Section 5. It is clear from the papers presented and from the rapporteur's statement that a great deal more work is needed for arriving at standardized techniques of analysis. The need for standardization in testing is also emphasized, and some valuable practical recommendations are made in the concluding statement.

The remainder of the book, Sections 6 and 7, deal respectively with primary and secondary fabrication. The papers concerned with flat rolling and forging review modern trends in primary fabrication although no major new development is reported. Much the same state of affairs is reflected in the four papers on machining, flow-turning, forming and joining.

Taken as a whole, the book is undoubtedly a valuable source of information for all workers in the field of refractory metals. L. W. Derry

STRUCTURE AND PROPERTIES OF UNSATURATED POLYESTER RESINS

Unsaturated Polyesters

Structure and Properties. By Herman V. Boenig. Pp. x+222. (Amsterdam, London and New York: Elsevier Publishing Company, 1964.) 55*s*.

TWENTY years have elapsed since the first commercial application of unsaturated polyester resins. In those twenty years much has been written about these resins, but so little about their chemistry and so much about their applications, that despite its apparently late appearance, Dr. H. V. Boenig's book, *Unsaturated Polyesters: Structure and Properties*, is welcome because of its emphasis on their chemistry.

Probably the main reason why earlier publications have concentrated on applications of unsaturated polyester resins, rather than their chemistry, is because they were the first thermo-setting resins which could be moulded under very low pressures. New methods could therefore be adopted for using them, and so authors, anxious that their new resins should be used as widely as possible, concentrated on making known how they could be used to produce such varied articles as radomes, boat hulls, car bodies and swimming pools. Often the chemistry of making these resins was dealt with in a single brief chapter. Dr. Boenig's book helps restore the balance.

After reviewing briefly the historical development of unsaturated polyesters, the author devotes three short chapters to their basic structure, how they cross-link, and what they are like when they are cross-linked. He then gets down to his main theme of relating structure to properties both before and after cross-linking or hardening.

This is dealt with in four much longer chapters, covering the effect on properties of variations in: (*a*) the additives, like promoters, inhibitors and retarders, made to the basic unsaturated polyester resins; (*b*) the polyester backbone; (*c*) the unsaturated monomers; (*d*) the means by which flame retardance can be achieved. The effect of these structural variations on such properties as the melting-point of the base resins, and the mechanical strength, hardness and heat-resistance of the cured resins, are described in some detail but, oddly, electrical properties are almost ignored. The final chapter of the book is devoted to polyester coating compositions, the emphasis being placed on means of preventing cure inhibition either by structural variations in the unsaturated polyester backbone or by the use of additives. The book is therefore more for those who make unsaturated polyester resins than for those who use them.

There are omissions and imperfections. On the subject of heat-resistance, data for exposures for periods of longer than 5 h at elevated temperatures are available and could with advantage have been included. There is barely a mention of the use of brominated acids or monomers to obtain flame retardance. It is not always clear whether the author is referring to saturated or unsaturated polyesters even though the title of the book seems to limit it to the unsaturated type. In other cases maleates and fumarates are referred to, when it is not clear whether the particular resin was made from maleic anhydride or fumaric acid, or whether the fumarate/maleate content

of the resin had been determined. There are misprints and the contents of Table 7, p. 45, are not what one expects from the title.

Despite the minor faults, the book does well in bringing together much scattered information and relating in a constructive way structure and performance, with useful references to the literature. Chemists working with polyester resins will be grateful to Dr. Boenig for his efforts. The book is well printed and presented, and is good value at 55*s*.

R. N. Lewis

FROM RADICALS TO RADIATION

A History of Chemistry

Vol. 4. By Prof. J. R. Partington. Pp. xxi+1007. (London: Macmillan and Co., Ltd.; New York: St. Martin's Press, Inc., 1964.) 210*s*. net.

FEW individuals in recent years have dared to undertake those massive works of learning that were borne so lightly, it seems, in the nineteenth century; and they have been scarce indeed in the history of science. In the United States, Sarton's *Introduction* and Thorndike's eight volumes, both the work of professionals, are clearly worthy members of this class. In Britain, this land of amateurs, we have the magnificent work on Chinese science by Dr. Joseph Needham which is still in progress, and now this massive history of chemistry by the emeritus professor of chemistry in the University of London, of which Volume 4 is the final volume in order and the third to be published. Prof. Partington has long prepared himself for his arduous task: apart from his own investigations in chemistry, his long experience of teaching at Queen Mary College, and his many scientific publications, he has given more than thirty years to the history of his science. For example, his *Short History of Chemistry* (1937) already contains a preliminary sketch of the present volume; and those who are acquainted with Prof. Partington in Cambridge (where he has lived since the War) know him as an untiring reader in the University Library.

This survey of the development of chemistry from the beginning of the nineteenth century into the nuclear age could only be the product of immense industry, since it is based on an exhaustive search through the scientific journals (the list of those cited fills eight and a half large pages). The indexes include more than 2,000 personal names. Moreover, in the final volume of this great enterprise, not only is the amount of chemical work covered far greater than that surveyed in the first three put together, but also the material is that which has been least collected and assessed by earlier historians. Indeed, nineteenth-century chemistry is virtually a virgin field, the fertile soil of which Prof. Partington has turned over for the first time. No one else could have proceeded with such patience, learning and judgement.

The chief feature of the structure of Volume 4 of *A History of Chemistry* is the assessment of the work of individual chemists, and of their influence. Thus, in Part 1 (565 pp.), all but three of the seventeen chapter-titles are personal names. Part 2 (physical chemistry, 178 pp.) is arranged by topics—affinity, thermodynamics, electro-chemistry, and so on; while Parts 3 and 4 (later organic and inorganic chemistry, 177 pp.) are again dominated by individuals. The last short section, on radiation and atomic structure, returns to topics.

Accordingly, though Prof. Partington has devoted roughly equal amounts of space to each half of his subject (before and after 1860), effectively the chemists of the early nineteenth century are treated in greater detail. This period was ruled by the triumvirate of Berzelius, Dumas and Liebig—often at odds with each other, but united in cutting down rivals to themselves. Judging by the length of the principal section concerned with him, Davy (41 pp.) emerges rather strangely as the most important chemist of the whole period, followed by Berzelius (35 pp.), Faraday (29 pp.) and Liebig (25 pp.). In the later nineteenth century Kekulé and Kolbe receive the limelight, though many other chemists and other types of chemistry are generously treated.

Here is a rich mine of information. But this is not an easy book: it has no continuous argument, no historical point of view, and very little time for the outsider. Largely it is history by a chemist, for chemists, setting out exactly and in detail who did what when. There is an explanation of the coining of the name 'aldehyde' (from '*alc*ohol *dehyd*rogenatus', p. 357) but none of the German predominance in chemistry. Occasionally the author takes note of a controversial question: the significance of Wöhler's synthesis of urea (1828), for example. This was claimed as an epoch-making discovery even in its own time, as a breach in the barrier between the organic and the inorganic, or in Liebig's words, "the first beginning of a truly scientific organic chemistry". Later critics have been less impressed, pointing out that Wöhler's reagents were actually derived from organic sources. Prof. Partington retorts that potassium cyanate had been prepared by Scheele and ammonia by Priestley from inorganic materials, so that Wöhler's was a true synthesis. (However, the fact remains that whatever Wöhler accomplished, he did not demonstrate the identity of a synthetic urea derived from inorganic reagents with the physiological product.) These excursions into debate are, like the dry human and personal reminiscences of the author, permitted all too rarely to penetrate into his pages.

It is now roughly a century since the chemical histories of Kopp and Hoefer appeared. They had a long, honourable innings, but their time for retirement had come, and Prof. Partington has given us a new work that is much fuller, much more accurate and up to date. His history, no doubt, will endure at least as long as theirs.

A. R. Hall

GENESIS OF HYDROTHERMAL ORE DEPOSITS

Complexing and Hydrothermal Ore Deposition

By Harold C. Helgeson. (International Series of Monographs on Earth Sciences, Vol. 17.) Pp. xiv+128. (London and New York: Pergamon Press, 1964.) 60*s*. net.

AMONG the problems confronting the geologist, none is more difficult or complex than that of the genesis of the hydrothermal ore deposits. That a close relationship exists between many occurrences of metalliferous minerals and of igneous rocks was recognized by Élie de Baumont in 1847, but the subsequent development of this branch of science has seen much controversy and fluctuation of opinion.

The mainstream of thought has continued to uphold the postulate that hot water is the vehicle for the carriage of metals such as copper, lead, zinc, silver and mercury into fissure veins. Thus, Lindgren[1] states, "the vein-forming solutions were probably liquid from the start, and often highly concentrated. They contained water as the principal constituent and were at all times in a mobile, non-viscous condition. In their constitution colloidal dispersions entered prominently". Bateman[2] held that "the thermal springs that transport metals at the [Earth's] surface are alkaline, and some of the metals have been carried as sulphides. Consequently, it is inferred that the ore-forming fluids also were alkaline".

Experimental investigations of phase and other relationships in relatively simple hydrated silicate systems, at temperatures beyond that of the critical point of water, have been made during the past several decades. It is only more recently that significant progress has been

made in the systematic application of the theoretical and practical methods of physical chemistry to equilibrium in aqueo-mineral systems at low temperatures. The position reached by 1960 was reviewed by Garrels[3].

In *Complexing and Hydrothermal Ore Deposition*, Dr. Helgeson discusses the internal structure of electrolyte solutions at temperatures lying, for the greater part, in the range 25°–400° C. He presents the results, obtained by the use of a digital computer, relating to the equilibria of complex formation in multicomponent systems, and is at pains to make clear that present thermodynamic knowledge falls far short of that required for the construction of a complete theory of hydrothermal ore deposition.

Helgeson, nevertheless, makes positive claims. He believes that his figures demonstrate, at least in the case of lead sulphide (galena), that the metal is carried as chloride complexes in an aqueous fluid containing alkali chlorides, and that the *p*H of such solutions is near to neutral or somewhat acid. This view will certainly draw the critical interest of the experimentalists.

All geochemists will welcome this contribution, though recognizing it as a progress report. Few geologists will be adequately prepared for the exposition of the principles on which the work is founded. It is much to be regretted that the introductory section of the book could not have been written with this readership more in mind.

The usefulness of a possible second edition would be greatly enhanced by an account of experimental methods used in the investigation of aqueous inorganic solutions at high temperatures; by a fuller discussion of hot-spring and of wall-rock analyses; by a review of relevant geothermometric observations based on isotopic and trace element distributions; by descriptions of chosen examples of metalliferous mineral deposits; and a discussion of the possible ultimate sources of the metals carried by hydrothermal complexing and also of the alkali chlorides necessary for this. Consideration could also be given to the genesis of the arsenides, sulph-arsenides and oxides frequently found in high-temperature hydrothermal (hypothermal) veins.

The price, 60*s*., is very high for the size of the book.

E. D. Lacy

[1] Lindgren, W., *Mineral Deposits* (McGraw-Hill, 1933).
[2] Bateman, A. M., *Economic Mineral Deposits*, second ed. (John Wiley and Sons, 1950).
[3] Garrels, R. M., *Mineral Equilibria* (Harper, 1960).

ANAXAGORAS REVISITED

Anaxagoras and the Birth of Physics

By Daniel E. Gershenson and Daniel A. Greenberg. (A History of Physics: Its Concepts, Methods, and Theories. Series I: Natural Philosophy Before Aristotle.) Pp. xxv+538. (New York and London: Blaisdell Publishing Company, a Division of Ginn and Company, 1964.) 10 dollars.

Anaxagoras and the Birth of Scientific Method

By Daniel E. Gershenson and Daniel A. Greenberg. Pp. xv+63. (New York and London: Blaisdell Publishing Company, a Division of Ginn and Company, 1964.) 1.45 dollars.

THERE are, apparently inevitably, recurring occasions in the history of scholarship when individuals feel compelled to revolutionize the study of some subject by rejecting traditional views in favour of a totally new approach. So, in fact, do new ideas penetrate the older disciplines. But a contemporary predicts at his peril whether any particular example of such an approach should be praised as useful innovation or damned as blatant impudence.

The authors of *Anaxagoras and the Birth of Physics* and *Anaxagoras and the Birth of Scientific Method*—two young Jewish-American scholars, one a classicist and the other a theoretical physicist—are professedly attempting two root and branch revolutions at the same time. They have planned a necessarily vast work, "A History of Physics: Its Concepts, Methods, and Theories", which is to be "a comprehensive history . . . intended for laymen as well as professional scientists", to be centred about great figures of the past. The authors do not say in what way they find the history of science as now practised deficient, nor why they feel the need to begin *de novo*; indeed, they do not use the phrase 'history of science' nor indicate that they are not the first to study physical concepts in historical perspective. In their review of the literature of Anaxagoras they give pretty short shrift to all the historians (half a dozen or so) whom they mention; but in fairness it must be said that they discuss only what relates to Anaxagoras, to whom historians of science have not, in general, devoted great space.

The authors have very properly begun at the beginning and *Anaxagoras and the Birth of Physics* is a part of Series 1 of their *History of Physics*, "Natural Philosophy before Aristotle" (*Anaxagoras and the Birth of Scientific Method* is Part 1 of *Anaxagoras and the Birth of Physics*, shorn of the more learned footnotes). This reveals the second revolution projected by Drs. Gershenson and Greenberg: an almost total revision of classical scholarship. They reject the work of Diels outright on two grounds: it is not chronologically arranged, and he cites as quotations statements not in Ionic dialect. The first comment is fair enough, and the authors have done a service in providing as Part 2 in their own translations and in chronological order all the statements they could find relating to Anaxagoras, from Plato in the fourth century B.C. to Simplicius in the sixth century A.D. The second criticism seems irrelevant: surely we cannot expect any ancient source to have survived intact, unaltered as to form by copyists and commentators who inevitably 'modernized' even their quotations.

The three parts of the longer work consist, first, of a brief appraisal of Anaxagoras's life and thought; secondly (the bulk of the work), the sources of Anaxagoras's ideas; and thirdly, a tendentious and detailed examination of ancient and modern traditions about Anaxagoras; of the authors cited, Drs. Gershenson and Greenberg place greatest reliance, not unreasonably, on Aristotle. This last part is learned, dogmatic, often characterized by a slightly naïve logic, and vaguely rabbinic in method. It must be for classicists to appraise the reliability of the evidence; they will certainly dislike the practice throughout the book of transliterating Greek—surely a foolish practice of service only to the printer.

The first part of the longer work (and the whole of the shorter) will appeal most to scientists. It is clearly and simply written, in a confident manner; the authors have made up their minds firmly on all relevant points and indulge in none of the doubts or hesitations which laymen find so irritating in the work of specialist scholars. The authors might have helped the reader by explaining the grounds for their prefatory sentence, "Anaxagoras was the first thinker of repute who was a scientist in the sense in which we use the word to-day". For while it is true that Anaxagoras was the first natural philosopher to live and teach in Athens, he had eminent predecessors in the Western Greek world, many of whose concepts, especially those relating to astronomy and cosmology, were more advanced than his. The authors appear to base their predilection for Anaxagoras as the first modern physicist on his theory of matter, which they find satisfyingly up to date. Basing their views as they do primarily on what Aristotle had to say about Anaxagoras enables them to fit Anaxagoras snugly into the atomic, logical, realistic, unified and dynamic tradition of physics. What one misses is the placing of Anaxagoras in the content of Greek thought and in the historical pattern of the development of natural philosophy.

Marie Boas Hall

COAL GAS TECHNIQUES

The Underground Gasification of Coal

(A Report on the work done in Great Britain, prepared for the National Coal Board by Sir Alexander Gibb and Partners, Consulting Engineers.) Pp. xiii + 205. (London: Sir Isaac Pitman and Sons, Ltd., 1964.) 70*s*. net.

THE underground gasification of coal is a subject that must appeal to the imagination of many interested in the production of power as a possible major development in the winning of coal, not actually of the coal itself, but of the energy that the coal is capable of providing. At first sight any measures that hold out the promise of successful techniques with such an objective are to be envisaged as being well worth while, and certainly, in this technological age, as being in the forefront of attractive projects.

The possibility of gasifying coal *in situ* underground has been known for nearly a century, for it was foreseen by Sir William Siemens in a paper to the Chemical Society in 1868, but the first practical attempt at underground gasification was made by Sir William Ramsay, who conducted small-scale experiments in Durham in 1914. The famous Russian chemist Mendeleef is credited with being the father of the idea in Russia about 1888. Large-scale experiments were conducted in the U.S.S.R. during the 1930's, and between 1946 and 1961 intensive efforts had led to the process being brought into commercial use. Several research institutes in the U.S.S.R. are carrying out applied research in the specific problems of underground gasification, and attention is being directed to engineering development and design in connexion with installations either under construction or projected. In the largest of these, at Kholmogorsk in Kuzbass, plans are in hand to use for gas a bituminous highly volatile coal, to generate power to the extent of 1,200 MW.

The present volume constitutes a report of the work done in Great Britain in this project, under the authorship of the consulting engineers, Sir Alexander Gibb and Partners, who carried out the engineering and development work for the Board, when in 1956 they took over the development work, which had been conducted since 1949 under the ægis of the Ministry of Fuel and Power.

The process of underground gasification of coal has the favourable feature that it may be applicable to quite substantial areas of inferior coal never likely to be worked by conventional techniques. The Report sets forth in detail a story that has now come to an end because the project is not now considered sufficiently promising to warrant any further expenditure. There are now more profitable lines of research opening up and they require the available funds. The publication of the Report will ensure that the record of the considerable work done in Britain should be available for those who are interested.

The basic conception of the underground gasification process is to sink two vertical boreholes from ground-level to the coal seam, and to form a link or channel in the seam to connect the two points where the boreholes enter the coal seam. Compressed air is admitted to one borehole, the seam is ignited, and the products of combustion, including some inflammable gas, are emitted at the other borehole. In the early stages this link between the boreholes was established by drilling along the seam from one base to the other.

Combustible gas is made in conventional plant by a number of different methods. In underground gasification these occur in combination and are subject to modifications arising from the special conditions of the process. In point of fact these conditions give rise to a system of considerable complexity. Combustible gaseous fuel is normally made from coal by means of oxygen by a process of partial combustion. The principal reactive elements in coal are carbon and hydrogen, and the products contain carbon monoxide, hydrogen, certain hydrocarbons, carbon dioxide and steam. The proportion of combustible gas in the products of the reaction depends on the degree to which the action of the oxygen has been taken, and the presence of hydrocarbons depends on the amount of carbonization that has accompanied the gasification process. Ideally, the active oxygen in the air should traverse a series of channels in the coal mass, thereby ensuring that there is always an excess of solid carbonaceous matter to react with any residual oxygen in the product gas. The pattern of flow of the gases and the character of the successive reactions, depending as they do on temperature, concentration and rate of flow of the gasifying stream, may become complicated. Yet everything must depend on the manner of breakdown of the solid coal in the seam. How may this be controlled from the surface? Many pages of this volume are taken up with descriptions of the various ingenious devices that have been applied in a solid bed of coal to produce the circumstances that are so readily obtained in a gas producer by suitable sizing of the fuel to form a permeable bed. Ignition has to be effected, and there follows the control of the many factors that determine the course of the process. The pattern of gasification has to be spread over a considerable area. The problem of drilling underground in a satisfactory manner and direction for considerable distances required much experimental effort and extensive practical trial, for coal seams have their wandering habits.

In many trials the quality of the gas, as determined by gross calorific value, varied between 60 and 115 B.Th.U. per standard cubic foot; this is scarcely a range of values to enthuse the prospective user. Most of the trials were carried out at Newman Spinney, near Chesterfield, and after 1950 at Bayton, near Bewdley, in Worcestershire.

An interesting chapter is devoted to the description of the combined performance of the gasification unit in the Sough seam at Newman Spinney together with an experimental power station, constructed on site by the Central Electricity Generating Board. Approximately 3½ million units of electricity were generated, 20 per cent of the total gas supplied being consumed, being 240 million cubic feet of calorific value 59 B.Th.U. per cubic foot. In practice the gas seldom reached the quality on which the design of the boiler was based. Oil fuel had to be used to stop the flame failure device coming into action unnecessarily. The trial was nevertheless regarded as satisfactory. From the data obtained in these trials which cost the National Coal Board £1·6 million it has been possible, following the Russian-type layouts of working from the surface, to derive cost estimates that make underground gasification look quite attractive. Nevertheless it seems that success is unattainable in the normal conception of an experimental-sized plant, and could only be achieved by working on a large scale with correspondingly high investment cost and risk. Even then there are disadvantages and imponderables inherent in the process, from which a direct and reliable economic estimate cannot be derived. It may be in countries outside Britain that there are seams thick enough to make the process really attractive.

To turn to the more rewarding aspects of the venture, many special techniques and apparatus were developed. This applies in particular to the drilling rigs, which had to be used for deflexion drilling, and were equally adaptable to accurate straight drilling in the horizontal direction. Scintillation counters had to be used for detecting radiation intensity in a vertical borehole. The experiences gained threw out suggestions for future progress in other spheres. The ignition devices were many and varied, such as high-tension sparking plugs to ignite the gas stream, chemical fluxes, low-tension electric cooker elements embedded in a wire basket of coal slack. A propane-fed ignition rig in connexion with an electric heater element formed a reliable combination.

Attention is given to the more fortunate development in the U.S.S.R., where the location, character, availability

and dimensions of the seams offered a more favourable set of circumstances for commercial development. Nevertheless, in those cases considerable pioneer work had to be undertaken before any feasible projects emerged.

The book is well produced. It is clearly printed and well illustrated. It would indeed be a boon to the scientific world if all projects of equal merit could be so well reported.

R. J. SARJANT

MATHEMATICS FOR PHYSICISTS

Mathematical Methods of Physics

By Dr. Jon Mathews and Dr. R. L. Walker. Pp. x+475. (New York and Amsterdam: W. A. Benjamin, Inc., 1964.) 13.75 dollars.

PART of the equipment of a modern physicist is a sound knowledge of a wide variety of techniques and theories in pure mathematics. If his interest is in theoretical physics he may have to be thoroughly expert in the theory of functions of several complex variables, Lie groups and functional analysis; even if his own work is primarily in the experimental field he still needs to have a 'reading' knowledge of a wide variety of mathematical methods if only to be able to read the theoretical papers relating to the subject of his own researches. This requirement places a heavy responsibility on teachers of mathematics to provide courses which are at the same time interesting, comprehensible and valuable to undergraduate and graduate students of physics. To anyone charged with such a task the present book will be of interest, since it shows one possible way of proceeding in the sense that within the space of 458 pages (sixteen chapters and an appendix) it attempts to present an account of the major branches of mathematics which are of interest to physicists. Since corresponding to each of the chapters but one there is at least one well-known monograph of roughly the same size as the present volume, it is obvious that the various topics discussed cannot be treated in a completely satisfactory fashion.

The book consists of the following chapters: (1) ordinary differential equations—a brief review but including a fairly full account of the Wentzel–Brillouin–Kramers method; (2) infinite series; (3) evaluation of integrals; (4) integral transforms—with an account of Fourier series and the Dirac delta function; (5) further applications of the theory of functions of a complex variable (conformal transformations and dispersion relations); (6) vectors and matrixes including (in 25 pages!) the theory of linear spaces and spaces of infinite dimensionality; (7) special functions (Legendre, Bessel, Hermite, Laguerre, hypergeometric, confluent hypergeometric and elliptic); (8) partial differential equations—mainly characteristics, separation of variables, use of integral transforms, Wiener-Hopf method; (9) eigenfunctions, eigenvaluos and Green's functions; (10) perturbation theory; (11) integral equations—with applications to dispersion theory; (12) calculus of variations—and its connexion with eigenvalue problems; (13) numerical methods (interpolation, numerical integration, numerical solution of differential equation, the summation of series; (14) statistics and probability; (15) tensor analysis and differential geometry, made up of sections on cartesian tensors in three-dimensional space, curves in three-dimensional space (the Frenet–Serret formulæ) and general tensor analysis; (16) groups and group representations consisting of sections on groups, group representations and characters, infinite groups. Finally there is an appendix of some properties of functions of a complex variable—provided as a review for most readers but as a study guide for readers unfamiliar with the subject already.

Both Dr. Mathews and Dr. Walker are physicists, and they have constructed the book from notes which they used in giving a course in mathematical methods in physics to first-year physics graduate students and senior physics undergraduates at the Californian Institute of Technology. This is reflected in the finished product. The choice of mathematical topics is dictated by their established usefulness in physics, the material is illustrated by examples chosen from mechanics, electricity and magnetism, and wave mechanics, and the level of rigour is that at present accepted in theoretical physics.

In a volume of this size covering so very many topics it is to be expected that most of the topics are treated in much the same way as they would be in an encyclopædia such as the *Encyclopædia Britannica* or the Pergamon Press *Encyclopædic Dictionary of Physics*; but it should be emphasized that they are first-rate articles. The basic definitions and points of theory are clearly stated, the relevance to physics brought out and illustrative examples provided; there are also problems provided by means of which the reader can test his comprehension of the text (but no answers are provided).

The authors are to be complimented in having covered so much material so well in a limited space and particularly in having shown (as so many books in this area fail to do) that there are algebraic methods valuable in physics as well as analytical ones. The nature of undergraduate and postgraduate courses in Britain is so different from the scheme set out in this book that it is unlikely to be of much value to students attending such courses—except as a valuable reference book. It should, however, be studied by university teachers conducting such courses and by scientists and students who have to study the subject on their own, for it will provide them with a clear statement of the fundamentals of most of the topics they are likely to be interested in and guidance towards a deeper study.

I. N. SNEDDON

MATHEMATICS OF RUSSIAN TECHNOLOGY

A Course of Higher Mathematics

By V. I. Smirnov. Vol. 1: Elementary Calculus. Pp. xiii+546. Vol. 2: Advanced Calculus. Pp. xiv+632. Translated by D. E. Brown. Translation edited by I. N. Sneddon. (International Series of Monographs in Pure and Applied Mathematics, Vols. 57 and 58.) (London and New York: Pergamon Press, 1964.) 90*s*. net each volume.

THESE are the first two of a set of five volumes, which cover the topics of mathematical analysis likely to be of use to the engineer and physicist, from the elements of calculus up to partial differential equations, integral equations, set theory, Stieltjes and Lebesgue integrals, normed spaces, and Hilbert space.

The book first appeared in 1924, a collaboration between Tamarkin and Smirnov; after the second edition, Smirnov's name appeared alone. The present version is a translation from the sixteenth Russian edition. Smirnov, though perhaps less well known in the West than Tamarkin, has had a distinguished career, with researches of importance in both pure and applied mathematics, as well as a wide teaching experience. This broad outlook prompted him to construct a comprehensive course of higher mathematics which should have an immediate appeal to the top-class engineer and physicist, without ignoring the demand of the pure mathematician for rigorous exposition.

Volumes 1 and 2 deal with the classical results of the infinitesimal calculus, including vector analysis, differential geometry, Fourier series, and the simpler partial differential equations of mathematical physics. In such a well-gleaned field, no great originality is to be expected, and the main interest lies in seeing how the author has coped with the two-fold demand for physical applicability and for mathematical rigour. The means adopted is the division of the text into two type-founts; in the large

type, we have the intuitive and sometimes informal introduction of calculus concepts, with a variety of physical applications as, for example, bending of beams, stream-lines, Maxwell's equations.

Where a strict account would require difficult theorems of pure mathematics, the results are stated, and we are referred to the relevant small-type sections for proofs, as well as for discussions of such rather more recondite topics as uniform convergence, basic theory of integration, and so on. In this way, by sticking to the large-type paragraphs, progress up to matters such as vector field theory, Green's function, Dirichlet's problem, can be rapid without being entirely superficial. The informal explanations are usually adequate, though there are places where attempts to convey the essence of an important technical concept in words rather than in symbols are not wholly successful; for example, from the statements about limits (1, p. 45) it would be possible to infer that $\sin(\pi/x)$ tends to zero as x tends to zero. These are dangers which beset any attempt to analyse in loose everyday language those concepts which demand extreme precision of definition. The first volume contains a good many exercises, chiefly of drill type, for the student; the second volume, regrettably perhaps, has none.

A treatise which is forty years old and has gone into a sixteenth edition must have solid merits, and must have exercised a substantial influence on the training of the present generation of Soviet scientists and on their splendid achievements. It will be of much interest to see how in the succeeding volumes the transition is made from concepts and techniques which go back to Cauchy, and even to Euler, to topics such as set theory and metric spaces which are essentially of this century. It will be in Volumes 3–5 that Western teachers are most likely to find methods of exposition worthy of close study and modes of presenting the tools of modern pure mathematics to the sometimes reluctant hands of the young technologist.

T. A. A. BROADBENT

CHEMICAL STRUCTURE OF THE UNIVERSE

Space Chemistry

By Dr. Paul W. Merrill. Pp. 166. (Ann Arbor: University of Michigan Press; London: Cresset Press, Ltd., 1963.) 5 dollars; 18*s*. net.

SPACE Chemistry, by the late Dr. Paul Merrill, is from the Ann Arbor Science Library, which also includes works on the Sun, the stars and the planet Earth. The author endeavours to present the vast field of astrochemistry in a form both compact and readily intelligible to the lay public. Very little scientific knowledge is assumed on the part of the reader, although some acquaintance with elementary physics would be of assistance.

The chapters form an orderly sequence, commencing with a review of the dual aspects of Nature, her uniformity and diversity, and continuing with an account of the history of stellar spectroscopy, a discussion of the properties of the chemical elements and molecules and their relationship to the physical structure of the Universe. The book concludes with a brief survey of the new techniques of rocket and satellite spectroscopy and describes a few of the observations made through these media. It is, perhaps, the first chapter that is the most controversial and it is hard to see how one can justify the use of such postulates as: "the uniformity of nature is God's greatest material gift to mankind" (p. 15), and: "the diversity of nature . . . is God's second material gift to mankind" (p. 17) in a scientific book directed towards a general audience. Surely, it is most dangerous to suggest that contemporary science is in a position to substantiate, or reject, such claims, or even to assume that it is its function to do so?

The historical survey is brief but informative and gently introduces the reader to the properties of light and the various types of spectra observed in Nature. In the following chapters, the components of the atom and individual elements and molecules of astrophysical importance are enumerated and described; but the definition of 'isotope' is delayed until the end of the book, and is then only included as a footnote. This is unfortunate, as the main text gives the impression that isotopes can exist only as stable forms of an element.

Only two chapters are devoted to the chemical composition of the Universe, and yet the author succeeds in conveying much information in this limited space. However, his concise style does lead to such questionable statements as: "it is now generally believed the numerous 'craters' on the moon are not of volcanic origin at all but were caused by the impact of large meteors" (p. 80). The agreement on this point is far from general, and it is misleading to imply that the question of the origin of the lunar craters has been settled. On p. 137 it is stated that the distant galaxies are receding from us, their velocities being deduced from shifts in their *H* and *K* lines; and yet no mention is given of the Doppler effect. This is an important omission.

The treatment of nuclear reactions and stellar evolution is simple and lucid. The sentence: ". . . in building still heavier elements, energy is subtracted rather than added, but the amount is relatively small and causes no appreciable cooling of the gases inside a star" presents too simplified a view of the situation. In the case of any particular star, account must be taken of its mass and the role of convection at the core, when deciding how important is the loss of energy through the most endothermic of these reactions.

The task of translating a highly specialized science into language acceptable to the general public is most difficult, and some loss of rigour is to be expected. Dr. Merrill has produced a book which should stimulate many to delve further into the ever-expanding realm of space chemistry.

J. A. DAWE

ASTRO-BIOLOGY

Life Sciences and Space Research II

A Session of the Fourth International Space Science Symposium. Edited by M. Florkin and A. Dollfus. Pp. viii + 440. (Amsterdam: North-Holland Publishing Company, 1964.) 50 guilders; 100*s*.; 14 dollars.

AMONG the many recent *mariages de convenance* between sciences hitherto rather shy of each other, the union of astronomy and biology to study life beyond the Earth may well be among the most enduring. This volume records one of the union's early offspring, the sessions on life sciences at the Warsaw Space Science Symposium in 1963.

The book begins with three review papers. The first, by S. I. Rasool and R. Jastrow, is a useful survey of what little is known about the atmospheres of Mars, Venus and Jupiter. The atmosphere of Mars is the best charted of the three, and it is interesting that although the surface pressure may be as low as 2 per cent of that on Earth, atmospheric pressure is greater on Mars than on Earth for heights above 60 km. The second review paper, by A. A. Imshenetsky, is entitled "Life and Space" and includes some valuable cautionary comments: for example, the author takes a sceptical look at the microbiological analysis of meteorites, and points out how little we know about micro-organisms in the upper atmosphere of the Earth itself. The third paper is a critical review of exobiology by C. Sagan. He remarks that although there is no generally accepted definition of life, the process of self-replication seems to be the critical event in the origin of life, and he

offers an illuminating discussion of the origin of life on Earth and its probable occurrence elsewhere.

The 41 specialized papers which occupy the remaining 400 pages of the book are divided into three groups, beginning with "Extraterrestrial Biology and Organic Chemistry". There are descriptions of 'Multivator', an automatic biochemical laboratory for seeking out life, for example, through detecting hydrolytic enzymes by fluorometry; and of 'Gulliver', which shoots out a sticky string to gather a sample of Martian soil and then examines it. M. H. Briggs and G. Mamikunian make a thorough report on the organic constituents of carbonaceous chondrites, and the possible contamination of these meteorites by terrestrial material. H. Yagoda examines the likely radiation at the surface of Mars, and concludes that the thin Martian atmosphere, far from being a shield, only intensifies the cosmic radiation. Several papers discuss whether terrestrial-type bacteria or other micro-organisms can survive on Mars, and conclude that they may well do so.

The next group of papers, mainly by Russian authors, discusses recent astronomical findings about the Moon and planets. V. D. Krotikov and V. S. Troitsky discuss the heat flow from the Moon's interior and conclude that the surface is porous, and has little dust, a finding confirmed in V. V. Sharonov's paper on the dust covers of the planets. Other subjects discussed include triangulation on the Moon, polarization of the thermal radio-emission of the Moon and planets, polarimetric lunar images, and lunar microrelief. O. N. Rzhiga gives the results of the 1962 radar observations of Venus, which indicated that the most likely rotation of Venus was retrograde with a period of about 300 days. Venus figures in several other papers, which deal with the microwave spectrum on the night side, radio observations, models of the ionosphere and the detection of water vapour. In a number of papers about Mars, the subjects covered include infra-red spectra, circulation in the atmosphere, brightness in the edge zone, polarimetric measurements and radar studies.

"Terrestrial Life in Space" is the title for the final and longest group of papers, which reflect the widespread concern among biologists about the debilitating effects of weightlessness: W. R. Adey discusses at length the effects of gravity on the functioning of the central nervous system; while the grave effects of cardiovascular deconditioning and changes in calcium metabolism are explained by D. E. Graveline and W. F. Neuman respectively. The latter remarks, a little gloomily perhaps, "For all we know of mice and men, when raised in space, they may assume a globular shape". It will apparently be vital to devise suitable exercises for spacemen—perhaps based on a pair of trampolines? Another crucial question-mark is over the effects of solitary confinement in an environment almost free of sensory stimuli, and further experiments on this topic are described by G. T. Hauty. His subjects, mainly military pilots who are said to have volunteered for the adventure, describe their hallucinations vividly. Like several other papers, this one has implications beyond the realms of spaceflight and is perhaps just as relevant to the study of medieval religious mystics. The other main topic in this section is the survival of micro-organisms in space and on other planets, and the best techniques for sterilization. The general impression is that spores are even tougher than was thought, and that, given a dust particle as shield, many a microbe could survive the worst rigours of solar radiation in space. Again there are wider implications here: if, as seems probable, the magnetosphere of Venus has an open tail, Venusian microbes (if any) will be escaping into the solar wind and some may be reaching the Earth.

The book is a handsome one, printed in pleasing type on good paper. All the papers are in English, mostly with Russian abstracts. Misprints are rather numerous, and the English in some of the translated papers is far from perfect. This has its compensations, however, for the language sometimes takes on an almost poetic fire, as when we learn that the "total heat flux out of the Moon's entrails" is $1{\cdot}6 \times 10^{19}$ cal/yr. The book records the latest results on space biology, and will be of interest not only to space scientists but also to those astronomers and biologists who do not intend always to remain earthbound.

D. G. King-Hele

PHYSICAL PROCESSES IN STARS

Stellar Interiors

By Prof. Donald H. Menzel, Prof. Prabhu Lal Bhatnagar and Hari K. Sen. (The International Astrophysics Series, Vol. 6.) Pp. xiii+317. (London: Chapman and Hall, Ltd., 1963.) 65*s*. net.

THERE have been two very good books published in recent years on different aspects of the theory of stellar interiors: *Structure and Evolution of the Stars* (Princeton University Press, 1958), by M. Schwarzschild, and *Physical Processes in Stellar Interiors* (Israel Program for Scientific Translations, 1962), by D. A. Frank-Kamenetskii. Together with Chandrasekhar's classic, *An Introduction to the Study of Stellar Structure* (Dover Publications, 1957), they form an excellent survey of the subject. A new book has to be very good if it is to do more than merely supplement these, although there are two suitable fields for further books: an introductory book for newcomers to the field, stressing the basic physical processes rather than the results of recent calculations, and monographs on specialized advanced topics.

Menzel, Bhatnagar and Sen have attempted to fill the former gap. Thus, more than half their book is concerned with the basic physics of stellar interiors and, although some space is devoted to discussion of detailed calculations, they have not attempted to keep up to date with recent advances in the field of stellar evolution; thus, most of their references to 'recent work' are to papers which appeared in the years 1954–57 and there are only a few references of later date than Schwarzschild's book.

In the first part of *Stellar Interiors* there are discussions of quantum statistics and degenerate matter, the equation of state of stellar material, radiative transfer and the laws of opacity and energy generation. Among topics which receive more attention than usual are the calculation of the mean molecular weight of a partially ionized gas and the related problem of its ratio of specific heats. The latter is of particular importance in the theory of stellar instability because the low value of the ratio of specific heats in a region of rapidly changing ionizations may lead to instability. Menzel *et al.* also give a detailed discussion of the conductive opacity of degenerate material and in their discussion of radiative opacity they direct attention to an error in the theory of electron scattering opacity which has been common for twenty-five years.

There are some minor omissions in their discussion. Their account of convection in stars is very brief, and the impression is given that convection in stars always occurs with a temperature gradient close to the adiabatic gradient and no account is given of convection in diffuse outer layers. The chapter on physical processes which is most liable to change is that on nuclear reactions. Advances in the knowledge of nuclear energy-levels and their spins have modified some of the detailed processes discussed by Menzel *et al.*; as an example a supposed resonance in the reaction $O^{16}(\alpha,\gamma)Ne^{20}$ has now been shown not to exist.

The authors' stated intention is that this should be regarded primarily as a text-book for students; but, as such, it seems to have some serious deficiencies. The first chapter, "Introduction and Brief Survey of Observational Data", is perhaps the most important chapter in such a book, and I find it disappointing. Several concepts are

introduced without being properly defined, and some of the definitions are muddled. As an example, the effective temperature of a star is not defined adequately. It occurs in an equation between luminosity and radius which is written down on the assumption that the star radiates as a black-body. It is not made clear that this is merely a definition of effective temperature (the temperature of the equivalent black-body) and shortly afterwards it is stated that the equation determines the radius of the star in terms of the luminosity and effective temperature. Overall the introduction fails, in my view, to paint a sufficiently clear picture of what are the basic problems, especially those concerned with comparison of theory and observation. It could usefully have been very much longer.

Although the book does not set out to keep up with the details of recent calculation it seems a pity that in a book published in 1963 two topics of very great interest have been omitted. In 1961 Hayashi published a paper that has completely changed views on the pre-main sequence contraction of stars and the Henyey-Le Levier-Levée theory has been largely superseded. For some years workers in the theory of stellar structure have been very concerned with energy losses from stars due to neutrino reactions and these also are not mentioned.

My conclusion is that the book will be read with interest by persons wishing to learn more about some of the basic physical processes in stars, but that it is not an ideal introductory text-book for beginners. It seems unlikely to me that (to quote from the fly-leaf) it will remain the standard text-book on stellar interiors for a long time.

R. J. TAYLER

SPACECRAFT ATTITUDE CONTROL

Torques and Attitude Sensing in Earth Satellites

Edited by Fred Singer. (Applied Mathematics and Mechanics, Vol. 7.) Pp. xiii+261. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1964.) 78*s*.

ONLY on reading to the end of the preface are suspicions confirmed that *Torques and Attitude Sensing in Earth Satellites* consists largely of a number of papers presented at a conference: the second Robert H. Goddard Memorial Symposium of the American Astronautical Society, Washington, D.C., 1962. It is therefore not surprising that the book does not contain a coherent, complete or exclusive account of "torques and attitude sensing in Earth satellites". The preface attempts to deny this statement, but openly contradicts the contents list when it states: ". . . the discussion does not deal directly with the design of attitude control systems, but only with the external torques and with the means of attitude sensing". Meteoroid impact is a notable absentee among the external torques, and detailed discussion of attitude sensing is limited to Earth horizon sensing. Star, Sun and inertial sensing methods receive at the most passing reference. Internal torques are discussed in relation to the damping of nutation of spin-stabilized satellites and of libration of gravity–gradient stabilized Earth-pointing satellites. Some aspects of attitude control system design are directly treated: passive gravity–gradient stabilization, magnetic attitude control of the *Tiros* satellites, satellite angular momentum removal utilizing the Earth's magnetic field. However, while the book does not provide "a scientific basis for the design of attitude control systems" (preface), the attitude control specialist will find, in a handy-sized volume, a useful collection of papers.

Chapter 1, by G. S. Reiter and W. T. Thomson, deals with rotational motion of passive space-vehicles (11 pp.). It discusses briefly the relation between nutation amplitude and energy for a spin-stabilized satellite, and gives a short review of nutation damping methods. R. E. Fischell, in "Passive Gravity–Gradient Stabilization for Earth Satellites" (18 pp.), summarizes the theory of gravity–gradient Earth-pointing stabilization for a 'dumb-bell' satellite configuration. A (largely) magnetic scheme for de-spin and attitude acquisition is described. The method has been applied in the *Traac* satellite, and structural and performance details are given of the 'lossy' spring and magnetic hysteresis libration damping.

In Chapter 3 (42 pp.) J. E. DeLisle, E. G. Ogletree and B. M. Hildebrant discuss gyrostabilizers mainly in relation to the provision of 'semi-passive' damping for 3-axis gravity–gradient Earth-pointing stabilization. Principles and optimization procedures are explained in considerable detail, though from a physical rather than a mathematical point of view. Under the title of "Generalized Gravity–Gradient Torques" (10 pp.) R. E. Roberson presents an interesting, if somewhat academic, answer to the question: What sort of gravity torques will be experienced by a space-vehicle if the gravitational field departs significantly from the inverse square form? In Chapter 5 (16 pp.) W. J. Evans gives a useful theoretical basis for the difficult task of accurate evaluation of disturbance torques due to the Earth's atmosphere and to solar radiation. Application of the theory is hampered by the lack of experimental data on the various reflexion and accommodation coefficients. Shadowing of one part of the satellite by another is discussed and noted as a major source of disturbance torque in an otherwise symmetrical satellite.

S. F. Singer deals with forces and torques due to Coulomb interaction with the magnetosphere (7 pp.) in Chapter 6 and points out that Coulomb forces may be relatively important sources of disturbance not only for small satellites (for example, West Ford needles experiment), but also for large satellites, if these are constructed of wire mesh. If the mesh size is sufficiently small and the mesh is at a suitable potential, it can present a 'solid' appearance to incident plasma. Ordinary aerodynamic and solar pressure forces may be comparatively negligible. A number of control and scientific applications are suggested for this effect. In the following chapter R. A. Lyttleton and S. F. Singer deal with some of the dynamical aspects of the West Ford experiment (9 pp.). This chapter contains a rather specialized application of vehicle dynamics and the effects of environmental disturbances, suggesting reasons for the non-detection of the first West Ford needle belt. Chapter 8 (10 pp.) is a very brief review of some ways in which the effects of magnetic torques might be used to obtain data on the geomagnetic field and satellite temperature, or to provide attitude control. The comparative wealth of illustrations, barely referred to, seems rather superfluous.

In their chapter entitled "Magnetic Attitude Control of the *Tiros* Satellites" (9 pp.) E. Hecht and W. P. Manger discuss the unexpected spin-axis precession observed in *Tiros I* and show how this was found to be due primarily to magnetic torques. A description follows of the simple magnetic system, used in *Tiros II* and *III*, to control the satellites against this effect. Satellite angular momentum removal utilizing the Earth's magnetic field is dealt with by R. J. McElvain in 22 pages. He discusses the use of magnetic torquing for unloading the angular momentum stored in reaction wheels, where these are used as the basic attitude control actuators. This scheme avoids the continual expulsion of mass which would occur with a jet unloading system. The general concept and attitude control scheme of the *Syncom* communications satellite is described in Chapter 11 by D. D. Williams (16 pp.). At synchronous altitude the disturbing torques are found to cause only very slow rates of spin-axis precession (a few degrees per year), making this simple form of stabilization attractive for the application.

Chapters 12 and 13 are concerned with the motion of *Explorer XI* round its centre of mass (16 pp.), and an

investigation of the observed torques acting on *Explorer XI* (15 pp.). These attempt to relate the observed motion of the *Explorer XI* satellite to the environmental factors causing the motion. The two chapters are complementary rather than overlapping. Chapter 13 gives some details of the attitude determination procedures, and examines the orders of magnitude of the various torques. Chapter 12 attempts a detailed explanation of the motion in terms of gravity and magnetic torques.

Chapters 14, 15 and 16 deal with various aspects of Earth horizon sensing. Chapter 15 surveys the physical phenomena and scanning methods which could be used, some mention being made of possibilities with regard to other planets and the Sun. Chapters 14 and 16 present, respectively, theoretical and experimental results concerning the infra-red radiation emitted by the Earth, the object being to discover which spectral bands should be used to give greatest reliability and accuracy in this method of attitude determination.

W. G. Hughes

LUNAR TOPOLOGY

The Lunar Surface Layer

Materials and Characteristics. Edited by John W. Salisbury and Peter E. Glaser. Pp. xxvi+532. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1964.) 86*s*.

THE lunar surface, which tantalizingly beckons the astronaut of the space age, has long been the subject of astronomical investigation at a distance (though we have probably never felt the limitations of this acquaintance more keenly than at the present time); but the urgency of engineering deadlines to be met in the next few years makes it desirable to take stock of our present knowledge in this field, to see what kind of physical picture it will make. A three-day symposium on the lunar surface layer, its materials and composition, was held in Boston in May 1963, the purpose of which was to bring together relevant astronomical observations and supporting laboratory data. The symposium was organized by the editors of *The Lunar Surface Layer*, and thirty-three different authors contributed to its proceedings; their communications are published in this volume.

A wide diversity of subjects relevant to the physics of the lunar surface were covered—including aspects of soil mechanics; the effects of micro-meteoroid bombardment and meteoritic impacts; spattering effects, electro-dynamical effects, photometric and thermal emission examinations, and many other lines of investigation which must be integrated in a coherent picture. Perhaps the most interesting contributions to the symposium were those in the field of photometric investigations and, in particular, on the interpretation of the lunar photometric function (unique among all bodies of the solar system) which makes the intensity of moonlight vary so steeply about the time of full Moon. Hapke's recent laboratory work on the dendritic growth *in vacuo* is both presented and critically discussed. Of other noteworthy contributions, a gradual understanding of the low albedo of the lunar globe has emerged from laboratory investigations of the radiation damage suffered by unprotected solid surfaces exposed to the full range of solar radiation —both electromagnetic and corpuscular.

The most significant feature of this symposium—and hence of the volume—was its attempt to blend together astronomical observations and laboratory investigations into a coherent physical and mechanical model of the lunar surface. It would be too much to hope that an undisputed picture of it would emerge in advance of the actual instrumental or manned landings—everyone seems to have managed so far to draw support from the recent photographs of *Ranger VII* for his preconceived opinions. Drs. Salisbury and Glaser are to be congratulated on a distinct measure of success in advancing this aim. Perhaps the best tribute one can pay to their efforts is to say that, although the parent symposium was held eighteen months ago, its *Proceedings* have lost little of their interest and relevance in the intervening time.

Zdeněk Kopal

A MEDIEVAL MASTER CRAFTSMAN

On Divers Arts

The Treatise of Theophilus: Translated from the Medieval Latin with introduction and notes by John G. Hawthorne and Cyril Stanley Smith. Pp. xxxv+216+16 plates. (Chicago and London: The University of Chicago Press, 1963.) 63*s*.; 8.50 dollars.

THE treatise *De diversis artibus* ascribed to Theophilus is the most interesting of the small group of craft handbooks surviving from the European Middle Ages; as such, it has been often edited, several times rendered into English, and also translated into French, German, and Polish. The text was most recently edited (and translated) by C. R. Dodwell (1961); the present book, though begun earlier, was able to make some use of its immediate predecessor. Its particular advantage is the metallurgical and historical experience of Prof. Cyril Stanley Smith, whose previous work of the same kind is well known.

The present translators agree with Dodwell in identifying the pseudonymous author with a Benedictine monk and metal-worker, Roger of Helmarhausen, examples of whose skill are preserved at Nuremberg and Paderborn. *On Divers Arts*, accordingly, was written in the first half of the twelfth century, at a time when the first renaissance of learning was commencing. The author, however, was no literary technologist nor compiler of traditional recipes; rather "when first written down his *Treatise* had a comprehensiveness and originality, a down-to-earth quality that makes it far superior to any other technological manuscript for some centuries to come". Theophilus, avoiding idleness and frivolity as productive of nothing but "slander, idle curiosity, drunkenness, brawling, fighting, murder, riotous living, thieving, sacrilege, perjury and other things of this kind", sought to be "the humble quiet man working in silence in the name of the Lord and obedient to the precept of the blessed apostle Paul" enjoining worthy labour. His experience extended to three of the arts most closely associated with the Church: painting on walls, wood panels, and vellum; glass-making including vessels, windows and faience glazes; and fine metal-working. The craftsman, of course, began at the beginning; he prepared his own pigments from mineral or herbal sources; fused glass from sand and beech-ash; made his glues from fish-bones, cheese, leather and horn; formed the mould in which the lead cames for his windows would be cast, built his own furnaces from clay and horse-dung, and so on. The art was in the preparing and the doing; there was no theory, no calculation, and very little design. No one need reasonably feel that there was some lost science behind medieval works of art, on either the small scale or the large.

The translation is agreeable to read, and is copiously supplied with the technical annotation without which the methods and processes described would be virtually unintelligible. Where necessary figures (derived largely from Theobald's German edition of 1933) have been added to improve intelligibility. A series of fine plates illustrates the works produced by Theophilus's techniques. There may be occasional slips (surely cinnabar was not "to Theophilus . . . artificial mercury sulphide"?), but these are few indeed in a volume which is—if a slight emphasis on the Gothick be accepted—splendidly produced.

A. R. Hall

CREATIVE THINKING

The Act of Creation

By Arthur Koestler. Pp. 751. (London: Hutchinson and Co. (Publishers), Ltd., 1964.) 42*s*.

THE rapid growth of knowledge which has taken place in the past fifty years or so has also enlarged our conception of science. Gradually it is being recognized that science, like art, is a creative activity. This has directed our attention to investigating the nature of scientific creativity.

Mr. Koestler has attempted in *The Act of Creation* to explain the creative process. His explanation is based on Freud's theory concerning the relation of wit to the unconscious. It is described in detail in the first part of his book; in the second part, Mr. Koestler cites the scientific evidence in support of his view.

Laughter and humour are therefore the first topics discussed. The pattern which underlies the joke is 'bisociative'—we perceive an idea or a situation simultaneously in two contexts that are usually incompatible. 'Bisociation' is the term invented by Mr. Koestler to distinguish between routine thinking, which is on one, single level, and creative thinking, which always operates on more than one level.

The transfer of thought from one matrix to another governed by a different set of rules arises from conflicting feelings. The ambivalence of our basic emotions, the aggressive–defensive mode with which we react to every happening, sets the pattern of all our activities. Thus, the 'bisociative' shock of humour which makes reasoning perform a somersault also occurs in scientific discovery, according to Koestler. Solving a problem is very similar to seeing a joke. Just as the joke liberates us from a tension which is discharged in laughter, so the creative act, too, resolves tension and enables us to attain a higher, more integrated, level of thought.

The moment of truth—the *Eureka* of Archimedes—characterizes the act of creation. This is illustrated by Mr. Koestler in many examples. Gutenberg's invention of the printing press, Kepler's discovery of the laws of planetary motion, and Darwin's formulation of the theory of evolution are discussed in detail. 'Thinking aside'—letting unconscious thoughts come up into the open—is the secret. Logical and intellectual reasoning is superficial and unproductive: it is, in any event, needed only later for verbalization once the discovery has been made. We have to 'regress' into more primitive, pre-verbal, unconscious modes of thought in order to progress to higher levels of thinking. "*Reculer pour mieux sauter*" is the proverb Koestler uses for describing the process.

The various mechanisms, such as displacement, condensation, etc., which Freud found operating in the dream can also be shown to be at work in scientific discovery. Scientists, like Poincaré and Kekulé, have in fact claimed to have arrived at their results in a dream. Unconscious phantasy is the source of all creativity. This is what we would expect if we regard the evolution of ideas as a natural process; for the unconscious phantasies are the mental representatives of our biological–genetic drives. A little 'calculated regression' into the realm of primitive imagination is needed; but our conscious mind tends to forbid this because infantile wishes and fears are always involved in unconscious processes. We must learn to be spontaneous and able to relax our conscious control. The moment of truth, then, occurs when unconscious and irrational thoughts are turned into conscious and rational knowledge.

The scientist is not a pedantic plodder; rather, he is a dreamer or a sleepwalker, as Koestler calls him. The scientist is just as emotionally involved in his work and impassioned as the artist. The earlier stages are the same as can be seen from the history, or pre-history, of both art and science. The æsthetic experience aroused by a work of art is derived from the same, bisociative processes that cannot be rendered in verbal language. The artist exploits these unconscious processes and externalizes them by following the rules which the conventions of a period or of a school prescribe. His originality, however, consists in directing attention to aspects previously ignored or in bringing out new relationships between *motif* and medium.

In the second part of the book, Mr. Koestler presents the evidence from biology and psychology in favour of the theory he has outlined in the first part. Pre-natal skills, the development of the nervous system, the generation and regeneration of primitive organisms, instinctual behaviour, perception and memory, motor skills, the pitfalls of learning theory, behaviourism these are the topics into which the author plunges with zest and erudition. Koestler's onslaught on the behaviourists who deny all creativity as something supernatural is savage though substantiated. The stimulus-response and rat experiments cannot suffice to explain the higher, more complex processes which are involved in creative work. Abstraction and concept-formation are the central problems of symbolic thinking. We cannot explain them unless we follow the slow growth of abstract symbolism from the 'concrete', physical reactions of the infant. This is very far removed from any stimulus-response theory of learning.

There are two criticisms which, I think, must be made. The first is that, for Koestler, creation is merely the new arrangement of already existing parts: a different synthesis rather than the emergence of genuine novelty. Novelty, however, must occur; this is shown, for example, by the mutations of a gene. There is discontinuity; and, indeed, evolution as a whole exhibits it. On the biological level, there is the appearance of new species: on the level of mental development, new ideas arise that go beyond what has previously been known.

The second criticism I would make is that wit is not a sufficiently complex phenomenon to serve as paradigm for the creative act. Freud never intended his explanation to cover so wide a range. Moreover, since his theory was published, just about sixty years ago, a great deal more has been said about creativity in art and in science by psycho-analytically oriented writers. Mr. Koestler does not mention this literature.

The merit of Mr. Koestler's book lies in pointing the way towards a better understanding of science. If science creates new knowledge, we cannot forget the unconscious processes from which it springs. We must reject the view still widely held that science progresses through intellectual and logical thinking and, in consequence, revise the narrow, mechanistic criteria which are used for judging whether a theory is acceptable or not.

E. H. HUTTEN

A GUIDE TO TECHNICAL TEACHING AND INSTRUCTION

Technical Teaching and Instruction

By Alexander Maclennan. Pp. 187. 8 in. × 5¼ in (20 cm × 13 cm). (London: Oldbourne Book Co., Ltd., 1963.) 18*s*.

TECHNICAL Teaching and Instruction is a very practical book by the director of the Training College for Technical Teachers at Huddersfield, and represents the fruit of nearly twenty-five years' varied experience. For new teachers it will serve as a sound guide, and the complacent established lecturer should heed its critical comment and cautionary points.

Two chapters provide a brief survey of primary, secondary and technical education, and are followed by others dealing much more fully with the learning process, factors in learning, the scheme of work, the preparation

of teaching material, the structure of the lesson, less-formal methods of teaching, class management, homework, students' notes and teachers' records. If all teachers in technical colleges mastered the essentials of good teaching here set out in detail, standards would manifestly rise, to the continuing advantage of students in full-time and sandwich courses, in part-time day and evening classes alike.

With so important a task to be accomplished, some shortcomings in the book must perforce be noted with regret. The style is terse, often with one fact per sentence, one sentence after another. The writing is frequently reiterative, with many sentences beginning alike, and only in the chapter on "Liberal Studies" is there felicity of thought and expression. Perhaps this is too much to expect in an essentially practical manual, but it is difficult to believe that enthusiasm and imaginative insight will be greatly stimulated by reading its well-filled pages.

Errors of fact and interpretation also occur which cannot be overlooked. For example, the Higher National Diploma is described as the second main course in the colleges of advanced technology: in 1963 out of 11,800 students in full-time and sandwich courses only 231 students were in Higher National Diploma courses. Some statistical treatment would have added precision to other generalizations. Again, very misleading interpretations could very justifiably be made of the tree diagram of the "Organization of a Technical College" (p. 111), especially as to the relative importance of the academic and administrative functions, and the line—staff relationships between principal, vice-principal and heads of departments. It is stated that thirty-two regional colleges have been designated in the past six years—the official number is twenty-five.

Despite its shortcomings, the book will be of undoubted value to teachers of students in craft and technicians courses and at similar levels, and is to be strongly recommended accordingly. Beyond these, however, the effectiveness of the book will be much less, as it lacks the philosophical treatment and speculative enquiry appropriate to teaching at higher levels. This is not a criticism and it may be unreasonable to expect so wide-ranging a task to be accomplished in one book. Nevertheless, this represents a lost or at least a deferred opportunity with the publication of the Hale Committee Report *. The need for better teaching will scarcely be less with degree courses sponsored in the technical colleges by the new Council for National Academic Awards, than with degree courses in the universities surveyed by the Hale Report, and courses in the new universities including the former colleges of advanced technology.

P. F. R. Venables

* Report of the Committee (chairman Sir Edward Hale), appointed by the University Grants Committee, on *University Teaching Methods* (H.M.S.O., Nov. 1964).

SOCIAL CONTEXT OF EDUCATION

Education and Environment

By Stephen Wiseman. Pp. xiv+202. (Manchester: The University Press, 1964.) 37*s*. 6*d*. net.

In this significant book, Dr. Stephen Wiseman describes four separate investigations carried out by the staff of the University of Manchester School of Education. Tests of reading comprehension, mechanical arithmetic and verbal intelligence were constructed and a massive testing programme involving 14,000 children of 14+ in 273 schools was carried out in the Manchester area.

The results must have been of particular interest to local educationists. There was a very great variation in the attainment of children in different schools, and the amount of overlap in attainment in arithmetic and reading in selective and non-selective schools was marked. Forty per cent of the best readers were found to be outside the grammar schools. In the second investigation the relationship between the educational attainments and social and environmental factors was examined. The results appear to have been disappointing. There were difficulties in deciding on the most important factors in the environment, but questions were raised and further research was suggested.

Chapter 4 cuts into the report of the 1951 survey, but in this chapter Dr. Wiseman makes a very useful and interesting selection of relevant researches into education and environment. Material concerning family background in researches not directly investigating educational attainment is described, but Dr. Wiseman seems reluctant to concede that his clinical colleagues might be able to assist him in the choice and definition of family variables. Dr. F. W. Warburton's report on the third investigation, in which he examines the relationship between attainment and the school environment, is concise and factual. 1,700 children were tested in 48 secondary schools in Salford excluding grammar schools. He finds that a factor which he describes as socio-economic has higher correlations with intelligence than with attainment tests, an association which Dr. Wiseman also found between intelligence and environment. The second factor seems to represent school atmosphere. Progressiveness in schools was shown to be associated with ability in reading comprehension. This result is noted by Dr. Wiseman in his final chapter as one of the most significant findings in the book. A third factor represented good teaching conditions which, as Dr. Warburton anticipated, were associated with higher attainment in arithmetic.

Dr. Wiseman goes on to describe the 1957 survey. He was well aware of the limitations of his previous survey, but apart from including grammar school children and adding 12 new variables which were thought to have some connexion with a 'maternal care' factor, the 1957 survey is very similar. About 1,400 children were tested from 20 schools in Manchester. A major improvement was found in the standards of reading and arithmetic and the proportion of illiterates and semi-illiterates had been nearly halved. Apparently it was not thought necessary even in this second survey to include a non-verbal intelligence test or to attempt to broaden the basis of measurement and to assess the effects of environment on the more liberal aspects of the secondary school curricula.

One interesting fact emerges concerning the interpretation of results in these surveys. The intelligence test results seem to be more affected by environmental factors than are the results from the other two tests. According to Dr. Wiseman these unexpected results occur when the unit of measurement and the basis of the correlations is not the individual child but the school, neighbourhood or town. Dr. Wiseman stresses the importance of the interactions of attitudes of pupils, teachers and parents in the education of a particular child, and he emphasizes the need "to buttress ecological surveys with investigations of individual children and individual families".

Many readers may wonder whether Dr. Wiseman and his colleagues might not have presented a more penetrating contribution to educational research had they made intensive studies of individual children and their families the starting point of their investigations. Some readers, too, may be disturbed by the application of scientific rigour to the analysis of human attributes and to the analysis of indices which are not directly measurable and may be confused by the statistical virtuosity displayed in certain chapters.

This is not an easy book. It is a book which poses more questions than it answers. It is, however, an important book which will certainly repay close study and which will provoke discussion and controversy and stimulate further research.

John Bradley

SHORT REVIEWS

Corridors of Power

By C. P. Snow. Pp. viii+408. (London: Macmillan and Co., Ltd.; New York: Charles Scribener Sons, 1964.) 25*s*. net.

THE title of Sir Charles Snow's latest novel in the *Strangers and Brothers* sequence appears to have been first used in one of his earlier novels some eight years ago, and not, as the currency of the phrase might now suggest, in his Godkin Lectures, "Science and Government". The phrase almost inevitably turns the mind of a scientist to those Lectures, and the novel might be described as an attempt to express in fiction the essential conflict which Sir Charles discussed in them and his subsequent postscript. There is, of course, much else, and the particular political issue on which the book centres is one of the place of nuclear weapons in defence policy, and not those of the Second World War. The setting of the whole drama of power and influence is unfolded with the same skill and insight which characterize earlier novels in the series, the accuracy of which, from the point of view of the scientist, Lord Rutherford once acknowledged. Quite apart from its merits as a novel, the book could well contribute something to the deeper understanding of the Civil Service by the scientist, perhaps also, especially in one passage which recalls A. H. Compton's description of the heart-searchings of the nuclear physicists in *Atomic Quest*, to the understanding of the scientist by the administrator and politician.

Office Economy by O and M

(A Preliminary Text-book on Methods.) By G. E. Milward and P. H. S. Wroe. Pp. ix+140. (London: Macmillan and Co., Ltd.; New York: St. Martin's Press, 1964.) 21*s*.

THE Organization and Methods Training Council was established in 1957 by nine companies intent on developing advanced training courses and preparing and testing a text-book on simple office methods, incorporating their experience and intended for the use of college students and others. Such a text, called *Organization and Methods*, was produced and has been eminently successful. On the whole, however, it covered too wide a field for the preliminary training required by industry, and *Office Economy by O and M* has been designed to deal particularly with methods training. It would provide an admirable basis for such training courses and, after describing the context of methods work, shows the fundamental approach to simplifying processes involving the choosing of the most suitable methods, the design of forms, various ways for reproducing and copying documents and information, clerical aids, the conduct of enquiry and procedure charts, office machinery, and the effects and evaluation of changes in methods. The book itself is an admirable example of how words can be used simply, economically and effectively. On its merits it should repay close examination by all who wish to improve the quality and efficiency of their office administration.

T. H. HAWKINS

Rural Life and Urbanized Society

By Lee Taylor and Arthur R. Jones, jun. Pp. xiv+493. (London and New York: Oxford University Press, 1964.) 60*s*.

THIS American text-book is aimed mainly, I should imagine, at professional rural sociologists or those in training. But it differs from many forerunners in that it is readable—in the armchair as well as at the office desk—and because it adheres closely to its central theme, that is, the marked changes which have occurred in the relationships between town and country people—especially the urbanization of social organizations—during the present century.

The material is arranged in four parts. The first brings out the changes, in intensity and kind, of rural–urban differences. The second part discusses the influences of farm, non-farm and suburban peoples and their work, as well as the increasing use made of the countryside for 'vacationing' of all kinds. The last actually has increased remarkably during the past decade and is becoming highly organized in many parts of the United States. The third section concentrates on the social aspects of food and fibre production; the present-day American farm is revealed as primarily and necessarily a business organization or, to use a present but horrible piece of American shorthand, an 'agribusiness'. The fourth and final group of chapters bring out the increasing dependence of rural people on urban-inspired and directed social institutions.

Numerous book references and notes bring together research material and works of reference from all over the United States. At the end of each chapter is a list of selective readings; here also is a summary of the chapter. I would have preferred these summaries to have been assembled as a concluding chapter so that the book and its authors might have come to a dignified conclusion rather than, as at present, a full stop. The text is well written, and the material points to many trends which we can see, or will be seeing, in the United Kingdom as we approach the state of one family one car (at least).

H. E. BRACEY

Human Histology

By Bruce Cruickshank, T. C. Dodds and Dugald L. Gardner. Pp. vii+268. (Edinburgh and London: E. and S. Livingstone, Ltd., 1964.) 70*s*.

AS the title of *Human Histology* implies, the authors have set out to use human rather than animal material for the production of about 300 colour photomicrographs which illustrate the text. These photographs show excellent colour rendering; but the detail obtained is less than it would have been in black-and-white photographs. The text is limited to a description of these plates. References to the physiological and biochemical significance of structures have usually been avoided. The term histology has been taken in a strict sense and tissues from human diseases of physiological interest are not described, nor are many of the changes occurring in different physiological conditions. Within these limitations, the aims of the authors have been admirably achieved, and despite its price it will undoubtedly be popular with medical students.

The limitations are such that the dissociation of structure and function is more marked than in some nineteenth-century text-books of histology. This, in my opinion, is really undesirable. It might at an early stage of a medical student's career inculcate an approach which is the antithesis of all that he will be made to learn later. Now that, under the influence of pathologists such as Cameron and Florey, the teaching of pathology integrates structural changes so closely with functional and biochemical ones, it does seem unwise to start a new text-book of histology

which does not emphasize, or even over-emphasize, this correlation. Is it reasonable to ask a student to memorize the differences between the cell types of the adenohypophysis without mentioning that they secrete hormones and without explaining that the staining differences are due to the chemical nature of those hormones? Why omit reference to the function of RNA in nerve cells, plasma cells and hæmocytoblasts? Surely nothing can be duller to teach or to learn than the differences in structure of different parts of the nephron; but, taken in conjunction with the structure shown by electron microscope and with modern theories of urine formation, the same differences are understandable, fascinating and easy to remember.

E. H. Leach

Seals of the World

By Judith E. King. Pp. 154. (London: British Museum (Natural History), 1964.) 11*s*.

Miss King has performed a most useful task in bringing together in succinct form so much information on the Pinnipedes. This group has sustained more attack from man than any other order of mammals, and only in this century has any attempt been made to preserve this valuable animal resource. Work carried out by American, British, Canadian, French, Australian and Russian biologists has gradually built up a body of knowledge which, within the foreseeable future, will allow a policy of management to be applied to most of the threatened species (see also *Nature*, 204, 522; 1964).

In the first half Miss King gives an account of the thirty-two species, including their classification and interrelationships and their distribution both past and present. Such features as the breeding biology, food, social behaviour, longevity and seasonal movements are included where they are known, and estimates of the world population are also given where possible. This part forms a most valuable synthesis of our present knowledge.

In the second half, aspects common to the group as a whole are dealt with. There are chapters on: distribution and ocean currents; body form and locomotion; skin, fur, moult and temperature control; skull and skeleton; dentition and age determination; alimentary, respiratory, vascular, nervous and reproductive systems; senses; and diving abilities. Two final chapters on the scarce fossil history and on the parasites recorded complete a really comprehensive survey. I applaud the inclusion of the reasons (origins) of the scientific names which brings to life for the layman what is often regarded as an arid field of zoology.

The maps and line drawings are excellent; clear and unambiguous. It is pleasant to find all the places mentioned in the text shown on the maps. The photographs of species are good but could perhaps have been more plentiful, although this would have raised the very moderate cost. The literature list might also have been longer, but it includes enough to lead to the rest and that is the main object in such a work as this, which should certainly be on the shelves of the libraries of all departments of zoology as well as those of the public libraries.

H. R. Hewer

Comptes rendus du 3ème Congrès d'EUCARPIA

(Association Européenne pour l'Amélioration des Plantes), Paris, 21–28 Mai, 1962. (Third Congress of the European Association for Research on Plant Breeding.) Pp. 336. (Paris: Institut National de la Recherche Agronomique, 1964.) 37 francs.

The Association Européenne pour l'Amélioration des Plantes provides annually one of the few opportunities for European plant breeders to assemble and to exchange ideas and experiences. This report of its third congress in Paris in 1964 is a full description of the proceedings of the meeting including the complete texts, with tables and illustrations, of the papers, twenty-one in all, delivered there.

In the first of eight sessions, R. Mayer described, in a historical fashion, the progress and organization of plant breeding in France. The themes of six following sessions were: dry matter production as an objective in plant breeding; disease resistance in plants; the fate of the mutated cell; induction of mutation and plant breeding; present views on the gene centre theory; and physiological aspects of quality. In a final session, P. l'Héritier gave an account of present-day views on cytoplasmic inheritance. The final part of this report deals mainly with the activity of the Association and its various sections during preceding years.

This volume conveniently brings together some of the recent findings and concepts of plant breeders and, as such, it will be of interest to a wide audience. In an otherwise very complete congress report, the only omission is the absence of records of discussions of the papers, the inclusion of which might have enabled the outsider to share more fully the benefit of the meeting of the Association.

The Architecture of the Germplasm

By Prof. Verne Grant. Pp. xv+236. (New York and London: John Wiley and Sons, Inc., 1964.) 75*s*.

The Architecture of the Germplasm is primarily concerned with the way in which genes interact in development, and how their linkage or independence in inheritance is related to this. After an introduction to Mendelian heredity there are useful discussions of multiple gene systems and of structural changes in chromosomes, particularly in relation to the evolution of close linkage of adaptive gene combinations. Examples have been chosen, where possible, from the flowering plants. In view of this, the decision of Prof. Grant largely to omit discussion of polyploidy in the interest of brevity is perhaps unfortunate. The book is addressed primarily to students in pure and applied genetics and to advanced biology students, and provides a clearly written account of a difficult subject.

A criticism of the book is that it is not up to date. The literature appears to have been well surveyed up to about the year 1957, but only superficially after that. This is particularly serious for the chapters concerned with the nature of the gene and with gene action. Progress in these fields has been so rapid in the past few years that an account based on what was known 6 or 8 years ago is of little value. Indeed, there are so many errors or misleading statements in these chapters (2 and 6) that readers would be well advised to omit them. Fortunately, such omission does not greatly mar the work as a whole, since the structure of the chromosome and the mechanisms of control of gene functioning in higher organisms are not yet understood in molecular terms. The recent advances on the boundary between biochemistry and genetics have therefore had little impact as yet on the ideas about gene interaction which form the main theme of the book. For the same reason, these ideas cannot be regarded as more than interesting hypotheses until substantiated by a clearer understanding of the chromosome and how it is organized.

H. L. K. Whitehouse

Selected Works in Organic Chemistry

By A. N. Nesmeyanov. Translated from the Russian by Dr. Avraham Birron and Z. S. Cole. Translation edited by David P. Gelfand. Pp. xvi+1172. (London and New York: Pergamon Press; Jerusalem: Academic Press, 1963.) 200*s*. net.

It is noted in the preface that the complete works of A. N. Nesmeyanov were published by the Academy of Sciences, U.S.S.R., in 1959, on the occasion of his sixtieth birthday—in the form of four volumes, embracing all his scientific papers which have appeared on various

occasions since 1929. The first three volumes were concerned with his experimental research and the resulting generalizations and theories, and the fourth contained papers of more general concern, such as review articles and public discussions in chemistry.

The selection of representative papers for the present volume was difficult, but it was decided, with Nesmeyanov's help, to exclude Vol. 4, and to distribute the contents of Vols. 1–3 under the headings: Part 1, "Organo-metallic Chemistry"; Part 2, "Elemento-organic Chemistry"; Part 3, "Organic Chemistry".

The meaning of "Elemento-organic Chemistry" may not be clear: Part 2 contains first a number of papers on 'onium' salts, particularly diaryl-chloronium, -bromonium, and -iodonium salts, and triaryl-oxonium salts. These are followed by a larger number of papers on the synthesis and reactions of alkyl titanates and other related titanium derivatives. Finally there are a few papers on the reaction of various silanes with olefines.

The whole volume contains about 180 papers, which, it should be emphasized, are printed in full with the complete experimental detail. Such a collection of papers, covering a very wide field, excellently translated and clearly printed, forms an impressive tribute to Prof. Nesmeyanov's work; furthermore, almost all organic chemists will find in this collection some papers having a direct bearing on their own work or chemical interests.

F. G. MANN

Modern Developments in Electron Microscopy

Edited by Benjamin M. Siegel. Pp. xiii+432. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1964.) 96*s*. 6*d*.

ELECTRON microscopy is now so diversely applied to the two principal fields of metal physics and microbiology that the usefulness of a single volume devoted to both is limited; any one laboratory is likely to use only half this book. The techniques of specimen preparation are different in the two fields, and the vocabulary of interpretation so different as to make the biological results practically unintelligible to the physicist, and *vice versa*.

However, those articles which I am competent to judge (those dealing with metal physics) are particularly well written. Chapter 5, by Dr. Pashley, constitutes an up-to-date review of the application of electron microscopy and diffraction to the defect structure of crystals, and forms a good introduction to the subject for a research student. Siegel's opening chapter on the electron microscope seems to fall between the stools of elementary exposition and technical monograph; but still it will be useful to microscopists as a source of references, and as a background for the instruction manual accompanying their microscopes. The remainder of the book is good browsing value. It is interesting to see that the problem of obtaining a three-dimensional knowledge of structures by systematic sectioning is common to physics and biology. Fawcett's advice at the end of Chapter 6, to the effect that a sharp, beautiful image is likely to be a truer representation of Nature than one which is coarse and indistinct, also seems to be of universal validity.

In short, although one would not recommend that every electron microscopist should buy this book, one can recommend it as bedside reading: it is a good book to have available.

L. M. BROWN

Fundamental Particles

By K. Nishijima. (Lecture Notes and Supplements in Physics.) Pp. xi+408. (New York and Amsterdam: W. A. Benjamin, Inc., 1963.) 11.85 dollars.

IT is difficult to conceive of an essentially better introduction to the theory of fundamental particles than Dr. Nishijima's *Fundamental Particles*. The first chapter is a historical survey of progress up to and including the discovery of pions. Chapter 2 presents, in clarity and detail, the transformations of space and time inversion and charge conjugation; Chapter 3 deals with charge independence, iso-spin invariance and G-conjugation. Each transformation or principle is illustrated by its application to an important experiment, so that by the end of Chapter 3 the author has given the experimental basis of the established quantum numbers of the pion. Chapters 4 and 5 are concerned with the dynamics of the pion–nucleon interaction covering, *inter alia*, perturbation theory, S-matrix theory, fixed-angle dispersion relations, pion–nucleon scattering up to the first resonance, multi-pion resonances, and nucleon form factors; the author mentions, but does not enter, the field of partial wave dispersion relations. Not only is there a thorough explanation of principle, but also (as indeed throughout the book) there are sufficient examples to make it a useful guide in many practical calculations. Chapter 6 deals with strange particles (including resonances) establishing quantum numbers and some interaction properties, while the final chapter deals with the principal points in weak interactions, again with a wealth of applications.

As is necessary, Dr. Nishijima makes considerable use of the language of field theory, and thus a good knowledge of quantum electrodynamics is desirable for the fullest appreciation of the book. From the point of view of the beginner it is (perhaps) unfortunate that most works on quantum electrodynamics are more difficult than the present book and cover some of the same ground with less force and clarity. However, those who wish to use the book without knowledge of field theory will be able to do so.

To be a most excellent introduction to the subject is not the only function of *Fundamental Particles*; it should, for many years, be a valuable handbook to experimental and theoretical research workers in the field.

R. G. MOORHOUSE

Ancillary Mathematics

By Sir H. S. W. Massey and H. Kestelman. Second edition. Pp. xviii+1053. (London: Sir Isaac Pitman and Sons, Ltd., 1964.) 65*s*. net.

THIS massive compendium, first issued in 1959 at 75*s*., was tailored to the mathematical requirements of those taking the London special honours degree in physics or chemistry. Its success has led to a second edition, at a reduced price, with an additional chapter on matrices. In 60 pages, there is a fairly comprehensive account of linear algebra and matrix theory, up to the Cayley-Hamilton theorem, eigenvectors, quadratic and Hermitian forms, unitary and orthogonal matrices, as well as nearly 100 good exercises for the student. Such a concentration is achieved partly by a terse exposition, demanding close attention, and partly by placing the chapter at the end of the book and so being able to make use of ideas on vectors and algebraic geometry from the earlier chapters. The science student may find the topic difficult, but he should be helped by his knowledge of its importance in theoretical physics to-day, and by the care the authors have taken to slant the account towards applications in this field.

T. A. A. BROADBENT

The Electronic Structure of Molecules

A New Approach. By J. W. Linnett. Pp. vii+167. (London: Methuen and Co., Ltd.; New York: John Wiley and Sons, Inc., 1964.) 25*s*.

DR. LINNETT and his group have been working for some years now on a concept of valency which differs from that of G. N. Lewis, and in which pairing of electrons plays a subsidiary role to another concept, namely, the division of electrons into two sets of opposite spin. Although these views have been developed mathematically in the journals, this book makes no demands on the mathematical background of the reader. The views are presented qualitatively, reference being made

at various points to the results of quantal investigations. The ideas as such, therefore, have to be judged by their success in interpreting known phenomena of molecular structure. Starting with simple systems, the author then leads the reader through the more complex areas of organic and inorganic chemistry, ending with a discussion of the structures of excited states of molecules. The mechanism of certain organic reactions is also described within the framework of the theory.

The position of the theory is clearly stated by the author in his final paragraph: "To summarize, it seems not unfair to conclude from the contents of the first half of this book, and from the examination of the simple systems considered in Chapters 3, 4 and 5, that there are sound reasons for believing that the hypothesis is firmly based and that there are advantages in making use of it for formulating at least some molecular electronic structures. This, together with the examples presented in Chapters 6, 7, 8 and 9, provide justification for also believing that the hypothesis deserves further study and investigation".

The book should appeal to a wide selection of readers, from the intelligent sixth-former to those concerned at all levels with the teaching and investigation of the structure of molecules. ALLAN MACCOLL

Physics of Atomic Collisions

By Dr. J. B. Hasted. (Butterworths Advanced Physics Series: Monographs on Ionization and Electrical Discharges of Gases.) Pp. ix+536. (London: Butterworth and Co. (Publishers), Ltd., 1964.) 130*s*.

PHYSICS of Atomic Collisions, the author states, is intended to aid the young experimental physicist entering into the investigation of ionized gases, or of other phenomena involving atomic collisions. This aim will be achieved admirably, provided the young scientist is willing to study with some intensity the very comprehensive data and theory given by Dr. Hasted. Indeed, the book will be valuable to the young physicist for many years, and no less to the worker already established in the field.

In recent years there has been a considerably increased interest in the investigation of atomic collision processes and allied subjects, so that there exists a large amount of published research which requires collation and unified presentation. Dr. Hasted has attempted this task with success.

In the book the author presents first a brief but careful survey of the basic concepts of swarm phenomena in gases and of the classical and quantum theory of collisions. This is followed by an extensive survey of experimental techniques used in collision physics, and this section is particularly valuable. Sources of molecular and atomic beams are described, as also are electron, ion and photon sources. Velocity selection and particle detection methods are surveyed. The major portion of the book is concerned with the description of the experiments and data available on some important collision phenomena. Chapters are given on electronic excitation of atoms and molecules; ionization by electrons; positive ion recombination; electron attachment and detachment; photon emission and absorption; elastic collisions between atomic particles; ionization and excitation by atomic particles; charge transfer processes; collisions of excited atoms and particles; and ionization interchange. In all these chapters the author presents, in a lucid manner, an up-to-date review of the subject-matter, giving the reader clear guidance on the importance of the data under examination and indicating the theoretical implications. There are many useful diagrams of modern data on collision cross-sections, excitation functions, potential functions, etc. Extensive bibliographies are given and should prove most helpful. There is a useful appendix of tables of atomic constants, ionization and excitation levels, selection rules and potential functions. In the text there are helpful review tables. The book can be recommended to all serious students of atomic collision processes and gas discharge physics. H. EDELS

The Nucleon–Nucleon Interaction

Experimental and Phenomenological Aspects. By Prof. Richard Wilson. (Interscience Tracts of Physics and Astronomy.) Pp. ix+249. (New York and London: Interscience Publishers, a division of John Wiley and Sons, 1963.) 45*s*.

A STAGE has been reached in the study of the nucleon–nucleon interaction where it is possible to give a fairly complete picture of the interaction up to nucleon energies of 300 MeV. All the general features seem to fit together, and it is natural therefore to seek a comprehensive review which can at the same time point the way to future experiments. This book sets out to discuss the nucleon–nucleon interaction from the "experimenter's point of view". On the theoretical side, a companion volume by H. P. Noyes is promised, and one by M. J. Moravcsik has already appeared.

The author is in an advantageous position to present the experimental side, having been engaged for the past fourteen years both in research and teaching in this field. However, this familiarity has led in a few instances to the presentation of a personal point of view, to a neglect of material, and to a greater number of mistakes in text and references than is permissible in a book aimed at the teaching of graduate students. It would also have been better to restrict the book to energies below a few hundred MeV, since data in the GeV range are accumulating so rapidly and their interpretation is so tentative that their inclusion adds nothing to the main purpose of the book. The 50 pages of experimental values, most of which have appeared in graphical form, can only be aimed at those who wish to make their own computer (phase shift ?) analysis of the data. Such serious students always refer to the original work and form their own independent assessments. This space could well have been devoted to a fuller development of the topics discussed, and in particular to a critical assessment of the points remaining to be resolved or tested, such as the application of charge independence.

In spite of these criticisms the book largely supplies a need in presenting the relevant data and its interpretation ranging from the ground state of the deuteron to the scattering of nucleons by light nuclei. It provides a useful starting point to be followed by reading the books stressing the theoretical side and the review talks given at annual conferences. A. E. TAYLOR

The Theory of Rings

By Neal H. McCoy. Pp. x+161. (New York: The Macmillan Company; London: Collier–Macmillan, Ltd., 1964.) 25*s*.

THE Carus monograph, *Rings and Ideals* (1948), by N. H. McCoy, has served as a good introduction to this branch of modern algebra, but the purpose of the Carus Foundation was to present mathematical topics to non-specialists. *The Theory of Rings*, however, is strictly professional, though only a minimum amount of previous knowledge is assumed. The main properties of rings, ideals and radicals are set out lucidly but tersely, with a good supply of exercises for the reader. The young student will find the book an excellent introduction to, say, the more detailed Jacobson classic, *Structure of Rings* (1956), and some account is also given of the more recent advances. The honours undergraduate with a taste for abstract algebra has now a number of valuable texts available; but if he wishes to proceed from a broad survey to deeper penetration of this narrower field, he should profit from this somewhat austere, but invigorating and up-to-date, account. T. A. A. BROADBENT

LETTERS TO THE EDITOR

ASTROPHYSICS

Two Sources of Cosmic X-rays in Scorpius and Sagittarius

We have observed two separate and intense sources of cosmic X-rays in the region of the constellations Scorpius and Sagittarius during a rocket experiment launched on August 28, 1964. This is the same general region of the sky from which non-solar cosmic X-rays were first detected in June 1962 by Giacconi *et al.*[1]. In that earlier work a Geiger tube detector of large area and wide angular response was mounted, looking out of the side of a spinning rocket. A strong maximum in the counting rate was observed when the detector pointed in a southerly direction. A detailed discussion of the results led to the conclusion that the X-rays were of non-terrestrial origin, and that they emanated from a source close to, but apparently not coincident with, the galactic centre. These conclusions were confirmed in two later rocket flights, also employing wide-angle detectors[2]. In April 1963 Bowyer *et al.* conducted a rocket experiment using a detector with a field of view 10° wide at half maximum intensity[3]. At the time of that flight the galactic centre was below the horizon. They gave a location for the source in Scorpius at about 16 h 15 min right ascension and −15° declination and stated that its angular diameter is less than 5°.

The results reported here are based on an analysis of data received from one of several detectors in a payload carried aloft by an *Aerobee* rocket from White Sands, New Mexico (latitude, 32·4°, longitude, 106° W.) at 05: 30 G.M.T. (sidereal time, 20 h 56 min). This particular detector was a bank of argon-filled Geiger tubes with 0·002 in. thick beryllium windows, and a total sensitive area of 70 cm². The X-ray sensitivity of the detector extends from 1 to 9 Å. A rectangular honeycomb collimator with a field of view of 15° × 21·5° at half maximum intensity was mounted in front of the detector, with its long dimension coplanar with the rocket spin axis. The normal to the detector made an angle of 80° with the rocket axis. The orientation of the rocket during the flight was established from data obtained by two star sensors which registered the passage of stars across narrow rectangular fields of view. During the observations the rocket motion was a rapid spin about its long axis, and a slow precession of the spin axis around a yaw cone of small opening angle.

The presence of two sources in the band of directions scanned by the X-ray detector is evident from the data presented in Fig. 1. In this figure the number of counts is plotted as a function of angle around the spin axis for each of several intervals around the yaw cycle. Fig. 2 shows the traces of the normal to the detector across the sky at the mid-point of each of the time intervals for which the data were compounded to give the results given in Fig. 1. The numbers along the traces correspond to the angle around the spin axis as shown in Fig. 1. The left-hand peak (between angles 175° and 200°) appears to be due to the source in Scorpius. The other peak (between 200° and 225°) is due to a source in Sagittarius and may be coincident with Sgr *A*, the radio centre of the galaxy. The shape of the double peak can be accounted for entirely in terms of the angular response of the detector, which implies that the angular widths of the sources are smaller than 5° in the scan direction.

The maximum counting rates recorded when the normal to the detector came closest to the two sources were 12·6 counts cm^{-2} sec^{-1} for the Scorpius source and 5·5 counts cm^{-2} sec^{-1} for the Sagittarius source. If we tentatively assume an effective wave-length of 3 Å (that is, a wave-length near the peak of the spectral response curves of the detectors) we compute for these rates energy fluxes of 1·0 × 10^{-7} and 0·44 × 10^{-7} ergs cm^{-2} sec^{-1}, respectively.

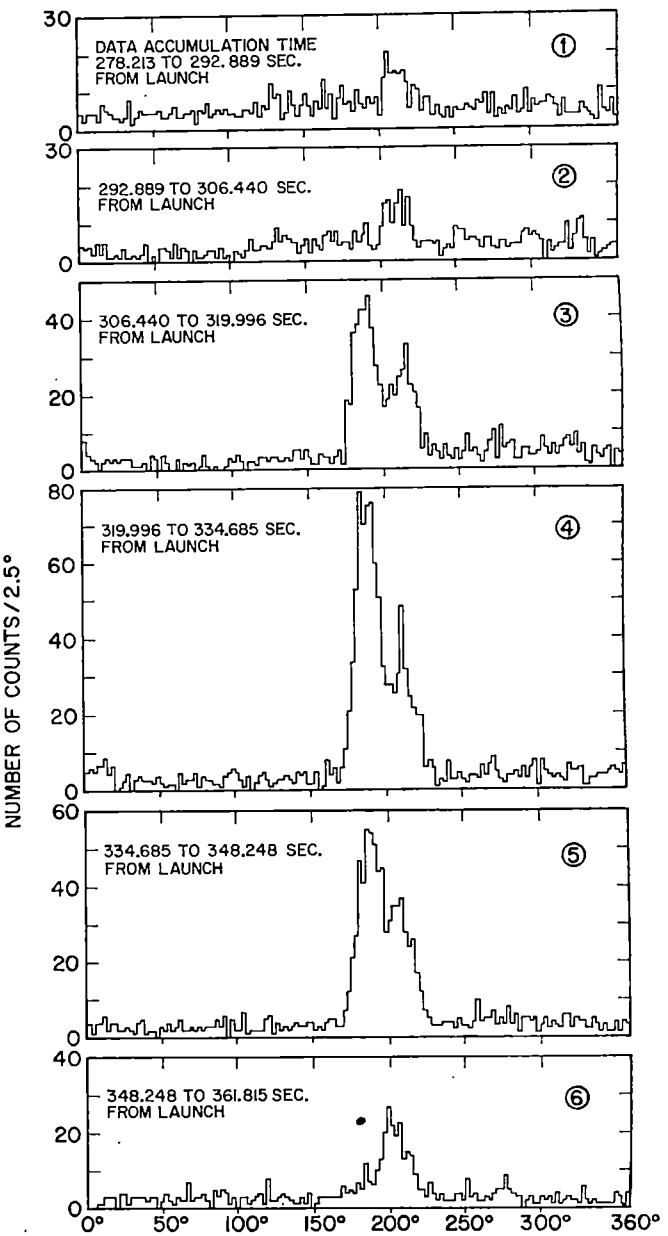


Fig. 1. Number of counts obtained in six time intervals plotted as a function of the angle around the rocket spin axis

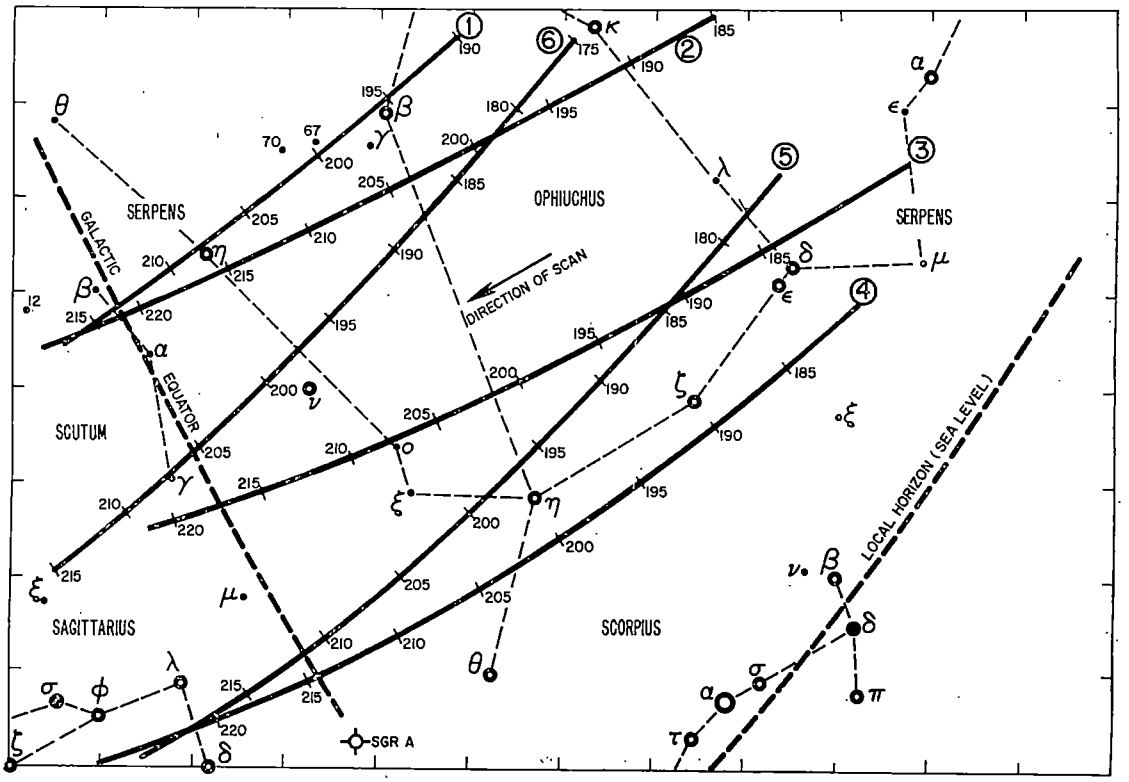


Fig. 2. Traces of the detector axis are shown in the region of the celestial sphere containing the two sources. The circled numbers refer to correspondingly numbered distributions in Fig. 1. The angles given along the traces correspond to those in Fig. 1

This work was supported by the U.S. National Aeronautics and Space Administration, Office of Space Sciences, under contract *NASw*–898.

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Upper Limit to Jupiter's X-ray Flux on September 30, 1962

During a search for celestial sources of X-ray emission, several opportunities arose to detect X-rays associated with Jupiter's radiation belts. The observations presented here were obtained from three successive scans of Jupiter made by a proportional counter flown on an *Aerobee* rocket launched from Wallops Island, Virginia. Although significant X-ray fluxes (which were time-dependent and apparently associated with or led to horizon effects) were observed, no X-ray flux from Jupiter was detected. The observations given in Fig. 1 below led to a conservative upper limit of $2{\cdot}4 \times 10^{-8}$ ergs/cm^2 sec for the flux of 4–8 keV (3–1·5 Å) photons from Jupiter between 0603 U.T. and 0605 U.T. on September 30, 1962.

The proportional counter employed had an effective aperture of 8 cm^2 provided by a beryllium window 0·005 in. thick set into the side of the counter. A sweeping magnet prevented electrons less energetic than 50 keV from impinging on the counter window. Collimation was provided by a simple aluminium baffle used to fix the field of view at (maximum widths of) 60° × 85°. The rocket rolled at a rate of 15°/sec and precessed in such a manner that the 60° dimension of the field of view scanned past Jupiter on three successive occasions early in the flight. Results related to galactic X-ray sources and details concerning instrumentation may be found in Fisher and Meyerott[1], and Fisher, Meyerott, Grench, Nobles and Reagan[2], respectively.

Fig. 1 gives the detector (labelled *K*–O–115) counting rate in two adjacent energy intervals for three complete rolls of the rocket as a function of time. A universal time of 0603 corresponds approximately to 100 sec after launching the rocket. The detector's field of view was inclined at an angle of 65° with respect to the rocket's nose, which was tipped down to the east by some 30° with respect to local vertical. As a result, the centre of the counter's field of view scanned along a path which rose above the northern horizon, passed approximately midway (near Cygnus) between zenith and the western horizon, descended to the southern horizon, and then scanned from south to north along the eastern horizon. The shaded areas in Fig. 1 separate regions in which the long (85°) dimension of the counter's field of view was completely above or below the rocket's horizontal horizon. Corrections made for counter dead time are such that instantaneous counting rates, for the combined energy intervals, greater than some 20 counts/cm^2 sec are not very precisely known. The 4–8 keV (3–1·5 Å) background of 9 photons/cm^2 sec (7·2 counts/cm^2 sec) was found by averaging data from the first two of the three available scans when the (85°) long central field of view dimension was completely above the horizon. The third scan was not used because Jupiter fell so near the edge of the counter's field of view that the detection sensitivity was only one-half that of the preceding two scans. Allowing for the 2 sec/scan that Jupiter fell within the counter's field of view at greater than 50 per cent detection efficiency, the upper limit for the 4–8 keV

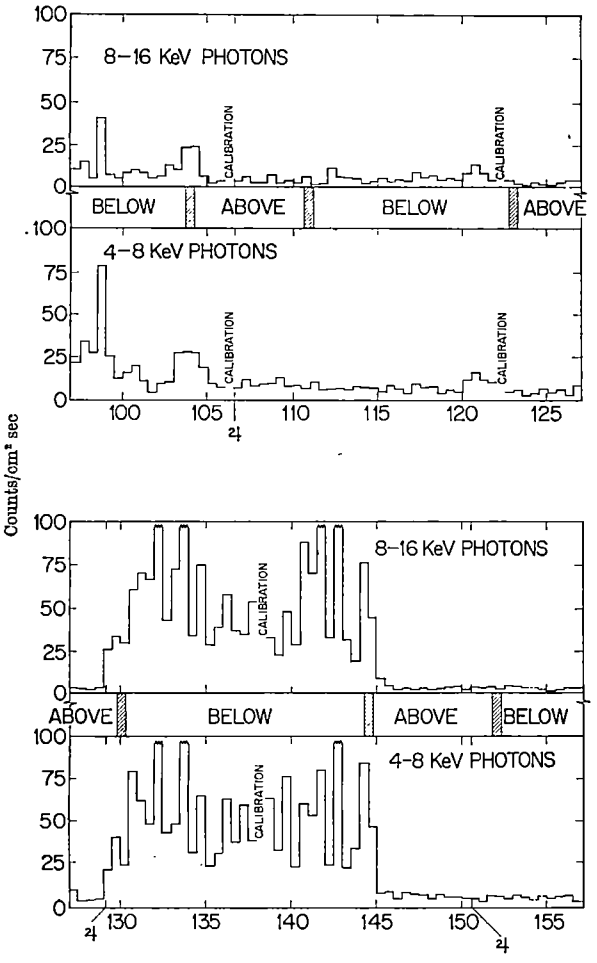


Fig. 1. Variation with time after launch of detector count rate in two adjacent energy-intervals

(3–1·5 Å) flux from Jupiter is 2·4 photons/cm² sec. This value corresponds to a signal three standard deviations above the average background level. Warwick[3] has estimated that Jupiter's X-ray flux might be of the order of 1/5,000 of the limit quoted here.

Further inspection of Fig. 1 establishes the fact that, during this part of the flight, instantaneous count rates significantly above the night sky background only occur when the detector's field of view includes some portion of the horizontal horizon. The complete northern auroral zone could be observed at one instant by the detector and measurements in that direction appear to produce the highest counting rate found during the whole flight. The time sequence given in the figure indicates a weak source near 104 sec of flight time, which was not present one roll later near 122 sec. However, at 145 sec, after the next complete revolution of the rocket, a source intense enough to jam the counter's electronics was found at approximately the same azimuth as the 104-sec source. The latest revolution indicates a source or sources extending along the whole eastern horizon, although the detected X-ray flux appeared to be most intense near the northern and southern extremities of the eastern horizon. Because of the rocket's precession, maximum sensitivity to the northern horizon source occurs at times separated by less than the 22-sec roll period of the rocket. No attempt has been made to associate the observed X-rays with the standard indicators of non-local ionospheric or auroral activity.

While significant and time-varying horizon related X-ray sources were found, no X-ray flux from Jupiter was observed. The upper limit to the 4–8 keV (3–1·5 Å) photon flux for the particular epoch investigated was 2·4 photons/cm² sec.

This work was supported by the National Aeronautics and Space Administration under contract *NAS* 5–1174, and by the Lockheed Independent Research Program.

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GEOPHYSICS

Downward Electron Flux at 1,000 km Altitude from Electron Content Measurement at Mid-latitudes

In a previous paper[1], describing diurnal variation of ionospheric electron content during November–December, 1961, at mid-latitudes, we reported the existence of a secondary maximum of ionization content below 1,000 km altitude for a period shortly after midnight. The data used in that paper were based on measurements of maximum inflexion Doppler slopes of simultaneous signals using high elevation orbits of the multiple-frequency navigational satellite 1961 *Omicron* I. Great care was taken to include in the statistics only those orbits for which the propagation paths of the various involved frequencies were nearly identical, that is, close to the overhead position. This reduces the available results considerably but increases the confidence in the correctness of the evaluation which assumes identical straight-line propagation. It could be seen that the diurnal contour of the content had some oscillations which need not indicate the presence of actual diurnal behaviour. Since the variation is based on two months of results the contour could include seasonal effects.

For this communication we have smoothed the obtained diurnal variation curve by normalizing each single observation of it with respect to the corresponding 10·7-cm radiation[2], $S_{10.7}$, which is an indicator of solar activity. The mean daily variation of the 10·7-cm flux for the period during which our observations were made is given in Fig. 1. This technique was previously used by Bauer and Blumle[3] for removing the seasonal effect superimposed on the diurnal variation of the topside ionosphere at fixed altitudes. Our curve is normalized to the average condition of $S_{10.7} = 92{\cdot}8 \times 10^{-22}$ W/m² (c/s) during observation time, making a proportional correction of the electron content to the corresponding (in date) variation of the 10·7-cm flux. The normalized curve is shown in Fig. 2. Although the diurnal curve was appreciably smoothed (indicating the correlation of the two quantities), the normalization failed to remove the midnight ionization content enhancement. This indicates that the content increase in regions below 1,000 km is real and deserves attention as to the mechanisms contributing to its production. At first, the correspondence of the content contour to the diurnal variation of the $h_{max}F_2$ for a similar period[4], as well as the approximate 12-h separation between the absolute content maximum (1400 E.S.T.) and the post-midnight maximum (0200 E.S.T.), suggested the influence of the semidiurnal solar tidal effects and the atmospheric natural mode of thermal oscillation whose period is close to 12[5] h in the creation of the midnight enhancement.

However, tidal and oscillatory effects cause vertically upward electron motion at noon and at midnight, whereas interpretation of the content curve must be described as

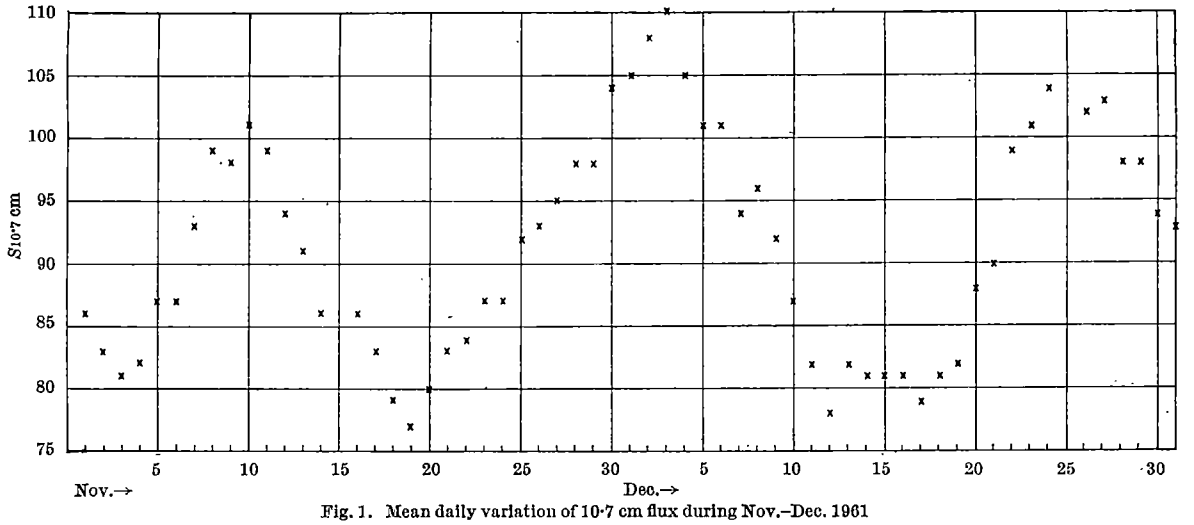


Fig. 1. Mean daily variation of 10·7 cm flux during Nov.–Dec. 1961

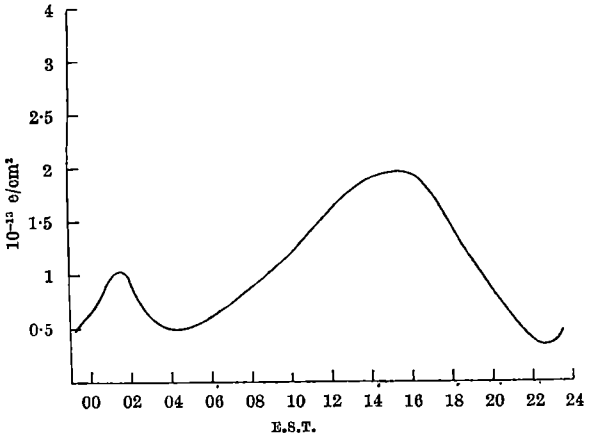


Fig. 2. Normalized diurnal variation of ionospheric electron content under 930 km during Nov.–Dec. 1961. (Note ionization content enhancement past midnight)

ionization drifts from ionospheric regions above the satellite (1,000 km) into ionospheric regions below it. In other words, the downward electron flux which is necessary to create the post-midnight maximum must be over and above the electron upward drift caused by tidal and oscillatory effects. (An analysis of the diurnal variation of the topside ionosphere electron density data at fixed altitudes (up to 1,000 km) points to a similar downward electron flux at night-time[6].)

The downward electron flux measured at the satellite's altitude is of the order of 5×10^8 electrons/cm^2/sec. The statistical uncertainties due to the lack of a great number of usable data (subject to the above-mentioned requirements of path equality, etc.) permit us to give only orders of magnitude rather than exact values. Using a value of 6×10^3 e/cm^3 at the satellite's average altitude, the above flux means that each second 1-km column of ionization of 1 cm^2 cross-section moves downward at the time of the post-midnight enhancement. It must be remembered, however, that the enhancement is over and above recombination and upward moving tidal and oscillatory motion. This consideration should increase the value for the downward flux by a factor of about two.

The flux seems to decrease substantially when the content contour reaches its secondary maximum at about 0130 E.S.T. From then on the content decreases with a rate which is equal to the decrease after sunset. (Note the parallelism of the content decay slopes after sunset and after the post-midnight enhancement.) The rate of decay at a certain time is proportional to the content value at that time, indicating a β-type attachment process for the two decreases. The secondary minimum (at 0400 E.S.T.) is slightly above the absolute minimum (2300 E.S.T.), indicating that not all the content-enhancement electrons recombined before the illumination of the topside ionosphere and the beginning of solar ionospheric ionization.

The mechanism responsible for the observed downward drift at high altitudes is not known. The possibility of horizontal ionization drifts from low latitudes into our latitude could also account, at least in part, for the observed ionization enhancement.

We thank Drs. S. J. Bauer of the National Aeronautics and Space Research Administration, Goddard Space Flight Center, and G. Goubau of our Institute for their advice. We also thank Mr. R. Silberstein of the U.S. Army Radio Propagation Agency for the 10·7-cm flux values, and Mrs. Myrna H. Meisner for performing the calculations leading to Figs. 1 and 2.

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Some Irregularities observed simultaneously in the Upper and Lower Ionosphere at Middle Latitudes

Results from a ground network of fast ionosondes over western Europe, together with simultaneous topside soundings from the satellite *Alouette*, have revealed the existence of irregularities extending to both the upper and lower ionosphere.

The ionograms (kindly communicated by Dr. J. W. King, Radio Research Station, Slough) on Fig. 1 correspond—on the left-hand side—to *Alouette* observations recorded by the telemetry station at Winkfield, Bucks (England), and—on the right-hand side—to a panoramic sequence taken from the ground station at Garchy (Nièvre, France). The date was April 1, 1963, and the time 11.30–11.50 U.T. at ground-level, and 11.09–11.11 U.T. for the satellite, which covers 3 geographical deg/min.

On the last three topside ionograms, a series of oblique propagation traces are visible. In a previous publication, Du Castel[1] has shown that such traces could be interpreted as due to ducted propagation, the ducting surface being the lower edge of an irregularity, characterized by an

increase of ionization along a magnetic line of force. The kind of abnormal trace present on the second ionogram, where both a lower and an upper frequency limit appear, as compared with the normal trace, corresponds to the satellite moving towards the outer, convex face of the ducting irregularity.

The kind of trace visible on the 3rd and 4th, which show only an upper frequency limit, corresponds to the satellite moving towards the inner, concave face of the duct. As the two types of traces occur on several ionograms running, it is possible to evaluate the position as well as the characteristics of the irregularities themselves (Fig. 2).

Calculation[2] shows these three sheets of ionization are 8 km thick transversally to the magnetic field, with maxima of 0·4 per cent in excess over the ambient plasma.

On the ground-based simultaneous ionograms, a fork appears at the upper end of the F_2 trace. This fork moves gradually downwards, first along the F normal trace, then along the E region trace, to disappear finally in a weak, short-lived sporadic E ionization.

These irregular traces are generally considered to be moving disturbances[3]. The simultaneous existence of such perturbations, on ground-based as well as topside ionograms, has already been mentioned[4]. When they are observed by ground-based network of ionosondes, the front is usually moving southwards, with an apparent speed over the ground of 100–200 m/s^{-1}. In the present case, three perturbations have been effectively observed (Fig. 2), with certain time-lags between the stations: Breisach (Bade, Germany), Garchy (Nièvre, France), Poitiers (Vienne, France) and Tortosa (Spain), from which it has been possible to deduce an apparent speed of 180 m/s^{-1}, and a direction at right-angles to the lines of isomagnetic inclination.

Thus the dimensions of these irregularities have been deduced from their special guiding effects on topside ionograms, and their movement from the ground-based network. Notice that their speed cannot be observed by the satellite, which travels more than 30 times faster.

Further, the irregularity corresponding to the first ducted echo on the *Alouette* ionograms has been very clearly observed, simultaneously at the stations Garchy and Poitiers, and 45 min later at Tortosa; it does not appear at Breisach. This shows that the longitudinal extension of such perturbations can be limited, and that they can be local in character.

The hypotheses which can account for such ionization irregularities at middle latitudes have recently been

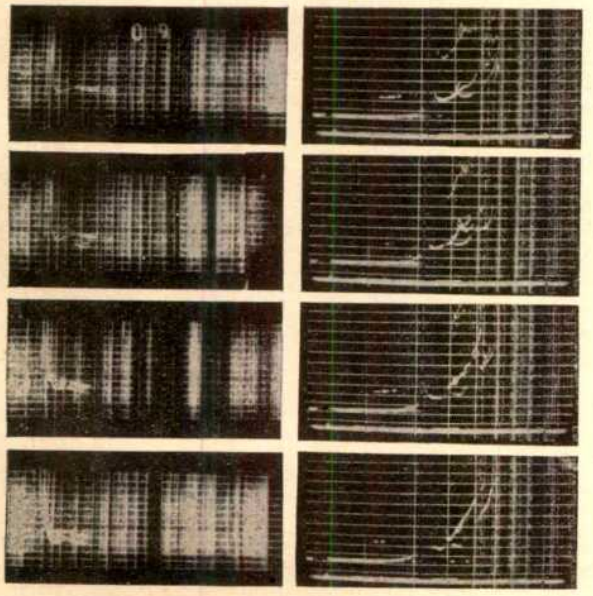
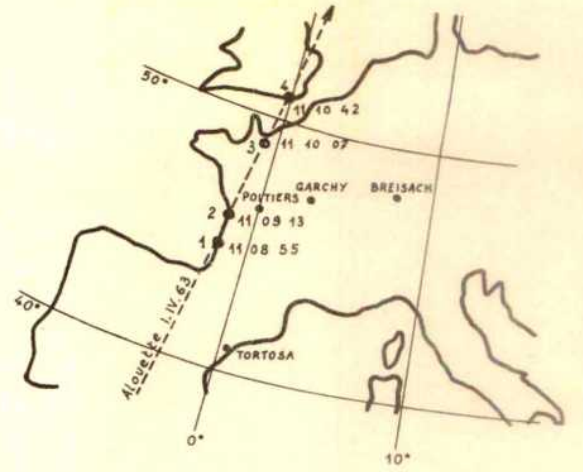


Fig. 1



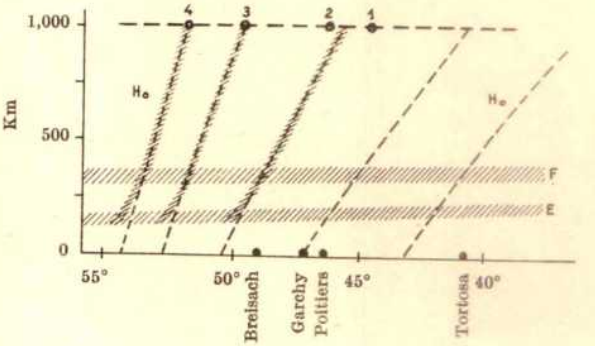


Fig. 2

examined[5]. It is reasonable to suppose that these irregularities originate at high latitudes, and then move towards equatorial regions under the influence of the S_q (currents) circulation. However, it seems impossible as yet to decide whether they have a magnetospheric origin from incident particles, or whether they are generated by this ionospheric S_q system.

Better understanding of these irregularities, obtained through further ground-based and topside observations similar to those mentioned in this communication, would be a help towards the interpretation of upper atmosphere phenomena.

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PHYSICS

Quadruple Frequency Multiplication of a 1·06-μ Neodymium-pulsed Laser

The fourth harmonic of a Q-spoiled high-intensity neodymium-in-glass laser was produced using the non-linear optic effect in two KDP (potassium dihydrogen phosphate) crystals. The laser output pulse at 1·06μ has been converted successively to 5300 Å and 2650 Å. The purpose of the communication is to report these experimen-

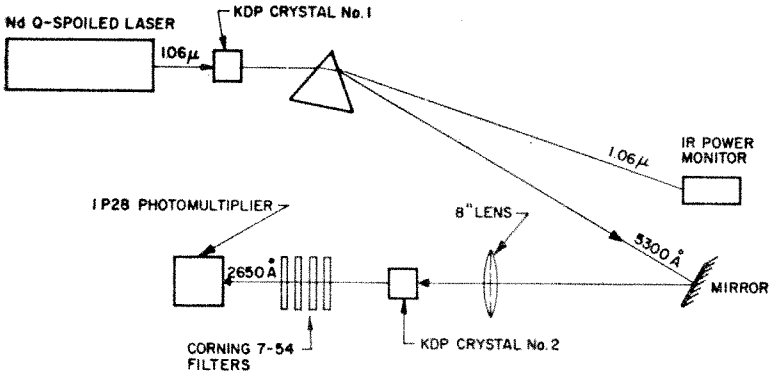


Fig. 1. Schematic diagram of experimental arrangement

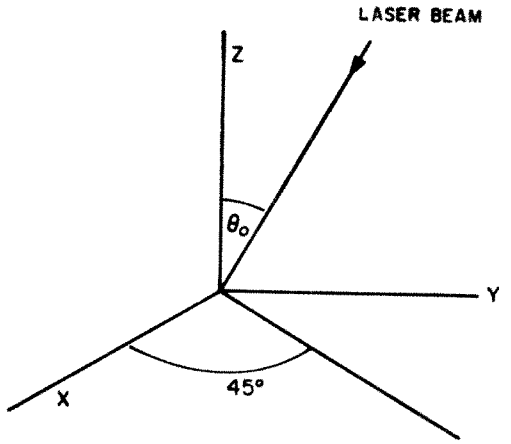


Fig. 2. Critical angles for harmonic generation

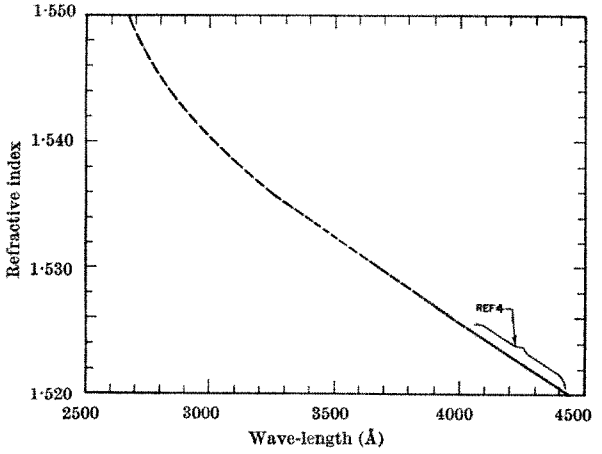


Fig. 3. Ordinary refractive index of KDP derived from the second harmonic generation results

tal results and to point out the potential importance of this high-intensity spatially coherent radiation at 2650 Å.

The experimental arrangement used is shown schematically in Fig. 1. The unfocused 10-MW output from the neodymium, *Q*-switched laser was passed through KDP crystal No. 1, which was properly index matched[1,2] for maximum energy conversion from 1·06μ to 5300 Å. A dispersive prism separated the 1·06μ and the green 5300 Å radiation. The green radiation was then reflected from a high-quality mirror and focused with an 8-in. lens on to KDP crystal No. 2. This crystal was again orientated in such a manner that the index matching the condition was satisfied for optimum energy transformation from 5300 Å to 2650 Å.

Table 1. Summary of Data on Frequency Quadrupling

Wave-length	Power	Pulse-width (nsec)	Index matching angle θ_o experimental
1·06 μ	10 MW	15	40° ± 1°
5300 Å	200 kW	10	
2650 Å	2·4 kW	—	77° 20′ ± 1°

In addition to choosing the appropriate polar angle, θ, and the azimuthal angle, Φ, proper care had to be taken so that the incident electric field components of the laser pulses were correctly polarized[3], that is, they were in the *xy* crystallographic plane and perpendicular to the bisector of the *xy* axis. Note the various changes in polarization. As the beam enters the crystal it is vertically polarized at 1·06μ. It then emerges as the extraordinary ray in crystal No. 1 at 5300 Å horizontally polarized, and again emerges as the ordinary ray vertically polarized at 2650 Å.

The results of power measurements at the respective wave-lengths are shown in Table 1. There was a noticeable pulse shortening at higher harmonics due to the quadratic nature of second harmonic generation. The conversion efficiency from infra-red to green is about 2 per cent and about 1 per cent from green to ultra-violet. It should be noted, however, that the laser beam is focused on KDP crystal No. 2 only, and not on No. 1, hence the apparent comparable conversion efficiencies. Higher conversion efficiencies, that is, 20 per cent, from infra-red to green would be expected if focusing were to be used in the first conversion. This was not done to avoid damage to the KDP crystal.

The critical angle, θ_o, which represents the angle subtended by the incoming laser beam and the optic axis, *z* (Fig. 2), and which allows the primary and second harmonic beams to travel at the same phase velocity[1,2] is given by the expression:

$$\sin^2\theta_o = \left(\frac{{}_en_2}{{}_on_1}\right)^2 \frac{({}_on_2 + {}_on_1)({}_on_2 - {}_on_1)}{({}_on_2 + {}_en_2)({}_on_2 - {}_en_2)} \qquad (1)$$

where the subscripts *e* and *o* refer to the extraordinary and ordinary index respectively and the subscripts 1 and 2 to the primary and second harmonic.

Equation (1) can be re-written to a good approximation for the region 2500–5000 Å:

$$\sin\theta_o = \left(\frac{{}_on_2 - {}_on_1}{{}_on_2 - {}_en_2}\right)^{\frac{1}{2}} \qquad (2)$$

Substitution of representative values of *n* in equation (2) shows that uncertainties in the index of refraction of one part per thousand causes an error in θ_o of the order of 1 per cent. Since θ_o can be measured to a precision of 10′, obviously more accurate values of *n* are obtainable by this technique. The quoted errors in θ_o arise because of experimental uncertainties in the X-ray crystallographic determination of the optic axis. We have measured the second harmonic generation in KDP over a wide range of wave-lengths, as shown in Table 2.

Table 2. Table of Wave-lengths and Refractive Indices

	Primary wave-length (Å)	Second harmonic wave-length (Å)	θ_o exp.	θ_o calc.	${}_on_1$	${}_on_2$	${}_en_2$
a	10,600	5300	40° ± 1°	40°	1·495$_5$	1·513	1·470$_5$
b	6943	3472	50° ± 1°	49° 44′	1·506	1·533	1·487
c	6328	3164	55° 40′ ± 1°	56° 6′	1·508$_8$	1·537	1·495$_5$
d	5300	2650	77° 20′ ± 1°	77° 12′	1·513	1·552	1·511

Note: All the experimental results in Table 2 were taken on a single crystal of KDP. The second harmonic generation of *a* and *b* has been reported also by other workers[1–3]; however, *c* and *d* represent new results[4].

All the second harmonic generation data were found consistent with the refractive index data[4], provided a small correction in the last significant place of the published refractive index data was made (which is well

within the stated error of their measurements). However, since recently two new measurements[5] on the second harmonic generation extending to the ultra-violet were made, it is possible to extrapolate values of the ordinary index of refraction to 2650 Å with a precision which is comparable to the published values in the visible region. See Fig. 3 for the results of the values of the refractive index.

The importance of these results points out the possibilities of new coherent optical sources with powers in the kW region and wave-lengths in the ultra-violet spectral region. A number of new experiments suggest themselves with such a source. The 2650 Å wave-length, for example, lies in the region of strong absorption for purine and pyrimidine[6] and other related compounds which are contained in living tissues. Thus, damage done to living cells may now be probed with this source.

I thank Drs. G. R. White, P. C. Fletcher and D. E. Rounds for their advice, W. C. Goss and M. J. Weinberg for their assistance, and J. W. Burns for performing the X-ray analysis.

This work was supported by the Laser Technology Branch, Air Force Avionics Laboratory, Wright–Patterson Air Force Base.

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Ionization of Mercury by Alkali Positive Ions

A NUMBER of workers have investigated ionization of gases by positive ions of low energies[1–5]. They have measured ionization electron current produced by positive ions by their impact with gas atoms. The greatest uncertainty in these investigations is due to the emission of secondary electrons from metals by positive ions and is an inherent weakness in most of these methods. The sensitivity of the apparatus used for detecting very small ionization electron current is another important consideration in these measurements. Disagreement in the results obtained by different workers may be ascribed mainly to these two factors. Values for ionization cross-sections and for on-set potentials vary from one observer to the other, but there is a general agreement concerning the belief that ionization of gases by positive ions of low energies is not caused by direct collisions between incident ions and electrons of the target atom. Satisfactory theory to explain the mechanism of this phenomenon in this energy-range is still lacking.

Accurate data for ionization on-set potentials and on cross-section measurements for slow ions in different gases are useful in understanding this process and are of importance and interest in plasma physics.

It has been observed[6] that K^+ ions of 220-V energy ionize mercury vapour. The ionization was detected by a photosensitive Geiger counter. In some earlier experiments[7] it was reported, however, that mercury atoms are not ionized by K^+ ions of energy less than 300 V.

In the work recorded here a much more sensitive device is used for detecting the radiation resulting from ionization of mercury. The object is to investigate if a lower on-set potential exists for this process to occur in this gas.

The ions are supplied from a Kunsman source. A 'Pyrex' ground-glass joint fused to a quartz chamber through a 'Pyrex'-quartz graded seal carries the K^+ ion source which is deposited on a directly heated tungsten filament. The ions are accelerated from the filament to a collimating slit system, carefully adjusted to be coaxial with the chamber, by low-voltage Exide batteries. The defined ion beam travels a distance of about 10 cm in the quartz chamber where collisions of K^+ ion occur with mercury vapour contained in it. The positive ion current is collected in a Faraday cup placed at a distance of about 16 cm from the source and is measured by a moving coil galvanometer of the sensitivity of 10^{-10} amp/mm. The collimating slits and the Faraday cup are held firmly in position by 'Pyrex'-tungsten seals fused into the body of the 'Pyrex' glass component of the apparatus. The interior of the chamber containing vapour of distilled mercury is well covered by a metal shield to check the scattered ions from striking against the quartz walls.

The apparatus was continuously pumped out through a liquid air trap by a two-stage mercury diffusion pump backed by a rotary oil pump. It was proved to be leak-tight before observations were made. The vacuum inside the apparatus was 10^{-6} mm mercury during the course of experiments and the vapour pressure of mercury in the chamber was maintained at $2–3 \times 10^{-3}$ mm mercury.

The radiation emitted by mercury vapour, excited and ionized by the impact of K^+ ions, was detected and measured by a quartz photoelectron multiplier, type E.M.I. 6255, which 'peeped' into the chamber through a wide window provided in the metal shield. The radiation was examined using ultra-violet interference filters supplied by Messrs. Barr and Stroud of Glasgow, the peak transmission of which varied from 14 to 23 per cent and band-width ranged between 150 Å and 200 Å.

The apparatus and the photomultiplier were placed in a closed light-tight enclosure in which a steady temperature was maintained, a condition which is necessary to keep mercury vapour pressure constant as well as to obtain reproducible observations with the photo-tube.

The photomultiplier pulses were fed through a cathode follower to a high gain amplifier the output of which was read in a scaler. A stabilized power unit provided the high voltage to the photo-tube.

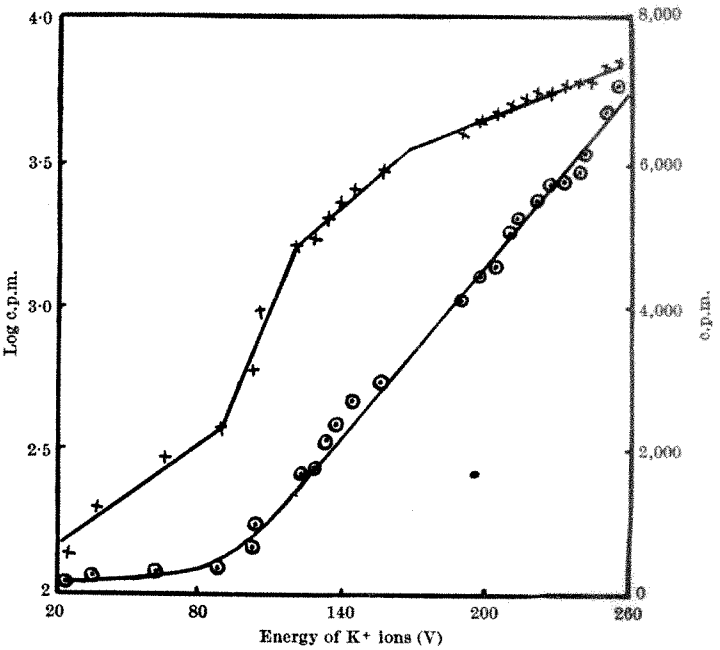


Fig. 1

Curve ⊙ of Fig. 1 shows the variation of the intensity of the radiation with ion energy for a constant K^+ ion current to the Faraday cup for mercury line 2537.

It is observed that the radiation is first emitted at an energy as low as 60 V; its intensity rises gradually with ion energy but there is an abrupt increase at 89–90 V. The rise in the intensity of the radiation is rapid above this energy.

We believe that the radiation detected by us at ion energies of 89–90 V and above is the recombination spectrum emitted by ionized mercury vapour. It is found that the strongest line in this radiation is 2537 and all other lines are comparatively very faint. This is what is observed in a low-pressure mercury arc spectrum[8].

The electric field distribution in the apparatus does not permit secondary electrons to gain a velocity high enough to produce excitation and ionization of mercury atoms. The fact that the radiation is emitted when the accelerating voltage is applied to ions and not without it ensures that photo-ionization due to ultra-violet radiation from heated filament carrying the source is negligible and beyond our detection. It was also observed that by putting a pad dipped in liquid air on the quartz chamber the counts of the photo-electron multiplier dropped to normal background.

Relation between the total intensity of radiation emitted by the ionized gas and the energy of K^+ ions is quite identical with curve ⊙, Fig. 1. A semi-log curve for this relation shows discrete kinks curve ×, Fig. 1.

It is probable that there are different modes of ionization of mercury atoms which may be excited at varying energies of K^+ ions. New groups of ionization electrons have, however, been detected[4] in ionization of argon, neon and krypton by K^+ ions of increasing energies, which support this assumption.

We thank Sir James Chadwick, Sir John Cockcroft and Sir Mark Oliphant for their advice, and CENTO administration for a fresh supply of ultra-violet filters.

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Identification of Free Radicals in Flash Photolysis

McGrath and Morrow[1] have recently reported on a spectrum near 3290 Å obtained in the flash photolysis of fulminic acid which they ascribe to the free CNO radical. It appears that this spectrum is the same as that found independently by Jennings and Linnett[2] and ourselves[3]. The spectrum has recently been analysed in detail and shown to be due to the free NCN radical[4]. All the strong bands listed by McGrath and Morrow agree with strong NCN bands listed in our paper and relative intensities are roughly the same. We believe that on the basis of the observed intensity alternation and the observed triplet splitting, there can be no doubt that our identification of this spectrum is correct.

The occurrence of the spectrum of NCN in the flash photolysis of fulminic acid is an example of the need for caution in the assignment of observed spectra on the basis of excitation conditions or chemical considerations only. The history of spectroscopy abounds in such examples. We only need to mention the spectrum observed in the flame produced by active nitrogen when BCl_3 is introduced into it. From the excitation conditions this spectrum was first believed to be due to BN until Mulliken[5] showed conclusively by observation of isotope shifts that it is due to BO. Similarly, in more recent years, the 4050 Å group of cometary spectra, since it occurs in a discharge through CH_4, was first thought[6] to be due to CH_2. However, isotopic investigations[7] showed it to be due to C_2. The observation of the same spectrum in the flash photolysis of diazomethane is equally surprising if one bases expectation on photochemical arguments. Many similar examples could be quoted.

It is indeed interesting that the spectrum of NCN appears in the flash photolysis of fulminic acid as well as in the flash photolysis of diazomethane and in the action of active nitrogen on hydrocarbons. This information may now be used to draw conclusions about the kinetics of these various reactions, but we feel it is not possible to draw definite conclusions about the nature of the carrier of a spectrum from photochemical evidence alone.

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In our previous communication[1] we gave an account of experiments on the flash photolysis of cyanogen/oxygen and cyanogen/ozone mixtures in which a new transient band system was obtained in absorption in the wavelength region 3250–3330 Å. Although the rotational structure of the band system could not be resolved because of the low dispersion used, the chemical and kinetic evidence obtained from these and related experiments indicated that the most likely absorbing species was the free fulminate radical (C—N—O). This same spectrum has recently been obtained by Herzberg and Travis[2] also by flash photolysis, using diazomethane and diazomethane/cyanogen mixtures. These authors were able to carry out a vibrational and rotational analysis of the band system and from their observations have concluded that the absorbing species is NCN. This conclusion of Herzberg and Travis is substantiated by their carbon isotope experiments showing the presence of one carbon atom and the observation of an intensity alternation indicating the presence of two identical nuclei of non-zero spin. The observed triplet splitting is also consistent with the assignment of the absorber to NCN. However, these workers have not attempted to give any kinetic mechanism for the formation of the NCN radical in their systems and did not carry out any isotopic experiments to disprove the presence of oxygen.

Although the spectroscopic evidence in favour of CN_2 is very strong, particularly the observation of the intensity alternation, it is not so easy to reconcile this assignment with the chemical kinetics of the systems in which it has been observed, and it would seem almost as dangerous to rely solely on spectroscopic evidence and not consider the possible chemical processes which could lead to the formation of the postulated absorber.

The most significant fact to emerge from the flash photolysis experiments on cyanogen mixtures which we have carried out is that oxygen must be present in the

reaction mixture in order to obtain the 3290 Å system, and that the intensity of this system is strongly dependent on the oxygen concentration. Unless rigorous precautions are taken to ensure total exclusion of oxygen from the reaction mixture the 3290 Å system can be detected when cyanogen/inert gas mixtures are flashed. The most intense spectrum was obtained when the oxygen concentration was 1/100 per cent that of the cyanogen concentration. Since it is easy to attain this if the cyanogen is insufficiently degassed or if high swamping concentrations of inert gas containing a few tenths of a per cent air or oxygen are used it would be easy to overlook the important function of oxygen in obtaining the spectrum. With higher concentrations of oxygen the 3290 Å system is weakened, being totally suppressed in a $(CN)_2/O_2$ mixture of ratio 4/1.

The kinetic mechanisms postulated for the production of the fulminate radical in these experiments and in the flash photolysis of fulminic acid have already been discussed[1]. If the radical responsible for the 3290 Å band system is actually NCN, as suggested by Herzberg and Travis, it is not so easy to provide a convincing reaction scheme, since it must satisfy the following conditions.

(1) A variety of cyanogen mixtures were investigated, including those containing oxygen, ozone, nitrogen dioxide, nitrous oxide, carbon disulphide, carbonyl sulphide, halogens, nitric oxide, carbon dioxide and carbon monoxide. Only in the cases of oxygen, ozone and nitrogen dioxide was the 3290 Å system observed.

(2) The 3290 Å system was not observed when pure cyanogen was flash photolysed. This latter fact indicates that if the 3290 Å spectrum is due to CN_2 it cannot arise by the reaction:

$$CN + N \equiv C - C \equiv N \rightarrow NCN + C_2N \qquad (1)$$

which is in any event to be expected from thermochemical and kinetic considerations. It is also in agreement with condition 1.

When a cyanogen/oxygen mixture is flashed only the cyanogen dissociates to give two CN radicals. These react with the oxygen to give the observed NCO radical according to:

$$CN + O_2 \rightarrow NCO + O \qquad (2)$$

For the production of NCN the most feasible process would be the further reaction of NCO with a CN radical.

$$CN + NCO \rightarrow NCN + CO \qquad (3)$$

In the cases of cyanogen/ozone/argon and cyanogen/nitrogen dioxide/argon experiments, in which the cyanogen was prevented from direct dissociation in the photolytic flash and only the ozone and nitrogen dioxide broke up to yield respectively ^1D and ^3P oxygen atoms, the reaction scheme would then be:

$$O(^3P \text{ or } ^1D) + (CN)_2 \rightarrow NCO + CN \qquad (4)$$

followed by (3).

If the observed 3290 Å system is, in fact, due to NCN then it must be concluded that reaction (3) is responsible for its production and that it is a very fast reaction. It is interesting to observe here, however, that the analogous reaction (5) in the case of the sulphur analogue NCS, produced in the flash photolysis of cyanogen/carbonyl sulphide mixtures, does not occur, that is:

$$CN + NCS \rightarrow NCN + CS \qquad (5)$$

since no sign of the 3290 Å system appeared in any of the cyanogen/carbonyl sulphide experiments. Reaction (5), however, would undoubtedly not be as thermochemically favoured as reaction (3).

Recent work in this laboratory using cyanogen/ethyl isocyanate mixtures was inclusive as regards the occurrence of reaction (3) because of the insufficient concentration of NCO obtainable. Mass spectrum analysis of the final products from flash photolysed cyanogen/oxygen/argon mixtures, however, did show the presence of a small concentration of a stable product of mass 80, which would correspond with $(NCN)_2$ along with the expected CO and N_2. This result is indicative of the presence of NCN in the reacting system but is not conclusive proof for the assignment of the 3290 Å absorber to NCN.

A final point of difficulty with the NCN hypothesis is how it can be fitted into a reaction scheme for the combustion of cyanogen which results in only CO_2, CO and N_2 as end-products.

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A General Expression for the Isentropic Stagnation Temperature of a Fluid

The stagnation temperature of a fluid is commonly represented by the expression:

$$T_0 = T_\infty + \frac{U_\infty^2}{2c_p} \qquad (1)$$

where T, U and c_p denote the temperature, velocity and specific heat at constant pressure, and the subscripts 0 and ∞ refer to the stagnation point and free stream, respectively.

It must be remembered, however, that this expression is true only for a perfect gas. For general application it is recommended that equation (1) be presented in the general form:

$$T_0 = T_\infty + \frac{T}{v}\left(\frac{\partial v}{\partial T}\right)_p \times \frac{U_\infty^2}{2c_p} \qquad (2)$$

where v denotes the specific volume.

This equation is valid for mono-phase fluids, liquids or gases, and is obtained by correlating the changes of pressure, kinetic energy and temperature in the assumed isentropic process of stagnation.

The steady flow energy equation applied to an isentropic flow gives the isentropic change of enthalpy,

$$(\mathrm{d}h)_s = v\,(\mathrm{d}p)_s = -U\mathrm{d}U \qquad (3)$$

From Maxwell's relations and the definition of specific heat at constant pressure:

$$\left(\frac{\partial T}{\partial P}\right)_s = \frac{T}{c_p} \times \left(\frac{\partial v}{\partial T}\right)_p \qquad (4)$$

By substituting for $(\mathrm{d}p)_s$ from equation (3), and rearranging, equation (4) becomes:

$$(\mathrm{d}T)_s = -\frac{T}{v} \times \left(\frac{\partial v}{\partial T}\right)_p \times \frac{U\mathrm{d}U}{c_p} \qquad (5)$$

Equation (2) is then obtained by integrating equation (5) between the free-stream and stagnation conditions, and assuming that $\frac{T}{vc_p} \times \left(\frac{\partial v}{\partial T}\right)_p$ is a constant (or adopting an average value).

For a fluid obeying the perfect gas law the stagnation coefficient $\frac{T}{v} \times \left(\frac{\partial v}{\partial T}\right)_p$ is equal to unity; only then is equation (1) applicable.

For water at atmospheric pressure, the coefficient varies from about 0·04 at 14° C to about 0·12 at 50° C, but at high pressures and temperatures it may exceed that of a perfect gas. For example, at 200 atmospheres and 300° C the coefficient reaches 1·43. An even more interesting fact is that, at temperatures between 0° C and 4° C and low pressures, the stagnation coefficient for water is negative (because $\left(\frac{\partial v}{\partial T}\right)_p$ is negative). Thus, contrary to expectation, the stagnation temperature is less than that of the free stream.

I thank Prof. T. R. F. Nonweiler and Dr. A. S. Thom of the Department of Aeronautics and Fluid Mechanics, University of Glasgow, for their advice.

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MINERALOGY

Structure of Opal

Since the work of Flörke[1] it has generally been accepted that natural opaline silicas fall into two broad categories: (1) specimens which give X-ray patterns indicative of an obviously, although in some cases poorly, crystalline structure; (2) specimens which give X-ray patterns showing only a few diffuse bands. Earlier work by some of us[2] supported these conclusions, and a comprehensive re-examination of natural opals has confirmed that the structure can vary from almost perfect α-cristobalite to apparently amorphous or near amorphous material. The X-ray diffractometer traces shown in Fig. 1 illustrate the variations encountered; it should be noted, however, that, of the diffuse bands visible on powder photographs, only that at about 4·1 Å is recorded in traces *G* and *H*. Most specimens which would be termed 'common opal' by mineralogists are crystalline. Rarely, these may be well crystallized, as in sample *B* (which may be compared with *A*, a synthetic α-cristobalite), or very poorly crystallized, as in *F*. Most samples, however, give traces similar to *C*, *D* and *E*. Most precious opals and their associated 'potch', as well as hyalite, give patterns like *G* and *H*.

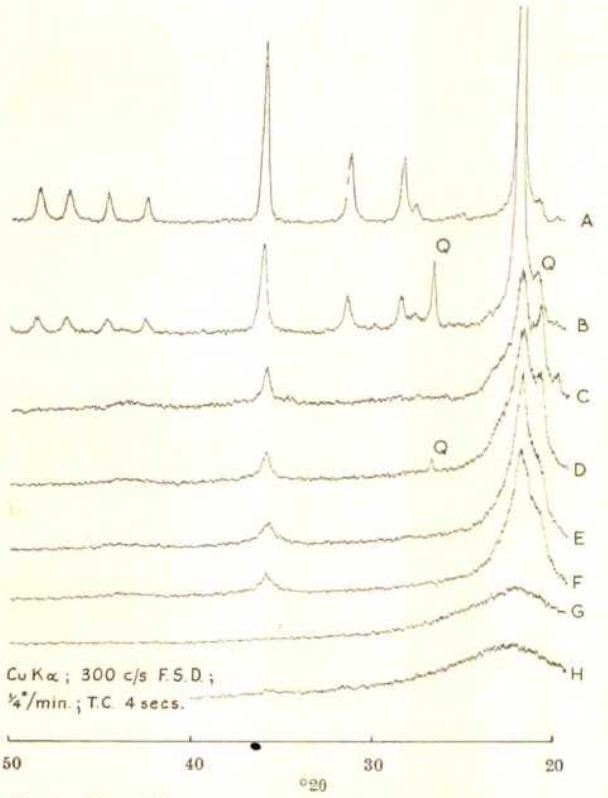


Fig. 1. X-ray diffractometer traces. *A*, α-cristobalite, prepared by heating opal from Coober Pedy, South Australia, at 1,200° C for 30 h; *B*, jasp-opal, Guanjuato, Mexico, No. 1664; *C*, common opal, Sunbury, Victoria, No. 19023; *D*, common opal, Kalgoorlie, Western Australia, No. 4680; *E*, common opal, Stony Creek, Victoria, No. 19020; *F*, jasp-opal, locality unknown, No. 2225; *G*, milky opal, locality unknown, No. 19000; *H*, precious opal, Coober Pedy, South Australia, No. 17015. Peaks marked *Q* are due to quartz. Numbers refer to specimens in the Geology Department Museum, the University of Adelaide

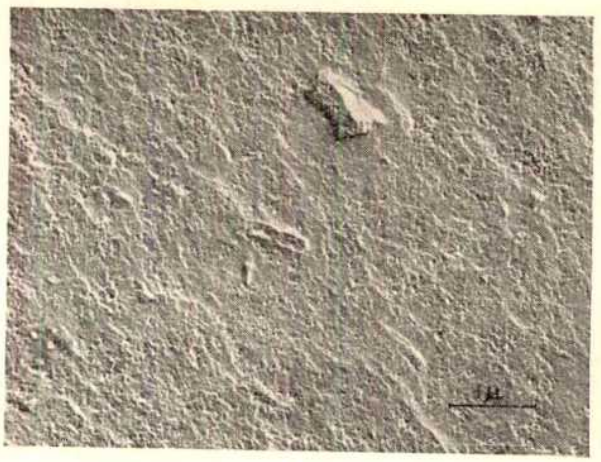


Fig. 2. Replica of fresh fracture surface of a transparent wood opal from Lake Eyre, South Australia. X-ray pattern of this sample is similar to *D*, Fig. 1, No. 1445

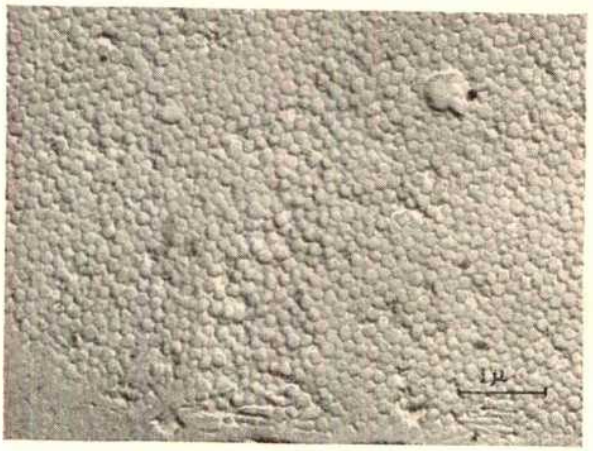


Fig. 3. Replica of transverse section of opalized wood, showing cell filled with silica spheres. Polished and lightly etched sample from White Cliffs, New South Wales. X-ray pattern similar to *H*, Fig. 1, No. 4662

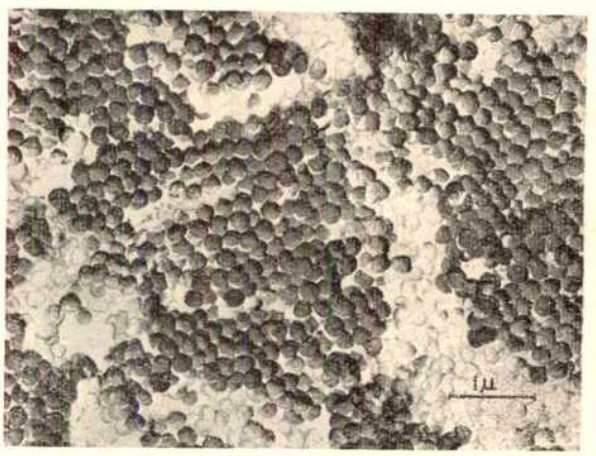


Fig. 4. Replica of etched surface of precious opal from Coober Pedy, South Australia. X-ray pattern *H*, Fig. 1, No. 17015

Although electron diffraction patterns of powdered samples generally confirmed the X-ray results, transmission electron micrographs showed only minor differences between the two groups of opal. By contrast, electron micrographs of surface replicas of freshly broken surfaces showed quite different gross structures. 'Crystalline' opals showed a relatively fine-grained uneven surface, the nature of which is still uncertain (Fig. 2). 'Amorphous'

opals, apart from glass-clear hyalites of low water content, showed discrete areas composed of close-packed aggregates of silica spheres of uniform size (Figs. 3 and 4). The diameter of the spheres is commonly in the range of 1500 Å–3500 Å.

The evidence presented here indicates a distinct break in structural properties between the two major groups of opals. It is believed that sufficient samples have been examined to indicate that this division is real, and may reflect differences in the genesis of the opals.

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CRYSTALLOGRAPHY

$TiAs_2$-type Phases

The structure of $TiAs_2$, which is of a new type, has recently been determined by Wenglowski *et al.*[1]. The orthorhombic unit cell contains eight formula units which comprise two kinds of titanium and four kinds of arsenic atoms. The co-ordination of Ti_I and Ti_{II} is very similar. Both have six As neighbours forming a trigonal prism and three more neighbours lying at the corners of a triangle perpendicular to the prism axis. The corners of this triangle are occupied by 3As in the case of Ti_I and by 2As + $1Ti_{II}$ in the case of Ti_{II}. As_I is located at the centre of a tetrahedron formed by $1Ti_{II} + 2Ti_{II} + 1As_I$. As_{II} and As_{III} lie at the centre of a distorted tetrahedron of $3Ti_I + 1Ti_{II}$. Finally, As_{IV} is placed at the centre of a trigonal prism of $2Ti_I + 4Ti_{II}$, a co-ordination which is also found in α-TiAs (TiP type) and β-TiAs (NiAs type). This co-ordination immediately reveals that $TiAs_2$ must be metallic though its composition would suggest the possibility of a semi-conductor of ionic formula $Ti^{4+}(As_2)^{4-}$. However, only one of the four different arsenic atoms forms pairs, but half the cations are connected as well. Assuming single bonds between Ti_{II}–Ti_{II} and As_I–As_I, we are left with 44 anion valence electrons per unit cell for the cation–anion bonds which cannot be balanced by the 28 available cation electrons and thus induce metallic conductivity. My resistivity measurements in fact confirm these conclusions.

$TiAs_2$ seems to be the only arsenide of this structure type, $ZrAs_2$ (ref. 2) and $HfAs_2$ (ref. 3) crystallizing in the *C*23 type of structure. We found this structure, however, in two antimonides, $ZrSb_2$ and α-$HfSb_2$ (low-temperature modification). Their lattice constants are listed in Table 1 together with the data derived for $TiAs_2$. Though Wenglowski *et al.*[1] deduced from their tensimetric analysis the existence of a broad homogeneity range for $TiAs_2$, we could not detect a difference in the X-ray patterns of different samples of all three compounds. $ZrSb_2$ and $HfSb_2$ also show metallic conduction and a small *p*-type thermoelectric power. Since no localized *d* electrons are left on the cations, a temperature-independent dia- or para-magnetism is to be expected in these compounds.

There is little doubt that $ZrBi_2$, the lattice constants of which have been reported by Bykov and Kazarnikov[4], also crystallizes in this structure. Our bismuthide samples did not allow a determination of the lattice constants but Guinier patterns give some indication that $HfBi_2$ is isostructural with $ZrBi_2$.

Table 1. Lattice Constants of $TiAs_2$-type Phases

	a (Å)	*b* (Å)	*c* (Å)
$ZrSb_2$	14·974 ± 14	9·968 ± 5	3·878 ± 3
α-$HfSb_2$	14·981 ± 12*	9·869 ± 5	3·848 ± 3
$TiAs_2$	13·220 ± 8	8·915 ± 4	3·478 ± 2
	13·27 (ref. 1)	8·96 (ref. 1)	3·50 (ref. 1)

I thank Mr. H. U. Boelsterli for assistance in sample preparation and X-ray work.

Note added in proof. For $HfBi_2$, which in powder form is as strongly pyrophoric as $ZrBi_2$, I obtained the following lattice constants: $a = 15{\cdot}7$ Å, $b = 10{\cdot}1$ Å, $c = 3{\cdot}93$, which are nearly the same as those of $ZrBi_2$ (ref. 4).

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CHEMISTRY

Chlorine Hyperfine Splittings in Phenoxy Free Radicals

Hollocher *et al.*[1] have presented evidence for chlorine hyperfine splittings of the electron spin resonance spectrum of 2-hydroxy-1,4-naphthosemiquinone chloro-derivatives. The observed splitting was quite small (0·28 gauss) and no attempt was made to present a value for a chlorine splitting constant. In order to obtain a larger splitting from the chlorine nuclear spin and perhaps also to obtain an estimate of the splitting constant, an electron spin resonance investigation of the chloro-derivatives of phenoxy radicals was made. The electron spin resonance spectrum of the unsubstituted phenoxy radical has been obtained by Stone and Waters[2], using a flow apparatus[3] in order to detect this short-lived free radical. The electron spin resonance spectra of the free radicals of phenol, *p*-chlorophenol, 2,6-dichlorophenol and 2,4-dichlorophenol have been detected using a similar system and are reported here.

The compounds were obtained from K. and K. Laboratories and from Eastman Kodak, and were used without further purification as 10^{-3} molar aqueous solution. Free radicals were generated by oxidizing these compounds with an acidic solution of ceric ammonium sulphate, and detected with a Varian 4,500 *X*-band spectrometer.

The electron spin resonance spectra obtained are shown in Fig. 1, and the hyperfine splittings assigned to these spectra are listed in Table 1.

Table 1. Hyperfine Splittings (Gauss)

Radical	Doublet	Triplet	Sextet
Phenoxy	10·1	6·6	
		1·8	
p-Chlorophenoxy		6·6	1·9
2,6-Dichlorophenoxy	9·7		
2,4-Dichlorophenoxy	7·9		1·9

A comparison of the hyperfine splittings enables us to assign them to specific protons or chlorine atoms in the different ring positions. For example, the large doublet splitting (~ 10 gauss) occurs in the electron spin resonance spectra of the phenoxy, and 2,6-dichlorophenoxy, radicals but not in the other two. Hence, we can assign the 10 gauss splitting to the 4 position proton of the phenoxy radical.

Similarly, in the phenoxy and *p*-chlorophenoxy radicals, we have triplet splittings of 6·5 gauss, but in the splittings for the 2,4-dichlorophenoxy radical we have a doublet of 7·9 gauss. Although these are somewhat different, we feel that it is reasonable to assign this splitting to the 2 and 6 position protons.

We have then assigned splittings to each of the ring protons in the phenoxy radical; the next step is to evaluate

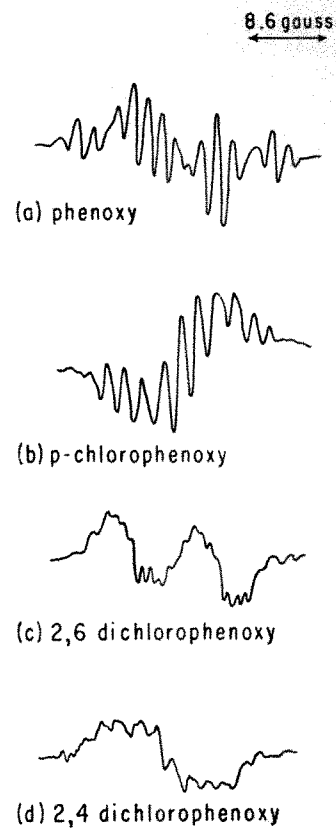


Fig. 1. Electron spin resonance spectra of (*a*) phenoxy, (*b*) *p*-chlorophenoxy, (*c*) 2,6-dichlorophenoxy, (*d*) 2,4-dichlorophenoxy radicals

the chlorine splittings. Comparing the splittings for phenoxy and *p*-chlorophenoxy radicals, we see that a doublet of 10·1 gauss and a triplet of 1·8 gauss in the phenoxy electron spin resonance spectrum are replaced by a sextet of 1·9 gauss in the *p*-chlorophenoxy radical spectrum. The sextet is due to an effective nucleus of spin quantum number 5/2, which it is reasonable to assume, to be made up of two equivalent protons (I = 1) and one equivalent chlorine nucleus (I = 3/2). This would mean that a chlorine in the 4 position of a phenoxy type radical produces a splitting of 1·9 gauss. A similar comparison with the electron spin resonance spectrum of 2,4-dichlorophenol shows the 4 position chlorine to give a splitting of 1·9 gauss. If we now made an assumption that the substituting of a Cl for an H does not change the distribution of the unpaired electron in the phenoxy radical drastically, we may make an estimate of the value of the ring chlorine splitting constant.

Using the equation[4]:

$$\Delta H_p = Q_p \rho$$

where ΔH_p is the splitting due to a ring proton, ρ is the unpaired electron density on the adjacent carbon atom, and Q_p is the ring proton splitting constant. We assume a similar relationship:

$$\Delta H_c = Q_c \rho$$

where Q_c is the ring chlorine coupling constant and ΔH_c the splitting it produces.

We may write then:

$$Q_c = Q_p \times \frac{\Delta H_c}{\Delta H_p}$$

$$Q_c = 0{\cdot}19\ Q_p$$

If we take Q_p to be 24·2 gauss[5], then:

$$Q_c = 4{\cdot}6 \text{ gauss} \pm 0{\cdot}6 \text{ gauss}$$

This value has been arrived at assuming there is no effect of the A on the π electron distribution.

This work was supported by grants from the American Cancer Society and the National Institutes of Health. An acknowledgement is due to the Wellcome Foundation (B. T. A.).

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Chemiluminescent Reactions of SO with O and with O_3

The spectrum of the sulphur dioxide afterglow was first investigated in detail by Gaydon[1], who reported that it appeared to be continuous over its entire range from 2400 Å into the visible, apart from some weak bands above 3800 Å which are now known to arise from the (0,0,0) and (0,1,0) levels of the $^3B_1$ state of SO_2 (refs. 2 and 3). More recently, Herman *et al.*[4] have investigated the afterglow at low pressures and found no evidence of banded structure; they confirmed Gaydon's view that the afterglow was due to the chemiluminescent combination of O and SO, but did not establish whether it required a third body.

Recently, we have used a fast flow system to examine the reactions occurring in the products of a radiofrequency discharge through sulphur dioxide in an argon carrier, and have shown that the intensity (I) of the sulphur dioxide afterglow obeys the relation:

$$I = I_0[\text{O}][\text{SO}]$$

It has not, however, been possible to determine whether the quantity I_0 depends on total pressure. This alone might not establish that the luminescent reaction requires a third body since for the analogous air-afterglow reaction involving the chemiluminescent combination of O and NO to form electronically excited NO_2, the quantity I_0 is independent of pressure over a considerable range[5,6]. In this case, kinetic evidence[5] and the presence of banded structure near the high-frequency limit of emission[7] support the view that a third body is involved.

We have observed weak but definite banded structure near the short wave-length limit of the sulphur dioxide afterglow at room temperature. In separate experiments we have established that sulphur dioxide does not absorb significantly in this region until the product of concentration and path length is twenty times the maximum that could have been present in our experiments. This provides clear evidence that a third body is required for the chemiluminescent combination reaction O + SO and that the 'continuum' which reaches its maximum intensity at about 2700 Å is a mass of overlapping bands arising from many vibrational levels in the excited state.

The origin of the triplet bands is of interest. Gaydon[1] observed that they were more intense at higher pressures; in addition, we find that they are greatly enhanced when additional sulphur dioxide is added downstream from the discharge. This establishes that these bands are not due to long-lived triplet SO_2 formed in the discharge. The observation that this enhancement is not accompanied by any detectable shift in the overall spectral distribution from the ultra-violet to the visible suggests that the effect of added SO_2 is to increase the rate of vibrational relaxation in the triplet state of SO_2, since the appearance of the bands from the lowest vibrational levels would then be accompanied by a matching reduction in the intensity of that part of the 'continuum' which has a

maximum near 4500 Å (ref. 4) and which presumably consists of transitions from higher vibrational levels of the triplet state. This interpretation is supported by the observation that the triplet bands are enhanced, albeit to a lesser extent, by the addition to the discharge products of CO_2 or SF_6, both of which normally have high efficiencies for vibrational quenching. Molecular oxygen does not show this effect.

At relatively high pressures, oxygen atoms decay much more rapidly than SO, the reactions of which can then be examined readily beyond the point at which the sulphur dioxide afterglow has faded out. Under these conditions, added ozone undergoes a rapid reaction accompanied by blue and ultra-violet chemiluminescence. This reaction can only be:

$$SO + O_3 = SO_2 + O_2 + 106 \text{ kcal/mole} \qquad (1)$$

since the spectrum consists of strong emission from the $^3B_1$ state of SO_2 (minimum excitation energy 74 kcal/mole) and weaker emission from the $^1B_2$ state (threshold energy 85 kcal/mole). The emission from the $^3B_1$ state comes mainly from vibrational levels (0,0,0)and (0,1,0), although transitions from the (1,0,0) level appear weakly.

The $^1B_2$ emission is very different in distribution from the SO_2 afterglow. It consists of a series of bands commencing near 3000 Å which under low dispersion merge into a pseudo-continuum at about 3300 Å. This 'continuum' reaches its maximum at about 3500 Å and extends to 4000 Å. The bands observed cannot be due to self-reversal; they correspond to those which appear in absorption in this region. This is to be expected, since the highest frequency bands must be transitions to the lowest levels of the ground state. The highest energy band so far observed corresponds to (3,6,0), (4,3,0) or (5,0,0) in Metropolis's scheme[8], and it seems clear that the pseudo-continuum at longer wave-lengths arises from overlapping bands mainly involving lower vibrational levels of the excited state.

A strict proportionality was always observed between the intensities of the singlet and the triplet bands; we therefore conclude that both states are populated by the same reaction and that the $^1B_2$ state is not populated in the collision of two $^3B_1$ molecules by a mechanism analogous to that of delayed fluorescence[9].

There is a clear similarity between reaction (1) and the chemiluminescent reaction[10,11]:

$$NO + O_3 = NO_2 + O_2 + 48 \text{ kcal/mole} \qquad (2)$$

which has recently been shown to populate lower vibrational levels of the excited state of NO_2, which is also responsible for the air-afterglow emission[11].

Reaction (2) has been shown to have a higher activation energy when it yields electronically excited NO_2 than when ground state NO_2 is formed[11]. On this basis[12], reaction (1) would be expected to have successively higher activation energies to yield SO_2 in its ground ($^1A_1$), $^3B_1$ and $^1B_2$ states. This would explain why the $^3B_1$ emission is roughly ten times more intense than the $^1B_2$ emission in the $SO + O_3$ reaction, while the reverse is true in the O + SO reaction, which is expected to have zero activation energy. The latter situation would be predicted from the radiative lives, which the relative intensities of the absorption spectra show to be much greater for the $^3B_1$ state than for the $^1B_2$ state.

For this reason it is unlikely that the observed vibrational energy distribution of molecules in the $^3B_1$ state resembles that with which they are formed. The radiative lives of the $^1B_2$ state[13] and of the emitting state of NO_2 (ref. 14) are much shorter; the vibrational energy distribution of the emitting states is therefore probably similar to that with which the excited molecules are formed. This distribution of energy together with Franck–Condon factors will determine the spectral distribution of the emission. To judge by the corresponding absorption spectra, the Franck–Condon factors make a large contribution to the observed spread of the emission, but these factors are unlikely to vary greatly from level to level in the upper state. Thus the difference in energy between a particular vibrational level and the most probable transition from it should be almost constant for a given excited state. Table 1 shows that, for the same emitter, the difference between the most probable energies of emission is equal to the difference between the exothermicities of the reactions forming it. This suggests that the difference between the exothermicity of the reaction and the most probable excitation energy is roughly constant.

Table 1

Reaction	Exothermicity (kcal/mole at 298° K)	Wave-length of max. emission (Å)	Energy of max. emission (kcal/mole)
$O + NO \rightarrow NO_2^*$	73	6000	48
$O_3 + NO \rightarrow NO_2^*$	48	12,000	24
$O + SO \rightarrow SO_2^*$ ($^1B_2$)	131	2700	106
$O_3 + SO \rightarrow SO_2^*$ ($^1B_2$)	106	3500	82

The general similarities between the four chemiluminescent reactions discussed here, the similarity of Franck–Condon factors suggested by the constant difference between columns two and four in Table 1, and the closeness of the measured radiative lives[13,14] and of the integrated absorption coefficients all suggest that the main visible and near ultra-violet absorption spectrum of NO_2 is analogous to the $^1B_2$–$^1A_1$ transition of SO_2, that is, that it is a $^2B_2 \leftarrow {}^2A_1$ transition (presumably $2b_1 \leftarrow 1a_2$) (ref. 15). The formation of $^1B_2SO_2$ molecules in reaction (1) is clearly accompanied by anomalous vibrational excitation. Detailed investigations of this and reaction (2) are being carried out since the examination of vibrational excitation in reactions which yield electronically excited species has advantages over the method of infra-red chemiluminescence[16]. These include the wider choice of detectors and the shorter radiative lives of the species involved, which makes it possible to work at higher pressures.

We thank the Department of Scientific and Industrial Research for a maintenance award to one of us (C. J. H.) and for a special research grant.

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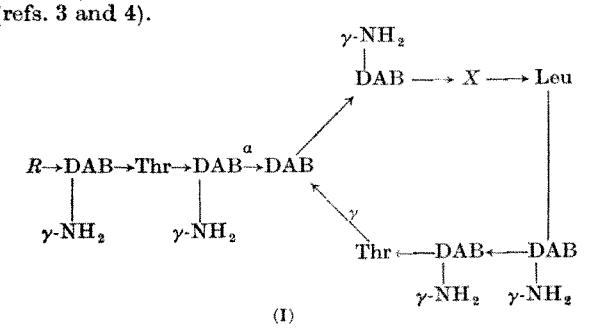
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Structures of Polymyxin *B2* and Polymyxin *E1*

In a previous communication[1], from the analysis of a partial acid hydrolysate, the structure of polymyxin *E1* was shown to be limited to two alternatives, the so-called 7α and 8γ formulæ. By applying the enzyme method of hydrolysis as devised by Suzuki *et al.* in their determ-

inations of the structures of polymyxin $B1$ (ref. 2) and colistin A (ref. 3), the problem has now been resolved.

We have isolated the peptides Ipel$\xrightarrow{\alpha}$DAB$\rightarrow$Thr and Ipel$\xrightarrow{\alpha}$DAB$\rightarrow$Thr$\xrightarrow{\alpha}$DAB from the hydrolysate of polymyxin $E1$ with the enzyme Nagarse (supplied by Teikoku Chemical Industry Co., Ltd., Osaka, Japan). The presence in particular of the second peptide eliminates the 8γ variant, and the structure of polymyxin $E1$ is therefore concluded to be (I; $R=(+)$ 6-methyloctanoyl, $X=$ D-Leu) and is therefore identical with that of colistin A (refs. 3 and 4).



(I)

Bands having the same electrophoretic mobilities as the two peptides described above are found in a comparable enzymatic hydrolysate of polymyxin $B2$ and therefore, in conjunction with the previous results of partial acid hydrolysis[1], this antibiotic is assigned the structure (I; $R=$ 6-methyl-heptanoyl, $X=$ D-Phe).

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BIOCHEMISTRY

Release of Carbohydrate from Fibrin Monomer during Clotting

Human fibrinogen contains a small amount of carbohydrate, consisting of about 3·2 per cent hexoses, about 1 per cent glucosamine and about 0·8 per cent sialic acid[1–4]. In the transition from fibrinogen to fibrin in clotting, a decrease in the carbohydrate content has been reported[1–4]. Thrombin, which catalyses the proteolytic cleavage of the fibrinopeptides at arginyl-glycine linkages[5], is not known to have any glycolytic function, although the release of carbohydrate is reported to parallel thrombin action during clotting[2]. Laki and Mester[6] showed that selective oxidation of the carbohydrate moiety of fibrinogen resulted in concomitant loss of clottability, although no changes in the fibrinogen molecule other than the oxidation of carbohydrate occurred. Recently, Laki and Chandrasekhar[7] found that selective enzymic alteration of fibrinogen by sialidase altered the ability to form 'insoluble' fibrin, but not the clottability of fibrinogen. 'Insoluble' fibrin, differing from 'soluble' fibrin by urea solubility as well as other characteristics[8], is formed through the reaction of a fibrinogen-like, plasma protein, fibrin stabilizing factor (FSF)[9], which in the presence of Ca^{++} converts what would otherwise be a 'soluble', weakly bonded gel into a covalently bonded, insoluble clot. Laki and Chandrasekhar[7] postulated, therefore, that the activity of FSF was related to sialic acid and the carbohydrate moiety of fibrinogen.

In order to investigate the proposed role of FSF activity in the release of carbohydrate from fibrinogen, preliminary experiments have been done in which the supernatant has been analysed for released carbohydrates after clotting a solution of purified fibrin monomer in the presence of FSF and Ca^{++} but in the absence of thrombin.

Human fibrinogen was fractionated by the method of Laki[10] and further purified by the procedure of Bergström and Wallén[11]. Fibrin monomer was prepared from this fibrinogen by the method of Donnelly *et al.*[12] as modified by Latallo *et al.*[13], keeping a constant concentration of 0·01-M EDTA in all solutions to prevent FSF activity during preparation of the pure material. The resultant preparation formed soluble clots even in the presence of Ca^{++}. Pure FSF was prepared by the method of Loewy *et al.*[14] and assayed at about 50 per cent of the activity of his preparation. An indole-sulphuric acid method for assaying hexoses and pentoses was used[15]. The method was found to be sensitive to 5 μg of sugar in the standard samples, giving a change of optical density 480 mμ = 0·035. The initial monomer concentration was 61 mg/ml. dissolved in 1 M sodium bromide in 0·05 M acetate buffer at *p*H 5·2. This was diluted 20-fold with 0·067 M sodium phosphate buffer at *p*H 6·1 to obtain a concentration of 3·05 mg/ml. in the clotting mixture.

Table 1. Analysis of Supernatant for Carbohydrate* after Clotting of Fibrin Monomer with Added FSF and Ca^{++}

Incubation time after dilution of monomer and initiation of clotting (min)	*O.D.* 480 mμ (corrected for blank with water)	Variation from baseline *O.D.* after dilution and before FSF addition
0 (before add. of FSF)	0·176	
0 (just after add. of FSF)	0·170	−0·006
5	0·157	−0·019
15	0·193	+0·017
30	0·179	+0·003
45	0·172	−0·004
75	0·180	+0·004
360	0·186	+0·010

* Indole-H_2SO_4 method of Ashwell (ref. 15).

Ten ml. aliquots of the clotting solution and clot supernatant were removed before addition of FSF (final concentration of 0·01 mg/ml.) and 0·75 mM Ca^{++}, immediately after the addition, and subsequently at 5, 15, 30, 45, 75 and 360 min. Each aliquot was mixed with absolute ethyl alcohol to stop any enzymatic process and to denature all protein. The mixture of test supernatant and alcohol was reduced to a 2-ml. volume in a negative-pressure desiccator to concentrate any sugar present, thus giving a final protein concentration (now denatured) of 15·25 mg/ml. This solution was centrifuged and 1 ml. of it used for carbohydrate determination.

As shown in Table 1, there is a random fluctuation of $\pm 0{\cdot}017$ $O.D._{480\ m\mu}$ during the time of incubation of the clots, but no significant increase in sugar is noted. A release of 1 mole of sugar per mole of fibrin should give, at the concentration used, a definite increase outside the limits of error. Our inability to find any hexoses or pentoses in the supernatant of a clotted solution of fibrin monomer (in the presence of FSF and Ca^{++}) is negative evidence for the function of FSF as an amylase. The carbohydrate lost from fibrinogen in clotting may be difficult to analyse due to absorption on to the clot, not lost as hexoses or pentases but as other carbohydrates, or lost from the fibrinogen molecule during its transformation into fibrin (despite the lack of glycolytic activity of thrombin) and thus presumably not cleaved by FSF.

Clotting in this work did occur at *p*H 6·1, perhaps not the optimum *p*H for FSF activity in the formation of insoluble clots under the same conditions and within the same time period. It would be of interest to analyse for differences in carbohydrate content between fibrinogen and fibrin monomer, as well as between fibrin monomer, soluble fibrin and insoluble fibrin in order to further elucidate the problem of the nature of FSF activity and the manner of the release of carbohydrate. Specific analysis of clot supernatant for sialic acid and for glucosamine must also be carried out.

This work was supported in part by U.S. Public Health Service grants *HTS*–5222 and *HE*–06314 from the Heart Institute.

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Chromatographic Identification of Lysins in Normal Tissues and Tumours of Mice

THE two hæmolytic materials found in the normal tissues of the mouse and man, and also in mouse and human tumours, have been partially identified as being higher fatty acids (or their soaps) and lysolecithin; the former give a characteristic ultra-violet fluorescence, and the latter are soluble in ethanol but not in cold ether[1,2]. This communication concerns their more complete identification by silica-gel thin-layer chromatography.

The separations were carried out on glass plates, 20 × 5 cm, coated with silica-gel according to standard procedure. Small quantities (0·01 ml.) of known solutions of fatty acids or lipids, or of unknown solutions (1 gm tissue in 5 ml. ethanol) of the ethanol soluble material of mouse tissues or tumours, are spread across the lower end of the plate; after drying, the plates are placed vertically in methanol–chloroform, which is allowed to ascend for about 18 cm. The plates are made in duplicate, so that one of each can be developed with iodine vapour to show the position of the unsaturated fatty acids, etc., and these positions are marked on the duplicate plates. Transverse strips, each 5 mm wide, are scraped off the plates at various levels, for example, at regions to which the fatty acids have migrated, and also at regions in which there is no reaction with iodine (blanks). The scraped-off material is received in test tubes, one for each scrape; 1 ml. of redistilled ethanol is added to each, and after 15 min the tubes are centrifuged and the supernatant fluid of each is poured off into porcelain cupels, one for each test-tube. The cupels are dried for 60 min at 60° C; 1 ml. of a suspension of washed red cells (the cells of 50 mm³ of blood to 10 ml. of saline) is added to each, and they are transferred to hæmolysis tubes. These are kept in a water-bath at 37° C, with hourly shaking, and the time for complete hæmolysis is determined for each.

The difficulty lies in avoiding the appearance of irregular lytic regions on the plates. Control plates (blanks) must show no hæmolytic regions, regions which do not stain with iodine should be negative, and many regions which stain with iodine are also not lytic. Avoidance of the appearance of irregular hæmolytic regions requires a standardization technique in which the samples do not come into contact with hands, a cell suspension buffered at *p*H 7, and redistilled ethanol for extraction.

The chromatographic plates usually show 6–8 components which stain with iodine vapour, but the only regions which produce hæmolysis in 10 h or less are: (*a*) a region which corresponds to the position of oleic acid; and (*b*) a region which corresponds to the position of lysolecithin.

Comparing plates of ethanol extracts of normal mouse tissue (liver, lung) with those of ethanol extracts of C_3H mouse tumour in the same concentration (g tissue/g extraction medium), the number and position of the regions which stain with iodine vapour are the same, and the same regions are hæmolytic, but the intensity and size of the regions corresponding to oleic acid and lysolecithin are greater for the extracts of liver and lung than for the extracts of tumours; measurement of fluorescence also shows the concentration of fluorescent material to be two to six times greater in the mouse liver and lung (male or female) than it is in the female breast tumour. The hæmolytic activity of the mouse tumour, however, is usually several times greater than that of normal mouse tissue[3,4]. That the lysin-inhibitor complex[5,6] in normal mouse tissues is more strongly bound than that in tumours is a possibility to be investigated.

This work was supported by a grant, *CA*–06604–02, from the U.S. Public Health Service, National Cancer Institute.

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Electrophoretic Patterns of Lysed Fibrin formed by Homologous and Heterologous Thrombin

THROMBIN[1] and fibrinogen[2] have been shown to exhibit species specificity. Recently attention has been directed to the possible removal of intravascular clots by fibrinolytic agents. Since high concentration of thrombin produces lysis of fibrin[3], it was of interest to investigate the effect of homologous and heterologous reagents.

In each of 2 tubes, equal volumes of fibrinogen (1 g/100 ml.) and thrombin (500 units/ml.) were quickly mixed and incubated at 37° C. Lysis was prevented in the control by the immediate addition of equal volume of 10 M urea (dissolves fibrin) in 0·1 M sodium acetate/acetic acid buffer *p*H 4·85, while in the test this reagent was only added after lysis had occurred, usually in 2½–4 h. In order to minimize the precipitation of fibrin and its lysed products, paper electrophoresis was carried out using 0·1 M sodium acetate/acetic acid buffer *p*H 4·85 (no final polymerization of fibrin at *p*H 4). For reasonable separation of components a potential of 260 V was applied for 28 h. After staining with azocarmine *B*, the strips were scanned with a chromoscan.

There was no demonstrable difference between the electrophoretic patterns of lysed and unlysed fibrin in urea on using homologous thrombin and fibrinogen (Table 1). On the other hand, the use of heterologous reagents resulted in the appearance of a new band moving towards the anode. This band was well illustrated on using bovine thrombin and human fibrin (Fig. 1). Increasing the concentration of thrombin did not alter the



Fig. 1. Change in electrophoretic pattern on using heterologous thrombin (bovine) and fibrinogen (human). (*a*) Unlysed fibrin dissolved in urea; (*b*) lysed fibrin mixed with urea

results. These findings prompted the following investigations:

(*a*) Thrombin–fibrinogen interaction without fibrin clot formation: this was achieved by maintaining the reaction at *p*H 4·85 throughout the experiments, thus allowing thrombin to act on fibrinogen but preventing the final formation of fibrin mesh[4]. The same concentrations of reagents mentioned here were used; urea being added after 48 h in order to compensate for the slowing of the thrombin–fibrinogen interaction occurring at *p*H 4·85. No electrophoretic changes could be elucidated on using heterologous reagents. It would therefore appear that the formation of fibrin is essential for the development of the new band.

Table 1. The Effect of Thrombin Species on the Electrophoretic Pattern of Lysed Fibrin

Species		
Thrombin*	Fibrinogen*	New band
Bovine	Human	Present
Human	Bovine	Present
Human	Human	Absent
Bovine	Bovine	Absent

* Freshly prepared solutions used after being clarified by centrifugation at 3,000 r.p.m. for 10 min.

(*b*) Other methods of lysis: for these, clotting of fibrinogen was induced by a lower concentration of thrombin, 10 units/ml. The lysis of this fibrin by the addition of streptokinase (activating plasminogen in fibrinogen solution) or by human plasmin 'Ortho-Actase' gave electrophoretic results similar to those described for the lysis of fibrin clot by thrombin. These findings indicated that the new electrophoretic band, appearing in case of heterologous reagents, was not related to the lytic agent used but rather to the molecular configuration of formed fibrin.

The exact nature of this electrophoretic band remains to be investigated. However, this variance in the electrophoretic pattern of lysed fibrin, depending on the source of thrombin, should be borne in mind when applying experiments on, or using reagents of, different species.

I thank Ortho Pharmaceutical, Ltd., for a gift of 'Actase'.

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PHYSIOLOGY

Simple Separation of Rabbit Marrow Myeloid Cells from Erythroid Cells

Mel has recently reviewed the sedimentation properties of nucleated and non-nucleated cells in normal rat bone marrow[1]. The complex nature of the factors that are involved in the separation of marrow cells by sedimentation technique are emphasized in his comprehensive paper. Tsuji reported a method for separation of guinea-pig bone marrow cells by using layers of gum acacia solutions of varying densities[2]. Myeloid cells were obtained in practically pure state. Gradient techniques involving several kinds of solutions, such as sucrose and bovine plasma albumin, have been used by others[3].

Procedures for separating bone marrow cells of the rabbit have been investigated in this laboratory for a number of months. Approximately 2 ml. of cells are obtained from the femurs, tibiae and humeri of one animal and suspended in isotonic NaCl solution. They are then filtered through a nylon filter (No. 9331, The MacBick Co., Cambridge, Mass.). After centrifugation and removal of buffy material the cells are placed in 10 ml. of 6 per cent sucrose solution and gently layered on top of seven other layered sucrose solutions, in concentrations from 15 per cent to 75 per cent, with an increment of 10 per cent in successive layers from above downwards. Each of these solutions is 10 ml. in volume and the test-tube is 6 in. in length and 1¼ in. in diameter. The cells in solution are spun at 350*g* for 6 min. The non-erythroid elements are distinctly banded in the lower 3–4 density zones. They can be reconstituted in isotonic NaCl solution and are practically free of erythroid forms. The latter, which are in the top layers, can be obtained by similar reconstitution.

Our most successful method, however, and one which does not involve subjecting the cells to distortion by hypertonic solutions is the simple procedure of adding 6 ml. of isotonic saline to 1·5–2·0 ml. of marrow cells in a 10 ml. centrifuge tube, mixing, and allowing the tube to stand undisturbed at 6° C for 1 h. Under the influence of 1*g* there is marked sedimentation of predominantly myeloid cells. The erythroid cells are almost entirely restricted to the top solution. The time is fairly critical since after 1 h the erythroid cells begin to settle on top of the myeloid cells. Similar successive resuspensions of the bottom section will result in practical isolation of non-erythroid and erythroid elements. This can be accomplished in about four such treatments.

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Presynaptic Inhibition induced by Muscle Stretch

The existence of a powerful inhibitory mechanism acting at the level of the primary afferent fibre has been well demonstrated[1]. The method by which this presynaptic inhibitory pathway acts is generally accepted to be through a prolonged depolarization of the central terminals of the primary afferent fibres with, as a consequence, depression of the amount of excitatory transmitter substance liberated by their synaptic terminals. This depolarization of the central terminals of a particular group of primary afferent fibres is effected in a rather specific manner by afferent impulses from some types of receptors and not from others. For example, the group I afferent impulses entering the spinal cord through the sensory fibres of the nerve to a flexor muscle (but not an extensor) will produce a prolonged depolarization of the central terminals of the primary afferent fibres from annulospiral endings of muscles of that limb, both flexors and extensors.

So far, presynaptic inhibition has been tested by electrically stimulating a given afferent nerve and noting the effects on the central terminals of other primary afferent fibres and on the excitatory synaptic action by

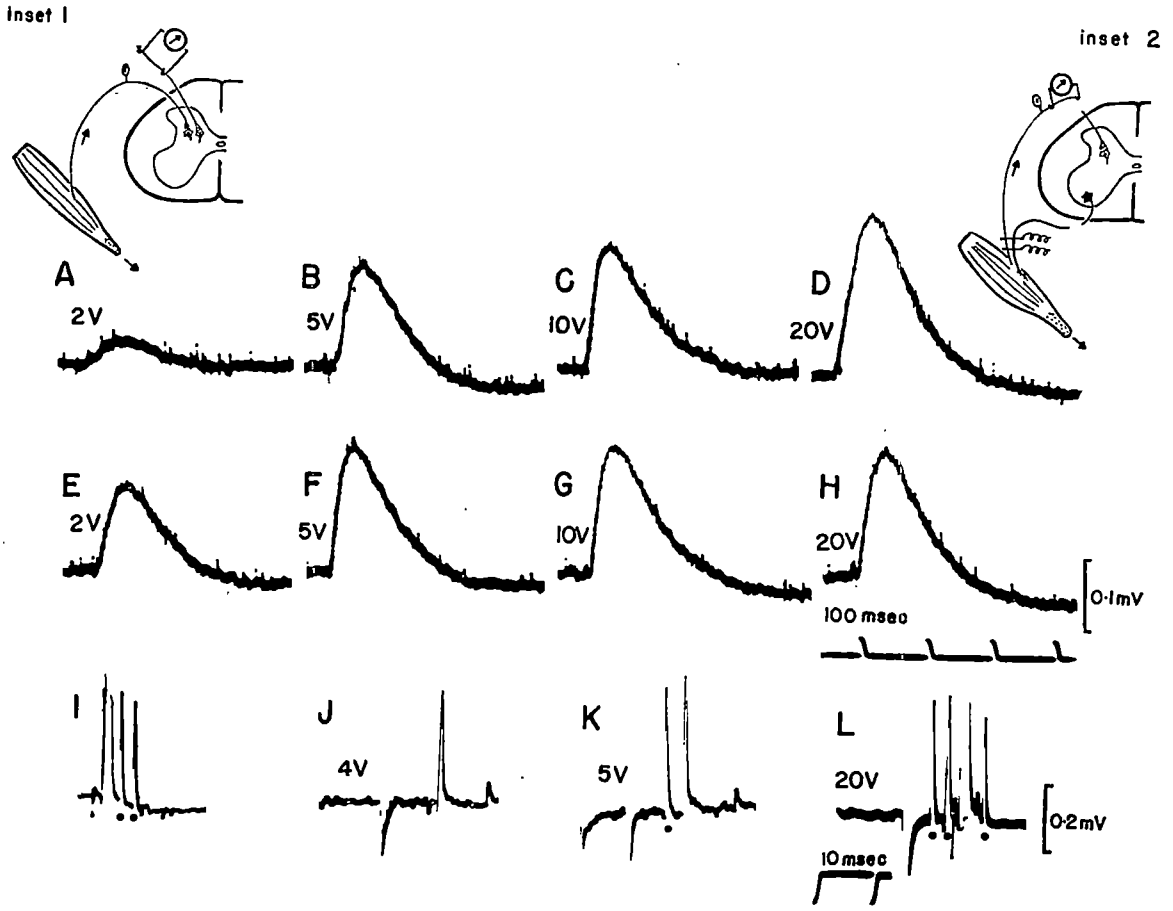


Fig. 1. *DRP*s recorded from an *L6* dorsal rootlet, as illustrated in inset 1. *A*, *B*, *C* and *D* show the *DRP*s produced when the *P* muscles were stretched, and *E*, *F*, *G* and *H* the *DRP*s when the *DP* muscles were stretched. The amplitude of the square pulse driving the vibrator is given in volts at the end of each *DRP*. *I* shows the impulses recorded in an *L7* dorsal root filament when the *DP* muscles were made to contract isotonically. *J* shows the beginning and end of the pull-artefact, using a 4-V pulse. In *K* and *L*, impulses (marked by dots) in the dorsal root filament, generated by a stretch of 5 V and 20 V respectively, are shown. Recording conditions are illustrated in inset 2

those fibres. These tests have thus employed conditioning by synchronous volleys and not the asynchronous repetitive discharges produced naturally by receptor organs. The present investigation has approximated much more closely to natural physiological conditions by investigating the presynaptic inhibitory action that is produced by the discharges of muscle receptor organs excited by brief stretches to the deep peroneal (*DP*) and peroneal (*P*) muscles.

Adult cats were anæsthetized with 'Nembutal', and the spinal cord was transected at the first lumbar segment. Both hind limbs were completely denervated except for the nerves to the peroneal and deep peroneal muscles on the left side. The muscles were pulled, using an electromagnetic vibrator, Goodman's model 390*A*, which was driven by a transistor d.c. power amplifier. The intensity of the stretch was determined by the amplitude of the square pulse driving the vibrator.

The depolarization of the central terminals of primary afferent fibres was recorded by two accepted techniques. In Fig. 1 (inset 1) it was recorded as an electrotonic spread into the dorsal rootlets, that is, the dorsal root potential (*DRP*)[2]. *A*–*D* show the *DRP*s produced when the *P* muscles were stretched, and *E*–*H* when the *DP* muscles were stretched. The intensity of the pull was progressively increased in each case, as shown by the amplitude of the square pulse used to drive the vibrator which is specified for

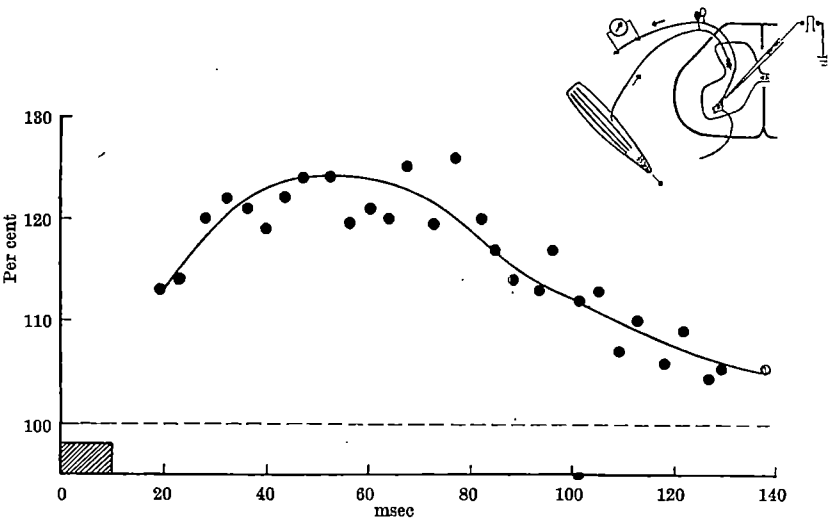


Fig. 2. The changes in excitability of the central terminals of the *Iα* afferent fibres of the gastrocnemius muscle were recorded as shown in the inset. The microelectrode is inserted near the *Iα* terminals of the *GS* nerve and connected to the square-pulse generator. The conditioning stimulus was an applied pull to the *DP* muscle setting up activity in the afferent fibres as shown by the arrow on the nerve into the cord; the other arrows indicate stretch of muscle and the direction of activity in the *GS* afferent nerve fibres. The time course of the change in excitability is plotted as a percentage (normal, 100) against time. The hatched rectangle denotes the duration of the pull (10 msec)

each *DRP*. To obtain a physiological measure of the pull intensity, small rootlets (inset 2) were dissected from the peripheral end of the cut upper *L*7 dorsal root so that they included only one large fibre from the muscle under examination. This fibre was identified as originating from a muscle spindle or a tendon organ by noting the discharge pattern when the muscle was allowed to contract isotonically. This identification was checked by application of a single stimulus to the peripheral nerve and by the measurement of the conduction velocity[3]. In I, the discharge of the I*b* fibre due to contraction of the *DP* muscles evoked by stimulation of the muscle nerve is shown, and in *K*, when the amplitude of the driving pulse was 5 V, the impulses in the I*b* fibres were just elicited by this pull. Correspondingly, the *DRP* showed a considerable increase when the pulse was increased from 2 V to 5 V, which indicates that the depolarization of the central terminals of primary afferent fibres was progressively increased as the muscle was stretched in order to stimulate the tendon organs in addition to the spindles. This agrees well with previous results[4].

In Fig. 2, another method of testing was used (inset). The change in excitability of the primary afferent fibres was recorded as described by Wall[5]. Since the stimulating microelectrode was placed at the motor nucleus of the gastrocnemius muscle, the primary afferent fibres tested were the I*a* fibres of the gastrocnemius nerve[6]. The figure illustrates that stretching of the *DP* muscle, using 10-V pulse to drive the vibrator, led to an increased excitability of the *GS* I*a* fibres which had a maximum at 50 msec and a duration of more than 140 msec. This excitability change indicates that the central terminals of the I*a* fibres of the gastrocnemius nerve were depolarized by impulses in the I*a* and I*b* fibres of the deep peroneal nerve. Again the present results are in agreement with those published previously[7].

It has also been observed that applied stretches to muscle diminished the size of intracellular monosynaptic EPSPs from motoneurones and were even more effective inhibitors of monosynaptic reflexes.

It is therefore concluded that stimulation of muscle receptors by stretch is an effective method of producing presynaptic inhibition.

We thank the Aeronautical Research Laboratories, Fisherman's Bend, Victoria, Australia, for the loan of the vibrator, Goodman's model 390*A*.

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Body Surface Area in Human Beings

Halliday and Hugo[1] reported on a new method for the determination of the radiation surface area of the human body. They concluded that "measurements on a conventionalized model of a human being have shown that a human subject in the spread-eagle position presents the largest radiation area that can be presented, and that this area is approximately 95 per cent of the true geometrical area". We were interested in testing this postulate.

A full-scale model of the integrating sphere proposed by Halliday and Hugo was constructed and calibrated for the Applied Physiology Laboratory. By the end of 1963 six Caucasian and four Bantu men had been extensively tested for body surface area. The spread-eagle position was used, and in Table 1 the results obtained are compared with those of a direct measuring technique in which the subjects' bodies were coated with a removable substance of which the true geometrical area could be determined by ordinary physical methods. Also included are the calculated surface areas using the conventional du Bois formula.

Table 1. Surface Area in m²

Subject*	Coating method	Photometric method	du Bois formula
1	1·81	1·73	1·70
2	1·82	1·72	1·70
3	1·96	1·89	1·81
4	1·85	1·75	1·72
5	2·55	2·42	2·38
6	2·07	1·94	1·95
7	1·89	1·77	1·77
8	1·85	1·77	1·75
9	1·94	1·86	1·78
10	1·96	1·87	1·85
Av.	1·97	1·87	1·84
S.D.	0·208	0·207	0·204

* Subjects 1–4 were Bantus.

It is obvious that the radiation surface determined by the new method with the men in a spread-eagle position is always less than the true geometrical area by ±5 per cent. This is in accord with the postulate of Halliday and Hugo[1]. Of more significance, though, is the large discrepancy between the geometrical surface area and those obtained by the du Bois formula. The latter underestimates the true surface area by 6·6 per cent, which is significant at the 5 per cent level. There is no difference between Bantu and European men in this regard. The finding is in agreement with those of Banerjee and Sen in India[2].

The error variances of the photometric and du Bois methods compared with the geometrical surface area obtained from coating the body are 0·000322 and 0·000306 respectively.

These results need to be validated in bigger samples but they direct attention to a probable systematic bias between the true geometrical surface area and that obtained from the du Bois formula which could be serious in the computation of basal metabolic rates, especially for primitive peoples. Also the actual radiation area is found to be larger than the du Bois surface area. Therefore, computations of radiation energy exchange for human beings using 95 per cent of the du Bois surface area, which is the usual procedure, are questionable.

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Microdroplet Assay of Human Serum Cytotoxins

Attempts to define leucocyte groups in man serologically have been hampered by the scarcity of immune human antisera and the capriciousness of the leucoagglutination reaction[1]. The method recorded here for assaying lymphocyte cytotoxins in a microscale was developed to circumvent these difficulties. Its extreme sensitivity permits performance of 1,000 or more tests with 1 ml. of antiserum. Furthermore, lymphocytes obtained from one finger-prick sample of blood are sufficient for 100 separate tests. The basic innovation in handling these small quantities of serum and cells and in gaining a greater than ten-fold increase in sensitivity over present-day methods is the performance of the reaction in microdroplets submerged under oil. This permits reduction in numbers of target cells to as few as 500 cells

in contrast to the 50,000 or so cells required for previously described cytotoxicity tests[2-4]. Since the sensitivity of cytotoxicity is inversely proportional to the number of cells used[2,3], an increase of sensitivity in the range of 10–100 times may be expected. Unlike the usual de Fonbrune oil chamber employed in micromanipulation, use of a 'dish' with a cover-glass bottom facilitated rapid addition of reagents and cells with a microsyringe.

Blood lymphocyte preparations containing less than 5–10 per cent granulocytes were isolated from fingertip blood in the following manner. Blood taken into 4–6 heparinized capillary tubes (1·4 mm diam.) was spun in a hæmatocrit centrifuge at 500*g* for 2 min, the supernatant plasma containing platelets was removed, and the tubes were spun at 12,000*g* for a further 2 min. After pipetting off the plasma, the tubes were cut just below the buffy coat layer under a dissection microscope. The buffy coat was then transferred to a polystyrene Petri dish together with about 0·1 ml. of fresh, type AB serum previously adjusted to *p*H 7 with carbon dioxide gas. A 45 mm × 50 mm cover-glass was put over the cells, which were enclosed by a rim of 'Vaseline' on the dish. For optimal adherence of polymorphonuclear leucocytes, a suspending medium containing either fresh serum or serum stored in liquid nitrogen was essential. Also maximal adherence could only be obtained in slightly acidic media of *p*H 6·5–7·0. Following incubation at 37° C for 15 min, the cells on the dish were examined under phase contrast microscopy to determine whether platelets, granulocytes and monocytes had firmly adhered. Granulocytes and monocytes should appear as large, flattened, dark granular cells. When in this condition, they will not wash off even with the most vigorous pipetting of solution over the surface of the dish. If the granulocyte contamination was greater than 10 per cent of the lymphocyte count in the wash, the sediment from centrifugation at 1,000*g* for 2 min was incubated either on another Petri dish or a glass slide. To remove contaminating erythrocytes which were present in a ratio of about 10 red blood cells to 1 lymphocyte at this stage, the cells were spun in narrow bore capillary tubes (1·0 mm diam.) at 12,000*g* for 2 min. Cutting off the buffy coat under a dissection microscope yielded the final suspension of cells which contained about equal numbers of erythrocytes and lymphocytes. Alternatively, erythrocyte contamination can be reduced to levels below 5 per cent of the lymphocytes by differential lysis in hypotonic solutions. As described earlier[5], 10–30 sec exposure to 1/3–1/5 isotonic Hanks's balanced salt solution was simple and effective. The hypotonic solution must be acidic (about *p*H 5) to lyse erythrocytes adequately. Water or hypotonic saline used by others[6,7] often resulted in formation of clot-like gels which could be prevented by trace amounts of Mg^{++} and Ca^{++} ions (we thank Prof. P. B. Medawar for this suggestion). Although lymphocytes treated in this manner were shown to yield almost exactly comparable results as the non-osmotically stressed cells, the latter were used exclusively for the present investigations. Similar principles of isolation were applied to larger volumes of blood following careful removal of only the uppermost layer of buffy coat cells as recommended by Jago[8].

For the test, oil chambers are prepared by affixing a 45 cm × 50 mm cover-glass to a 60 mm square 'picture frame' with ordinary cellophane tape. If the outside frame is made out of heavy aluminium foil, the foil was crimped at the corners to form a dish of 3 mm height. A stainless steel frame of similar construction was preferred for rigidity. After filling the chamber with mineral oil, antisera were placed on the cover-glass with a flat-tipped needle from a microsyringe of 0·01 ml. or 0·001 ml. capacity (Hamilton Syringe Co., Anaheim, California). To antiserum dilutions of 0·003–0·00001 ml., 0·003 ml. of pooled rabbit complement (*C′*) and 2,000 lymphocytes (in 0·001 ml.) were added. Rabbit *C′* was previously absorbed twice with 1 : 10 washed human erythrocytes and once with human leucocytes (10,000 per ml.) at 0° C for 5 min and separated by centrifugation at 0°–3° C. The *C′* was stored at −196° C since activity declined rapidly within a few days at −20° C. Though the numbers of cells added to each droplet can be as low as 500 cells, for routine work 2,000 cells provided a more readily countable number of cells per high-power observation field. Since the temperature of the microdroplet could not be easily maintained at 37° C because of excessive fluctuations in the temperature of the oil, the chambers were incubated at room temperature for 4 h.

Cell viability was assessed by the morphological appearance of lymphocytes when examined with an inverted phase contrast microscope at 620 or 820 times magnifications. Comparisons of viability by dye exclusion methods with time-lapse cinematography have shown that one prominent morphological change occurs on death of the cell. With phase contrast the cell nucleus, which is not visible in a living cell, becomes unmistakably recognizable in dead cells. Presumably injury to the cell membrane followed by leakage of intracellular substances reduces the refractility of the cytoplasm. Though this criterion of viability cannot be used for all types of cells (for example, monocytes, granulocytes, and fibroblasts), it appears to be applicable to a few other cell types besides lymphocytes (epidermal cells and certain tumour cells). It was found that the morphology of living and dead cells could be permanently fixed by the addition of 0·002 ml. of 37 per cent formalin. This provided a means of stopping the reaction at standard times for later thorough examination.

In confirming our earlier observation that normal human sera, even across ABO incompatibilities, are not cytotoxic[5], tests of 70 normal sera with lymphocytes from a type *B* and two type *A* individuals were entirely negative in the 210 combinations tested. In the course of other experiments, roughly a similar number of combinations used as controls were also negative. In contrast, many sera obtained from women with multiple pregnancies were cytotoxic. Though only a relatively few sera have thus far been tested, antibodies were detected in 49 per cent of sera (30 out of 61) from women with five or more pregnancies. A lesser proportion (16 per cent) of sera from women with 1–4 pregnancies was found in the 38 serum samples tested. In this limited series, the correlation of cytotoxic activity with number of pregnancies was better than with number of abortions. The high incidence of positive sera from multigravid women provides an abundant 'natural' source of allogeneic antibodies, obviating the need for deliberate immunization of human volunteers. Payne[9], van Rood[10] and Jensen[11] have earlier uncovered this source of allogeneic antisera as measured by leucoagglutination. However, the incidence of positive leucoagglutinating sera in these investigations ranged from 6 per cent[10] to 20 per cent[9].

The sensitivity of the present microdroplet assay can be gauged from the fact that out of the 49 antisera tested, 11 reacted to a maximum titre of 0·00001 ml. (50 per cent or more of cells killed). Further, the maximum titre was 0·0001–0·00001 ml. for 34 antisera and 0·001–0·0001 ml. for 15 sera.

The frequency with which each antisera reacted positively (50 per cent cytotoxicity) is given in Table 1. Based on the reactions of lymphocytes from 10–35 persons, the number of sera reacting at different levels was somewhat evenly scattered. Two antisera were cytotoxic to all lymphocytes thus far tested. The antisera which were cytotoxic to a high proportion of lymphocytes also tended to be those which acted to high dilutions. Therefore, frequency data with this type of sera must be interpreted with caution since the frequency may be dependent on concentration. Though non-specificity of cytotoxicity may therefore be suspected, it should be noted that often a serum highly toxic to

Table 1. Frequency of Cytotoxicity of 52 Antisera, each tested with Lymphocytes of 10–35 Individuals

Per cent of positive reactions	0–10	11–20	21–30	31–40	41–50	51–60	61–70	71–80	81–90	91–100
Total No. of antisera in each category	2	2	11	5	7	4	7	6	3	5

lymphocytes of one individual was not toxic to those of another with a 300-fold increase in antiserum volume.

To test reproducibility, lymphocytes of three individuals were reacted with 48 antisera in six dilution steps (by 1 : 3·3). The percentages of viable cells were estimated in multiples of 10 and duplicate titrations on a second day were compared by noting the difference in number of dilution steps ('tubes' or 'droplets') by which an equivalent degree of cytotoxicity was attained. Only deviations in multiples of 1/2 'tube' were counted in this rough estimation. Totalling up the differences obtained, which was never more than 1·5 dilution steps, the average dilution step variation for the 48 antisera was 0·16, 0·23 and 0·39. Duplicate tests with lymphocytes from one individual isolated with and without osmotic lysis and tested on one day resulted in an average discrepancy of 0·08 dilution droplet. Actual counting of 200 cells and dilution of sera by 1 : 2 or by 1 : 1·5 would increase the precision and accuracy obtainable, but was not deemed necessary for this initial investigation.

In summary, the microdroplet method has most of the features necessary for a practical human lymphocyte typing test. It is reproducible, rapid, and extremely sensitive. Since only semiquantitative data can be obtained, the principal future refinement needed will be a quantitative assay similar to the Coulter Counter method[4], which would be effective at this sensitivity range.

Results of using this assay in matching lymphocytes of long-term kidney homograft survivors and their respective donors have been described[12]. Evidence that the actual donor possessed less incompatible antigens than other random hypothetical donors was obtained.

This work was supported by grants from the U.S. Public Health Service *AM*–02375, *AI*–04444, and a U.S. Public Health Service research career programme award No. *AM*–7513 from the National Institute of Arthritis and Metabolic Diseases.

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PHARMACOLOGY

Release of Adenine Nucleotide from Nerve Axons

Recent experiments have shown that adenine nucleotides antagonize the neural effects of calcium deficiency[1] and procaine[2]. These findings suggest that adenine nucleotides play some part in normal axon physiology and, specifically, a nucleotide-protein complex in the excitable membrane of the axon has been postulated[1,3]. Nerve stimulation and certain alterations of the ionic environment may affect axon function by causing dissociation of this complex and, consequently, an increased rate of nucleotide release. In this communication we present evidence in support of this hypothesis.

The sciatic nerve of the frog, *Rana pipiens*, was isolated and immersed with the cut ends tied in buffered Ringer's solution (*p*H 7·2) at room temperature (22°–24° C). The nerve was then soaked for 2 h in 1 ml. Ringer containing 8×10^{-5} M tritium-labelled adenosine-5′-monophosphate (^{3}H-AMP), 50 μc./ml. After rinsing for 20 sec in 50 ml. of non-radioactive Ringer's solution, the time course of ^{3}H-AMP efflux into normal Ringer was determined for a period of 6 h. During this time the nerve was successively immersed into a series of scintillation sample vials, each containing 2 ml. Ringer, for intervals of 5–20 min. The total radioactivity in each vial, and that of the acid extract of sonicated nerve obtained at the end of the experiment, was measured in a liquid scintillation counter. The scintillation solvents and fluors were added directly to the collection fluid in each vial just prior to counting. Immediately after the collection procedure, and before sonication, the nerve was stimulated with supramaximal square wave shocks and action potentials recorded in order to evaluate the functional state of the nerve. To measure the efflux of ^{3}H-AMP from the epineural connective tissue sheath, the procedure was the same as already described here. The efflux of AMP labelled with carbon-14 from the nerve was also studied, and the results were the same as those obtained from the experiments with ^{3}H-AMP.

A curve describing the time course of decline of nerve ^{3}H-AMP is shown in Fig. 1. An initial rapid phase of loss of nucleotide is followed by a slower exponential loss. The rapid phase probably represents mainly the emergence of nucleotide from the extra-axonal spaces, and the slow component is assumed to represent mainly the efflux from axonal membranes. Although some of the nucleotide emerging from the nerve after 240 min comes from the epineural sheath, this comprises a relatively small fraction of the total. Comparison of the desaturation curves for nerve and sheath shown in Fig. 1 reveals that both the

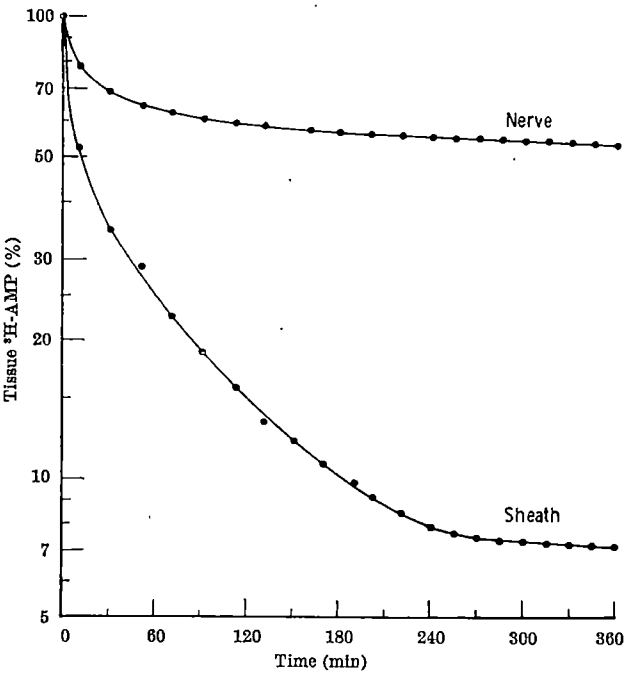


Fig. 1. Time course of decline of ^{3}H-AMP content of whole nerve and of epineural sheath during immersion in normal Ringer's solution. Nerve and sheath desaturation curves become exponential at 180 and 280 min respectively

ucleotide and the actual amount lost to the are much greater in the sheath. Even after normal Ringer's solution for 48 h, about 10 e initial radioactivity is present in the nerve, elatively tight binding of AMP to cellular

ct of $2{\cdot}5 \times 10^{-3}$ M ethylenediamine tetraacetic TA) was ascertained on the rate of loss of during its exponential phase of emergence. ent for 1 h with this concentration of EDTA was to have no effect on electrophysiological parameters n function. We showed that electrical stimulation ve causes increased rate of nucleotide release into al Ringer (Fig. 2) and, therefore, it was desirable to d a concentration of EDTA which causes depolariza- n or spontaneous discharge during the test period. Immediately after the immersion of a nerve into the DTA–Ringer's solution, the rate of nucleotide loss in- reased, and the total amount lost during the test period was a large fraction of the initial uptake (Fig. 3). The EDTA also caused an increase in rate of nucleotide release from the epineural sheath but the effect was no greater in magnitude than that obtained with the whole nerve. This is interesting because the results shown in Fig. 1 indicate relatively weak binding of AMP to the sheath. Although the striking effect of $2{\cdot}5 \times 10^{-3}$ M EDTA on nucleotide release is unrelated temporally to measurable changes in axonal electrophysiological parameters, the effect is probably related to molecular events in the excitable membrane which lead ultimately to spontaneous discharge (after about 75 min treatment) and axon depolarization.

The effect of calcium deficiency is not limited to the release of adenine nucleotide. Other experiments[3] indicate,

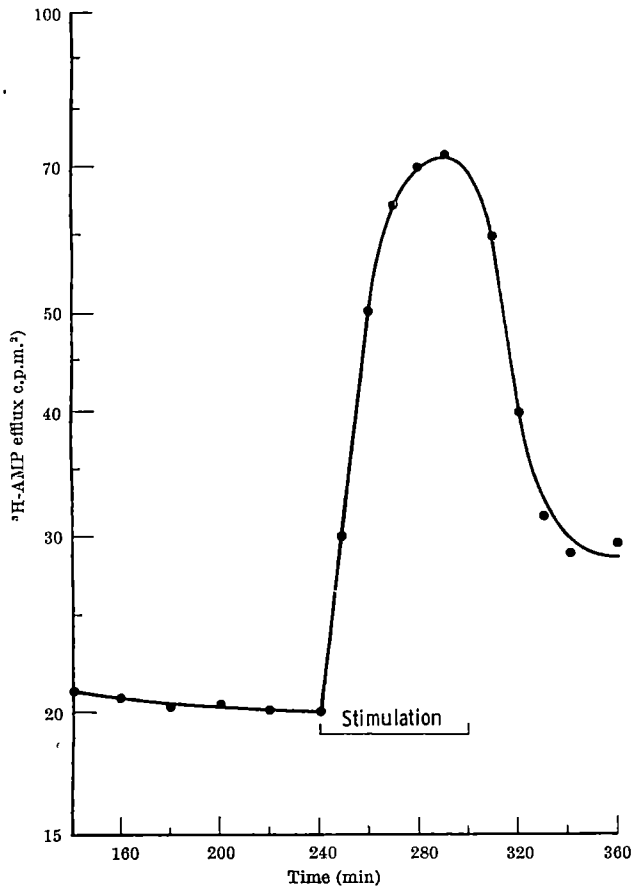


Fig. 2. Effect of electrical stimulation on rate of efflux on ^{3}H-AMP from nerve into normal Ringer's. Stimuli were applied at frequency of 50 per sec through bipolar platinum electrodes; voltage was supramaximal for the α-A fibres

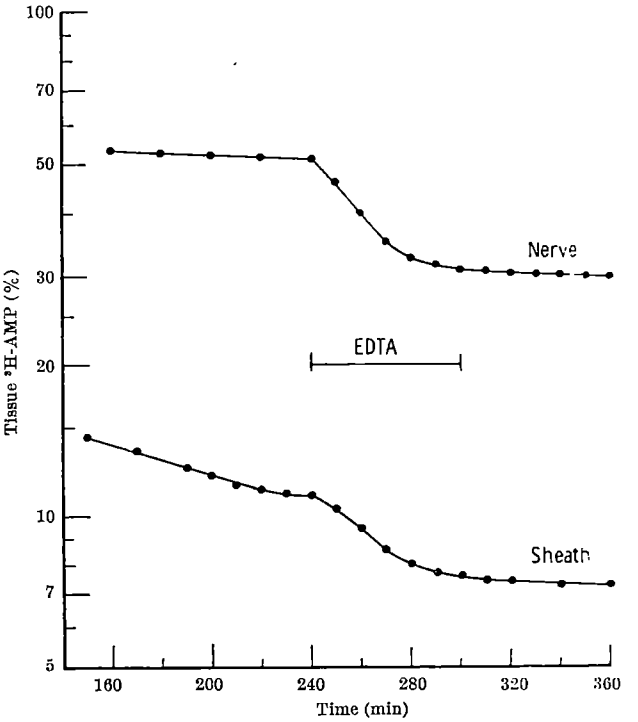


Fig. 3. Effect of $2{\cdot}5 \times 10^{-3}$ M EDTA on the rate of loss of ^{3}H-AMP from whole nerve and from epineural sheath

for example, that phosphocreatine is released to a much greater extent than nucleotide during exposure of a nerve to EDTA–Ringer's. Also, we have shown that EDTA causes a marked increase in the rate of loss of ^{3}H-adenosine. The fact remains, however, that neither phosphocreatine nor adenosine antagonizes the axonal effects of calcium deficiency, whereas the adenine nucleotides do. Thus, the effect of EDTA on the release of nucleotide from axons is probably related to a primary effect of calcium depletion.

These results are interpreted to indicate: (1) that extracellular AMP binds to receptors presumably located in the excitable membrane; (2) that this AMP binding is weakened by conditions which affect axon function. Other experiments in this laboratory have shown that ATP behaves in a similar way and, in fact, exchanges freely with axonal AMP. By reporting only the AMP results, we emphasize a role of adenine nucleotide in axon function which does not involve hydrolysis of pyrophosphate bonds. The conditions which affect nucleotide release also increase the rate of calcium efflux from the membrane[4], suggesting that nucleotide binding to membrane receptors depends on the presence of calcium. Conceivably, the configuration of a protein receptor in the excitable membrane is regulated primarily by the degree of binding to nucleotide and calcium; conditions which affect this binding will, accordingly, alter protein configuration in a manner which leads to changes in axon function.

This work was supported by research grant *B* 3597 from the National Institutes of Neurological Diseases and Blindness, U.S. Public Health Service.

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HÆMATOLOGY

Xg Blood Groups of Chinese

SAMPLES of blood from 64 normal unrelated Chinese people, mostly from Singapore, were tested for the X-linked blood group antigen Xg^a with the following results:

	Xg(a+)	Xg(a−)	total
Males	16	19	35
Females	20	9	29

The number is small because the supply of anti-Xg^a plasma is being used mainly for the investigation of X-linkage and of abnormalities of sex, but the results are sufficient to show that the antigen is less common in Chinese than in Europeans.

Chinese gene frequencies calculated from the male and female results are: Xg^a 0·46 and Xg 0·54. These gene frequencies re-applied to the 64 show the male and female results to be mutually concordant (Table 1), and this suggests that the gene frequencies may be about right despite the small number of tests.

Table 1. MUTUAL CONCORDANCE OF THE CHINESE MALE AND FEMALE PHENOTYPE FREQUENCIES IN THE SAMPLE OF 64

	Expected proportion	Expected absolute	Observed
Males Xg(a+)	0·46	16·1	16
Males Xg(a−)	0·54	18·9	19
Females Xg(a+)	0·71	20·6	20
Females Xg(a−)	0·29	8·4	9

Estimates of the gene frequencies of people so far tested with anti-Xg^a are given in Table 2. The Bombay Indian frequencies are recalculated from the male and female figures in Bhatia's results[1]. The Chinese sample differs significantly from the Caucasian and almost significantly from the Indian, but it is too small to show whether it differs significantly from that of the Negroes.

Table 2. Xg GENE FREQUENCIES IN VARIOUS PEOPLE CALCULATED FROM THE MALE AND FEMALE RESULTS

	No. tested	Xg^a	Xg
Caucasians (ref. 2)	2,000	0·662	0·338
Bombay Indians (ref. 1)	100	0·65	0·35
Negroes (ref. 3)	219	0·55	0·45
Chinese	64	0·46	0·54

We thank Dr. A. Cahan of Knickerbocker Biologics, New York, and Dr. J. D. Mann of Butterworth Hospital, Grand Rapids, for gifts of the anti-Xg^a plasma.

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Dose-response Curve of Phytohæmagglutinin in Tissue Culture of Normal Human Leucocytes

PHYTOHÆMAGGLUTININ (PHA) stimulates division of normal lymphocytes from venous blood *in vitro*[1]. Little or no cell division occurs when it is omitted from culture[2]. Other workers[3] have suggested a relationship between the amount of protein in the culture and the rate of cell division, but have not reported a systematic study. The relationship between a single dose of phytohæmagglutinin and the kinetics of cell division has been d
communication reports the effect of various
on cell division with other conditions consta
of the investigation is a reproducible biologi
the induction of cell division.

Phytohæmagglutinin *P* (PHA-*P*, Difco La
batch 457925), a partially purified protein,
8·5 mg protein/ml. by the Kjeldahl method.
blood from six normal human subjects was in
with dextran (Abbott Laboratories, 6 per cent dex
normal saline) in a final concentration of 1 per cent
blood was allowed to stand 45 min at room temper
Two ml. of leucocyte-rich supernatant plasma wer
moved and mixed with 8 ml. tissue culture Medium
Varying concentrations of PHA-*P* were added in appr
mately 0·2 ml. buffered saline. Fifty aliquot cultures w
made. Cultures were incubated at 37° C and uniform
terminated at three days. Colchicine 10^{-7} M was added fo
2 h prior to death of the culture and air-dried metaphas
preparations made. From each culture 1–10 × 10^3 cells
were counted and the number of mitoses tabulated. The
mitotic index was expressed as percentage mitoses per hour exposure to colchicine.

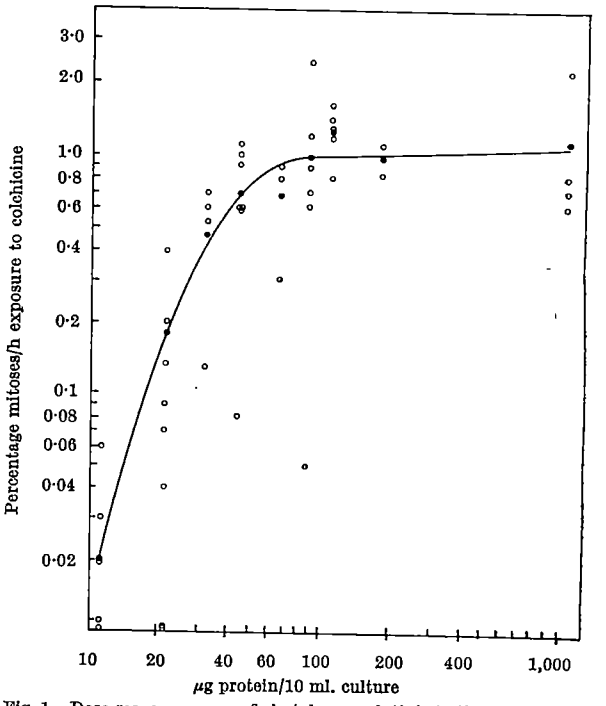


Fig. 1. Dose-response curve of phytohæmagglutinin in tissue cultures of human peripheral blood. Open circles represent individual mitotic indices at various concentrations of PHA; solid circles represent mean values. Log_{10} log_{10} plot

The lower four points were linear using probit analysis. The curve shows that the concentration of the protein and the proportion of cells which divide are directly related. The maximum response was obtained with 85 μg, and a half-response with 30 μg protein. One per cent mitosis was the maximum response which could be obtained by varying the concentration of protein. There was no detectable threshold of mitosis-stimulating activity. Fig. 1 shows the mitotic index plotted against the dose. The variations in mitotic index were attributed to large deviations from the mean which occur at low percentages[4], and variations among normal subjects.

The measurement chosen did not involve gradation. Either the cell was dividing or it appeared dormant. The assay resembles pharmacological investigations in which mortality or other single event is measured. It differs from the mortality investigations because cell division rather than death is its end-point.

work suggests that PHA-*P* may function as an Cells are known to divide in the presence of *ivo*[7]. PHA is not unique in its ability to induce in tissue culture. A wide variety of heterologous ave been demonstrated to stimulate mitosis HA has several unusual features: ribonuclease tes it[8]. Its activity is associated with a leucoag utinating property or substance[9]. Primary immunization to PHA has not been proved necessary to leucocyte proliferation. The precise mechanism by which PHA stimulates cell division is still uncertain.

This work was performed during a clinical investigatorship sponsored by the Veterans Administration.

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PATHOLOGY

Establishment of a Turkey Flock Free of 'N' Strain *Mycoplasma*

Mycoplasma of the 'N' strain are frequently isolated from turkey poults affected with airsacculitis but free of *Mycoplasma gallisepticum*[1]. Research on the 'N' strain has been impeded by the presence of a high proportion of turkey poults with airsacculitis in all breeding flocks examined[1,2,4,5], by the difficulty of culturing and identifying the *Mycoplasma* strains, and by the lack of a practical serologic test. This communication describes the establishment of a small nucleus of 'N'-free turkeys from a commercial breeding flock known to carry 'N' *Mycoplasma*. Hens and toms for the production of 'N'-free poults were selected from the parent flock by serology and culture.

An antigen was prepared from an 'N' *Mycoplasma* designated 'strain 529'. This strain was isolated from the sinus exudate of a turkey negative to *Mycoplasma gallisepticum*. Unlike other 'N'-type isolates which grew poorly and on agar medium only, this isolate grew readily in broth, facilitating antigen production.

Virgin female and male turkeys were screened serologically at 14 and 6 days prior to the first insemination. Swabs of the mucosa from the female oviduct taken by eversion of the cloaca, and semen from the males, were also collected for culture. In a test pen, 12 females diagnosed as negative by serology and culture were inseminated artificially with semen from males which were also negative. In a control pen, 19 females diagnosed as positive by serology or culture were mated to males which were positive.

Because a previous experience indicated that airsacculitis possibly caused by 'N' *Mycoplasma* was lowest in the poults from early hatches[3], only the first hatches were considered. All 'dead-in-shell', pipped eggs, cull poults and half the normal poults from the first hatch from each group were killed and dissected, and specimens taken for culture. Ten eggs candled out at 14 days, four cull poults, six pipped eggs, and 21 normal poults were examined from the negative test pen. No evidence of airsacculitis was found in the poults, and all cultures were negative for *Mycoplasma*. The remaining 19 birds in the hatch were raised in isolation and serologically tested at frequent intervals with 'strain 529' antigen. At 27 weeks of age, and after 11 separate bleedings, nine females and six males were negative. The four birds which died or were culled from this group were free of airsacculitis and 'N' *Mycoplasma* as diagnosed by examination and culture.

The culture of ten eggs candled out at 14 days incubation from the positive control pen revealed one isolation of an 'N' strain *Mycoplasma*. Four cull poults examined were found negative for airsacculitis and *Mycoplasma* on culture. Twenty 1-day-old poults were examined; one had airsacculitis from which 'N' *Mycoplasma* was cultured. Of 17 additional poults held for further investigation, one of nine that died within a 21-day period had airsacculitis from which *Mycoplasma* was recovered. The remaining 8 poults killed at 21 days of age were all negative for airsacculitis and *Mycoplasma* of the 'N' strain.

The fertility of the eggs in the second hatch from the negative test females was compared with that of the positive controls. The percentage of eggs candled out as infertile at 12 days incubation was 4·5 for those laid by the negative hens and 13·2 for those from the positive hens. In 'break out' examination of these, 1/11 from the negative hens was considered truly infertile, compared with 12/37 from the positive group. The remaining eggs were considered to be cases of early embryonic death.

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Inhibition of a Transplantable Murine Leukæmia by a Lymphocytopenic Virus

IN our laboratory the isolation of a viral agent with lymphocytopenic action *in vivo* directed our attention to its possible anti-leukæmic action. Anti-tumour properties of this virus are briefly described herein.

We have described the physical and chemical properties and the pathology of this lymphocytopenic virus which was isolated from a non-tumorigenic tissue culture line of Ehrlich's ascites carcinoma[1]. Parenteral introduction of this virus into adult mice of Swiss and several inbred strains (*C_3H*, *DBA/1*, *A/He*, *$C_{57}Bl/6$* and *Balb/c*) produces an acute infection within 7–10 days, involving primarily a reduction of lymphocytes in lymph node, thymus and spleen, secondarily some fatty degeneration of the liver, and is accompanied by the development of small amounts of ascitic fluid. The most susceptible Swiss strain usually develops both ascitic and pleural fluids and was chosen for routine passage of this virus in its 28th passage on August 18, 1964. The transplantable lymphoid leukæmia[2] in its 166th passage, on August 18, 1964, was used as a source of leukæmic cells. This lymphoid leukæmia was originally induced by 3,4,9,10-dibenzpyrene treatment to *Balb/c* mice in 1960. The concentration of leukæmic cells was determined with a Coulter Counter, followed by serial dilution with sterile phosphate-buffered saline (*p*H 7·4) to reach the desired number of cells in a 0·25-ml. inoculum.

Each experimental group was concurrently injected with a standard dose of the lymphocytopenic virus. A standard dose of lymphocytopenic virus was arbitrarily set as 0·25 ml. of a filtrate obtained from a 1 : 5 dilution of liver homogenate with phosphate-buffered saline (*p*H 7·4) obtained on the 7th day of infection in Swiss mice. Experimental mice were compared with control groups

receiving leukæmic cells only. A control group receiving virus only was also included.

Treatment of leukæmic mice with the viral agent resulted in complete remission in some mice (Table 1), still alive. They are grossly normal 60 days after the start of the experiment. Survival times were extended when the viral agent was administered to the leukæmic mice. All the control groups receiving leukæmic cells died within 7–12 days. Each group contained 12 mice to start, equally divided among males and females. Some mice from each group were killed during the course of the experiment to verify that infective action of the viral agent was occurring and to follow the course of the infections. These pathological and histological data support the gross observation; no evidence of the presence of leukæmic cells was found in the surviving mice, and 100 per cent survival after viral infection was observed in the viral control group.

In a similar experimental design, administration of a standard dose of lymphocytopenic virus concurrently with counted numbers of Ehrlich's ascites carcinoma cells intraperitoneally in *Balb/c* mice resulted in inhibition of tumour growth (Table 2). This Ehrlich's ascites carcinoma was obtained from Dr. K. Sigiura in November 1960 and is carried routinely in C_3H mice.

Law and Dunn[3] have described a lymphocytopenic virus isolated from *L*1210 leukæmia carried in *DBA*/1 mice which produced a statistically significant lengthening of the life of mice bearing subcutaneous implants of the infected *L*1210 sub-line and suggested that more striking results in survival time would be expected by titrating the dose of virus.

The lymphocytopenic virus in the present study has demonstrated an inhibition of the growth of both transplantable lymphoid leukæmia and Ehrlich's ascites carcinoma. Additional experiments currently under way will determine the condition under which increases in the titre of this virus may have further inhibitory activity against more concentrated tumour challenges for both lymphoid leukæmia, and Ehrlich's ascites carcinoma and other tumours.

Oncolytic viruses have been reported previously, and were reviewed recently by Southam[4] and Love[5]. The virus described in this article has the interesting properties of inducing complete remission of a transplantable lymphoid leukæmia and Ehrlich ascites carcinoma. The mice also recover from the viral infection, and are immune to further infection with the lymphocytopenic virus. These immune mice, however, do not have the ability to inhibit the growth of subsequent transplants of Ehrlich's ascites or leukæmia (Table 3). This suggests that the viral agent directly affects the tumour cells *in vivo*.

This virus can be repeatedly isolated from the tissue culture line of Ehrlich's ascites carcinoma. Feulgen-stained smears of tissue culture cells reveal a few lymphoid-type cells distributed among the predominant spindle-shaped cells containing large nuclei typical of Ehrlich's carcinoma[1]. The virus is non-cytopathic for these tissue culture cells. On injection into mice, the virus caused reduction of lymphocyte population during the acute phase of the infection and necrotic foci were secondarily induced in liver cell parenchyma. The inhibition of lymphoid leukæmia and Ehrlich ascites carcinoma are probably produced by similar cytopathic activity of this virus.

This virus for the two tumours tested so far demonstrates significant inhibition of the growth of the tumour graft. Pilot investigations now being evaluated have shown viral infection in suckling rats. Adult rats show reduction in spleen size.

The investigations described herein were supported in part by the National Institutes of Health, research grant *CA*–07114-01, and by Mrs. John E. Nicholson and family of Manhasset, New York.

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Table 1. Effect of a Lymphocytopenic Virus on the Survival of *Balb/c* Mice concurrently injected with a Transplantable Lymphoid Leukæmia

No. of leukæmic cells	No. virus doses	No. surviving/ Total	Time of death	% Survival	% Increase in median survival beyond control†
5×10^4	None	0/10	12 ± 0·48	0	
5×10^3	None	0/11	11 ± 0·34	0	
5×10^4	None	0/11	9·3 ± 0·24	0	
5×10^5	None	0/12	7·3 ± 0·13	0	
5×10^6		0/12	7·4 ± 0·14	0	
5×10^2	1	5/9	17·1 ± 0·72	55	42
5×10^3	1	1/10	15·2 ± 0·26	10	38
5×10^4	1	0/11	12·4 ± 0·38	0	33
5×10^5	1	0/10	10·4 ± 0·16	0	42
5×10^6	1	0/10	10·4 ± 0·37	0	41
5×10^6	1*	0/10	10·2 ± 0·17	0	38
None	1	10/10	—	100	—
None	1	5/5	—	100	—

* Virus dose administered 24 h after leukæmic transplant.
† ($t/c \times 100-100$).

Table 2. Effect of a Lymphocytopenic Virus on the Survival of *BALB/c* Mice concurrently injected i.p. with Ehrlich's Ascites Carcinoma

No. of Ehrlich's ascites cells	No. of virus doses	No. mice/ Surviving mice	Survival time (days)
5×10^5	0	0/5	14·2 ± 0·44
5×10^4	0	0/5	17·2 ± 0·76
5×10^3	0	1/6	22·2 ± 0·85
500	0	2/6	25
5×10^5	1	1/5	12·5 ± 2·2
5×10^4	1	1/5	36·3 ± 1·5
5×10^3	1	4/5	37
500	1	5/5	
None	1	10/10	

Table 3. Survival of Mice immune to Lymphocytopenic Virus implanted with Lymphoid Leukæmia

No. leukæmic cells	Status of mice	Survival time (days)	Surviving/ total
2,500	Virus free	10·3 ± 0·16	0/11
1,500	,, ,,	10·2 ± 0·08	0/12
500	,, ,,	12·4 ± 0·26	0/11
100	,, ,,	13·0 ± 0·28	0/12
2,500	Recovered from viral infection	10	0/3
1,500	,, ,,	10	0/3
500	,, ,,	13·66 ± 0·56	0/3

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Partial Protection by an Ox Liver Extract against Rat Liver Carcinogenesis by 4-Dimethylaminoazobenzene

The fraction of ox liver which contains those components soluble both in water and in acetone was found by Maisin and Lambert to have a powerful prophylactic effect against the liver carcinogen 4-dimethylaminoazobenzene (DMAB) when both were given in the diet[1]. It has been found in these laboratories that a diet supplemented with copper acetate affords a good degree of protection against this carcinogen[2] and it was decided to test the ox liver fraction under our conditions so that the efficacy of the copper salt could be compared with that of the fraction.

The fraction, prepared as described[1] and stored in a stoppered dark bottle at 4° C, was added to a diet of maize meal containing 0·09 per cent DMAB at the level of 5 ml. per rat per day. In a preliminary experiment, this diet was given to 6 female rats for 5 days each week, proprietary rat cube being given on the other 2 days of the week to provide the vitamins and other growth factors deficient in maize.

Only poor protection resulted, and the rats all developed tumours after an induction time of 10·5 months, com-

pared with 8·5 months for control animals given maize plus DMAB only.

The material utilized in this experiment was prepared in batches every 12 weeks, and it was observed that during this time the content of glucose, as measured by the specific glucose oxidase test, fell from an initial value of 2·93 to 0·16 mg/100 ml. Bacteriological examination showed the presence of large numbers of yeasts and some Gram-negative bacilli.

The original work had been carried out on such a large scale that fresh batches of liver extract were prepared daily[3], and accordingly our fraction was thereafter stored in 15-ml. quantities in sealed vials in the deep freeze before use. Under these conditions, contaminating organisms would be prevented from metabolizing the fraction by the low temperature.

When the feeding experiment was repeated using liver extract stored in this way, a much better degree of protection (mean tumour induction time 14 months) was observed although: (1) each of the 6 rats eventually developed tumours; (2) the protection was not so pronounced as that given by copper acetate, where tumours only develop in rare cases (in about 10 per cent of rats after 500 days on a diet containing 0·09 per cent DMAB + 0·5 per cent cupric oxyacetate).

Copper acetate and the two liver fraction preparations are compared as protective agents in Table 1. The criteria are the reductions in changes in three parameters which are affected by DMAB carcinogenesis. These are the decreases in the ratio of body-weight to liver-weight, liver protein content and liver succinoxidase activity, after an arbitrary time of 350 days.

Table 1. Ratio of Body-weight to Liver-weight, Liver Protein Content and Liver Succinic Dehydrogenase Activity after 350 Days

Diet	Weight ratio	Liver protein (mg/100 mg pulp)	Liver enzyme activity (manometric units/ 100 mg pulp)
M	26·5	3·41	115
M + DMAB	7·6	1·59	27
M + DMAB + F_1	11·5	2·16	53
M + DMAB + F_2	15·9	2·69	79
M + DMAB + CuAc	19·8	2·96	91

M, maize; DMAB, 0·09 per cent carcinogen; F_1 and F_2, first and second beef liver fraction preparations, 5 ml./rat/day; CuAc, 0·5 per cent cupric oxyacetate hydrate; pulp, minced whole rat liver filtered through a 1-mm stainless steel mesh to retain connective and vascular elements.

Maisin and Lambert found that their preparation contained about 1 per cent protein and 0·8–1 mg per cent riboflavin. FAD is known to give partial protection against DMAB carcinogenesis but the protective effect of the liver extract was much greater than could be accounted for by its riboflavin content. The fraction was also found to retain some activity after deproteinization.

From the results of the experiments reported here, it would seem that the growth of bacterial contamination lowers the activity of the fraction. Chemical changes observed during this process were: (1) glucose fell from 2·93 to 0·16 mg per cent; (2) protein fell from 1·34 to 1·17 g per cent; (3) riboflavin fell from 1·43 to 1·27 mg per cent.

The original concentration of all these components were higher than those reported by the earlier workers, and when fraction F_2 was given our animals presumably received the unknown protective agents also at a higher dosage-level.

The slight falls in riboflavin and protein may be discounted as reasons for the loss of activity. There was no change in the free amino-acid content, and two-dimensional paper chromatograms showed further that no particular amino-acids were selectively decreased or increased.

Glucose was the only sugar which could be detected in the fraction by paper chromatography and by paper electrophoresis, both in the freshly prepared fraction and after storing. It may be postulated either that glucose is a contributory factor to the protective mechanism or that its metabolism products, mostly alcohol and acetic acid, inhibit the process, possibly by the change in *p*H.

Alternatively, the loss of efficacy may be concerned with some other necessary factor being destroyed by the yeasts or by some substance being formed which opposes the protective mechanism against DMAB.

My thanks are due to Dr. D. L. Woodhouse for advice given during this work, which was carried out during the tenure of a Medical Research Council training scholarship and supported by the Birmingham Branch of the British Empire Cancer Campaign for Research.

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RADIOBIOLOGY

Sensitization of Anoxic Bacteria to X-rays by Di-t-butyl Nitroxide and Analogues

Few compounds are known to sensitize living organisms to the lethal effects of ionizing radiation. Since those that do include oxygen[1] and nitric oxide[2,3], both of which are stable free radicals, it was considered of interest to see if certain substituted nitroxides[4], which are water-soluble stable free radicals, would exhibit similar properties.

Escherichia coli B/r were grown with aeration to stationary phase in nutrient broth and then diluted to approximately 2×10^6/ml. in ice-cold buffered saline (0·13 M sodium chloride, 0·02 M phosphate; *p*H 6·8) for irradiation. The radiation vessels were shallow 'Pyrex' cylinders equipped with inlet and outlet tubes to permit the flow of gas over the bacterial suspension, which was stirred magnetically to promote equilibration of the dissolved gases throughout the experiment. Anoxia was

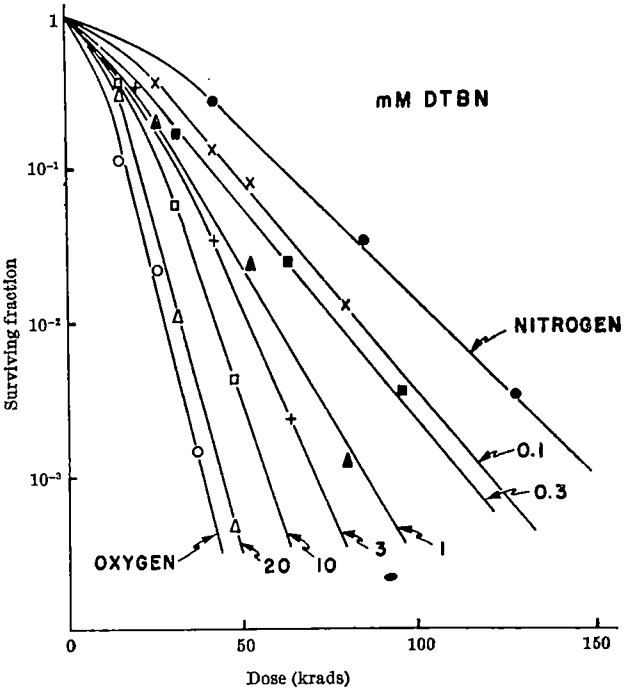


Fig. 1. The fraction of *E. coli B/r* that retains the capacity for colony formation when plated on nutrient agar, as a function of the X-ray dose delivered in the presence of various concentrations (mM) of di-*t*-butyl nitroxide under anoxia

maintained by passing nitrogen (containing less than 8 parts per 10^6 of oxygen) at a flow-rate of 12 ml./sec over the bacterial suspension for a period of 30 min prior to, and also during, irradiation. Irradiations were carried out at ice temperature with a 250-kV X-ray tube operating at 15 m.amp with a 1-mm aluminium filter. The mean dose-rate, determined by ferrous sulphate dosimetry, was $5{\cdot}4 \times 10^3$ rads/min. Samples were withdrawn after suitable X-ray doses, and after appropriate dilution the number of surviving bacteria was determined by plating on nutrient agar and counting the number of colonies produced on incubation overnight at 37° C.

Typical survival curves obtained for anoxic bacteria irradiated in the presence of various concentrations of di-*t*-butyl nitroxide (DTBN) are shown in Fig. 1. For comparison the survival curves under pure oxygen and pure nitrogen are also shown. Under the conditions of the experiment the free radicals showed no toxicity in the absence of radiation. It can be seen that there is a progressive increase in sensitivity to X-rays with increasing free radical concentration. At 2×10^{-2} M, the sensitivity is comparable with that in 100 per cent oxygen.

Similar results were obtained when the experiment was repeated with tetra-methyl-penta-methylene nitroxide (TMPMN). A third substituted nitroxide, *t*-butyl-2,6-dimethoxyphenyl nitroxide (TBDMPN), showed the same sensitizing action within the limits of its rather low solubility. The dose-reduction factors for various concentrations of the three nitroxides are shown in Table 1.

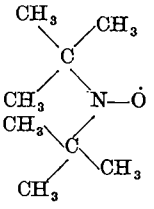
Table 1. X-ray Dose Reduction Factor for Anoxic *E. coli B/r* irradiated in the Presence of Various Concentrations of Substituted Nitroxides

Conc. (M)	Dose reduction factor* DTBN	TMPMN	TBDMPN
10^{-4}	1·3	1·2	1·2
3×10^{-4}	1·4	/	/
10^{-3}	1·9	2·0	/
3×10^{-3}	2·0	2·2	/
10^{-2}	2·4	2·4	/
2×10^{-2}	3·2	/	/

* Defined as the ratio of doses required to kill 99 per cent of the cells in the absence of and in the presence of the nitroxide.

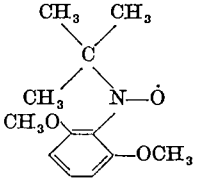
The structures of the substituted nitroxides are as follows:

DTBN



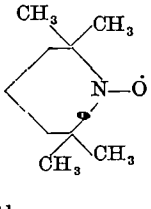
Di-*t*-butyl nitroxide

TBDMPN



t-Butyl-2,6-dimethoxy-phenyl nitroxide

TMPMN



Tetra-methyl-penta-methylene nitroxide

Chemically, these compounds resemble nitric oxide by virtue of the unpaired electron associated with the nitrogen–oxygen bond, but differ in that the nitrogen co-ordination number is two higher, which presumably explains the failure of the compounds to react with oxygen to form the peroxides. In comparison with nitric oxide or oxygen, much higher concentrations of the nitroxides are required for any given increase in sensitivity to X-rays, 1 mM DTBN being roughly as effective as 5 μM of oxygen in this system. The need for a higher concentration may reflect steric hindrance at the site of reaction or a lower rate of reaction, or—less likely—a difficulty in penetration into the cell. Nevertheless, the substituted nitroxides appear to be an important new class of substances for sensitizing anoxic cells to X-rays and may be of particular interest because of their stability to oxygen[4] and their apparent lack of toxicity.

We thank Dr. A. K. Hoffmann of the American Cyanamid Co. for the gift of the substituted nitroxides, and Dr. Ivar Johansen for advice.

This work was supported by U.S. Public Health Service grants *CA*–06519 and *AM–K6*–9397.

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Gamma Emitters in a Fin Whale

Baleen whales are prodigious collectors of marine zooplankton. Zooplankton in turn concentrate radionuclides. Both fission products from fall-out, and neutron-induced radionuclides (mostly zinc-65) from Hanford Laboratories, Washington, have been reported in marine zooplankton off Oregon[1,2]. An opportunity to measure the same radionuclides in various tissues of a plankton-feeding mammal occurred when a fin whale, *Balaenoptera physalus* Linnaeus, was captured west of Depoe Bay, Oregon, on September 19, 1963.

Tissue samples of a 55-ft. (16·8 m) male fin whale were removed from a fresh animal at the Bioproducts Inc. facility near Astoria, Oregon. Most samples were preserved by freezing, but stomach contents, liver, eye-ball and intestine were fixed in formalin. Samples were weighed, freeze-dried, and ashed at about 500° C. Oily tissues were burned in an open flame before ashing. Residual ash was analysed in the well of a 5 in. × 5 in. (12·7 cm) sodium iodide (Tl) crystal using a 256-channel pulse height analyser, with 100–200 min counting times. The eye was an exception. It was counted whole, lens downward, on top of the crystal. The external location of the eye-ball and its shape complicated the counting geometry and its value is subject to this additional error.

Zinc-65 was the most prominent peak in spectra of all whale tissues analysed (Fig. 1), and the large variations among tissues are probably related to differences in stable zinc and in individual turnover rates[3]. Potassium-40, the naturally radioactive isotope of potassium, was next in prominence, followed by manganeso-54, cæsium-137, zirconium-95, niobium-95, and cerium-141–144, in some tissues. Unlabelled peaks at 0·5 MeV arise from both ruthenium 103–106 and zinc-65.

The spectrum of muscle had a small peak due to ^{137}Cs, in addition to ^{65}Zn and ^{40}K, and evidence of $^{141-144}$Ce and ^{95}Zr–^{95}Nb (Fig. 1*a*). Cæsium is chemically similar to potassium and is probably taken up in the same way. The abundance of potassium in the sea reduces uptake of ^{137}Cs by marine animals, compared with terrestrial and freshwater animals.

The tongue contained much less ^{65}Zn than did other tissues in Fig. 1. Fission products (^{95}Zr–^{95}Nb and $^{141-144}$Ce) were present, with some indication of ^{137}Cs,

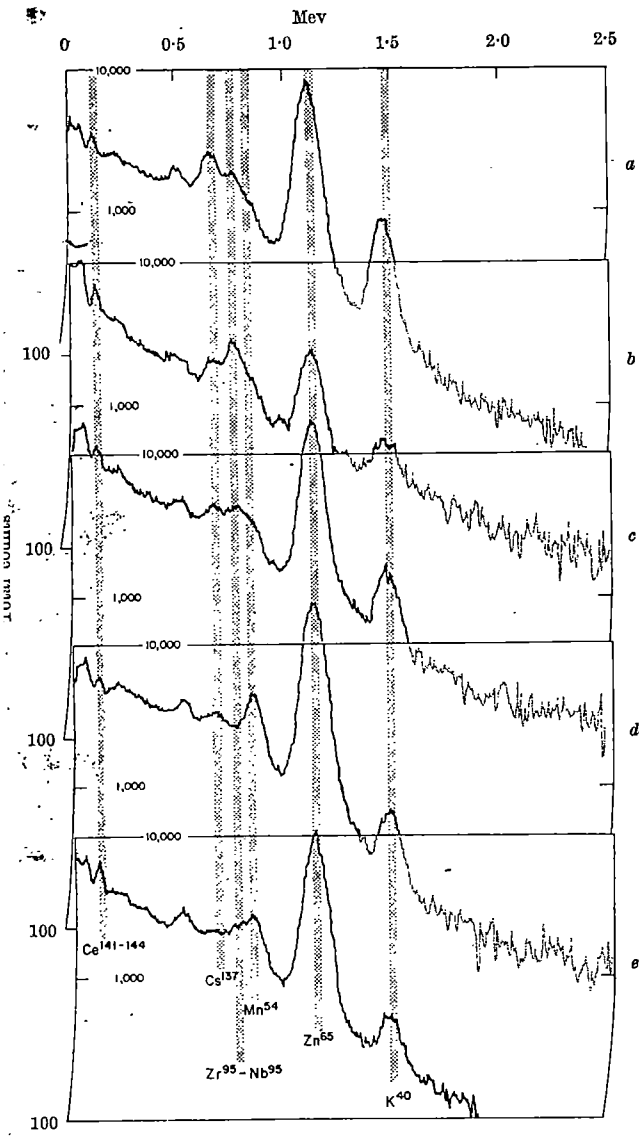


Fig. 1. γ-ray spectra of whale tissues (*a*, *b*, *c*, *d*) and stomach contents (*e*) all adjusted to the same wet weight (949 g): (*a*) muscle, (*b*) tongue, (*c*) lungs, (*d*) liver, and (*e*) euphausiids from the whale's stomach. Grey bands show location of γ emitters permitting direct comparison of relative amounts of radionuclides in each tissue. Dotted tracings of spectra indicate overlap. Counting times were 100 min with no background subtracted

even though the amount of ^{40}K was quite low (Fig. 1*b*). While the ^{95}Zr–^{95}Nb peak may result from surface adsorption, neither the spectrum of the skin (both dermis and epidermis) nor lungs (Fig. 1*c*) shows comparable evidence of this air-borne fall-out radionuclide. The spectrum of the lungs is most nearly typical of the spectra of tissues which are not individually illustrated, for example, testes, intestine, skin and blubber.

The liver contained ^{65}Zn, ^{40}K, traces of ^{137}Cs and $^{141-144}$Ce and a distinct peak of ^{54}Mn (Fig. 1*d*). Folsom *et al.*[4] reported ^{54}Mn both in sea-water and in marine organisms from off southern California. Osterberg *et al.*[5] found much larger ^{54}Mn peaks in the liver of albacore, *Thunnus alalunga*, from off Oregon, and freshwater clams, *Anodonta wahlamatenses*, from the Columbia River. Although ^{54}Mn is present in fall-out[4], it may be even more abundant in the vicinity of the Columbia River; but no direct comparisons have been made.

The stomach of the whale contained tremendous quantities of zooplankton ('krill'). Only 4 l. (primarily *Euphausia pacifica*) were removed, and a portion analysed. The resulting spectrum (Fig. 1*e*) contained mostly ^{65}Zn and

Table 1. ZINC-65 IN TISSUES OF A WHALE
The fish, used as a comparison, were various lantern fish from near the same area in which the whale was captured

Sample	^{65}Zn (pc./g wet wt.)
Liver	7·4
Lung	5·2
Stomach contents	4·0
Testes	3·6
Intestine	3·1
Muscle	2·7
Skin	2·3
Tongue	0·7
Eye (whole)	0·4
Blubber	0·2
Mesopelagic fishes	1·7–7·3

^{40}K, but $^{141-144}$Ce and ^{54}Mn and possibly a trace of ^{95}Zr–^{95}Nb were present.

In general, spectra of tissues from the fin whale resembled those of small mesopelagic fishes from the same trophic level[2]. Since fall-out is normally concentrated by herbivorous zooplankton and is discriminated against at higher trophic levels[2], the presence of ^{95}Zr–^{95}Nb in the whale tongue seems anomalous. Little, if any, appeared in zooplankton taken from the whale's stomach. Radioanalyses of euphausiids collected off Newport in October 1963 verified the virtual absence of fall-out radionuclides in euphausiids at this time. Levels of fall-out in plankton from surface waters off Oregon are much reduced compared with those observed during the past two years[5]. Therefore, the tongue may have been contaminated from terrestrial sources after capture of the whale, or by fall-out retained from a previous exposure. Since fin whales are highly mobile and our knowledge of the fall-out pattern at sea is limited, neither alternative can be readily discounted.

The activity of ^{65}Zn/g wet wt. of the various tissues is given in Table 1. Liver, lungs, testes and intestine contained the greatest amounts of this radionuclide, while the eye-ball and blubber contained the least. Maximum and minimum values for various small mesopelagic fishes, typical of levels of ^{65}Zn usually found in marine plankton and nekton in this area, are comparable with those in the whale. Since the Columbia River is the principal source of this radionuclide in the north-east Pacific Ocean[6], whales apparently accumulate ^{65}Zn rapidly during their sojourn in Oregon waters. Levels are extremely low and no health hazard exists. However, if whales retain this radioactive souvenir of the Columbia River for a sufficiently long period, the ^{65}Zn should provide a useful tracer for migration investigations of these ocean wanderers.

These preliminary results warrant further investigation, particularly since the great size of the whale provides samples of almost all tissues in quantities suitable for radioanalysis. Unfortunately, the small 90-ft. (27·4 m) whaling boat was damaged when it prematurely tied to an 80-ft. (24·4 m) whale[7]. This required a delay for repairs, and the subsequent onset of winter storms ended the whaling season.

We thank R. Carruthers and L. Anderson, of Bioproducts, Inc., and L. Hubbard for their assistance, and J. McCauley for identification of our samples.

This work was supported by grants *AT*(45–1) 1726 and *AT*(45–1) 1750 with the U.S. Atomic Energy Commission.

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Autoradiographic Evidence of Synthesis of an Arginine-rich Histone during Spermiogenesis in *Drosophila melanogaster*

The DNA-associated protein of the animal spermatozoon customarily undergoes two major changes in the course of differentiation of this cell during spermatogenesis. These changes, as have been established in a variety of organisms, are cytochemically delineable by the alkaline-fast-green, picric acid-bromophenol blue, and ammoniacal-silver staining methods[1–4], when used in conjunction with the procedures of deamination and acetylation[5–7]. The first of these changes relates to the replacement of a lysine-rich histone by one rich in arginine in the spermatid stage of development of the spermatozoon, and the second change reflects the acquisition of protamine in place of the arginine-rich histone by the maturing spermatozoon. In *Drosophila melanogaster*, however, there is observed only the first of these protein transformations[8,9], and this occurs, according to our observations, at the terminal stages of spermiogenesis, shortly before eclosion of the fly (within 24 h or so at 29° C); that is, the time of the arginine-histone shift during sperm development in *D. melanogaster* coincides with the second stage of these chemical alterations in some other organisms, and there is no subsequent change to protamine in the mature spermatozoon.

The implications of the sequential transition of chromosomal proteins during development of the spermatozoon must necessarily be considered at both the biological and biochemical levels. We have previously ventured to speculate in some measure on the biological significance of such chemical transformations as are operative during animal spermatogenesis[8,9]. We now wish to report the preliminary results of autoradiographic studies which were undertaken to elucidate the nature of the chemical processes that underlie protein transition in *D. melanogaster*.

Testes of the Swedish-*b* stock were removed from pupæ shortly before the time of emergence of the adults, by dissecting in a modified Ephrussi–Beadle medium (EBM) made by adding 0·7 g NaCl, 0·035 g KCl, and 10 mg glutathione to 100 ml. of 0·0125 M sodium phosphate buffer at *p*H 6·8. Groups of 12–15 pairs of testes were excised in the above solution and transferred for treatment to one of the following two solutions (designated as *a* and *b*), where they remained at either room temperature (about 22° C), at 25° C, or at 37° C for 3–5 h: (*a*) tritiated-arginine (0·1 ml. tritiated-arginine of specific activity, 105 mc./m.mole to 0·4 ml. EBM); and (*b*) puromycin (0·3 ml. of 0·01–0·1 mg/ml. puromycin to 0·1 ml. tritiated-arginine and 0·1 ml. EBM adjusted to *p*H 7·2–7·4).

These intact treated testes and other groups that served as untreated controls were then transferred to 10 per cent neutral buffered formalin and allowed to remain in the fixative for 6–12 h at room temperature. After washing and dehydrating, the testes were embedded in paraplast. Sections were then cut at 3–5μ. Following deceration and hydration, the sections were rinsed in cold perchloric acid to remove acid-soluble, low-molecular-weight precursors. Some of the sections from experiment *a* were stained specifically for arginine-rich histone. All sections were then covered with '*AR*-10' autoradiographic stripping film (obtained from Kodak, Ltd., England). The film was exposed for 4–6 weeks, after which the autoradiographs were developed and processed by the usual method.

Our previous cytochemical investigations of spermatogenesis in *D. melanogaster* revealed that a shift from a lysine-rich to an arginine-rich histone occurs during the final stages of maturation of the spermatozoon[8,9]. This transition was presumed, by analogy with the experimental studies of Bloch and Hew[2,3] on *Helix aspera*, to reflect a synthesis of new protein molecules. Confirmation has now been obtained by investigating the pattern of incorporation of tritiated-arginine.



Fig. 1. Longitudinal section of pupal testis of *Drosophila melanogaster*. Arrows indicate bundles of mature spermatozoa the heads of which show heavy incorporation of tritiated-arginine

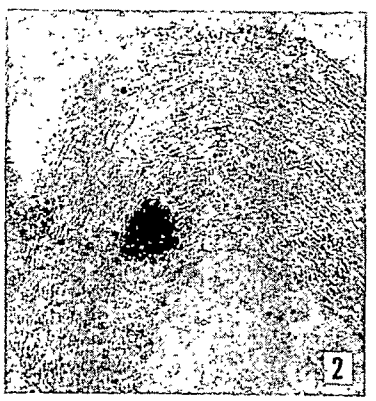


Fig. 2. A single bundle of spermatozoa at high magnification showing that tritiated-arginine has been incorporated in sperm heads

Microscopic examination of sections of testes offers clear evidence that tritiated-arginine is built into the nuclei (or heads) of maturing spermatozoa. The over-all distribution of the incorporated radioactive arginine parallels that of the arginine-rich histone, as determined by the specific colour reaction for nuclear basic proteins, namely, alkaline-fast-green stainability after deamination. For example, in some of the sections from experiment *a*, it could unmistakably be observed that the heaviest concentration of silver grains lies over nuclei of spermatozoa that exhibit by their staining reactions the presence of an arginine-rich histone (Figs. 1 and 2). However, in some bundles of mature spermatozoa showing alkaline-fast-green stainability after deamination, no evidence of incorporation of tritiated-arginine was detected, and in other bundles only a small fraction of the spermatozoa appeared to be labelled. We assume that these patterns reflect differences in the original distribution of the isotope within the testis, or differences in its uptake by spermatozoa with dissimilar physiological properties.

The incorporation of amino-acid may not in itself be considered as offering sufficient proof of protein synthesis, unless evidence can be presented of a net production of new protein molecules[10]. We have found that uptake of amino-acid becomes more pronounced (increasing beyond the threshold values) as the time and temperature of treatment increase. Moreover, uptake is inhibited in the

presence of puromycin. These findings suggest that the incorporation of tritiated-arginine into the spermatozoon of *Drosophila* reflects a true synthesis, which is expressed in the production of new molecules of arginine-containing proteins.

Synthesis and incorporation during the terminal stages of spermiogenesis involve a unique system, since the nucleus of the mature spermatozoon consists primarily of DNA-protein, and lacks any nucleolus-synthesized RNA of recent origin (the nucleoli having broken down during spermiogenesis[11]) as well as a large share of the cytoplasmic RNA that was present during earlier stages of transformation. The exact mechanism controlling the incorporation of amino-acids into DNA-associated protein within the head of the spermatozoon of *D. melanogaster* remains to be determined, and experiments designed to provide an answer are now in progress. In the grasshopper, *Chortophaga viridifasciata*, according to a recent report by Bloch and Brack[12], the arginine-containing histone is synthesized in association with RNA granules in the cytoplasm of the spermatid and then migrates into the nucleus where it combines with the DNA.

This work was supported in part by institutional research grant *IN–40E* to the University of Michigan from the American Cancer Society, and in part by research grant *GM–10499* from the National Institutes of Health, U.S. Public Health Service.

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BIOLOGY

Schistosoma mansoni: Humoral Transfer of Protective Factors produced by Irradiated Cercariæ

Previous investigations[1,2] have produced information concerning the manner in which prior exposure of mice to irradiated cercariæ of *Schistosoma mansoni* affects the course of the challenge infection. The results indicated that the principal effect of the aborted infection with irradiated cercariæ was to slow the migration and development of worms from the challenge infection. The delay in accumulation of recoverable worms and eggs suggested the probability of inhibitory factors being produced by the aborted infection with irradiated cercariæ, although their relation to specific acquired resistance could not be defined.

The experiments described here produced additional information concerning the nature of the changes induced by irradiated cercariæ.

Mice were exposed on three occasions on alternate days to 500 mixed cercariæ irradiated with 2,000 r., thus making the total 1,500 cercariæ. The mice infected with

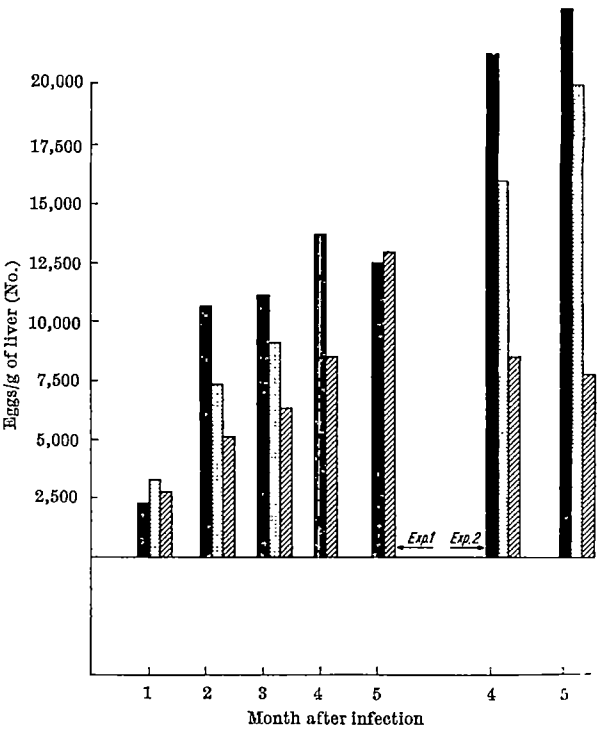


Fig. 1. Effect of humoral transfer of factors produced by irradiated cercariæ on liver–egg accumulation following infection with *S. mansoni*. Each bar represents the mean of counts based on 5–10 mice. Egg counts were compared by Wilcoxon two-sample test: immune serum versus control: Exp. 1; 3 months, P, 0·016, 5 months, P, 0·05. Exp. 2; 5 months, $P < 0·01$, 6 months, $P < 0·01$. Normal serum versus control: Exp. 1; 3 months, P, 0·016. Exp. 2; 5 months, P, 0·048. Immune versus normal: Exp. 2; 5 months, P, 0·054, 6 months, $P < 0·01$. The remaining comparisons did not show differences significant at the 10 per cent level. Black, control; stippled, receiving normal serum prior to infection; hatched, receiving 'immune' serum prior to infection

the irradiated cercariæ were bled 60 and 90 days after the last exposure and the sera used in immunization tests. The time chosen for bleedings were based on previous investigations[1,2]. The pooled sera from the 60- and 90-day bleedings were used in Exps. 1 and 2 respectively. In both experiments female white Swiss mice weighing 20–24 g were given 0·8 ml. of pooled serum intraperitoneally and then exposed to 70 sex-determined (35 female + 35 male) normal cercariæ. Evidence of humoral immunity transferred to fresh animals was sought in worm counts, in liver–egg counts and mortalities as compared with results from control mice. All procedures used for exposure of cercariæ to radiation, for infection of animals, and for evaluation of the infections have been described[1,2].

Data from both experiments are presented in Fig. 1. When mice were examined five months after infection, the egg counts from mice that received 'immune' serum prior to infection were significantly lower than egg counts from control mice (solid bars). While the counts rose in Exp. 1 and by month 6 after infection were not different from those of control mice, they continued much lower than counts from control mice in Exp. 2. As these two experiments differed only in that the 'immune' serum used in Exp. 1 was obtained from mice 30 days earlier than that used in Exp. 2, it appears that the protective factors produced by the irradiated cercariæ were more potent or at a higher titre 3 months after exposure than after 2 months.

The numbers of worm pairs in the portal system of mice that received immune serum prior to infection was also lower than those from control mice, and these counts did not rise as the infection became older. Thus, 30 days after infection the worm pair return in the portal system of control mice was 13 per cent and only 8–9 per cent in test mice. Ninety days after infection the worm pair return reached a maximum of 18–20 per cent in control

Table 1. The Effect of Humoral Transfer of Factors produced by Irradiated Cercariæ on Deaths of Mice Five Months following exposure to *S. mansoni*

Mice receiving	Exp. 1 * No. of mice used	Exp. 1 * Percent cumulative deaths	*P*	Exp. 2 † No. of mice used	Exp. 2 † Percent cumulative deaths	*P*
'Immune' serum	20	30	= 0·014	20	20	= 0·024
No serum	27	70		26	50	
Normal serum	15	73	> 0·10	20	35	> 0·10

* 'Immune' serum versus normal serum; *P*, 0·026.
† 'Immune' serum versus normal serum; *P* > 0·10.

mice receiving either normal serum or no serum at all, and remained unchanged in the immunized mice (8·6 per cent).

The cumulative deaths among test and control animals were compared with the use of a χ^2 test (and in one case, Fisher exact test), and results obtained are shown in Table 1. It is clear from these results that mice receiving 'immune' serum prior to infection tended to live longer than control mice receiving no serum or 'non-immune' serum. The answer to the question whether death of mice was prevented or only delayed is beyond the scope of those short-lasting experiments.

It is noteworthy that the numbers of eggs recovered from the livers of the control mice receiving the normal serum prior to infection were also lower than the egg counts from the control mice receiving no serum. However, they rose more steadily with the passage of time than those from mice receiving 'immune' serum prior to infection. The decreased mortality among mice receiving normal serum prior to infection, as seen in Exp. 2, suggests that specific humoral antibodies may not be essential for the observed protection against *S. mansoni* infection.

The results obtained concerning egg recovery seemed to parallel those previously reported[1,2], showing once again that the effect of humoral factors produced by irradiated cercariæ was to slow the liver egg accumulation after exposure to normal cercariæ. However, the effect on worm return differed. The worm return in mice pre-infected with irradiated cercariæ increased as the challenge infection became older, whereas in mice receiving 'immune serum' no such increase occurred. Furthermore, pre-infection with irradiated cercariæ neither delayed nor prevented death caused by a challenge infection. In mice receiving 'immune serum' mortality was significantly reduced during the period of observation.

Further experiments will be necessary to assess the potential importance of humoral protective antibodies in immunizing procedures against schistosomes.

I thank Dr. David W. Alling for making the statistical tests, and Mr. L. T. Cannon for his assistance.

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Influence of Serum of Children and Adolescents on the Growth of Plants

Macht and Livingston introduced in 1922 a root-growth test for the investigation of the interaction between the physiology of the animal and vegetable kingdoms. Macht demonstrated in a large number of papers that, under standard experimental conditions, sera from healthy persons inhibit the growth of *Lupinus albus* roots. The sera from patients suffering from various diseases, especially cancer, were also investigated by Rønnike[1]. So far as we know, no experiments of this type have previously been made in children. From this point of view, we investigated 113 sera from children and healthy adolescents as well as 273 sera from patients affected by various diseases. In 113 sera from healthy individuals aged 9 months to 23 years we found that, with sera from individuals of increasing age and height, the growth of roots of *Lupinus albus* was increasingly inhibited. We found also that there is no substantial difference between the average inhibition caused by sera from boys or girls. Fig. 1 summarizes our experiments.

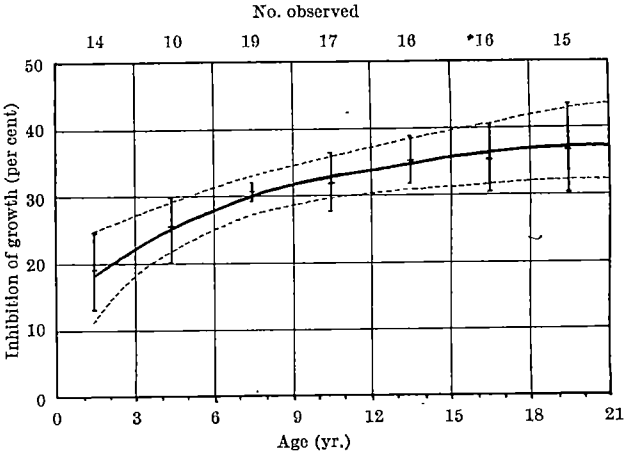


Fig. 1. Inhibition of growth of roots of *Lupinus albus* by serum from children and adolescents. Vertical lines, 95 per cent confidence limits of observed means; solid line, theoretical curve of mean values; broken lines, 95 per cent confidence limits of theoretical means

We have analysed the inhibitory effect of sera of healthy children as well as children treated for prolonged periods with corticosteroids, and children suffering from malignant growths. These results will be published in a later communication.

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Hardening Treatment of Seeds as a means of increasing Yields under Conditions of Inadequate Moisture

Genkel *et al.*, as well as a number of other investigators, have claimed that 'hardening' treatment of seeds increases the drought, heat and frost resistance of plants developing from the treated seeds[1,2]. This 'hardening' treatment or 'P. A. Genkel's method' is nothing else but the repeated soaking and drying of seeds before sowing, a method already reported by Will[3] in 1883 as being effective in increasing drought and frost resistance. It is claimed that plants made drought resistant in this way yield higher yields under drought conditions. As an example, we cite Mart'yanova[4], who reports for barley grown in 1957 a yield of 28 centner/hectare of treated and of 18 centner/hectare for untreated plants.

Zubenko[5], working with corn, concludes that this method "could be recommended for wide use in crop production . . . when growing it in conditions of inadequate moisture without sprinkling and also with irrigation". In a recent extensive survey of the Russian work, May, Milthorpe and Milthorpe[6] have stressed the need of exploring the practical value of this technique because the 'hardening treatment', if successful, would be one of the

most important improvements of arid zone agriculture. It seemed, therefore, advisable to repeat the 'hardening' experiments. It was not intended to test drought resistance or tolerance which, according to recent tests of Waisel[7] and Jacoby and Oppenheimer[8], with wheat and sorghum, is not increased by the hardening treatment. Our experimental aim was a purely practical one as we only wanted to find out if 'hardened' plants when grown under water stress caused by insufficient soil moisture develop better and yield more than untreated controls.

In summer 1962, grains of *Sorghum* var. 'Early Haggary' were soaked at room temperature in distilled water until they had taken up an amount of water equivalent to 50 per cent of their imbibition water, after the maximum of water taken up by the grains during imbibition had been determined in a previous experiment. The moist grain was then spread out in a thin layer and air dried until it had reached its initial weight. The procedure was repeated three times. This method of pre-treatment was chosen after preliminary experiments had shown that germination and seedling development of pre-treated grains were normal.

Table 1. Average Height (cm) of all the Plants contained in the Ten Tins of Each Treatment, Total Number of Flowering and Fruiting Stalks per Each Treatment

Treatment	Average height (cm) per plant	No. of flowering stalks of all plants	No. of fruiting stalks of all plants
$N+$	45·7	10	20
$N-$	52·3	13	16
$N/2+$	26·9	1	0
$N/2-$	24·8	2	0

After the last drying, the grains were sown on May 29, 1962, into tins (22 cm × 22 cm × 22 cm), 10 grains to one tin. The tins were filled with 10 kg of loess soil each, collected from our desert farm in Avdath[9]. Before the sowing, the loess was watered to full saturation and was kept at field capacity for the following three weeks until the grains had germinated and the seedlings had started to grow. At that time the seedlings were thinned out and only five seedlings per tin were left. The tins were then divided into two lots. Lot N, consisting of 20 tins, received twice a week 1 l. of water per tin, which was sufficient to keep the soil wet, whereas lot $N/2$ (20 tins) received at the same times only 0·5 l. of water per tin. This last treatment put the plants under considerable water stress as can be seen from the results (Table 2). Ten tins of each N and $N/2$ contained pre-treated ($N+$, $N/2+$) and 10 tins untreated grain ($N-$, $N/2-$) serving as controls. All the tins were placed in a randomized block design on a plot in the experimental field of the department. As the experiment was carried out during the dry season, the tins received no rain.

Table 2. Average Height, Fresh and Dry Weight of Vegetative Parts, and Average Dry Weight of Grains of all the Plants of Each Treatment and Number of Fruiting Stalks per Treatment

Treatment	Average height (cm) per plant	Average fresh weight (g) per plant	Average dry weight (g) per plant	No. of fruiting stalks of all plants	Average dry weight of grains (g) per plant
$N+$	46·6 ± 7·15	7·9 ± 1·7	2·9 ± 0·41	42	1·70 ± 0·81
$N-$	56·0 ± 7·84	9·2 ± 1·73	3·4 ± 1·79	42	2·30 ± 0·57
$N/2+$	20·6 ± 3·69	2·1 ± 0·72	1·1 ± 0·26	6	0·23 ± 0·14
$N/2-$	19·4 ± 4·84	2·1 ± 0·84	1·3 ± 0·62	5	0·64 ± 0·05

The first flowers were formed by the N plants in the first week of August, whereas at that time the $N/2$ plants were still in the vegetative state. On August 21, 1962, the average height of the plants and the number of flowering and fruiting stalks were determined (Table 1).

On September 10, 1962, the plants were harvested, that is, cut at the soil surface, their height measured, fresh and dry weight of vegetative parts and grains determined (Table 2).

A statistical analysis has shown that all the differences between $N+$ and $N/2+$ and those between $N-$ and $N/2-$ are highly significant at the 5 per cent level. The differences in average height, average fresh weight of vegetative parts and average dry weight of grains between $N+$ and $N-$ are significant on the same level. There is no significant difference of average height and average fresh weight between $N/2+$ and $N/2-$, whereas the difference in dry weight of grains is highly significant at the 5 per cent level.

It is, therefore, obvious that under the conditions of our experiment with *Sorghum* the 'hardening' treatment of the seeds does not increase grain yields in either the normally watered plants or those which suffered from lack of water. The contrary is true, that is, in both cases the grain yield was negatively affected by the treatment.

In the case of the normally watered plants, height, fresh and dry weight of vegetative parts was also significantly lower in the 'hardened' than in the untreated plants.

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Dependency of Pollination on Patterns in *Monarda* (Labiatae)

The members of the genus *Monarda* are usually pollinated by bees and wasps. The latter will also nip holes into the backs of the expanded corolla tubes to take the nectar directly and thus circumvent the process of pollination. Some corollas, like those of *M. clinopodia* and *M. lindheimeri*, are very rich in nectar. Nectaries are located in the throat of the corolla tube from the opening downward toward the base, and consist of short hair-like glands. They are protected by a dense ring of non-glandular pubescence at the inner throat opening. In the small and abruptly expanded corolla tubes of the sub-genus *Cheilyctis* the nectaries extend over a more restricted area than in the larger and more evenly expanded corolla tubes of most species in the sub-genus *Monarda*.

Many species have so-called 'nectar guides', which are composed of rows of spots on the lower corolla lip. Most taxa in the sub-genus *Cheilyctis* possess them, and *M. russeliana* and *M. bradburiana* in the sub-genus *Monarda*. These nectar guides lead to the throat opening. In one section of the sub-genus *Cheilyctis* there are, in the back of the throat, 3–5 additional distinct round spots of pigment above the nectaries. In the other section there are, instead of the dots, 3–5 thin vertical lines. The upper corolla lips in many species are also marked. These markings, I assume, increase the pattern of irregularity[1], and thus attract the insects. With the exception of *M. russeliana* and *M. bradburiana*, which are woodland species, all taxa with markings exude a strong fragrance. This undoubtedly is due often to their high oil content of thymol and carvacrol and its vaporization in nature. It is facilitated in those species which are exposed to direct sunlight. I believe that this fragrance attracts the pollinating insects from a distance. Once attracted near the plant by odour, the insect may be induced by colour and pattern to visit it. The insect then follows the nectar guides, especially in the sub-genus *Cheilyctis* with the very arcuate corolla.

Experimentation with colchicine, a mutagenic reagent, to induce teratologies in order to learn about evolutionary homologies produced flowers of *M. punctata* var. *arkansana* with entirely unspotted corollas. These, when grown in a nursery row, showed during periods of several weeks that bees and wasps would alight on the lower lip, but apparently being at a loss would always leave again without feeding. When these unspotted plants, however, were grown among ordinary spotted ones in nursery rows, the insects would ignore them altogether, but would visit the flowers of the spotted ones and enter the throat.

I believe that part of the *Blumenstetigkeit* in bees is not only due to distance, fragrance and colour of the flowers as such, but also to a definite colour pattern. The bees, therefore, would ignore the unspotted plants growing together with the spotted ones, if conditioned to or expecting spotted flowers. Their visits to the non-spotted flowers growing by themselves might have been due to random or accidental visits. It is also interesting to note that certain species of *Monarda* have developed a colour in their bracts subtending the glomerules which approaches that of the corolla. Some have even originated additional spotting on these bracts similar to that of the corollas, thus increasing the total effect of flower pattern.

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VIROLOGY

Inhibition of Multiplication of Foot-and-Mouth Disease Virus by Guanidine Hydrochloride

It has recently been shown that the multiplication of many picornaviruses can be specifically inhibited by non-cytotoxic concentrations of guanidine hydrochloride and 2-(α-hydroxybenzyl)-benzimidazole (HBB)[1]. These inhibitors appear to act by interfering with the production of virus-induced RNA-polymerase[2].

Tamm and Eggers[1] have produced a classification of some small RNA viruses on the basis of their response to these two inhibitors. It was of interest, therefore, to determine the effect of these inhibitors on foot-and-mouth disease virus (FMDV) and to establish the position of FMDV in this classification.

Virus strains of all seven immunological types of FMDV were examined: strains 119–1 (type *A*), *C*997–1 (type *C*), *M*11–30 (type *O*), *Tur* 43/62–1 (*SAT* 1), *Rho* 1–285/1 and *Kenya* 3–476 (different sub-types of type *SAT* 2), *RV* 7–323 (type *SAT* 3) and *Pak* 1–1 (type *Asia* 1). These strains are all laboratory strains maintained by passage in pig-kidney monolayers, except *Tur* 43/62–1 which was passaged in *BHK* tissue.

Pig-kidney and *BHK*-21 cells (ref. 3) were used for virus assay, either as monolayers, suspended cell plates or in the metabolic inhibition test (pig-kidney only)[4]. The standard tissue culture procedure for FMDV was followed[5]. The results obtained were substantially similar whichever host cell system was employed and only the pig-kidney data will be quoted.

Guanidine hydrochloride and HBB (supplied by courtesy of Dr. K. Folkers of Merck, Sharp and Dohme, New Jersey) were incorporated into either solid overlay or fluid medium. HBB was not completely soluble and was treated as recommended by Tamm and Eggers[1].

It was found that HBB had no effect on the multiplication of the eight strains of FMDV tested by the methods of assay used (plaque inhibition, suppression of cytopathic effect and virus yield) at concentrations as high as 500 μg/ml. No HBB-susceptible virus was available to test the potency of the HBB sample used, but there was no reason for supposing that it was in any way defective. Moreover, unsubstituted benzimidazole had no effect on FMDV multiplication at concentrations (0·25–1 mg/ml.) which have been reported to reduce significantly yields of poliovirus[6].

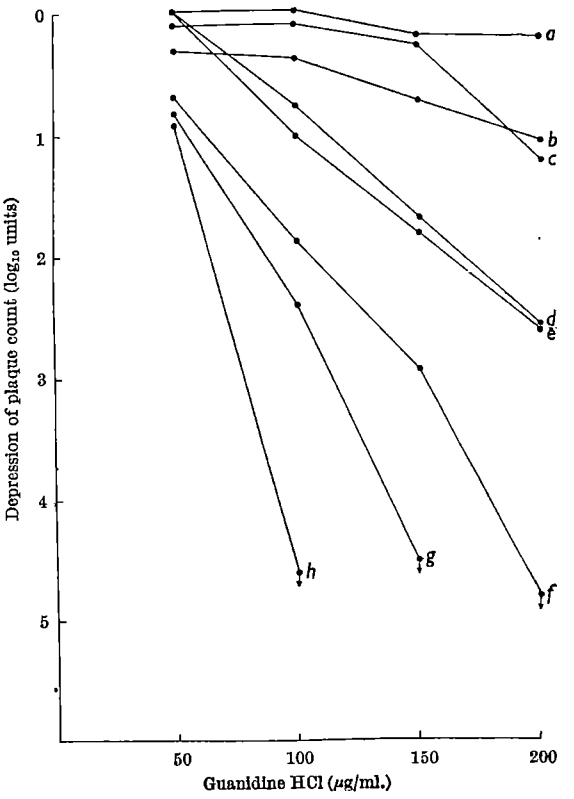


Fig. 1. Effect of increasing guanidine concentration on plaque count. Determined by titration on pig-kidney monolayers with guanidine hydrochloride incorporated in the overlay. *a*, C997–1 (type *C*); *b*, *M*11–30 (type *O*); *c*, 119–1 (type *A*); *d*, Kenya 3–476 (type *SAT* 2); *e*, *Pak* 1–1 (type *Asia* 1); *f*, *Rho* 1–285/1 (type *SAT* 2); *g*, *Tur* 43/62–1 (type *SAT* 1); *h*, *RV* 7–323 (type *SAT* 3)

On the other hand, it was found that these eight strains of FMDV were susceptible to guanidine inhibition. Three of the strains—119–1, *M*11–30 and *C*997–1—only exhibited appreciable inhibition at relatively high guanidine concentrations (200–600 μg/ml.). The remainder were susceptible at lower concentrations, and Fig. 1 indicates the relative susceptibilities of the eight strains in the concentration range 50–200 μg/ml. Guanidine hydrochloride was not observed to have any inactivating effect on the virus at these concentrations. Inhibition was reversible, and prolonged (24 h) pre-exposure of tissue to guanidine hydrochloride (200 μg/ml.) did not subsequently affect adsorption and virus yield even in the case of the most susceptible strains.

Although only a few strains have been examined it is of interest that the most susceptible strains were those of immunological types *SAT* 1, *SAT* 2, *SAT* 3 and *Asia* 1, whereas those of types *A*, *C* and *O* were much less susceptible. The fact that the former types are at present restricted to the African and Asian continents, whereas the latter are widespread, may be indicative of a taxonomic relationship.

Otherwise the different levels of guanidine susceptibility observed showed no obvious correlation with other virus properties. No difference in guanidine susceptibility was detected between an early virulent passage level of the *Tur* 43/62 strain and a late attenuated passage level (kindly made available by G. N. Mowat of this Institute).

It has been possible to isolate stable variants of increased resistance from the more susceptible strains. For example,

[illegible]ts resistant to 400 μg/ml. guanidine hydrochloride [illegible]been isolated from strains *Rho* 1–285/1 and *Kenya* [illegible]. Mutation of this marker was not associated with [illegible]on of any other of the marker characteristics [illegible] in these strains (heat resistance, capacity to [illegible]y at elevated temperature, adult mouse patho-[illegible]y). Estimates of the mutation rate of this charac-[illegible] by fluctuation test have been made and will be [illegible]ed elsewhere[7]. It has not as yet been possible to [illegible]e guanidine-dependent variants.

[illegible]n the basis of its response to these inhibitors FMDV [illegible]mbles the coxsackie *A* viruses[1]. Similarity between [illegible]e coxsackie viruses and FMDV in respect of their [illegible]athological effect in infant mice has previously been [illegible]noted[8].

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Comparative Immunological, Biological and Biophysical Behaviour of Meningopneumonitis Viral Antigenic Preparations

THE Psittacosis-LGV-Trachoma (*PLT*) group of viruses is mainly characterized by their complement-fixing (*CF*) activity[1–5]. We have recently reported multi-functional, that is, *CF*, precipitating and leuco-agglutinating meningo-pneumonitis viral *MPV* sub-units[6]. These immunological sub-units differ from those observed by electron microscopy after negative staining. They could easily be obtained by genetron extraction of the virus (*FEM*)[6]. We now wish to report immunological, biological and biophysical behaviour of *FEM* sub-units in comparison with several other antigenic preparations reported in the past.

Fig. 1 shows the infra-red spectrum of *FEM* extracted with ether. The dip at 2·8μ is probably due to peptide N–H bond stretching and the band at 3·4–3·5μ is due to C–H bond stretching. Strong absorption at 5·8μ is strongly indicative of lipid. Protein is indicated by peptide absorption at 6·05 and 6·5μ. Bands between 9 and 10μ are due to nucleic acid. The curve in Fig. 1 is very similar to that of phenol-insoluble *MPV* antigen and of methanol–chloroform extract of *MPV* as published by Benedict[7]. This observation further substantiated the results obtained by enzymatic degradation of the precipitinogens, indicating a lipid, protein and nucleic acid composition[6]. The curve (Fig. 1) obtained from the ether extract of normal egg factor (prepared in the same way as *FEM*) exhibits few absorption dips, but it does indicate the N–H bond stretching at 2·8μ and a small amount of absorption at 5·9μ due to lipid.

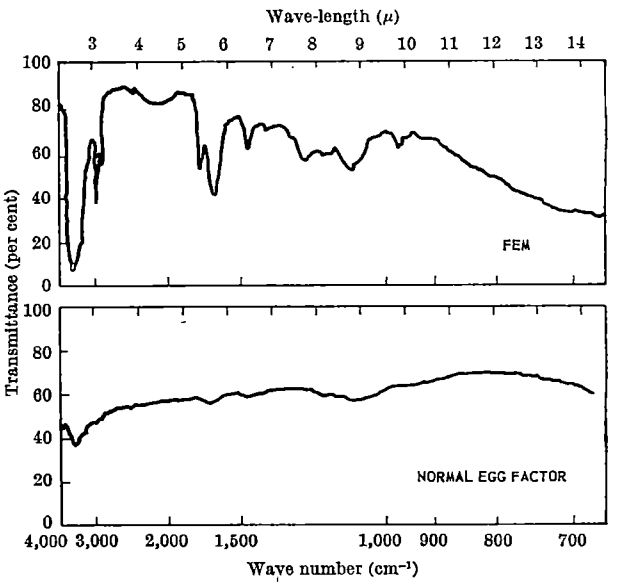


Fig. 1. Infra-red spectra of fluorocarbon-extracted meningopneumonitis virus (*FEM*) and normal egg factor antigens

CF abilities of *FEM* and its ether extract were compared with those of several antigenic preparations. *FEM* showed *CF* activity at a dilution of 1 : 512 with group-specific psittacosis antiserum. On ether extraction a 40-fold increase (1 : 20,480) in *CF* activity with type-specific antiserum and a 20-fold increase with group-specific antiserum were obtained (Table 1). It has previously been reported that species-specific activity has been unmasked by destruction of group-specific antigens with periodate or lecithinase[8–10]. Ross and Gogolak reported a titre of 16,000 in ether extracts of feline pneumonitis and psittacosis virus disrupted by ultra-sonication[9,10]. Ether-insoluble groups and type-specific *CF* antigens were isolated by making use of their alkali solubility. Cell walls of *MPV* isolated by sodium deoxycholate also had a type and group-specific *CF* activity of 1 : 1,280 titre. The deoxycholate-soluble fraction also contained both antigens. Ross and Jenkins[11] have reported only type-specific *CF* antigen in cell wall; our *MPV* cell wall preparations, however, contained two precipitinogens, one in position 1 and the other in position 7; the other five components were extracted in the sodium deoxycholate (Fig. 2).

All antigenic preparations, except the alkali-soluble one, showed multi-functional viral sub-units having precipitating or leuco-agglutinating and *CF* activities.

The thermal inactivation curves presented in Fig. 3 show that the five virus preparations listed could be divided into three groups based on the susceptibility of their *CF* ability to heat inactivation above 60° C. Group 1: Antigenic preparations characterized by thermolabile component(s) only, which showed linear inactivation with no constant residual *CF* activity above 80° or 90° C. Only the alkali-soluble, ether-insoluble extract of *MPV* exhibited thermolability of this nature. Group 2: Pre-

Table 1. COMPARISON OF COMPLEMENT-FIXING, PRECIPITATING AND AGGLUTINATING ACTIVITIES OF VARIOUS ANTIGENIC PREPARATIONS OF MENINGOPNEUMONITIS VIRUS

Antigenic preparations	Complement-fixing titre* of antigens		Ouchterlony's reaction (precipitating lines)	Agglutination of rabbit pseudo-eosinophils
	MPV specific-antiserum	Psittacosis group antiserum		
Genetron extract of *MPV* (*FEM*)	512	256	All (1–7)	+
Ether extract of *FEM*	20,480	5,120	All	+
Alkali-soluble, ether-insoluble *MPV*	1,280	640	None	−
MPV cell wall preparation	1,280	1,280	1 and 7	+
Deoxycholate extract (without cell wall) of *MPV*	640	640	2, 3, 4, 5 and 6	−

* Titre indicating reciprocal of highest dilution of antigenic preparations.

parations which exhibited a constant residual activity above 80° or 90° C, dependent on the concentration of the thermolabile components. The genetron extract (*FEM*), its ether extract and the preparations of *MPV* minus cell wall fell into this category. Group 3: Preparations relatively thermostable up to 90° C. The *MPV* cell walls alone showed this type of heat resistance.

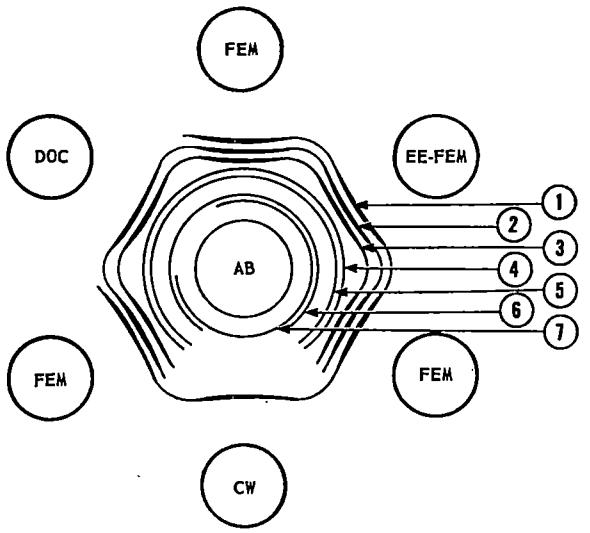


Fig. 2. Diagrammatic representation of precipitinogens of *MPV* by the Ouchterlony technique. *FEM*, undiluted fluorocarbon extract of the concentrated infected yolk sac; *EE-FEM*, ether extract of *FEM*; *CW*, cell wall of *MPV*; *DOC*, deoxycholate extract (without cell wall); *AB*, pooled undiluted rabbit antibody to *FEM*. Numbers 1–7 indicate precipitating lines from the antigenic cup to the antibody cup

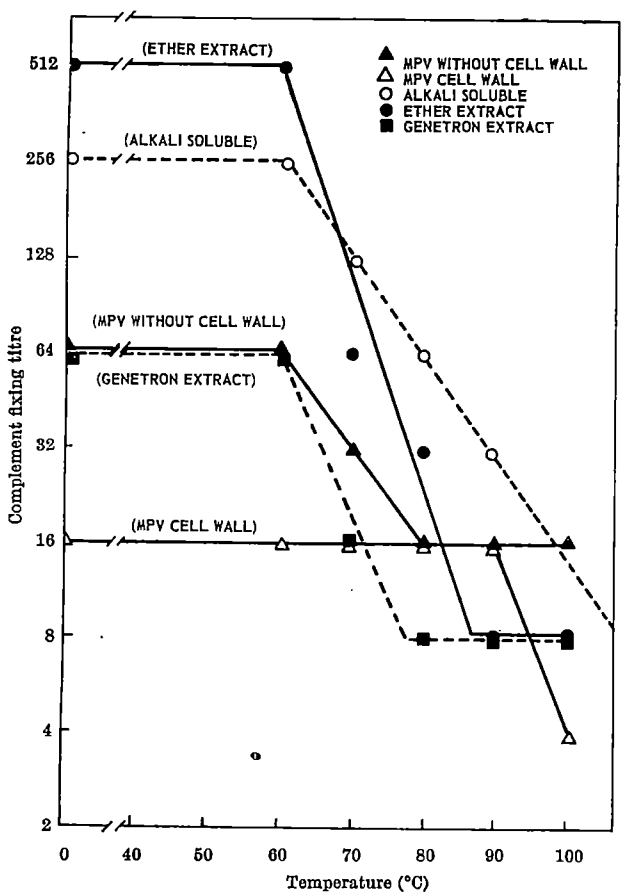


Fig. 3. Thermal curve of complement-fixing activities of various antigenic preparations of *MPV*

Thus, by careful inspection of the thermal inacti curves it is possible to suggest the location of the *CF* antigens in the virus particles. There appea at least two species of thermostable antigens in elementary bodies, one of which is located in or virus cell wall, and another which lies within the pa The thermostable antigen of the cell wall also seem responsible for leuco-agglutination and some deg precipitating ability (Table 1). The finding that th cell wall yielded a relatively thermostable antigen agreement with the report by Ross and Gogolak[9,10] similar antigen in *MPV*. *FEM* exhibits both ther stable and thermolabile antigens in addition to leu agglutinating ability. On ether extraction of *FEM* tw significant events take place. First, the thermostable antigen is completely taken up by the ether, and secondly, the thermolabile antigen undergoes some sort of unfolding or fission to yield two (or more) antigens, one of which is ether soluble and yields an eight-fold higher titre than the original *FEM*; this extract also shows leuco-agglutinating ability. Thus, it can be seen that the thermostable and thermolabile complement-fixing activity and precipitinogen formation at positions 1 and 7 (Fig. 2; Table 1) are associated with the cell wall and/or surface of the virus particle. In summary, then, the multifunctional *MPV* antigen responsible for leucoagglutination appears to be either a protein-lipid-carbohydrate-DNA complex which exhibits group specificity and is responsible for precipitinogen line 1 or a protein-lipid-RNA complex which is responsible for line 7, but which is not group specific (see ref. 6 for biochemical composition).

Similar thermal inactivation investigations have been carried out with psittacosis *CF* antigens. Early work by Bedson[2] showed that *CF* antigens derived from psittacosis infectious material could be separated into a group-specific antigen which was stable at 100° C, and a type-specific antigen which was heat-labile. More recently Volkert and Christensen[12] and Benedict and O'Brien[3] have separated psittacosis *CF* antigens into ether-soluble and ether-insoluble fractions, both of which contained *CF* activity. Ross and Gogolak[9,10] showed that although the alkali-soluble fraction and the ether-soluble fraction were serologically related, differences were observed both in their hæmagglutination properties and their gross heat stability. Kinetic studies of the thermal destruction of influenza *A* virus hæmagglutination have been reported by Lauffler and Carnelly[13]. Thermal differentiation of the adenovirus *CF* antigens showed that the *H* values derived from thermal constants of the virus and its *CF* antigens are not similar[14].

This work was partly supported by training grant *E*–3175–*C*2 from the Institute for Allergy and Infectious Diseases, and contract *Nonr*–3127(00), Office of Naval Research.

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, December 7

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 10 a.m. and 2.30 p.m.—Colloquium on "Programmed Test Equipment and Techniques".

BRITISH SOCIETY FOR THE PHILOSOPHY OF SCIENCE (in the Joint Staff Common Room, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Mr. G. C. Archibald: "Refutation or Comparison?"

INSTITUTION OF ELECTRICAL ENGINEERS, SCIENCE AND GENERAL DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Prof. J. C. West: "Non-Linear Circuit Theory".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 6 p.m.—"The Bridge" (Geographical Films).

UNIVERSITY OF LONDON (at Birkbeck College, Malet Street, London, W.C.1), at 6 p.m.—Prof. J. Th. Runnenburg (University of Amsterdam): "Some Practical Problems Solved with Simple Tricks from Probability Theory and Analysis".*

SOCIETY FOR ANALYTICAL CHEMISTRY, MICROCHEMICAL METHODS GROUP (at "The Feathers", Tudor Street, London, E.C.4), at 6.30 p.m.—Discussion Meeting on "The Characterization of Organic Compounds" opened by Dr. D. W. Mathieson and Mr. L. C. Cross.

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (joint meeting with the Paper and Textile Chemicals Group, at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Mr. I. F. Hendry and Mr. J. A. S. Newman: "New Developments in Papermaking".

Tuesday, December 8

BRITISH INSTITUTE OF RADIOLOGY (in the Edward Lumley Hall of the Royal College of Surgeons, Lincoln's Inn Fields, London, W.C.2), at 10 a.m. —Symposium on "The Scientific Bases of Permissible Levels".

ZOOLOGICAL SOCIETY OF LONDON (at the Zoological Gardens, Regent's Park, London, N.W.1), at 5 p.m.—Scientific Papers.

INSTITUTION OF THE RUBBER INDUSTRY, LONDON SECTION (at the Royal Society of Tropical Medicine, 26 Portland Place, London, W.1), at 5.30 p.m. —Miss B. E. Sabey: "Hysteresis in Rubber". 7 p.m.—Mr. A. Dibbo: "Oscillating Disc Rheometer"; Mr. R. H. Norman: "Process Control and Cross-Linking Studies using the Wallace-Shawbury Curometer".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.— Mr. P. N. T. Wells: "Ultrasonics in Clinical Diagnosis". (Thirteenth of fourteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)*

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.— Prof. J. J. Pritchard: "The Skeleton and Homeostasis".*

UNIVERSITY OF LONDON (at the Institute of Archaeology, 31–34 Gordon Square, London, W.C.1), at 5.45 p.m.—Dr. M. V. Seton-Williams: "Tell Rifa'at".*

INSTITUTION OF MECHANICAL ENGINEERS, AUTOMOBILE DIVISION (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Mr. T. Priede, Mr. A. E. W. Austen and Mr. E. C. Grover: "Effect of Engine Structure on Noise of Diesel Engines".

UNIVERSITY OF LONDON (in the Engineering Theatre, University College, Gower Street, London, W.C.1), at 6 p.m.—Prof. Stephen H. Crandall (Massachusetts Institute of Technology): "Random Vibration". (Further lectures on December 9, 10 and 11.)*

Wednesday, December 9

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Du Cane Road, London, W.12), at 2 p.m.—Dr. I. Holden: "Toxicology".*

GEOLOGICAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. K. Coe: "Intrusive Tuffs of West Cork, Ireland"; Dr. G. P. L. Walker and Dr. D. H. Blake: "The Formation of a Palagonite Breccia Mass Beneath a Valley Glacier".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. R. E. Young: "Telemetry—The Present Position and Future Trends".

INSTITUTION OF ELECTRICAL ENGINEERS, SCIENCE AND GENERAL DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "The Electrometer Amplifier—Its Design and Applications" opened by Mr. G. I. Hitchcox and Mr. G. R. Taylor.

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Annual General Meeting. 7 p.m.—Colonel G. W. Raby, C.B.E.: Presidential Address.

INSTITUTION OF MECHANICAL ENGINEERS, INDUSTRIAL ADMINISTRATION AND ENGINEERING PRODUCTION GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Trends in Machine-Tool Design and Development".

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (at Shell-Mex House, Strand, London, W.C.2), at 6 p.m.—Annual General Meeting.

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Sir Rex Cohen, K.B.E.: "Leisure in an Affluent Society".

UNIVERSITY OF LONDON (at Birkbeck College, Malet Street, London, W.C.1), at 6 p.m.—Prof. J. Th. Runnenburg (University of Amsterdam): "The Influence of Markov-Dependence of the Arrival Intervals on a Simple Queue".*

INSTITUTE OF INFORMATION SCIENTISTS (at the Swedenborg Society Hall, 20 Bloomsbury Way, London, W.C.1), at 6.15 p.m.—Dr. B. C. Christian: "Report Writing and Editing in an Industrial Research Laboratory".

OIL AND COLOUR CHEMISTS ASSOCIATION (at the Royal Society of Tropical Medicine and Hygiene, 26 Portland Place, London, W.1), at 7 p.m.—Mr. V. Jenkins: "The Use of Furoic Acid in Alkyds".

PHARMACEUTICAL SOCIETY OF GREAT BRITAIN (at 17 Bloomsbury Square, London, W.C.1), at 7.30 p.m.—Mr. D. Stephenson: "Experiences and Experiments in Tabletting" (Harrison Memorial Lecture).

ROYAL AERONAUTICAL SOCIETY, GRADUATES' AND STUDENTS' SECTION (at 4 Hamilton Place, London, W.1), at 7.30 p.m.—Mr. D. C. Armstrong: "Ultra-Light Aircraft".

Thursday, December 10

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Prof. B. Katz, F.R.S., and Mr. R. Miledi: "Propagation in Impulses and the Release of Transmitter in Motor Nerve Endings".

INSTITUTION OF CIVIL ENGINEERS, HYDROLOGICAL GROUP (at Great George Street, London, S.W.1), at 5.30 p.m.—Informal Discussion on "The Prediction of River Flows" introduced by Mr. T. O'Donnell.

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. D. Jones: "Resistive Earthing of Electrical Distribution Systems".

ROYAL INSTITUTION, LIBRARY CIRCLE (at 21 Albemarle Street, London, W.1), at 5.30 p.m.—Mr. Edward Ironmonger: "Tyndall in America".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.— Prof. R. B. Fisher: "Chemical Transfer Across Membranes". (Last of fourteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)*

UNIVERSITY OF LONDON (at the School of Pharmacy, 29/39 Brunswick Square, London, W.C.1), at 5.30 p.m.—Prof. Rita Levi-Montalcini (Washington University): "Growth Control of Sympathetic Nerve Cells by a Protein and Its Antiserum".*

UNIVERSITY OF LONDON (in the Physiology Lecture Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. R. J. Rossiter (University of Western Ontario): "Biosynthesis of Phospholipids" (Further lecture on December 15).*

INSTITUTION OF MECHANICAL ENGINEERS, NUCLEAR ENERGY GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Fast Start-Up of Gas-Cooled Reactors".

ROYAL AERONAUTICAL SOCIETY (at 4 Hamilton Place, London, W.1), at 6 p.m.—Mr. E. H. Heinemann: "Aviation in Perspective" (Fifty-third Wilbur Wright Memorial Lecture).

SOCIETY FOR ANALYTICAL CHEMISTRY, BIOLOGICAL METHODS GROUP (at the 'Feathers' Restaurant, Tudor Street, London, E.C.4), at 6.30 p.m.— Twentieth Annual General Meeting, followed by Informal Discussion on "Present Position with Regard to Microbiological Assay" opened by Dr. E. C. Barton-Wright.

RADAR AND ELECTRONICS ASSOCIATION (at the Royal Society of Arts, John Adam Street, Adelphi, London, W.C.2), at 7 p.m.—Mr. K. A. Russell: "Pay Television Systems".

TELEVISION SOCIETY (in the Conference Suite, I.T.A., 70 Brompton Road, London, S.W.3), at 7 p.m.—Discussion Meeting on "Television Servicing—Is it Satisfactory?"

Friday, December 11

ASSOCIATION OF APPLIED BIOLOGISTS (in the Lecture Hall of the British Museum (Natural History), Cromwell Road, London, S.W.7), at 10.50 a.m.— Meeting on "Bacterial Plant Pathology".

BIOCHEMICAL SOCIETY (in the Department of Chemistry and Biochemistry, St. Thomas's Hospital Medical School, London, S.E.1), at 11 a.m.—Scientific Papers.

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Du Cane Road, London, W.12), at 4 p.m.—Dr. I. MacIntyre: "Magnesium Deficiency".*

OCCAM SOCIETY (at the Northampton College of Advanced Technology, St. John Street, London, E.C.1), at 6.15 p.m.—Prof. R. O. Kapp: "Some Problems of Chairmanship".

Saturday, December 12

LONDON COUNTY COUNCIL (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Mr. A. Robinson: "Bird Life of South-East London".*

Monday, December 14

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Du Cane Road, London, W.12), at 4 p.m.—Lord Cohen of Birkenhead: "The Lipidoses".*

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Mr. J. G. Attfield and Mr. D. A. Wheeler: "Vapona Insecticide".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.15 p.m.—Prof. E. H. Thompson, O.B.E.: "An Itinerant Scientist in China".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT LECTURER IN GEOLOGY—The Registrar, The University, Manchester, 13 (December 10).

EXPERIMENTAL OFFICER (with at least a B.Sc. degree in biology and physics) for the Tropical Stored Products Centre based at the Pest Infestation Laboratory, Slough, for research investigations on the importance of moisture in stored products and the design and suitability of new types of storage containers—The Research Department, Ministry of Overseas Development, Eland House, Stag Place, London, S.W.1, quoting Ref. RE 255/230/01 (December 11).

LECTURER (with an interest in either experimental or theoretical physics) IN PHYSICS—The Registrar, Queen Mary College (University of London), London, E.1 (December 11).

MYCOLOGIST (with a degree in agricultural botany or botany or equivalent qualifications) IN THE POTATO BRANCH, to assist in the assessment of disease resistance in potatoes—The Secretary, National Institute of Agricultural Botany, Huntingdon Road, Cambridge (December 11).

ASSISTANT LECTURERS (2) IN PATHOLOGY—The Secretary, St. Thomas's Hospital Medical School, London, S.E.1 (December 12).

SENIOR LECTURER OR ASSISTANT LECTURER IN PHYSICS IN THE SCHOOL OF MATHEMATICS AND PHYSICS—The Registrar, University of East Anglia, Earlham Hall, Norwich, NOR 05G (December 18).

SENIOR LECTURERS, LECTURERS OR ASSISTANT LECTURERS IN MATHEMATICS IN THE SCHOOL OF MATHEMATICS AND PHYSICS—The Registrar, University of East Anglia, Earlham Hall, Norwich, NOR 05G (December 18).

LECTURER (Grade II) IN THE DEPARTMENT OF MECHANICAL ENGINEERING—The Registrar and Secretary, The University, Bristol (December 21).

ASSOCIATE PROFESSORS OR PROFESSORS IN THE DEPARTMENTS OF MATHEMATICS AND PSYCHOLOGY; and LECTURERS OR ASSOCIATE PROFESSORS IN CHEMISTRY, PHYSICS, SOCIOLOGY, GEOLOGY AND GEOGRAPHY—Dean of the Faculty of Arts and Science, Laurentian University, Sudbury, Ontario, Canada (December 31).

CHAIR OF PHYSICS at the University of New England, Armidale, New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (December 31).

LECTURER/SENIOR LECTURER IN THE DEPARTMENT OF CLINICAL PSYCHOLOGY, School of Applied Psychology, The University of New South Wales—The Agent General for New South Wales, 56–57 Strand, London, W.C.2; and The Appointments Section, The University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia (December 31).

PROFESSOR OF ENGINEERING PRODUCTION—The Registrar, Ref. 60Y/E, Bradford Institute of Technology, Bradford, 7 (December 31).

PROFESSOR OF STATISTICS AND OPERATIONAL RESEARCH—The Registrar, Ref. 61Y/E, Bradford Institute of Technology, Bradford, 7 (December 31).

LECTURER (with some teaching experience and qualifications in the theory of education) IN EDUCATION—The Registrar and Secretary, University of Durham, Old Shire Hall, Durham (January 1).

LECTURER OR RESEARCH ASSISTANT IN GEOGRAPHY—The Registrar and Secretary, University of Durham, Old Shire Hall, Durham (January 2).

CHAIRS (2) OF PURE MATHEMATICS—The Registrar and Secretary, University of Durham, Old Shire Hall, Durham (January 4).

CHAIR OF SOCIOLOGY OR SOCIAL ANTHROPOLOGY—The Registrar, Room (T), The University, Hull (January 8).

RESEARCH FELLOW in the Ion Research Unit of the Research School of Physical Sciences, Institute of Advanced Studies, Australian National University—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 8).

LECTURER/SENIOR LECTURER (with a Ph.D. in chemical engineering and several years industrial experience) IN CHEMICAL ENGINEERING at the University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 15).

CHAIR OF PHARMACOLOGY at the University of Ibadan, Nigeria—The Secretary, Senate Committee on Higher Education Overseas, University of London, Senate House, London, W.C.1 (January 18).

ASSISTANT IN MATHEMATICS—The Secretary of the University Court, The University, Glasgow (January 30).

LECTURER (preferably interested in numerical analysis or a related field) IN PURE MATHEMATICS in the Faculty of Science—The Registrar, The University, Manchester, 13 (January 30).

RESEARCH FELLOW IN MATHEMATICS—The Secretary of the University Court, The University, Glasgow (January 30).

CHAIR OF BIOLOGICAL SCIENCES (Botany) at Massey University of Manawatu, Palmerston North, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, January 31).

LECTURER IN THE DEPARTMENT OF PSYCHOLOGY, University of Melbourne, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 31).

SENIOR LECTURER OR LECTURER IN THE DEPARTMENT OF MATHEMATICS, University of Melbourne, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 31).

UNIVERSITY LECTURER IN GEOCHEMISTRY IN THE DEPARTMENT OF GEOLOGY AND MINERALOGY—The Professor of Geology, Department of Geology and Mineralogy, University Museum, Oxford (January 31).

BIOLOGIST (well qualified) to teach up to University Scholarship standard and to be Head of the Department—The Warden, St. Edward's School, Oxford.

EXPERIMENTAL RESEARCH ASSISTANT (with a Ph.D. degree or approximately equivalent research experience) IN THE PHYSICS DEPARTMENT, to join a group working on liquid metal physics under Dr. N. E. Cusack—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1.

OIL PALM AGRONOMIST (with a degree in agriculture and postgraduate training, preferably with previous experience as an agronomist) in Sarawak, to open up and establi h a new experimental Station, mainly to be concerned with oil palm and other crops new to Sarawak, for example, cocoa and hemp—The Appointment Officer, Room 301, Ministry of Overseas Development, Elanc House, Stag Place, London, S.W.1, quoting Ref. RC 213/155/08.

POST-DOCTORAL RESEARCH FELLOW IN PHYSICAL CHEMISTRY, for work concerned with the application of radiotracers and other methods to the study of the defect solid state, under the direction of Dr. J. N. Sherwood—Prof. M. Gordon, University of Strathclyde, George Street, Glasgow, C.1.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Agricultural Research Council. Memoirs of the Soil Survey of Great Britain—England and Wales. The Soils and Land Use of the District Around Aylesbury and Hemel Hempstead. By B. W. Avery. Pp. vii + 216 + 11 plates. (London: H.M. Stationery Office, 1964.) 35*s.* net. [610

Institute of Personnel Management. Industrial Behaviour and Personnel Management. By Prof Tom Lupton. (Industrial Relations Series.) Pp. 59. (London: Institute of Personnel Management, 1964.) 7*s.* 6*d.* [610

Agricultural Research Council. A Bibliography of Farm Buildings Research. Part 5: Buildings for the Drying and Storage of Grain, 1st Supplement, 1958–1961. Pp. 35. (London: Agricultural Research Council, 1964.) 2*s.* 6*d.* [610

Friends of the Lake District. Report and News Letter, August, 1964. Pp. 21. (Ulverston: Friends of the Lake District, 1964.) [610

Other Countries

Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Geological Map 1083A: St. Peters, Richmond County, Cape Breton Island, Nova Scotia. Geological Maps—Preliminary Series. Map 49–1963: Alberni Area, British Columbia. Map 3–1964: Kasheshibaw Lake, West Half, Newfoundland–Quebec. (Ottawa: Director, Geological Survey of Canada, 1963 and 1964.) [610

Transactions of the American Philosophical Society. New Series, Vol. 54, Part 5: The Formative Cultures of the Carolina Piedmont. By Joffre Lanning Coe. Pp. 130. (Philadelphia: The American Philosophical Society, 1964.) 3.50 dollars. [610

Proceedings of the UNESCO/FAO Regional Symposium on Pulp and Paper Research and Technology in the Middle East and North Africa, 10–14 December 1962, Beirut, Lebanon. Pp. ix + 479. (Cairo: Unesco Middle East Science Co-operation Office, 1963.) [610

Comptes Rendus des Travaux du Laboratoire Carlsberg. Vol. 34, No. 4–5: The Limited Proteolysis of Native Ovalbumin by *Bacillus subtilis* Proteinases. By M. Ottsen and Birte Østergaard. The Amino Acid Compositions of Three Proteinases from *Bacillus subtilis*. By Gordon Johansen and Martin Ottesen. Pp. 187–210. 4 kr. Vol. 34, No. 6: Differential Uptake of Protein and Glucose by Pinocytosis in *Amoeba proteus*. By Cicily Chapman-Andresen and Heinz Holter. Pp. 211–226 + plate 1.4 kr. Vol. 34, No. 7: Photo-Recording of Numbers in Small Protozoan Populations. By G. W. Salt. Pp. 227–235. 2 kr. (Copenhague: Danish Science Press, Ltd., 1964.) [610

Institut Royal Météorologique de Belgique. Publications, Série A, No. 48: La Variation Séculaire du Champ Magnétique Terrestre en Belgique, Période 1955–1960. Par Dr. A. de Vuyst. Pp. 11. Observations Ionospheriques—Station de Dourbes. Juin 1964. Pp. 26. Juillet 1964. Pp. 26. (Uccle-Bruxelles: Institut Royal Météorologique de Belgique, 1964.) [610

Comité International des Poids et Mesures. Procès-Verbaux des Séances, 52e Session—1963 (1–4 Octobre). 2e Série—Tome 31. Pp. 106. (Paris: Gauthier-Villars et Cie, 1964.) [610

Kungl Sjöfartsstyrelsen, Stockholm. Lovö Geomagnetic Observatory—Year Book 1961. Pp. 34. (Stockholm: Kungl Sjöfartsstyrelsen, 1964.) [610

Fisheries Research Board of Canada. Bulletin No. 143: Life History and Present Status of British Columbia Herring Stocks. By F. H. C. Taylor. Pp. viii + 81. (Ottawa: Queen's Printer, 1964.) 1.75 dollars. [610

United States Department of Agriculture. Index-Catalogue of Medical and Veterinary Zoology. Subjects: Trematoda and Trematode Diseases. Part 2: Supergenera and Genera C. By Mildred A. Doss, assisted by Katharine F. Roach and Virginia L. Breen. Pp. v + 159–358. (Washington, D.C.: Government Printing Office, 1964.) 60 cents. [610

The Design Synthesis of Biological, Chemical, and Physical Response Systems. By Jule Neville Dews. Pp. 25. (Frederick, Md.: Apropos' Press, P.O. Box 213, 1964.) 75 cents. [610

Commonwealth of Australia: Department of Supply. Annual Report of the Defence Standards Laboratories, 1963–1964. Pp. 50. (Ascot Vale, Victoria: Defence Standards Laboratories, 1964.) [610

Ten Years' Research at the Battelle Institute, Geneva. Pp. 71. (Geneva: Battelle Institute, 1964.) [610

World Health Organization. Technical Report Series. No. 286: Research in Immunology: Report of Five Scientific Groups convened by the Director-General of the World Health Organization. Pp. 97. 5 Sw. francs; 8*s* 6*d.*; 1.75 dollars. Public Health Papers. No. 24: Care of Children in Day Centres. By F. Davidson and 7 other authors. Pp. 189. 7 Sw. francs; 12*s.*; 2.25 dollars. No. 25: Housing Programmes—The Role of Public Health Agencies. By A. Andrzejewski and 7 other authors. Pp. 197. 8 Sw. francs; 13*s.* 4*d.*; 2.75 dollars. (Geneva: World Health Organization; London: H.M. Stationery Office, 1964.) [610

Geological Survey, Borneo Region, Malaysia. Memoir 17: The Geology and Mineral Resources of the Labuan and Padas Valley Area, Sabah, Malaysia. By R. A. M. Wilson and N. P. Y. Wong. Pp. xiii + 150 + 45 plates. (Kuching, Sarawak: Geological Survey Department, 1964.) 6 Malayan dollars; 14*s.* [610

Inter-American Tropical Tuna Commission. Annual Report for the year 1963. Pp. 89. (La Jolla, California: Inter-American Tropical Tuna Commission, 1964.) [610

Geological Survey of New South Wales. Bulletin No. 18: The Botany Basin. By R. J. Griffin. Pp. 110 + 6 maps. The Mineral Industry of New South Wales, No. 18: Gemstones. Compiled by G. Rose. Pp. 60. (Sydney: Government Printer, 1960 and 1963.) [610

United States Department of the Interior: Fish and Wildlife Service. Bureau of Sport Fisheries and Wildlife. Research Report No. 63: Sea-Water Systems for Experimental Aquariums—a Collection of Papers. Edited by John R. Clark and Roberta L. Clark. Pp. vi + 192. (Washington, D.C.: Government Printing Office, 1964.) 1.25 dollars. [610

Editorial and Publishing Offices of "NATURE"
MACMILLAN (JOURNALS) LIMITED
ST. MARTIN'S STREET, LONDON, W.C.2
Telephone Number: Whitehall 0081. Telegrams: Phusis London W.C.2

Annual subscription: Inland £13, Overseas £13 15s., payable in advance, postage paid to any part of the world.

Advertisements only should be addressed to
T. G. Scott & Son, Ltd., 1 Clement's Inn, London, W.C.2.
Telephone Number: Holborn 4743

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INVENTION AND DISCOVERY

PROF. I. D. RATTEE'S inaugural lecture in the chair of Colour Chemistry and Dyeing at the University of Leeds (now published*) deserves wide attention, not only for its percipient and highly stimulating discussion of the relations between discovery and invention, but also for its reasoned refutation of the false idea which is widely held that technology as an intellectual activity is inferior to pure science. Apart from this, he is particularly concerned as to the danger that applied science may become increasingly the activity of those in whom there is not fusion of the scientific, social and technological disciplines which are so necessary, and who tend to regard that fusion as neither necessary nor desirable. It is, as Prof. Rattee observes, very important that scientists should enter the applied sciences because of the valuable contribution they can make, but it is vital that applied science should never be dominated by the pure-science attitude as it largely exists to-day. This attitude of superiority may well persist, if the evidence of a recent survey of intending university entrants made by the Department of Education of the University of Oxford is any guide.

Prof. Rattee does not think that there is any rapid solution to these problems, although much could and should be done to discourage the prevailing attitude among prospective university students towards applied science and technology. Much could also be done to avoid the present imbalance in disciplines and to break down the divisions between pure and applied science within the universities themselves. He believes that in the long run it will be demonstration rather than hypothesis which will prevail: the greater inventiveness of university-trained applied scientists and the penetration of problems by university-based research will play the greatest part in making the change.

The plea for effective combination of scientific and technological disciplines is not based solely on its value in attempts to develop new and improved products for industrial use. The combination is also of particular value in fields which would generally be regarded as falling within the province of academic research—the type of problem, it might be added, which is sometimes the subject of 'fundamental' investigations by a research association or an industrial research department. It is this, Prof. Rattee suggests, that gives a particular excitement to work in the field of applied science, an excitement which is associated, on one hand, with the application of knowledge in a way which may benefit society, and on the other, with an intellectual challenge and inspiration directed towards discovery as in pure science. The inventor needs curiosity about things no less than the discoverer, as well as curiosity about his fellow men, about the sociological and technological background to his problem, and knowledge about the historical reasons for existing practices.

Prof. Rattee's exposition of the way in which the right teaching of technology develops a broad and not a narrow outlook could not easily be bettered, and it is to be hoped that it will not be overlooked by the Minister of State for Education and Science, Lord Bowden, some of whose recent utterances regarding higher education have been disturbingly neglectful of this question of quality. Moreover, the statement gains added force from the skilful way in which it is built up from a discussion of the relations between discovery and invention, and from a lucid account of the way in which a scientific approach to the behaviour of dyes has been developed in Britain under the influence of the Department of Colour Chemistry at the University of Leeds and comparable centres in other institutions, and not in Europe where organic chemistry dominates the fields of dyes and dyeing. This tribute should be noted by those who are ever ready to disparage Britain's readiness to develop new knowledge. The gap between application and discovery is seldom closed without bringing together the efforts of people whose interest bridges the gap between science and industrial practice. In its absence, subsidiary or even wrong objectives may be pursued and the difference between invention and discovery confused.

Prof. Rattee does not seek in this lecture to defend the triviality of some university research, and the whole tenor of his lecture is in keeping with that of an inaugural lecture by Prof. T. H. Cotterill in Edinburgh and numerous papers read at the recent conference on "Materials Science" organized by the Materials Science Club (*Nature*, 204, 831; 1964). He recognizes that there has always been a difference of approach to research in industry and in the universities—not necessarily in quality so much as in attitude and objective. He is concerned that what could be regarded as a reasonable and desirable differentiation is becoming a rift, and that great difficulty is often being encountered in establishing common ground between workers in the two fields. This is a highly dangerous situation, in which the academic teacher may fail to recognize his broad social responsibilities while, on the side of industry, the rate of technical progress may be impaired by shortcomings not in the numbers but in the kind of scientist or technologist trained.

Prof. Rattee does not accept the extreme industrialist view that academic research lacks a clear objective: on the contrary it is clear and praiseworthy, if difficult to describe in specific terms. It is discovery. Confusion arises not from the lack of motivation but from a failure to appreciate the difference between discovery and invention, and to some extent the failure of both sides to grasp the fact that these are objectives of equal stature and challenge, sustained by the use of the same sort of methods, but from two distinct lines of approach. Discovery, he suggests, may be regarded as the revelation of what exists in our environment and the study of the laws which condition it, while invention is the utilization of the results of discovery, new or old, in order to change our environment.

Thus, to take an inventive step, a discovery must be made, or old discoveries juxtaposed in a new relation. A discovery may be examined no more than is necessary to achieve the practical objective, though significant inventions usually stimulate fundamental work which enlarges the understanding of their basis, and may lead to a further invention. Nevertheless, it is unreasonable to argue that because it goes deeper, fundamental research possesses a higher intellectual standard than inventive research. The differences arise purely from the kind of issue and the way of thinking involved—from the

* *Discovery or Invention?* (Inaugural Lecture delivered in the University of Leeds on January 20, 1964.) By Prof. I. D. Rattee. Pp. 23. (Leeds: The University Press, 1964.) 2*s*. 6*d*.

differences between those seeking to understand the world and those seeking to change it.

Clearly the two modes of thought are interdependent but not identical, and in almost any scientist both instincts may operate. Prof. Rattee again illustrates his argument by reference to the dyestuffs and pigments industry and its tendency to overlook the difference between discovery and invention. His critical examination of the development of new products leads him to conclude that research directed at specific targets has proved restrictive in the sense that deeper examinations, which might well have given rise to a greater number of discoveries, could not be pursued: however, research is not primarily directed towards invention. This is a serious situation because it can generate attitudes leading to the restriction, and even the virtual cessation, of research on grounds of efficiency, economy and financial deployment. A similar situation is to be found in the synthetic fibre industry.

Prof. Rattee does not pursue this aspect beyond pointing out that it falls within the scope of managerial methods in industrial research. Before commenting on his analysis, however, it may be as well to consider his discussion of the other factors involved in the organization of invention. It is important that the research worker should possess an understanding of and a feeling for the technical and economic environment in which the results of his work will be used, so that he may see in correct terms the significance of his observations or the validity of the technical targets with which he is presented. Quite correctly Prof. Rattee maintains that this condition was not met in the dyestuffs industry—at any rate in general or at one time. He argues that in the chemical field interest in the product of the experiment is the beginning of the inventive step—a step that leads from discovery to invention. The view is plausible though a little superficial compared with C. Nicolle's study *Biologie de l'Invention*, but, by and large, his comments as to the rarity with which a student who prepares a dye evinces curiosity regarding its dyeing behaviour and the extent to which research objectives are based in present practice are well founded. He emphasizes the latter point by describing how the solution to a continuous dyeing problem in the shape of dyes which react with the fibre was found outside the normal processes of industrial research.

Historically, Prof. Rattee appears to rely on a secondary source of information, the soundness of which is open to challenge on various grounds. Further, the figures quoted for the appearance of new commercial dyes at least within the period from 1915 until perhaps 1930 take little account of the circumstances in which the British industry made its research effort at that time. If it had not concentrated on supplying its customers with the dyes with which they were familiar, there would have been no renaissance of the British industry at all. The Dyestuffs Development Committee has paid adequate tribute to the achievements of the British industry in entering new fields and, so far as the new synthetic fibres are concerned, those achievements necessitated a fresh look at methods of dyeing. This was true to some extent of viscose rayon as well as of acetate rayon. In the research which led to the development of new level dyeing products there was close co-operation between the dyestuffs research laboratory and the rayon manufacturer. In the main the search was for dyestuffs to be applied by conventional methods, but it may be mentioned that at about that time a suggestion was made that the difficulty could be overcome by pigmenting the rayon before it was spun—a suggestion which was discouraged on grounds of policy.

Prof. Rattee's argument is thus generally substantiated, and since his lecture was published in October there has been welcome evidence that his words may have fallen on receptive ears. It has been announced that an experimental course is to be established at the University of Liverpool to bridge the gap between academic life and industry. This is to start next year, and will be for post-graduate students in science and engineering. The course, which will run for two years, is being organized jointly by the University and Imperial Chemical Industries, Ltd., who are seconding Mr. R. D. S. Glascodine, research works manager and research engineer in the Mond Division, as director of industrial studies: a board of industrial studies has been created. If successful, the course will be renewed annually and the support of other industrial organizations included. Specifically it will aim at easing the transition from the research-orientated attitudes of a university to those required in modern industry, and will deal with general industrial problems, including motivations for university research and industrial research and development, and collaboration between industry and the universities in matters affecting research and design. Clearly it is designed to heal that rift between industrial and academic research with which Prof. Rattee is most concerned, and the benefits should be mutual.

It has long been held by experienced directors of industrial research that in its closer contact with production the research department of an individual firm has a great advantage over the normal industrial research association. Prof. Rattee obviously thinks that that contact may sometimes have a restrictive effect on research. Whether that is so or not, his argument points to one direction in which the research association could have an important advantage if there is really effective contact and co-operation between its members. The wider range and variety of experience which a research association represents should stimulate a much wider outlook and help to correct the weaknesses of target-directed research when excessively influenced by existing practices.

This is an aspect of co-operative industrial research which deserves closer examination. It is related, moreover, to the educational functions of the research associations. Whatever decisions may be taken as to the future of these associations, their functions and future do not concern either the Department of Technology or the Ministry of Education and Science alone. Both should be concerned with the independent appraisal and the creative thinking which Prof. Rattee's lecture is so well designed to stimulate.

RECENT ADVANCES IN THE RARE EARTH ELEMENTS

Progress in the Science and Technology of the Rare Earths

Vol. 1. Edited by LeRoy Eyring. Pp. 532. (Oxford, London, New York and Paris: Pergamon Press, 1964.) 120*s*.

THE rare earth elements are to some extent a misnomer. As pointed out in the final chapter of this new book, problems of dumping of the rare earths as by-products of thorium production occurred first in the

Welsbach mantle industry, and later (1940's and 1950's) in connexion with atomic energy activity. However, only with the development since 1947 of modern methods of separation, ion-exchange columns and solvent extraction methods have the individual rare earth elements become easily available to research workers.

The elements themselves, while possessing very similar chemical properties, because of the filling up of the partly filled 4*f* shell, show a variation in physical properties of the greatest scientific interest. Following on their availability, these elements have found numerous applications in industry. Because of the importance of these activities for science and technology, the present volume, the first of an annual series, is very much to be welcomed.

Prof. LeRoy Eyring, as editor, has wisely decided to provide a firm basis by starting with a review of the six years 1955–61. It cannot be too much emphasized that a review is little use unless it is comprehensive in scope (this does not mean that it should not select the wheat from the chaff) and covers a definite period, as here. A list of authors and chapters would occupy too much space, but it may be said that the coverage of subjects in sixteen chapters is comprehensive and authoritative. Starting with geochemistry, passing through separation methods, solution and solid-state chemistry, analytical chemistry and physical properties, it ends with an excellently documented chapter on "Uses and Applications". I was able to test out my own special interest, catalytic properties, on this last chapter. While this comprises only thirteen lines out of thirty-seven pages, there is a list of eighty references which I found of the greatest value in this work.

The book is well edited and printed, and a really useful production. It will no doubt justifiably join the ever-growing family of 'Advances' and 'Progress' volumes on the library shelves. At its price it is not expensive. In modern times the provision of lucid review volumes is more a public service than the proliferation of new journals, and workers in this particular field will be grateful for the present effort. D. D. ELEY

POPULAR ASTRONOMY

Pictorial Astronomy

By Dr. Dinsmore Alter, Dr. Clarence H. Cleminshaw and Dr. John G. Phillips. Pp. 312. (London: Museum Press, Ltd., 1964.) 42*s*. net.

Astronomy with Binoculars

By James Muirden. Pp. 146. (London: Faber and Faber, 1963.) 25*s*.

BOOKS dealing with popular astronomy are now very common. Most of them follow a set pattern, and it is therefore refreshing to find accounts which have something new to say.

This applies to *Pictorial Astronomy*, written jointly by three American astronomers, of whom Alter and Cleminshaw are associated with the Griffith Planetarium in Los Angeles. The influence of planetarium presentation is very evident, since although the book covers a wide range there are several unusual chapters—such as "The Visit of Arcturus to the Sun", in which the appearance of Arcturus as seen from our Earth is traced from the remote past to the equally remote future. The general impression is that of a series of articles instead of a wholly connected text. This is said as a comment, not as a criticism, since it leads to a very interesting book even though some aspects of astronomy are necessarily treated rather briefly. The line drawings are excellent, and the numerous photographs are reasonably well produced, even though black sky too often appears as a grey. The amateur with a little previous knowledge of astronomy will find the book very rewarding, particularly in view of the fact that it is extremely well written. There are some sections which are already somewhat out of date, but this is inevitable, and neither authors nor publisher can be blamed. Recent developments will presumably be included in any future edition.

Muirden's *Astronomy with Binoculars* is of a different type. Its range is aptly summed up by its title; the author caters for the interested beginner who possesses no equipment other than a pair of binoculars, but who is anxious to use a very modest optical aid to the best advantage. He considers that some useful work may be accomplished in such a way, and, as he points out, telescopes are of little use for observing auroræ, while binoculars are ideal for observing bright comets (though not, of course, for measuring comet positions). After dealing with fundamentals, he turns to the bodies of the Solar System, describing what can and what cannot be seen with binoculars, after which he turns to the changing skies; there are chapters on the stars of spring, summer, autumn and winter, illustrated by clear star-maps which should enable the beginner to identify the various constellations without much difficulty. Stellar objects within binocular range are shown and described. Here, too, the writing style is good, and is well calculated to hold the reader's attention.

It is true to say that the range of amateur astronomers to-day is considerably more limited than was the case even ten years ago; the opportunities for serious and useful research have lessened, though in some fields they still exist and are likely to continue indefinitely. Yet not all enthusiasts aspire to carry out such research; there are many who are content to do no more than look at the skies and learn what they can without making any attempt to delve into technicalities. To these people, Muirden's book will be of great interest, and for this reason alone it has been well worth writing. Moreover, it may well persuade some of those who read it to take a more positive interest in astronomy, to their inevitable benefit. Generally speaking, the advice given is perfectly sound, but it is most unwise to recommend that direct observation of the Sun may be carried out using darkened filters placed over the object-glasses of binoculars. Admittedly, the procedure is safe enough provided that sufficient care is taken; but the book is aimed at the complete beginner in astronomy, who may not be able to judge what is harmless and what is not.

PATRICK MOORE

ATLAS OF LIVER STRUCTURE

Submicroscopic Ortho- and Patho-Morphology of the Liver

(Submikroskopische Ortho- und Patho-Morphologie der Leber). By Dr. Heinz David. Text volume: Pp. 412. Atlas: 361 illustrations. (London and New York: Pergamon Press, 1964.) 500*s*. net per set.

THE study of the ultrastructure of the liver cell is still proceeding apace in many countries in the world. Electron microscopy is playing a leading part in this field. However, the interpretation of many of the findings is difficult and in recent investigations such interpretations have often been aided by an experimental approach or by the use of biochemical or histochemical techniques. Gradually some aspects of normal cellular functions can be related to structure, and abnormal structure found in spontaneous or experimental lesions interpreted in the light of normal structure and function. The subject is developing so rapidly that any attempt at a comprehensive account must inevitably be out of date before it appears. Dr. H. David mentions this difficulty in his introduction to *Submicroscopic Ortho- and Patho-Morphology of the Liver*, but this does not obviate it and it remains a serious defect particularly since the introduc-

tion is dated February 1962 and the text appears to be of about the same date but the date of publication is 1964. A supplementary list of references up to August 1963 is given, but the papers in this list are not discussed in the text. Partly as a result of the delay between writing and publication, the book presents a rather unsatisfactory account of this developing field.

The account of lysosomes, cytosomes and microbodies is confused. The author includes the work of Daems in his additional list of references, but he does not consider it in the text in relation to his own. Fig. 77 and 78 of vacuoles containing Periston are identical to the structures found by Daems after injection of dextran. Daems showed that these structures are lysosomes containing the injected material and not dilated endoplasmic reticulum as David suggests. Many other aspects of the subject have changed significantly since the book was written. The use of histochemical techniques is dismissed in two paragraphs. The work of Novikoff and of Sabatini and his colleagues is included in the additional list of references and again not considered in the text.

The material in the volume is of interest and the technical standard of the electromicrographs is good. The presentation, however, is most extraordinary; the work is in two volumes, the text in one and the electronmicrographs in the other. The text is in German followed by an English and then a Russian translation; however, the English translation is stilted and betrays a lack of familiarity with the subject-matter. The legends to the illustrations are in the text volume, but the figures are not referred to in the body of the text when a point illustrated is alluded to, but are given as a list at the end of each chapter. In reading the book one has an exasperating time referring rapidly from one volume to the other. The structures in electronmicrographs are profusely labelled with abbreviations of their German names. It was only after looking through the book many times that I discovered the key to the abbreviations hidden under the dust cover inside the back cover. Probably the most extraordinary thing of all about the two volumes is their price, £25.

D. B. Brewer

MECHANISMS OF ENDOTOXIN ACTION

Bacterial Endotoxins

Edited by Maurice Landy and Werner Braun. (Proceedings of a Symposium held at the Institute of Microbiology of Rutgers University.) Pp. xvi+691. (New Brunswick, N.J.: Institute of Microbiology, Rutgers, The State University, 1964.) 12 dollars.

In September 1963 a three-day symposium devoted to the problems of bacterial endotoxins was held at Rutgers University. Following the example set by the Society for General Microbiology the texts of the principal papers were circulated to the 212 participants and this greatly increased the time available for discussion. The symposium has been published with commendable speed and consists of the original papers grouped into eight sections, each one being followed by an edited version of the discussion. The habit of publishing the discussion has its critics but in this volume it reads well and does supplement the preceding section.

There are a total of 58 papers which vary in length and quality. Some are well-documented reviews of a particular problem while others are short reports of a few experiments. There is a variation in the treatment of the references in that in some papers the bibliography gives the titles of the works cited while others do not. Many readers prefer to see the titles quoted, and it seems a pity that all the papers did not follow this pattern.

There is an extremely interesting introduction by Dr. I. L. Bennett which clearly describes the type of problem which is to be discussed. As he points out, endotoxins have a complex chemical structure which attracts considerable interest in its own right. On the biological side their range of activities is not only vast, it is confusing, since to take two examples they may raise or lower body temperature and increase or decrease resistance to bacterial or viral disease. While they are antigenically specific, in many biological tests they are surprisingly non-specific. Endotoxins, while not so potent as some exotoxins, are very toxic and have been used clinically to produce artificial fever. Repeated injection may, however, lead either to tolerance or hypersensitivity. These few pages set the scene for the papers that follow.

The first section is devoted to the chemical research that is taking place and covers such problems as the structure of bacterial cell walls and the relationship between structure and biological activity. The pharmacological effects are discussed in two sub-sections and the attempt is made to relate the action of endotoxins to the fundamental processes in the cells involved.

Parts 4 and 5, devoted to immunological phenomena and the effects on host resistance to infection, overlap to some extent and may be taken together. The problems of natural antibodies and recovery from infection figure largely in these sections. In Part 6 there are eleven papers devoted to the conflicting problems of tolerance and hyperreactivity. The symposium closes with two sections devoted to the mode of action of endotoxins and in many ways this is the most interesting.

In terms of research interest for many years endotoxins have been the poor relations of the exotoxins. *Bacterial Endotoxins* will, I think, do a great deal to redress the balance and may be warmly recommended to a wide range of readers.

R. H. Gorrill

THE POGONOPHORES

Pogonophora

By A. V. Ivanov. Translated from the Russian and edited by D. B. Carlisle. With additional material by Eve C. Southward. Pp. xvi+479. (London: Academic Press, Inc. (London), Ltd., 1963.) 90*s*.

Pogonophora, although rather common marine animals, have until recently been almost unknown. In 1962 only three species were recorded against the eighty species described by 1964. Such a sudden acquisition of knowledge of a widespread group of animals is unprecedented in the history of zoology, and it is all the more surprising in a phylum related to the Protochordata. During the 1920's the R.R.S. *Discovery* dredged enormous masses of apparent fibres from the abyssal depths of Antarctic and sub-Antarctic waters, and many tons of unrecognized Pogonophora in their tubes were shovelled overboard. These amazing animals, lacking a gut and feeding exclusively by means of tentacles, form the dominant element in the fauna of quite large stretches of the sea bed, but elsewhere they appear to be entirely absent.

The examination of Pogonophora is now an active pursuit in the marine laboratories of many countries. These animals have been exhibited alive in the rooms of the Royal Society in London. But it is the intensive collection by Soviet oceanographical expeditions and the subsequent investigation of this material by Prof. Ivanov in Leningrad which has provided the wealth of information concerning structure, manner of life, life-cycle and systematics of these creatures.

Prof. Ivanov's beautifully illustrated monograph on the Pogonophora, issued in Russian in 1960, has now been translated by Dr. D. B. Carlisle, and this English edition of the *Pogonophora* has been brought up to date by the inclusion of diagnoses of new species described between 1960 and 1963 and of further data concerning geographical distribution and general biology, so pro-

ducing a book half as long again as the original. Much of the new material has been provided by Prof. Ivanov himself, and also by Dr. Eve C. Southward, Dr. Olga Hartman and Dr. Carlisle.

Abundant black-and-white drawings of excellent quality depict the structure of the species described. Coloured stereograms and diagrams illustrate the basic organization of different types of pogonophore, and some photographs in colour of living pogonophores, by Dr. A. J. Southward, have been included. This English edition provides also very many additional line drawings.

The systematic account, well illustrated and provided with keys and tables, occupies more than half the book. Part 1 (147 pp.) presents a general account of the Pogonophora, illustrated by 86 figures, 3 plates and a map showing global distribution. These chapters meet the needs of the general zoologist besides those of the specialist.

The vermiform cœlomate body is divided, usually by grooves, into three regions. A tentacle-bearing cephalic lobe or protosoma contains an undivided cœlom opening to the exterior by paired excretory cœlomoducts, the exits corresponding with the proboscis pores of the Hemichordata, etc. A short mesosoma always bears cuticular keels, the 'bridle', which probably permits the animal to cling to the inner surface of the tube. Internally lie a large pair of cœlomic cavities. The metasoma, or trunk, forms the greater part of the body, and may be very long indeed. A pair of cœlomic spaces traverses the whole. A few girdles of toothed platelets divide the anterior from the posterior metasoma, and serve no doubt to fix the middle part of the body in the tube. Adhesive papillæ, which are frequently secondarily metameric in arrangement, lie in front of the girdles. Behind them the papillæ are irregularly distributed. The sexes are separate; a single pair of gonads lies in the metacœls, the cœlomoducts of which are transformed into gonoducts opening on the metasoma. There appears to be no asexual budding. A heart is formed by elaborations of the ventral blood vessel, and lies between the proto- and meso-cœls. Red blood containing hæmoglobin is pumped forwards, through the tentacular apparatus, which is digestive, absorptive and respiratory, there being no gut. Well-developed longitudinal muscles (unstriated) are associated with giant nerve fibres situated in the dorsal nerve trunk —features doubtless associated with an ability for 'panic withdrawal' of the anterior part of the body into the protection of the tube, the toothed bristles of the girdles fixing the middle part of the body to the tube wall. The nervous system is entirely ectodermal in position, as in the Hemichordata and Phoronidea, resembling also the ecto-neural system of the echinoderms.

Heavily yolked eggs are laid into the distal part of the tubes. The embryonic development resembles that of the echinoderms and chordates in the radial, indeterminate cleavage, the association of the blastopore with the anus and the trisegmental body plan.

The long unbranched tubes secreted by pogonophores are several times the length of their owners. Transverse lamellæ are usually apparent, although the superficial appearance of the tubes is very varied. Chitin, and protein hardened by tanning, are present in the tube wall. The organic remains of graptolites have similarly yielded glucosamine.

Accounts of the habits of life, ecology and geographical distribution provide most interesting reading, together with a discussion on secondary metamerism in Pogonophora and of other phyla, the affinities and systematic position of the Pogonophora and evolution within the group. The evidence fully supports the conclusion that the "pogonophores are an independent phylum within the Deuterostomia".

The monograph as a whole is a masterly production brought out in record time, and Dr. Carlisle's translation and amplification supplies an English edition of great worth. Passing references to matters outside the echinoderm–protochordate–chordate animals, however, are less fortunate. I know of no copepod or other crustacean which lacks a blood system, as stated. Small Crustacea exhibit a variety of ways of effecting blood circulation without a heart, using sometimes a pulsating pericardial floor, but the hæmocœl and associated structures are not absent[1]. Few invertebrate embryologists would agree with the claim that the fate of the blastopore of, say, the Arthropoda, a group exceeding in numbers of species the whole of the rest of the multi-cellular animal kingdom, can be explained away as "merely the result of secondary changes". No evidence supports such a sweeping generalization. The objections to the use of the term 'Prosterostomia' and its implied community in blastoporal fate throughout most of the Invertebrata still stand[2,3]. The determinate spirally cleaving invertebrates are probably related to one another, but not because of the various fates of the blastopore. These matters are by the way, and the book as a whole is an excellent and much wanted contribution to our libraries.

S. M. MANTON

[1] Cannon, H. G., *Discovery Reps.*, 2, 435 (1931).
[2] Manton, S. M., *Nature*, 181, 748 (1958).
[3] Manton, S. M., *Phil. Trans. Roy. Soc.*, B, 233, 483 (1949).

A LIZARD FOR TEACHING AND RESEARCH

The Life of the Rainbow Lizard

By Dr. Vernon A. Harris. (Hutchinson Tropical Monographs.) Pp. 174. (London: Hutchinson and Co. (Publishers), Ltd., 1964.) 21*s*.

I AM pleased to see this work on three grounds. First, the author recognizes an important need in any developing country—educational materials which bear some relation to the experience of the pupils concerned. I well remember a country secondary school in the West Indies in which the sixth-form pupils knew all about the English frog in the text-book but did not even realize that the frogs in the school yard were different. Secondly, the choice of material is admirable: diurnal lizards enter into our daily life in the tropics in a manner which is matched by no wild vertebrate in the temperate regions. Thirdly, the book is easy to read.

The anthropomorphic but unsentimental style gives "life to the narrative" without being irritating. These lizards share our gardens and our houses; Dr. Harris shows how we can get to know them as individuals. As the book is largely concerned with behaviour, I think that it would have been useful if the author had given details of how to discriminate between *Agama agama* and other related species. The book shows how much can be learned by careful observation of individually marked lizards. I was particularly intrigued by the way in which the author was able to take advantage of the regularity with which each lizard defæcates at a particular time and place, to collect the scats and analyse the feeding habits of different age groups under undisturbed conditions. He demonstrates a number of relationships between the activities of the lizards and variations of the environment; for example, he interprets their geographical distribution in Nigeria in terms of their thermal requirements in relation to features of climate and vegetation. Dr. Harris also shows what can be done by simple experiments, many of which could be repeated in a school yard. Simple models were, for example, used to elucidate the importance of colour pattern and behaviour in defence of territory, courtship and mating.

References are given throughout the book to work on other lizards so that we have some idea of how far the behaviour of *Agama* is representative of lizards in general. The chapter that I found the weakest was that on colour change. In the general discussion the three major

factors influencing colour change were not brought out clearly: background response mediated by the intermediate lobe of the pituitary, response to stress mediated by the autonomic nervous system and adrenal gland and direct response of the chromatophores to temperature. I remain uncertain as to whether *Agama agama* shows a background response.

I like to think that those interested in animal behaviour will feel that lizards offer a splendid and almost untapped field of study. We can observe almost every phase in the life of a diurnal lizard; some of them seem to thrive as commensals of man. The fact that they, like us, are eye-minded animals makes it easier for us to appreciate much that is important in the life of such lizards. This book should be used by every school in Africa; indeed, it furnishes an argument for the teaching of biology in every school in Africa. I would like further to commend the book to every biology teacher in those warm countries in which lizards abound. Your common lizards will certainly differ from *Agama agama*, but Dr. Harris's book will give you a wealth of suggestions as to the kind of studies that can be made.

GARTH UNDERWOOD

AMPHIBIAN PHYSIOLOGY

Physiology of the Amphibia

Edited by John A. Moore. Pp. xii+654. (New York and London: Academic Press, 1964.) 128*s*. 6*d*.

OWING to their wide range of habitat and their poikilothermy, the Amphibia are a group of cardinal importance in the comparative physiology of the vertebrates. A second, and too often quite distinct, claim to fame is that some members of the group, the frog in particular, have provided generations of physiologists with convenient, readily available, preparations. It is to be hoped that *Physiology of the Amphibia*, with its clear indications of the many gaps in our present knowledge, will help to shift the emphasis from the latter role and accelerate progress toward an integrated physiology of these animals in relation to their varied environments.

The book is intended to be useful to both the amphibian and the general physiologist, and there can be no doubt that it performs a signal service for the former, for chapters on the digestive system (W. G. Reeder), blood and respiration (G. E. H. Foxon), water balance and kidney (I. J. Deyrup), and amphibian metabolism (G. W. Brown) may be instanced as providing lucid and effective syntheses of work which, with the possible exception of endocrine aspects of water balance, was sorely in need of collection and evaluation. For good measure, the book concludes with two chapters of a rather more specialized nature on developmental physiology (C. J. Barth) and regeneration (S. M. Rose). The general physiologist, on the other hand, will be primarily interested in this book because it makes work on the Amphibia more readily accessible, although there are two chapters ("Amphibian Muscle", B. C. Abbot and A. J. Brady; "The Amphibian Heart", A. J. Brady) which deal with fields where the frog has usually provided the experimental material without itself being the animal under investigation. Both contributions, which are heavily biased on the electrophysiological side, deal with work which is regularly reviewed at greater length than has been possible here, and it is therefore a pity that, apart from the slow fibre system in muscle, the opportunity has not been taken to include peculiarly amphibian aspects, such as seasonal phenomena.

The general editorial plan has not completely overcome the problems inherent in any attempt to produce a physiological treatise on a single group of animals. Several contributors feel that it is necessary to cover the whole range of their subject, at least in outline, even when nothing is derived from the Amphibia directly. This leads to the inclusion of several pages which can surely contain nothing but gratuitous information for most readers. Is this the type of book to which even a student would turn for an account of the tricarboxylic cycle or the β-oxidation of fatty acids? There is also a certain amount of overlap between some contributions, this being particularly evident in the endocrine field. The control of blood-glucose level is considered in a wide-ranging article on amphibian metabolism (G. W. Brown) and the hypothalamic control of pituitary secretion in a thoughtful section on amphibian metamorphosis (W. Etkin). Both these subjects are at least partially covered by A. Gorbman in the chapter on amphibian endocrinology, yet the important field of reproductive endocrinology is only given passing mention as one which has attracted the attention of many investigators.

The text is adequately illustrated, reasonably free from typographical errors and, despite the above strictures, the book is a valuable addition to the literature which should supplement Noble's earlier classic and be welcomed by all who wish to learn of the amphibian way of life.

C. L. SMITH

CROCIDURA

A Systematic Revision of the Shrew Genus *Crocidura* in Southern Africa

By Jurgens Meester. (Memoir No. 13.) Pp. vii+127. (Pretoria: Transvaal Museum, 1963.) n.p.

THE shrews of the genus *Crocidura*, and of related genera, constitute one of the most difficult taxonomic groups among the Mammalia. The difficulty lies mainly in the fact that the observable interspecific differences are so slight as to be obscured, if not equalled, by regional intraspecific variation. ('Observable' is the critical word—the shrews presumably recognize each other by scent, and if we could learn to do likewise many taxonomic problems would be resolved.) The taxonomist aiming to delimit the species is faced in reality with two tasks: first, the detection of the essential characters, leading to the recognition of the species as biological entities; and secondly, the description of those specific characters that are of practical value for identification of the species.

The revision of a genus can only be regarded as definitive when it is considered simultaneously throughout its entire range. Such a task is premature at present for *Crocidura*, ranging as it does from the Cape of Good Hope to Japan, but this volume, dealing with the southern part of Africa (south of the Zambesi), is a very valuable contribution towards that end. Nine species are recognized, and there are serious doubts about the validity of only one of them. The diagnoses, based almost entirely on size, colour of the pelage and dentition, inspire confidence in the validity of the results, but do not give a great deal of help to the non-taxonomist trying to identify species. A more detailed study of such external features as ears, nose, feet and scent-glands might have contributed some additional characters of practical as well as theoretical value. A valuable feature is the inclusion of distribution maps of each species, but the interpretation of the distribution is made unnecessarily devious by the attempt to correlate distribution primarily with rainfall and only secondarily with vegetation zones. The fact that the homoiothermic vertebrates are much more directly dependent on the biotic than the climatic component of their environment has been curiously overlooked by generations of biogeographers.

The introductory chapters contain a general account of the genus *Crocidura* and very detailed illustrated description of the skull and dentition of one species, *C. flavescens*. This should prove invaluable to the less experienced, or less specialized, taxonomist tackling other parts of this fascinating but frustrating group.

G. B. CORBET

Virological Technique

By D. W. G. Busby, W. House and J. R. Macdonald. Pp. x+218. (London: J. and A. Churchill, Ltd., 1964.) 30*s*. net.

THERE is, undoubtedly, need for an up-to-date handbook describing the techniques used in applied virology, and the aim of this one has been to provide a "reliable guide to laboratory practices in everyday use". The laboratories in mind are clearly those engaged in the routine isolation and identification of human pathogens and the measurement of immune responses to them.

There is a concise and lucid section on the cleaning and sterilization of apparatus, while filtration is described shortly. In discussing disinfectants it is difficult to see why ether and glycerol are mentioned and why more emphasis is not given to the proven virucidal range of agents recommended. Apparatus such as egg incubators, gassed incubators, homogenizers and the high-speed centrifuge are described in some detail.

The section on light microscopy is sometimes unhelpful; for example, the only use mentioned for inverted microscopes is to "examine cell monolayers growing in Petri dishes . . .". Electron microscopy is given brief introductory treatment. The account of inclusion bodies is outdated and contains some inaccuracies; for example, basophilic elementary bodies are described in the cytoplasm of cells infected with herpes febrilis and type *B*, intranuclear inclusions are attributed to herpes febrilis and zoster, both NITA viruses.

The chapter on animal isolation and inoculation techniques is clear and simple, though it is difficult to see why arterial cannulation is preferred to cardiac puncture for obtaining chicken plasma. Embryonated egg techniques, cell-culture methods and media receive good attention, but there are disturbing generalizations; for example, "Results obtained from cell cultures are invariably quicker . . . than those from laboratory animals". There is a brief general description of serological tests.

A very useful practice throughout the book is to append footnotes giving the addresses of suppliers for recommended items.

Systematic virology is dealt with in the final third of the book. In view of the apparent aim to cater particularly for workers in Britain, it is occasionally difficult to understand the relative allocation of space. For example, there are more than two pages devoted to a list of arboviruses, but no mention of rubella. The final chapter is a short account of freeze-drying techniques—not intended to be a practical guide to those required to carry them out but directing attention to more comprehensive reference works.

This well-produced volume will be very useful in its intended sphere and goes a considerable way towards filling the previous hiatus.

The Nature of Parasitism

The Relationship of Some Metazoan Parasites and Their Hosts. By W. P. Rogers. (Theoretical and Experimental Biology: an International Series of Monographs, Vol. 2.) Pp. ix+287. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1962.) n.p.

THE first edition of this compact, original and attractively-written book earned for itself a unique place in the considerable and growing literature about parasitology. It is not a text-book, nor is it a book for those who wish merely to know about the structure of parasites and how to identify them, or about the diseases they cause and the treatment and control of these diseases. The aim of the book is expressed rather in its sub-title. It is written for those who wish to understand the biochemical and other conditions which govern the way of life called parasitism as revealed especially by the nematodes and flatworms parasitic in Metazoan hosts.

As the author rightly observes, the mere entry of a parasite into a host does not constitute parasitism. Once a parasite has successfully overcome the many difficulties that may prevent it from reaching the right host and succeeded in entering it, it is confronted by many powerful factors challenging its very presence and continued existence inside the host. Against these adversities it must feed, grow and reproduce its kind and, if it can, overcome the biochemical, physiological or immunological resistance reactions of the host.

The Nature of Parasitism contains an excellent account of our knowledge of the biochemistry of these factors which govern the relationship of parasite and host. These are illustrated by reference to the more common and better known parasites of man and domesticated animals. To understand the text the reader will require a considerable knowledge of biochemistry and its technical terms and at least an elementary knowledge of the structure and life-histories of the parasites used to illustrate Dr. Rogers's views. He discusses in detail the remarkable effects of parasitism on the parasite and gives much space to a discussion of the basis, especially the biochemical basis, of the key problems of the host-specificity of parasites, the biology of their infective stages, and the modifications of the reproductive processes of the parasite, including the structure, formation and biochemistry of the parasite's egg-shell, its production of eggs and sex ratio and other factors which enable it to ensure reasonable chances of infection of the right hosts. The later chapters of the book discuss the ecological factors which influence the origin of parasitism and Dr. Rogers's own interesting views on the evolution of this mode of life.

The book deserves, and will no doubt achieve, many more editions, to which all parasitologists who wish to understand the way of life they are concerned with will eagerly look forward.

G. LAPAGE

Cats of the World

(World Wildlife Series No. 1.) By Armand Denis. Pp. 119+36 illustrations. (London: Constable and Co., Ltd., 1964.) 30*s*.

Rhinos Belong to Everybody

By Dr. Bernhard Grzimek. Translated by Oliver Coburn. Pp. 207 (138 photographs). (London: William Collins, Sons and Co., Ltd., 1964.) 63*s*. net.

THESE are two animal picture books by television personalities; both emphasize the impending extermination of much of the world's fauna and plead for effective conservation before it is too late. It remains to be seen whether such conservation is possible while the human population pressure increases at its present rate. Unless war, pestilence or famine reduces the number of men in the comparatively near future there will literally be no room for wild animals before many years have passed. Although politicians refuse to face the one problem of overwhelming importance to mankind, and fritter their efforts away on parochial controversies and international paper-pushing, they will before the end of this century be forced in all civilized countries to abandon subsidizing human breeding, to institute family planning permission and the imposition of heavy taxes on families of more than two children. The most optimistic estimates of the size of the world population in A.D. 2000 show that through the whole of the twenty-first century there will be food-rationing all over the world for at least a hundred years; the ration-book and the food queue will be as much a normal part of life to our great-grandchildren as the motor-car and the television are to us. Conservation of the fauna, ardently as it is desired by zoologists, is no more than a pipe-dream if the population explosion is not very soon extinguished.

Mr. Denis acknowledges the help of his editor, Dr. Bruce Campbell, in writing his book, and indeed it seems

that Dr. Campbell's hand can be recognized on most pages. It would therefore have been more gracious had the book not been written throughout in the first person singular. There is a useful check-list of all the species of the Felidae in an appendix, and there are illustrations of all species. The format and production are not distinguished. In contrast, Dr. Grzimek's book is beautifully produced and contains a collection of colour and monochrome photographs printed in Germany and second to none, all of them interesting and many of great beauty. The text, which is mainly a personal narrative of travels in Africa, has been skilfully translated by Oliver Coburn. L. Harrison Matthews

Theory of Excitons

By Robert S. Knox. (Supplement 5 of Solid State Physics: Advances in Research and Applications.) Pp. vii+207. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1963.) 68*s*.

THE absorption-edge spectrum of a semiconductor or insulator often shows a complicated structure which can, in many cases, be explained by exciton theory. An exciton is the simplest kind of electronic excited state of a crystal, in which a single electron has been excited across the forbidden energy gap to form an electron-hole pair.

The book by Robert S. Knox is a review of exciton theory covering the period from its first formulation by Frenkel in 1931 up to the present. Although the exciton has been investigated for more than thirty years, the papers referred to in the book—almost 400 in number—have mostly been published in the past decade. The increased interest in recent years has been stimulated by the large number of optical measurements on semiconductors, as it is for these crystals that exciton theory has had most success in explaining the features of experimental absorption spectra. According to Dr. Knox, a plateau has now been reached, with the electronic structure of the exciton well understood, but with much work still to be done on its transport and statistical behaviour.

The theoretical discussions of the book are based on a thorough account of the one-electron theory of the ground state and simple excited states of an insulating crystal. Dr. Knox then shows how this theory leads in different limits to the tight-binding Frenkel exciton and to the weak-binding Wannier exciton, the connexions between the two cases being emphasized. The theory of the effects of electric and magnetic fields and of mechanical strain on the exciton energy-levels is well presented, and the supporting experimental results are compared with theoretical predictions. The importance of excitons is almost entirely due to their influence on the absorption spectra of crystals, and a large and excellent section of the book is devoted to the theory of the optical properties of excitons. The final main section of the book deals with the theory of exciton transport.

Dr. Knox's book is well written, with the theory clearly presented and with the large volume of published work on excitons exhaustively covered. It can be wholeheartedly recommended both as a detailed account of the present state of exciton theory and as a stimulating source of ideas for experimental and theoretical research worth pursuing in the future. R. Loudon

Recommendations of the International Commission on Radiological Protection

(As Amended 1959 and Revised 1962). (ICRP Publication No. 6.) Pp. v+70. (London and New York: Pergamon Press, 1963. Published for the International Commission on Radiological Protection.) 25*s*. net.

PUBLICATION No. 6 of the International Commission on Radiological Protection contains the amendments made in 1962 by the Commission to its recommendations first adopted in 1958 and amended in 1959.

The greater understanding of the interactions and effects of radiations, resulting from the large volume of research in this field, makes it possible for more precise recommendations on radiation protection to be drawn up. The need for continuous revision arises from the considerable expense involved in maintaining the necessary standard of protection; any relaxation of the requirements compatible with safety is, therefore, of great practical value.

Apart from introducing the concept of Dose Equivalent (*DE*), to be measured in rems, which takes into account the difference in the biological effects of radiation by using the Quality Factor (*QF*), the main changes to the previous recommendations concern exposure of women of reproductive age. For those occupationally exposed, the maximum permissible exposure during any 13-week period has been reduced from 3 rems to 1·3 rems, although the annual maximum permissible exposure of 5 rems remains unchanged. This reduction was necessitated by evidence that the embryo is more sensitive to radiation, particularly in the early stages of pregnancy. For the same reason, all women of reproductive age should avoid X-ray exposure of the lower abdomen, except during the ten-day interval from the start of menstruation, when it is virtually certain that there is no pregnancy. Other modifications concern the lens of the eye, short-term exposures and emergency work, dose-rate effects, and the addition of external and internal exposures.

The maximum permissible concentrations of strontium-90 in air and water have been relaxed by a factor of 4, without reducing the maximum permissible body burden; this again follows a better understanding of the metabolism of strontium. New data are also given for many transuranium elements, up to fermium. These and other modifications to maximum permissible concentrations are tabulated in the book as a supplement to the previously published tables. Considerable confusion may arise from the existence of two sets of data and it would have been advisable to reproduce the tables in full, even though this may have resulted in the book costing slightly more.

J. Rotblat

Economic Development

By Prof. John Kenneth Galbraith. Pp. xiii+109. (Cambridge, Mass.: Harvard University Press; London: Oxford University Press, 1964.) 12*s*. 6*d*. net.

THREE chapters of *Economic Development*, on education and economic development, on developing and developed, and on development and the industrial corporation, are substantially unchanged from Mr. Galbraith's earlier book, *Economic Development in Perspective*, but the titles of the second two are changed. Substantial parts of a fourth chapter, "Development as a Process", are also taken unchanged from the first and title chapter of the earlier book. For the rest, though the main theme is the same, this is virtually a new book, rewritten and expanded with all the wit and realism that characterized the first. It carries, too, the same warning of the danger of any rigid classification of the nations into developed or underdeveloped nations: almost all, to some extent, fall into both categories, as well as into a third category of developing nations, to which most also belong. There are now eight instead of five chapters, the others discussing the process of economic development, the causes of poverty, and the choice. There is also a postscript on population and a brilliant introduction which explains the origin of the book and its evolution. Although concerned mainly with the underdeveloped or developing nations, and the ways in which technical or economic assistance can most effectively be given, the refreshing challenge of the book to doctrinaire thinking of any order makes it a stimulating and pertinent contribution to constructive thought about economic development in Britain or anywhere else. R. Brightman

SOCIAL ORGANIZATION OF INFORMATION*

By The Right Hon. Lord SHACKLETON, O.B.E.

President of the Association of Special Libraries and Information Bureaux

IN using the above title I mean organization by and on behalf of society or the community. This is a somewhat ambiguous title, but unfortunately we are operating in a field where many of the words are for us going to have a special significance, while popularly retaining a more general meaning; even the word 'information' has come to have a specialized meaning to us. I rather think the time has come when we need a single word to describe all these matters in which we are dealing. Otherwise we shall need a sentence, indeed a definition covering such words as 'information', 'documentation', 'storage', 'retrieval', 'dissemination', 'communication'. It is arguable we should perhaps have the definition before we have the word, but this need not deter one from the game of word invention.

I do not know how Norbert Wiener, if he was really the author, came to invent the name 'cybernetics' but it is a convenient and appropriate term with an impeccable derivation from the Greek work 'guberneo' (Greek letters γυβερνέω)—a most evocative derivation for the purpose. Inspired by this thought, I hastened to the Exeter University Library where, aided by their system of instant retrieval and by a Liddell and Scott Greek Lexicon, I devised the word 'exagellectics' from the Greek 'exagello' (Greek letters ἐξαγγελλω), meaning 'to inform'. I am not sure that the word is an entirely happy one, indeed it is certainly open to misinterpretation, but so is the title Aslib itself (being an abbreviation for Association of Special Libraries and Information Bureaux), and at least it is no uglier than 'ergonomics'. I think we might say that exagellectics is a branch of epistemology but must stop playing a game that any linguistic philosopher would scorn. However, I still urge the need for a single word to describe our study and our craft.

There is, alas, no professorial chair at a university with a sustained study of this subject and, although much of the thinking and some research is being carried on, I cannot discuss the whole of what will have to be the subject of major research in the future. However, in the absence of a professor it is perhaps not a bad idea for a politician or even a businessman to attack the subject.

The broad purpose of this article is to argue the case for action by the new Government in accepting responsibility for establishing machinery on a national footing for the provision and organization of scientific and all other research information and documentation. I will state right at the beginning that the purpose of a body operating in this way will be to define and identify, to advise, promote and co-ordinate, and to investigate and administer in the information field.

There are some grounds for supposing that the progress which can be made by any civilization is limited by the speed and effectiveness with which knowledge can be communicated. In Egypt hieroglyphic writing on papyrus made it possible for knowledge to be communicated to others and stored; but the process was so slow that the limit was soon reached. The remarkable flowering of Greek culture must have had some association with the Greek form of writing which made the transfer of knowledge more rapid and precise. though fortunately for contemporary classical research many accounts were kept on tablets. There was little progress from Roman times until the invention of printing, and we to-day must make sure that the progress we are capable of achieving by the new methods of communicating, storing and retrieving knowledge is not halted through failure to develop system or organization.

Scientific knowledge is doubling itself every 10–15 years and so are the documents within which it is contained. We need to be a great deal more concerned about the scientific explosion and its recording if we are to hope to find the means of making use of it.

While probably few would dissent from the proposition that much of a nation's wealth lies in its accumulation of specialized knowledge, I suspect that, for many people, it would be a very long step from that position to acceptance of the fact that, for those sections of a nation's wealth to be converted into real terms, an integral part of national economic planning must consist of the development of systematic arrangements for the control and exploitation of the specialized knowledge generated by mankind. (The word 'control' is a loaded one when used in its popular sense, but I am here using it in a technical and precise sense.) They would look at you with slightly pained surprise if you suggested that the range of operations involved in this process should have both an expertise and a philosophy of its own, capable of development and refinement by the processes of research and testing and discussion, and that this process of refining and testing is a discipline that could be of real value in itself. We in Aslib have come to accept this, but now we have a very much harder task of pressing our beliefs on those who have the power to convert them into the mainstreams of national vigour.

While we have been accustomed to think and plan information activities—in so far as we do either—in terms of harnessing scientific and technical knowledge. we must be ready to acknowledge the needs of other disciplines in the near future. The country has not yet faced up to the importance of the social sciences and equally we have not yet fully come to grips with their information needs. No doubt when the Heyworth Committee reports we shall see the formation of a Social Science Council and of course this will give impetus to research in these subjects. The need for a proper national organization of information services concerned with the social sciences will then become much more apparent. and I hope that Aslib is giving urgent attention to ways of adapting to this field at least some of the lessons learned in relation to the natural sciences.

Nor can I see why we should overlook the humanities. I can see no reason why, for example, historical or linguistic studies should not be modernized and streamlined by the adoption of the most modern information techniques. Nor might I think that some university librarians are shocked by the suggestion that the computer might on

* Substance of the Presidential Address delivered at the thirty-eighth conference of Aslib held in the University of Exeter on September 28. The full address will be published in *Aslib Proceedings*, 16, No. 12, December, 1964.

occasion replace the quill. After all, Canon Morton did much of his researches into the Epistles of St. Paul with the aid of a computer, though this was not strictly speaking an information technique. Librarians must of course see that the benefits of their techniques and work show themselves much more evidently and cogently in a steadily widening range of subjects. But there is more to it than this. Not only must they develop and adapt techniques of operation that should transform their own image into a combination of what is best in classical scholarship and modern technology; they must also, and so must we all, recognize that their activity is fundamental to political and economic planning. As a legislator I know from experience how very patchy is much of our real, organized knowledge in the field of economics, education and psychology—indeed, in all aspects of industrial and civil life, not to mention the law. We know, too, that new techniques are being developed that may revolutionize government—for example, by Prof. J. R. N. Stone of the Department of Applied Economics at Cambridge who is seeking, with the aid of a computer, to feed the multitudinous economic variables into a cybernetic model. The structure of government is still rather old-fashioned, but as life gets more complex it is bound, I hope, to adjust its machinery so as to improve both departmental and legislative decision making. Two scientific clerks to the House of Commons Library have recently been appointed, on the initiative of Mr. Austen Albu, one of the few MPs who understands this problem. This is at least a sign of the times.

There is, of course, already much first-class information provision—for example, in the Board of Trade, Ministry of Pensions, Ministry of Public Building and Works, and, not least, the Commissioners of Inland Revenue. Much of this has produced valuable raw material for all forms of research, including sociological research. As more and more is produced, a way has to be found of ensuring that this information is not only made available but is also known to be available and, furthermore, retrievable, and this needs both machinery and properly trained people to organize it. A special problem arises from the growth of new sciences and especially of inter-disciplinary studies where even the words used carry different meanings to different specialists. The meaning of words has already created difficulties in medical engineering. Furthermore, as the specialist reaches out into other fields, he finds the information resources of his own subject inadequate and possibly wholly lacking in important respects for his new purpose and he is unable to find a new starting point. An example of this is the investigation of gerontology where the searcher may be concerned not only with radiobiology, psychology, and biochemistry, but even zoology and botany, and he may well be unable to find (as was indeed the case in a particular instance in a well-known medical library) suitable reference information in the latter two fields. There was only one course open to my frustrated research friend and that was to join Aslib. This worked well enough but serves to illustrate the problem, which increasingly will call for radical solutions.

It must be apparent that a much more far-reaching revolution in the work of documentation and information research is called for than the modest steps which Aslib has been quietly and painstakingly leading during the last thirty or forty years. All this implies a radical re-thinking of our approach to information and documentation services. What has often been regarded as just a series of technical operations incidental to particular research and development must be recognized as a study in its own right. Increasing effort must go into developing a theory and philosophy. Cybernetic concepts are already available and are of some value. Much of the theory is at an early stage, but other disciplines even as far afield as ecology may provide useful analogies.

The President's Science Advisory Committee of the United States in its report on *Science, Government and Information* has said: "The flow of undigested information will surely engulf our science and shatter it into isolated fragments unless we change the traditional methods that we use to handle the flood". To do this we are enjoined to examine the information transfer chain and seek desirable qualities such as compatibility between one system and another. Only thus can information, like the living species in an ecological system, survive and fulfil its role. We must recognize the complexity of systems, and accept that we are not confronted with just a narrow professional problem. We are dealing with a hybrid discipline. Documentation and control of information are something that can straddle all cultures and help to set Sir Charles (now Lord) Snow's mind at rest.

In the past Aslib has spearheaded this attack with considerable assistance from the Department of Scientific and Industrial Research within the limitations of grant aiding, but I am doubtful as to whether the research association scheme, admirable though it is in many ways with its linking of grant-aid with subscription income, has been particularly suitable for the financing of work which is essentially of interest not just to those few people who are aware of it, but also to the nation at large.

During the past years Aslib, despite shoe-string resources, has achieved a great deal and we owe much to its officers, its Director, Leslie Wilson, and his dedicatep staff. Aslib has united in a common purpose and with, I understand, a really remarkable degree of loyalty, organizations from industry, education and government; and I think we have probably been much freer from the frictions that other associations have suffered. Intellectually, leadership by Aslib is acknowledged in the international field despite the fact that there are more massive resources available in other countries, especially the United States and U.S.S.R. I think, in fact I am sure, that no other national information organization has won—whether they campaign for it or not—the membership support of six hundred organizations in countries other than its own, in addition to its home membership, as well as many more regular subscribers to its publications. This is the point at which we always pride ourselves that this is the typically British creation, and we follow our time-honoured practice of appointing a chosen instrument and then forgetting to give it the resources that the task demands.

We have, however, now reached a stage when the new Government must accept full responsibility for establishing on a national footing machinery for the proper organization of information and documentation. The Government must do so not only for its own purposes but also because it, alone, can harness the diverse activities and support them on an adequate scale. Support must be found for more research, particularly if we are to achieve economy and cogency in the recording of information by using various forms of mechanization. I am glad that Aslib is putting mechanization firmly at the head of its research programme. There may be a need to concentrate much more of our information resources into a relatively small number of information centres under expert staffs. But the primary need is for a national authority of some kind which must accept responsibility for identifying national information needs, for ensuring that they are filled and for helping to develop the information system according to whatever tasks the national interest demands. "Hitherto the Government's attitude in Britain has been negative rather than constructive", according to an editorial in *Nature* of May 23, 1964. The editorial goes on to say that "some central authority competent to take an overall national view and identify weaknesses as well as having the resources and influence to rectify such weaknesses is obviously imperative". This is

precisely what Aslib said to the Minister for Science concerning the Trend Committee Report which, in this respect, was a rather disappointing document. Certainly Trend reckoned that the Minister for Science should assume general responsibility in regard to methods of disseminating research information, but that is only one aspect of the matter. While the Committee proposes that the Industrial Research and Development Association should have a division responsible for co-ordinating information activities within its own sphere, there is no reference to the need to encourage information studies within either the Science Research Council or the Natural Resources Research Council. Nor must we overlook the enormous interest of both private and public industry in obtaining this information.

Now that responsibility for science and education is concentrated in one Ministry and under one Minister, and this includes universities and public libraries, it is logical for this Ministry to accept responsibility for establishing an authoritative body with undivided responsibility for development in the information field. So far, with our limited resources, we have not done too badly, but we are beginning to lag behind. The United States has established the Office of Science Information Service of the National Science Foundation in Washington, and in Moscow there is the All-Union Institute of Scientific and Technical Information. In Britain I hope we shall develop a system that is both more subtle and more far-reaching and that it will cover all the 'cultures'.

Aslib has always preached the oneness of information and has rightly seen it as an incipient discipline with philosophies, techniques and aspirations of its own. While I believe that a centralized approach is essential to the solution of these problems, any national body that is set up must recognize that there is immense diversity in this field. There will need to be links with libraries of all kinds, public and private. In particular, the needs of users both corporate and individual must be known and this means more research. The professional in this field will need not only training but also education on a broad inter-disciplinary basis—a requirement indeed for any good librarian. While he may often be required to be a technologist too, he must be ready to draw on the older wisdoms. To achieve all this, the authoritative body to be set up must have adequate resources and must be able to initiate or itself carry out research.

Granted that the principle is accepted—and there are signs that these needs are becoming recognized—it is important to establish the right organization. We must approach this great issue of communication and information in a questioning mind. We do not want just a short bout of crystal gazing by a few wise men, which has so often been the basis of government decision. But of one thing I am certain, that if—and this is the central theme of my article—this service is to be the unifying force that I hope it will be in regard to knowledge and research of all kinds, it cannot be tied to science and technology alone. It must be made crystal clear that this body would not seek to exercise control over the subject of study though it will, I hope, influence the individuals concerned, especially some of the professors, to accept a greater degree of responsibility for 'exagellectics' or information used in their investigations, for no one is better equipped as a rule than the professor to do this and to guide and assist the professional librarian.

My own view is that something is needed of the nature of an independent Research Council. The Government cannot directly run everything, nor would we want it to. The example of the Nature Conservancy which with limited resources has achieved an authoritative position is before us. It has spread much of its thinking and influence far beyond its main conservation role, especially in establishing ecological concepts. A Research Council would fit in well with the post-Trend situation. It could have flexibility of staffing and occupation and some measure of freedom and independence in its day-to-day activities, while at the same time retaining essential links with the Government. There may be other ways of tackling the problem but I hope that we shall no longer dodge the issue. I will not attempt to describe how the organization will work. Certainly much consideration will have to be given to abstracting services and to documentation on an international basis. Some measure of co-ordination is necessary in relation to existing national scientific libraries such as the National Lending Library for Science and Technology at Boston Spa and the new National Reference Library of Science and Invention. The present relationship is a reflexion of the piecemeal 'ad hoccery' of Government decision. The membership, I would beg, should not be wholly scientific and technological. Let us by all means have people who cross the cultural frontiers whether it be the mathematician, the social scientist, the philosopher or even simply the historian. Among the scientists, I would have particular reasons for advocating the membership of zoologists. The Research Council will of course need a pluralistic approach, and will certainly need to maintain links with the users, for the body will exist for them.

Where would Aslib fit in? This is difficult to say, but it is certainly too early to consider assimilating Aslib completely into the organization. Aslib will need the final strength to carry on and to continue to approach the gap in its traditional semi-voluntary way. Aslib has successfully brought together information interests in all fields—government, private and public industry, university colleges, learned societies, publishers and the rest. Nor must we be afraid of some overlap, though this need not mean that there is excessive duplication of effort—merely variety in approach and particularly in new ideas. It would be quite wrong in my view to contemplate, certainly at this stage, a monolithic structure.

We are dealing here with one of those elusive subjects, the full extent of whose import is apt to be missed. In any project or activity, communication, liaison, information—all these are things which people tend to deal with as an afterthought. This can no longer continue. The time has come when we must ensure that Aslib's accumulated knowledge and experience together with that gained through other bodies with an interest, even a peripheral one, is used for the benefit of all. Sooner or later we shall need something of the standing of an independent research council and it may be that what is needed in the long run is what is needed in the short run. Its functions should be to define and identify needs and problems, to advise, promote and to co-ordinate, to carry out research and to administer directly where this is appropriate national institutions such as the National Lending Library for Science and Technology and perhaps even the National Reference Library of Science and Invention.

Perhaps some will think that what I am proposing is premature and that other solutions can be found. Perhaps in the next thousand years, perhaps in the next million, we shall all become cybernetic men with instant recall and telepathic aids to communication. Yet now with our limited, inadequately used brains we must seek to organize the full utilization of the product of those brains. This is a field in which, in every way, it would be fatal to be guilty of under-investment, yet we squander nothing so prodigally as knowledge. Money, metals, farm produce—waste these and the balance sheet, the scrap yard, and the muck heap stand as monuments to folly. But treat knowledge as a luxury, a frill to gracious living, the product of an intellectual exercise to be tossed back and forth between experts and finally allowed to drop into the tranquil pool of things that do not count because they cannot be measured—and we can live for years with never an eyebrow raised. Or can we? Well, perhaps we could. But we shall not do so again.

COMPARISON OF RADIO AND OPTICAL POSITIONS OF SOME IDENTIFIED RADIO SOURCES

By R. L. ADGIE
Royal Radar Establishment, Great Malvern

THE identification of radio sources is one of the most vital problems in radio astronomy and demands the utmost precision in the determination of positions. Recent observations of lunar occultation of radio sources have made it possible to determine certain angular positions to an accuracy of about 1 sec of arc. This is potentially the most precise method of measuring position, but is unfortunately limited by the path of the Moon to a band of declination close to the ecliptic, and it takes several years to cover this band completely. For the majority of radio sources, the positions have to be measured with an aerial system of high resolving power, such as a two-aerial interferometer.

It is very difficult to make an absolute measurement of position with these aerial systems, because of the uncertainty about the precise location of the radio phase-centres of the aerials and the exact phase changes through the electronic equipment and cables. But provided these parameters are stable, the systems are capable of measuring the relative positions between sources very accurately, and can be calibrated by observing sources the positions of which are already known. Obviously the sources observed by lunar occultation provide ideal calibrators over a limited range of declination close to the ecliptic, but others are needed throughout the whole declination range under observation, and these must be taken from the optically identified radio sources. The optical positions of these sources are known very precisely, and they can be used as calibrators provided the radio and optical positions are the same. In general this is likely to be a valid assumption, especially as agreement in position is the initial basis of an identification, but there could be exceptions. This problem arose recently during the calibration of the interferometer at the Royal Radar Establishment (R.R.E.); fortunately there are many more optically identified sources available than are strictly necessary for the calibration, so a comparison can be made between the different calibrators to check the agreement between the radio and optical positions.

Observation of radio sources. The R.R.E. interferometer[1] consists of two steerable paraboloids, 25 metres in diameter, mounted on movable carriages, which can be separated up to 750 metres in any direction. The present operating frequency is 610 Mc/s, giving a lobe-width of 140 sec of arc at maximum spacing. Two separate sets of observations had to be made to determine positions in two co-ordinates. A baseline of about 750 m was oriented approximately east–west to measure the right ascension and near north–south to measure the declination.

Variations of the phase changes through the amplifiers and cables have been minimized by careful design and temperature control, and the shift in the interferometer pattern corresponds to only 1 per cent of a lobe-width over a few days. The errors due to random noise on the records have been reduced to a negligible value by averaging over several periods of the interferometer pattern. However, the accuracy of the determination of the positions of weak sources is influenced by the confusion of the numerous other sources within the aerial beam. The root mean square value of the confusion is 0·5 flux units* with this equipment, which corresponds to a directional error of 1·6 per cent of a lobe-width when observing a source of 5 flux units. All the identified sources observed have flux densities greater than 5 flux units, so the accuracy of the relative positions should vary from ± 4 sec of arc root mean square, to better than ± 2 sec of arc for the most intense sources.

Only sources having a small angular diameter can be measured with this large aerial spacing. The selection has been limited to those the apparent amplitude of which does not fall below half the amplitude at close spacing, which imposes a limit of 60 sec of arc diameter for a single source and 40 sec of arc separation for a double source. In any event, there is less significance in determining the effective centres of large diameter sources to an accuracy of a few sec of arc; measurements of the positions of such sources can be made with sufficient accuracy with a smaller aerial spacing.

The measurements have been corrected where necessary for ionospheric refraction, using values of total electron content from the critical frequency data. These corrections are less than 3 sec of arc, and can be estimated so as not to contribute a significant error.

Calibration procedure. The calibration of the system was determined by the examination of 18 sources fulfilling the required conditions of small diameter, flux density greater than 5 units, and with information available on the positions of optical objects that may be identified with the radio sources. For three of the sources, radio positions are known from lunar occultations[2,3]. The remainder are included in Griffin's list[4], giving positions of optical objects in the vicinity of radio sources with an accuracy of better than 0·5 sec of arc.

An approximation of the true callibration was first obtained by direct geometrical measurement of the co-ordinates of the baseline. The measurements were then put into the appropriate equations to give a first approximation to the radio positions, which can then be compared with the optical or occultation positions. In general the positions will differ, because of errors in the approximate calibration, and the differences will be a function of declination.

These differences, for both right ascension and declination, are plotted in Fig. 1. For convenience, the differences in right ascension have been converted to true angular displacements in secs of arc by multiplying seconds of time by 15 cos δ, where δ is the declination. The true calibrations are represented by curves through these points satisfying the correct form of the equations of calibration. These equations contain three unknown constants, so the curves can be made to fit points at each end and at the centre of the declination range. The curves drawn in Fig. 1 are considered to be the best fit to the observational data, and their departure from straight lines parallel to the declination axis indicates the extent to which the approximate calibrations were in error. One of the unknown constants in the declination equation is the wave-length, and since this cannot be measured sufficiently accurately it has been adjusted to give the best fit in the calibration.

* One flux unit is taken as 10^{-26} Wm^{-2} $(c/s)^{-1}$.

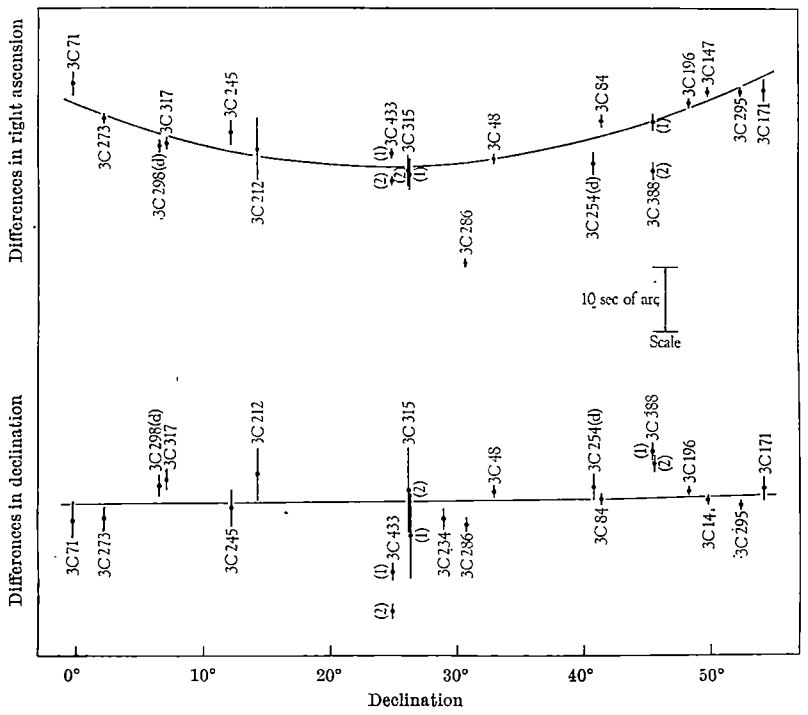


Fig. 1

It is clear that the calibration curves cannot be drawn through all the points and that certain discrepancies exist between the radio and optical positions. The two groups of sources at each end of the declination range appear to be self-consistent, and it is gratifying to find that the three radio occultation positions (3*C* 273, 3*C* 212 and 3*C* 245) fit the calibration curves. But at the centre of the declination range, where the agreement is rather less close, the determination of the calibration curves relies rather heavily on the true position of 3*C* 48. Despite this, it is unlikely that the calibration curves are in error by more than 1 sec of arc throughout the range.

Once the calibration curves have been determined then the true radio positions can be calculated. Table 1 gives a list of the differences between these radio positions and the corresponding optical or occultation positions, both in the right ascension direction, Δ(R.A.), and declination, Δ(Dec.). To appreciate the significance of these differences they should be compared with the tabulated estimates of the root mean square errors derived from the equipment stability, confusion and the fitting of the calibration curves. Errors for the occultation sources also include the uncertainties in the radio occultation positions. Significant differences, greater than 4 times the root mean square error, exist for four sources (3*C* 234, 3*C* 286, 3*C* 388 and 3*C* 433), but for the remaining sources the differences are within the experimental limits of error. As discussed later, the source 3*C* 298 would show a large discrepancy on the basis of a previous identification, but good agreement is now obtained on substituting a revised identification.

To obtain a good agreement for the majority of the sources indicates that the prime object of calibrating the interferometer has been achieved. It also means that in general the radio and optical positions agree to within about 2 sec of arc. The radio positions represent the 'radio centre of gravity' of the sources, many of which have angular diameters greater than 2 sec of arc. The angular diameters of the radio sources are given in Table 1, information being obtained from references 5–7 and from the present observations.

Table 1. Position Differences: Radio Minus Optical

3*C* No.	Right ascension Δ(R.A.)[a]	R.m.s. error	Diam.	Declination Δ(Dec.)	R.m.s. error	Diam	Optical object
48	0·0″	±1·1″	< 1″	+1·3″	±1·4″	<20″	Q.S.R.S.
71	+2·9	±1·8	10	−2·4	±3·1	<30	Nucleus of *NGC* 1068
84	+2·5	±1·2	~10	0·0	±1·1	<20	Nucleus of *NGC* 1275
147	+1·3	±0·8	1·5	−0·4	±0·9	<20	Q.S.R.S.
171	−2·3	±1·8	15	+1·0	±1·9	<30	Faint single galaxy
196	+0·7	±0·8	6	+1·0	±0·9	<20	Q.S.R.S.
212	+1·0[b]	+4·9	*c*	+4·7[b]	±4·6	*c*	Q.S.R.S.
234	−36·5	±2·9	?	−2·6	±2·0	45	Single galaxy
245	+2·8[b]	±2·1	*c*	−0·7[b]	±3·0	*c*	Q.S.R.S.
254	−3·8	±2·0	~6	+1·8	±2·2	<20	Assumed to be Q.S.R.S.[d]
273	−0·4[b]	±0·7	*c*	−2·3[b]	±2·0	*c*	Q.S.R.S.
286	−15·6	±1·2	<1·5	−3·6	±1·5	<20	Q.S.R.S.
295	−0·8	±0·8	3	−1·5	±0·9	3	Faint single galaxy
298	−2·0	±1·1	2	+2·9	±1·8	<20	Assumed to be Q.S.R.S.[e]
315	−0·9	±2·6	55	−1·5	±6·6	95	Double galaxy[f]
317	−1·2	±1·1	20	+3·7	±1·8	30	Single galaxy
388	−5·7	±1·6	30	+6·3	±1·5	25	Double galaxy[f]
433	−0·1	±1·1	15	−13·8	±1·5	30	Double galaxy[f]

Notes: (*a*) to convert to seconds of time, divide by 15 cos δ. (*b*) These differences are relative to the radio occultation positions and not to the optical. (*c*) The occultation measurements give a detailed picture of the structure of these sources. (*d*) Optical object '*d*' (3*C* 254) in Griffin's list[4]. (*e*) Optical object '*d*' (3*C* 298) in Griffin's list[4]. (*f*) The optical position has been taken as the mean of the two galaxies.

The results will now be discussed in greater detail, grouping the sources according to type.

3*C* 71 *and* 3*C* 84. These have been identified with the two Seyfert galaxies *NGC* 1068 and *NGC* 1275. Seyfert galaxies have very small bright nuclei, and in these two cases the agreement between radio and optical positions suggests that the nuclei are responsible for the radio emission. Although the radio diameters are larger than the optical nuclei they are less than the overall size of the optical galaxies. (3*C* 84 has also a radio halo of 26 min of arc diameter.)

3*C* 212, 3*C* 245 *and* 3*C* 273. The radio occultation and optical positions[2,3] are in good agreement, and these three sources have been identified as 'quasi-stellar' radio sources (Q.S.R.S.s). For the interferometer calibration the radio occultation positions have been used, after making allowance for the change with frequency in the relative amplitude of the two components in the case of 3*C* 273. The interferometer and occultation positions agree within the experimental limits.

3*C* 48, 3*C* 147, 3*C* 196 *and* 3*C* 286. These are the remaining sources in the list which have also been identified as Q.S.R.S.s, and the present observations show a good agreement between radio and optical positions, except for 3*C* 286. The discrepancy in right ascension in this case is 15·6 sec of arc (or 1·21 sec of time), which is well beyond the experimental limits. Earlier observations at 400 m spacing had shown a similar discrepancy[8].

The measurement of radio diameter of 3*C* 286 at 159 Mc/s (ref. 5) indicates a single source of less than 1·5 sec of arc diameter in the right ascension co-ordinate. So the puzzling picture is of a small diameter radio source, displaced from a quasi-stellar optical object. Before the previously claimed identification is dismissed as false it is interesting to examine 3*C* 273 again. This has two radio components separated by 20 sec of arc which are coincident with a very bright small object and the end of a faint jet. At 610 Mc/s the radio component associated with the jet is the strongest, and in fact the effective centre of gravity at this frequency is 12 sec of arc from the bright optical object. If the jet were appreciably fainter it would not be seen and the situation would then be similar to the case of 3*C* 286. Some clue to the problem of 3*C* 286 might be given by

accurate position measurements at other frequencies to find if the effective centre is frequency dependent as it is for 3*C* 273.

In general the Q.S.R.S.s have seemed likely to be the ideal optical position calibrators for radio astronomy; they have small radio angular diameters and the identifications can be established from their unusual optical spectra. But the evidence of a substantial discrepancy, for 2 sources out of 7, between the radio position and the position of the brightest optical component shows they must be treated with caution.

3C 254 and 3C 298. 3*C* 298 had previously been identified as a galaxy, but the optical position differs by 43 sec of arc in declination from the radio position given by these observations, and it seems very unlikely that this is the correct identification. No positive identification has been stated for 3*C* 254. Both these radio sources agree in position with stars listed by Griffin, the chance of finding an optical object of comparable brightness within the experimental limits being less than 1 in a 1,000. In addition, they have small radio diameters, which tend to support their possible identification as Q.S.R.S.s.

3C 171, 3C 234, 3C 295 and 3C 317. These are all single optical galaxies. 3*C* 171 and 3*C* 295 agree closely in optical and radio positions. 3*C* 295 is a double radio source with the components separated by only 4 sec of arc[9], probably symmetrically placed about the optical galaxy. Although 3*C* 234 agrees in declination it disagrees too widely in right ascension for inclusion in Fig. 1; but this is probably because the radio source is double, thus introducing a phase change and consequently a misleading position at this wide interferometer spacing. The agreement in declination for 3*C* 317 is not good but the difference is only about 25 per cent of the radio angular radius so is well within the limits of the radio source.

3C 315, 3C 388 and 3C 433. This group consists of double optical galaxies which are too close together to be resolved by the radio measurements. The separate optical positions have been plotted in Fig. 1, and it is clear that the identifications cannot be attributed to only one of the optical components. In view of this uncertainty the mean optical positions have been adopted in compiling Table 1. The agreement is not good, but the differences are still within the limits of the radio diameters. The diameter of 3*C* 315 in the declination direction is really too great to justify its inclusion in Table 1, although in this event the agreement is satisfactory. The large reduction in apparent amplitude at this wide spacing accounts for the abnormal error.

We conclude that the interferometer system at the Royal Radar Establishment has achieved a directional accuracy of about 2 sec of arc at 610 Mc/s in the measurements of the positions of radio sources in both angular co-ordinates. Calibration of the system by optically identified sources has proved a satisfactory procedure. For the majority of the chosen calibrator sources, the differences between optical and radio positions are within the estimated experimental errors. For three radio sources, 3*C* 234, 3*C* 388 and 3*C* 433, larger differences are explicable in terms of the angular size and complexity of the sources. The previous identification of the source 3*C* 286 with a quasi-stellar radio source may need modification, as a significant difference in positions has been revealed. It is also shown that the previous identification of 3*C* 298 with a galaxy has been erroneous. New identifications are suggested for this source and for 3*C* 254, and both of these appear to be quasi-stellar radio sources.

The calibration measurements described above now enable accurate positions of other sources to be determined. The analysis of observations of about 100 small diameter sources is nearly complete and will be published later.

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POLYMER RESEARCH FOR INDUSTRY

By WENDY HEAP

Rubber and Plastics Research Association of Great Britain, Shawbury, Shrewsbury

ON September 29, Mr. P. C. Allen, president of the British Plastics Federation, opened the new laboratories and library of the Rubber and Plastics Research Association (RAPRA) at Shawbury, Shropshire. This important event resulted from the formation in 1960 of a joint Research Association for the Rubber and Plastics Industries developed from that of the British Rubber Manufacturers (RABRM), which had been formed as far back as 1919 and which moved to its present premises at Shawbury in 1954.

Research Association for the Plastics Industry

During the 1950's the rapidly developing plastics industry began to feel the need for a research association and the British Plastics Federation investigated the possibility. With the increasing use of flexible thermoplastics by the rubber industry, such as polyethylene and PVC, together with the fact that rubbers and plastics are both polymeric materials and so the approach to their basic physical and chemical properties is similar, it became apparent that the needs of the plastics industry could be most economically served by a joint research association with the rubber industry.

The existing establishment (RABRM) had much of the equipment and knowledge necessary for dealing with polymers and its interest in plastics had already been shown, for example, by the investigation in the mid-1950's of the frictional properties of PVC. To cope with the challenge brought by the plastics industry new buildings and more staff were acquired. The new laboratories house the main physics and chemistry departments and the process laboratory, while a new library wing has been built to accommodate much of the information department.

The increased scope of the work of the Association is reflected in the activities of all the departments. New scientific and technological research projects on plastics are arising by the side of those on rubbers, new testing methods are being examined, new problems are being overcome, and the scientific basis of the plastics industry is being strengthened.

Information Centre

Although more than 50 per cent of the Association's annual income is spent on research, and the most exciting developments appear to occur in this field, the horizons

of the information department have also widened. The work of the Association in acting as an information centre for the industry which will help research workers and technologists to keep up to date with the literature in their fields is extremely valuable. To this end, RAPRA surveys the technical literature and publishes abstracts of all that is relevant. An efficient classification system makes all the information speedily available to deal with members' enquiries.

In a very far-seeing move information has been collected on flexible thermoplastics ever since it was published, thus there is already a considerable body of information on plastics in the library. As from 1965 the scope of the work of the information division will be extended to include rigid plastics.

An arrangement with the British Plastics Federation has resulted in its information service discontinuing and in the transfer of its library facilities to RAPRA, where information will consequently be available to cover the whole field of rubbers and plastics. New developments resulting from this arrangement include the publication of two series of abstracts, one covering rubbers and the other plastics, and the adoption of a new classification system. This was necessary since the old system, while allowing the inclusion of thermoplastics, would not adequately cover the addition of thermosets. This unification of information on rubber and plastics makes an important contribution to the establishment of RAPRA as a central co-ordinating body for both industries.

Scientific Research

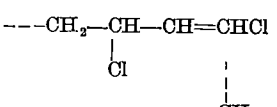
The fundamental scientific problems which are chosen to be investigated are those which can be expected to produce results which may eventually be of practical advantage to industry. This is necessitated by the very nature of the Association, and may be illustrated by some examples of present research projects.

Thermal degradation of PVC. Temperatures of up to 200° C are attained during the processing of PVC, which would result in degradation of the material if no stabilizer was added. Since little is known about the mechanism of degradation, the method of choosing stabilizers is mainly empirical. In an attempt to rationalize their use, and to develop cheaper and more efficient stabilizers, RAPRA is investigating why PVC does not possess the thermal properties expected from its ideal structure.

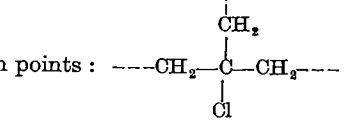
$$\text{------}(CH_2 \cdot CHCl)_n\text{------}$$

The effects of degradation, which are cross-linking and coloration of the material with evolution of hydrogen chloride, suggest that the reaction may be initiated by a chlorine atom. This, by attacking the chain, starts a 'zipper' reaction in which hydrogen chloride is eliminated. The resulting double bond system absorbs in the visible region of the spectrum producing the coloration.

The importance of the chlorine atom in this process makes its origin an obvious point of interest. In an attempt to find out where it originates consideration is being given to two possible points of weakness in the PVC chain—the chain ends :



and the branch points :



The possibility that it is produced as a result of free radical impurities from the catalysts used in polymerization is not excluded.

The problem is being approached kinetically with measurements of viscosity, molecular weights, evolution of hydrogen chloride, and ultra-violet and visible absorption spectrophotometry. It is also hoped that model compounds of low molecular weight will be made which will contain a high proportion of possible weak points. From an examination of the degradation rates of these the required information about the chlorine atom should be obtained, although there will be difficulty in relating the results to the complex commercial materials available.

Glass-reinforced plastics. An increasing interest is being shown in glass-reinforced plastics for constructional work. Their major weakness, a loss of up to half their strength after prolonged contact with water, is unimportant in many cases since more material than is strictly necessary for the required strength must be used to obtain rigidity. If maximum strength for weight is required, as for example in the aircraft industry, this factor becomes a grave disadvantage.

Under contract to the Ministry of Aviation, RAPRA is investigating the nature and strength of the glass/resin interface, the failure of which contributes to the loss in strength (Fig. 1).

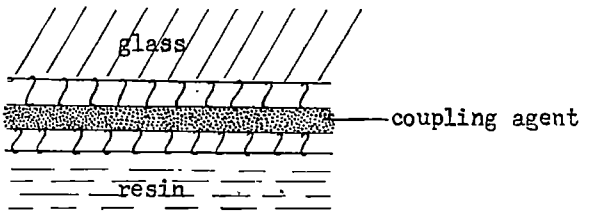
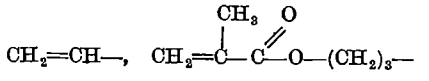


Fig. 1. Diagram of glass/resin interface

The glass fibre/polyester resin interface with and without coupling agents present has been studied by photomicrographs before and after boiling the specimen in water. The pattern of failure indicates that the presence of a coupling agent decelerates the water attack at the interface and that it is the coupling agent/resin bond, and not the coupling agent/glass bond, along which failure gradually occurs.

Since the presence of a coupling agent retards water attack its coverage of the glass is obviously important. This will be investigated by contact angle measurements on a glass filament in a drop of water. The presence of the coupling agent will be clearly indicated by this method since the difference between the contact angle for water and clear glass, water and covered filament is about 80°.

The silanes, R^1 Si $(OR)_3$, are usually used as coupling agents, the R^1 group to react with the resin, for example:



and the OR group, where R can be CH_3, C_2H_5, C_2H_4. OCH_3, to react with the glass. Various coupling agents must be investigated for good covering and bonding properties.

Electron microscopy. The acquisition of an electron microscope in the near future by the physics department will assist in their approach to the problems of the glass reinforced plastics. It is also an important asset to the rubber and plastics industries since it will give RAPRA increased facilities for research which few of the members possess for themselves.

Rheology of elasto-viscous fluids. Another instrument which RAPRA has recently obtained is the Weissenberg rheogoniometer which will be used to study the flow properties of polymer melts. The sample is sheared between a cone and a horizontal plate and two forces are measured, the resistance to rotation and the vertical force exerted on the plate, the latter effect being peculiar to elasto-viscous fluids.

By interpreting these results it is hoped to elucidate the present theories on the flow properties of elasto-viscous fluids. Two basic approaches are involved, that on the molecular level which although useful qualitatively is almost impossible to apply quantitatively, and that which treats the fluid as a continuous medium.

Three polymers which represent different systems are being studied: polystyrene, high-density polyethylene, and PVC. Since much of the processing of plastics, such as extruding and moulding, depends on their flow properties, this investigation should contribute directly to improving productivity.

Technological Research

The emphasis in the technology division is on practical assistance to the member firms, by helping them to increase the quality of their products and thus their productivity. Many industrial techniques are based only on practical experience and many of the problems arising are common to several firms. Frequently the technical services of the individual concerns have neither time nor resources to investigate the fundamental problems involved in processing, whereas the Association by tackling these can save duplication of effort by the individual firms and assist industry as a whole. Several projects which are being undertaken at the moment specifically for the plastics industry are: the moulding of polypropylene, an assessment of acrylonitrile/butadiene/styrene terpolymer, and an investigation into the effects of catalysts on the curing of polyester resins.

The Future

The Association's work on plastics is only just beginning, the research projects are in their early stages, and the information division is not yet besieged with enquiries on rigid plastics. Far from neglecting the needs of the rubber industry RAPRA has been stimulated by the new challenge to increase its endeavours in all aspects of its work. The scope of the Association's work has been extended, not to the detriment of the rubber industry but to the advantage of both the rubber and the plastics industries of the future.

SEX CHROMOSOMES AND SEX CHROMATIN*

By Dr. URSULA MITTWOCH
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BEFORE defining the terms 'sex chromosomes' and 'sex chromatin', it is appropriate to quote the following remarks by the American embryologist, Frank Lillie[1]: "There is no such biological entity as sex. . . . What exists in Nature is a dimorphism within species into male and female individuals, which differ with respect to contrasting characters, for each of which in any given species we recognize a male and a female whether these characters be classed as of the biological, or psychological, or social order. Sex is not a force that produces these contrasts; it is merely a name for our total impression of the differences".

Although no attempt will be made to discontinue the use of the word 'sex', its meaning in the present context should be clearly stated: namely, the differences exhibited by male and female organisms within the same species.

There can be no doubt that the causes underlying the differences between the sexes is of fundamental importance in biology, for in many animals, as well as some plants, such differences are much more striking and far-reaching than are normally found in closely-related organisms.

Theoretically, the cause which brings about the divergence of the sexes might be either inherent in the organism or moulded on it by its environment, and until about sixty years ago there was no means of deciding which of the two alternatives was right. At the beginning of the present century the sex chromosomes were discovered, and by now their existence is common knowledge. It is known that the sex of an embryo is determined at the moment of fertilization, by the sex chromosomes which are contributed by the sperm of the father and the ovum of the mother. What is the evidence underlying this now commonplace statement?

During the last quarter of the nineteenth century it was found that when a cell is about to undergo division its nucleus resolves itself into a definite number of threads, which have a special affinity for certain dyes, and for this reason were given the name 'chromosomes', or 'coloured bodies'[2]. Our knowledge of sex chromosomes is based very largely on discoveries made on insects. In 1891, Henking[3] studied the chromosomes during the development of spermatozoa in a bug, *Pyrrhocoris apterus*, and found one of the chromosomes behaved differently from the rest. This chromosome was more darkly stained and moved at a different rate from the others. At the end of cell division, this chromosome passed undivided into one of the daughter nuclei, with the result that only half the spermatozoa finally contained this chromosome. Henking arrived at no very clear idea about the nature of this chromosome and in his drawings he merely labelled it with an *X*. The idea that this chromosome might be concerned with the determination of sex was first formulated by McClung in 1902 (ref. 4); McClung suggested that the two types of spermatozoa, which were produced in equal numbers, would give rise to two equal classes of individuals of different sex. The truth of this hypothesis was proved within the next few years, particularly by the cytologist, E. B. Wilson[5]. Wilson showed that, in a variety of insects, the males produced two types of spermatozoa, which differed in their chromosome constitution, that is to say, half the sperm contained an *X*-chromosome, while the other half lacked it; but all the eggs of the females were alike in this respect, since they all contained an *X*-chromosome. Wilson further showed that different insects could be divided into two classes, namely those in which the *X*-chromosome was present on its own, and a second group, in which the *X*-chromosome was partnered by another, but different-looking, chromosome, which was called the *Y*-chromosome. This second group of insects, therefore, produces two types of spermatozoa, those containing an *X*-chromosome and those containing a *Y*-chromosome; on fertilization these give rise to female and male offspring, respectively. Since the *X*- and the *Y*-chromosome differ in males and females they are called 'sex chromosomes'[6]; all the other chromosomes are the same in both sexes and are called 'autosomes'[7].

Evidence for the existence of an *X*- and a *Y*-chromosome in human males was put forward by Painter in 1921 (ref. 8), but final agreement on this matter could not be reached until thirty-five years later[9,10] when, as a result of improved techniques in tissue culture and cytology, the establishment of the chromosome constitution in man became a commonplace investigation.

The unequivocal demonstration of the sex chromosomes in man is a remarkable discovery made in recent years; but there have been others equally remarkable. The

* Substance of a lunch-hour lecture delivered in University College, London, on November 10.

chromosomes can be seen only in cells which are dividing; but in 1949 Barr and Bertram[11] announced that they had found a sex difference in the nuclei of the neurones of the cat, and soon afterwards it was shown that such a sex difference could be demonstrated in many types of cells and in many species, including man[12–14]. It consists of a small, very intensely staining body, which is most commonly situated at the periphery of the nucleus, just inside the nuclear membrane. Such a body is present only in females and is absent in cells from males and has been called the sex chromatin body. There is now conclusive evidence, which will be elaborated later, that the sex chromatin consists of one of the two *X*-chromosomes in a heavily condensed form[15]. Whereas the chromosomes can be seen only in dividing cells, the sex chromatin can be seen only in non-dividing or so-called 'interphase' nuclei. The simplest way of demonstrating sex chromatin is by scraping off some cells from the inside of the cheek, where the sex chromatin will be seen in cells from females but not from males.

Yet another sex difference was described by Davidson and Smith[16] in polymorphonuclear leucocytes of the blood. Some of these nuclei in cells from females bear a special appendage, which is known as a drumstick. A drumstick consists of a darkly staining head which is attached to the nucleus by a stalk. Drumsticks do not occur in blood cells from males, and it is now known that they also represent one of the two *X*-chromosomes of a female[17,18]. Drumsticks are, therefore, equivalent to sex chromatin, and their distinctive shape is merely a reflexion of the peculiar shape of the nuclei of the polymorphonuclear leucocytes.

Our knowledge of sex chromosomes is thus based on three separate events: (1) the discovery of the sex chromosomes at the beginning of the century; (2) the discovery of sex chromatin which began in the late 1940's; (3) the unequivocal demonstration of the sex chromosomes in man, which happened in the second half of the 1950's. It may be of interest to note that the discovery of the sex chromosomes in insects was directly related to the perfection of the microscope, that is to say with the invention of apochromatic and oil immersion lenses, during the 1880's (ref. 19). By contrast, the demonstration of the sex chromosomes in man had no connexion with any advances in scientific technology; rather, it was made possible by certain improvements in scientific cookery, as a result of which the chromosomes, instead of being glued together in an indigestible lump, appeared nicely separated, like the grains of rice which has been properly cooked. The discovery of sex chromatin did not even require any advance in cookery. In their first publication on this subject, Barr and Bertram[11] wrote: "It appears not to be generally known that the sex of a somatic cell as highly differentiated as a neurone may be detected with no more elaborate equipment than a compound microscope following staining of the tissue with the routine Nissl method". When the discovery of drumsticks was announced in 1954 (ref. 12), people could scarcely believe that such an obvious structure could have been overlooked for so long, in view of the large numbers of blood films which are examined daily both in medical practice and in research.

These discoveries have already had a very profound effect in clinical medicine as well as on our knowledge of the theoretical aspects of chromosomes. To begin with the more practical aspect, it is now well known that in a small proportion of cases the process of sex determination is faulty, so that persons with an abnormal sex chromosome constitution result. Thus a person with two *X*- and one *Y*-chromosome suffers from Klinefelter's syndrome[20]. Such patients are phenotypically male, but the testes remain small and spermatogenesis does not occur. By contrast, a patient having only a single *X*- and no *Y*-chromosome (Turner's syndrome)[21] is phenotypically female, having rudimentary ovaries in which oogenesis does not occur. Patients with three *X*-chromosomes[22] are also females; they may have various abnormalities but some are essentially normal and fertile.

The mere existence of patients with abnormal sex chromosomes has given us some very important information about the nature of the sex chromosomes. If we had nothing but normal males and females, it would be impossible to know whether male development is due to the presence of only a single *X*-chromosome or the presence of a *Y*-chromosome; and, similarly, whether female development is brought about by the presence of two *X*-chromosomes or the absence of a *Y*-chromosome. It is now clear that a single *X*-chromosome in the absence of a *Y*-chromosome gives rise to a female, while two *X*-chromosomes in conjunction with a *Y*-chromosome cause development to be male. This proves that in man the *Y*-chromosome is essential in ensuring male development. Nevertheless, if a normal male is to be formed, the *Y*-chromosome must be associated with a single *X*-chromosone; similarly to ensure normal female development two *X*-chromosomes are required.

Furthermore, these patients have clarified our ideas about the nature of sex chromatin. All patients with abnormal *X*-chromosomes have a characteristic sex chromatin pattern, and, indeed, the sex chromatin test is of considerable value in diagnosing such patients. Patients with Turner's syndrome have no sex chromatin, although they are female; patients with Klinefelter's syndrome have sex chromatin, although they are male. Persons with three *X*-chromosomes have two sex chromatin bodies. More rarely, patients are found with four *X*-chromosomes and even five *X*-chromosomes[23,24] and they have three and four sex chromatin bodies. It is clear that basically sex chromatin has no connexion with sex, or male or female development, but merely reflects the number of *X*-chromosomes present in the nucleus. If only one *X*-chromosome is present, no sex chromatin body is formed, but any *X*-chromosomes in addition to the first may form a sex chromatin body.

The relationship of sex chromatin to the *X*-chromosomes has been strikingly confirmed as a result of labelling chromosomes with radioactive markers. Although the chromosomes can be seen only during cell division, they synthesize their DNA in the interphase prior to cell division, when the chromosomes are dispersed and invisible. One of the necessary components of DNA synthesis is thymidine and, by offering the cells radioactive thymidine, the time at which this substance is taken up can be accurately determined. In this way it has been found that in normal females one of the *X*-chromosomes synthesizes its DNA later than the other chromosomes[25]. Such a late replicating *X*-chromosome is not found in normal males; it is, however, present in males with Klinefelter's syndrome who have two *X*-chromosomes. Moreover, in patients with multiple *X*-chromosomes, the number of late-replicating *X*-chromosomes is equal to the number of *X*-chromosomes minus one[26]—just like the number of sex chromatin bodies.

There is evidence, too, that the *Y*-chromosome replicates late[27], but the *Y*-chromosome is too small to be seen as a separate body in interphase nuclei. By contrast, the single *X*-chromosome of males and one of the *X*-chromosomes of females replicate at the same time as the autosomes—indeed, it might be justified to call them autosomes, for they behave like autosomes and they are the same in males and females.

Now that the origin of sex chromatin has been explained, its existence prompts the following rather naïve question: Why are sex chromosomes different from all other chromosomes? All other chromosomes replicate their DNA more or less in unison, but the sex chromosomes replicate late. All other chromosomes become dispersed and invisible during interphase; but the sex chromosomes remain compact and in a discrete body. Any answers to these questions must at present be speculative—even though the problem is by no means new.

Chromosomes and parts of chromosomes which thus behave differently from the majority are said to be composed of 'heterochromatin'[28], in contrast to all the rest of the chromosomes which are made up of 'euchromatin'[28]. The subject of heterochromatin has had a somewhat unhappy childhood, but there is every prospect that its future will be brighter. Before this subject is discussed further, brief mention should be made of sex-linked genes.

For practical purposes, sex-linked genes are those borne on the *X*-chromosome. In man, the two best-known sex-linked genes are those for colour blindness and hæmophilia; they are characterized by a peculiar mode of inheritance, which is governed by the mode of transmission of the *X*-chromosome. On the human *X*-chromosome there are many other genes, apart from colour blindness and hæmophilia, which are inherited in essentially the same way. In fact, more than 50 genes have been described the location of which on the *X*-chromosome is known or postulated with varying degrees of probability[29]. They include genes for an enzyme deficiency causing hæmolytic crises, for a type of muscular dystrophy, for eye diseases as well as a normal blood-group variant. None of the genes appears to affect sex determination although some sex-linked genes may interfere with normal sex development. The absence of a gene affecting sex determination is even more significant in *Drosophila*. Here, more than one hundred genes are known to be located on the *X*-chromosomes, and their actual place on the chromosome is known with great accuracy[30]. It is also known that the *X*-chromosome in *Drosophila* determines female development[31]. An intensive search has been made to try to identify such female-determining genes in *Drosophila*[32]. As Goldschmidt[33] wrote: "The first unequivocal result is that no single female sex gene could be located"; and the most obvious conclusion is that such sex genes do not exist.

The *X*-chromosome, therefore, fulfils two functions, namely, to ensure normal female development and, secondly, to carry a large number of genes affecting a variety of characters. For normal female development, the presence of two *X*-chromosomes is required; and in mammals this is determined in early embryonic life. Beyond this stage it is likely that one *X*-chromosome is sufficient to carry out the functions of the genes, since this function is adequately fulfilled by the single *X*-chromosomes of males. It has been postulated by Lyon[34–36] that it may be the function of the sex chromatin to put the second *X*-chromosomes of females out of action by curling it up into a tight bag. This hypothesis is in agreement with traditional views that heterochromatin is genetically relatively inactive. At present, much work is in progress trying to test the Lyon hypothesis and to see to what extent it is applicable to human cells.

Finally, how do the sex chromosomes carry out their task of determining whether an organism is to develop into a male or female? Nowadays it is a commonplace notion that the chromosomes act via the genes which they carry, and it is one of the triumphs of present-day biochemistry to have discovered the pathway from the DNA of the gene to the protein of the gene product. There can be little doubt that characters such as hæmophilia and colour blindness, and all the other characters which are determined by genes borne on the *X*-chromosome, will be found to be controlled in this manner. However, there seems to be strong evidence that the inheritance of sex may follow a different route, which is controlled not by individual genes but by whole chromosomes or at least large parts of chromosomes. This conclusion is based on three sets of facts: (1) the different behaviour of the sex chromosomes; (2) the absence of genes determining sex; (3) the apparent absence of a specific substance which differentiates the sexes; for the sex hormones, which are so powerful in promoting sexual differentiation, act not by their presence or absence but by the relative proportion with which they occur in either sex. It is a remarkable fact that the human testis produces, in addition to androgens, also small amounts of œstrogen, while the ovary is capable of secreting androgens[37]. There appears to be every indication that the basic difference between the sexes is of a quantitative, rather than a qualitative, nature.

Commoner[38] has recently put forward the view that DNA participates in two systems of genetic control. One of these systems accounts for the characters showing typical Mendelian inheritance, which may be assumed to be due to specific biochemical differences. This system is localized in the euchromatic regions of the chromosomes. In the second system, DNA gives rise to the genetic regulation of certain metabolic processes, thus determining quantitative differences such as cell size and the rate of oxidative metabolism. This second system is assumed to be localized in the heterochromatic portions of the chromosomes. It would appear that the sex chromosome mechanism of mammals provides the most striking example of this type of dual genetic control. The first *X*-chromosome is euchromatic and presumably exerts its control via the sex-linked genes which it carries. The second *X*-chromosome is heterochromatic (whether this is so in every cell is not certain) and ensures normal female development; but, if present in addition to a *Y*-chromosome, this type of *X*-chromosome precludes normal male development.

The age-old question of what determines the sex of an individual has been formally answered in terms of the sex chromosome mechanism. The subject now provides a challenge to discover the biological realities underlying this mechanism—and a promise that progress in the years ahead will be at least as rewarding as that which we have just witnessed.

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OBITUARY

Prof. I. A. Preece

PROF. I. A. PREECE, who died suddenly at the age of fifty-seven on August 12 while on holiday in Switzerland, was a leading figure in the world of brewing science. Born in Birmingham in 1907, he graduated at the University of Birmingham in 1927 and was later awarded the degree of D.Sc. (Birmingham) in 1950. Meanwhile, following three years on the staff of the Department of Biochemistry in Birmingham, he was successively lecturer (1931–45), head of the Department of Brewing (1950) and professor in the Department of Brewing and Applied Biochemistry (1950–64) in the Heriot-Watt College, Edinburgh.

Prof. Preece found in brewing, and particularly in malting, a fertile field for his early and abiding interests in plant biochemistry. Structural problems in connexion with the carbohydrates of barley and of malt led him to undertake with exemplary precision work on numerous difficult topics such as the hemicelluloses and phytin, on the nature and function of the amylases, and on proteolytic and cytolytic enzymes. None of these subjects would attract anyone easily repulsed by difficulties, for all abounded in preparative and analytical problems. Nevertheless, many of Preece's papers, especially those dealing with the cytases, phosphatases and amylases of germinating barley, are clearly destined to become indispensable to future advances. His interests extended, however, far beyond his personal research. Prof. Preece was a gifted teacher, as evidenced by his postgraduate students, who came from many parts of the world, and by many audiences at home and abroad, who knew him as an expert lecturer whose lectures were, quite apart from their invariably substantial scientific content, always memorable for their lucidity and nice distinction of language.

Among many professional and educational activities, Prof. Preece played a prominent part in the Royal Institute of Chemistry and the Institute of Brewing. He had since 1949 been editor of the *Journal of the Institute of Brewing*, the high quality of which over recent years owes much to his meticulous standards. Prof. Preece was elected a Fellow of the Royal Society of Edinburgh in 1949 and early in 1964 was awarded the Horace Brown Medal as the highest honour which the Institute of Brewing could bestow. Perhaps, however, his most effective memorial is to be found in the large number of brewers whom he trained. Preece probably more than anyone else, at least in the English-speaking world, inspired them with the possibilities of scientific understanding and control in what is in some respects still a markedly traditional industry. A. H. COOK

NEWS and VIEWS

The Royal Society: Officers for 1964

AT the Anniversary Meeting held on November 30, Sir Howard Florey, Provost of The Queen's College, Oxford, and formerly professor of pathology in the University of Oxford, was re-elected president of the Royal Society. The other officers re-elected for the ensuing year were: *Treasurer*, Lord Fleck, formerly chairman of Imperial Chemical Industries, Ltd.; *Physical Secretary*, Sir William Hodge, Master of Pembroke College and Lowndean professor of astronomy and geometry in the University of Cambridge; *Biological Secretary*, Prof. A. A. Miles, director of the Lister Institute and professor of experimental pathology in the University of London; *Foreign Secretary*, Sir Patrick Linstead, Rector of the Imperial College of Science and Technology. The other members of Council elected (or re-elected, marked *) were: Sir Eric Ashby, Master of Clare College, Cambridge; Prof. D. H. R. Barton, professor of organic chemistry in the Imperial College of Science and Technology; *Prof. P. M. S. Blackett, professor of physics in the Imperial College of Science and Technology; Sir William Cook, deputy chief scientific adviser, Ministry of Defence; *Prof. A. H. Cottrell, Goldsmiths' professor of metallurgy in the University of Cambridge; *Prof. C. A. Coulson, Rouse Ball professor of mathematics in the University of Oxford; Prof. F. S. Dainton, professor of physical chemistry in the University of Leeds; Prof. K. C. Dunham, professor of geology in the University of Durham; Prof. G. W. Harris, Dr. Lee's professor of anatomy in the University of Oxford; Prof. W. O. James, professor of botany in the Imperial College of Science and Technology; *Prof. B. Katz, professor of biophysics in University College, London; Sir Hans Krebs, Whitley professor of biochemistry in the University of Oxford; Dr. N. Kurti, reader in physics in the University of Oxford, and Senior Research Fellow, Brasenose College; *Sir Bernard Lovell, professor of radio astronomy in the University of Manchester and director of the Nuffield Radio Astronomy Laboratories, Jodrell Bank; Prof. K. Mather, professor of genetics in the University of Birmingham and Vice-Chancellor elect of the University of Southampton; *Prof. J. W. S. Pringle, Linacre professor of zoology in the University of Oxford.

Crystallography in the University of Birmingham: Prof. A. J. C. Wilson, F.R.S.

PROF. A. J. C. WILSON, at present professor of physics in the University College of South Wales and Monmouthshire, Cardiff, has been appointed to the newly established chair of crystallography in the Department of Physics in the University of Birmingham. Born and educated in Canada, Prof. Wilson was engaged in research at the Massachusetts Institute of Technology before joining the Cavendish Laboratory, Cambridge; he holds Ph.D. degrees of both the Massachusetts Institute of Technology and the University of Cambridge. In 1945 he moved to University College, Cardiff, and became head of the physics department there in 1954. Prof. Wilson is well known for his distinguished contributions to X-ray analysis in the elucidation of the atomic structure of crystals. He was elected a Fellow of the Royal Society of London in 1963. His new appointment will take effect from October 1, 1965.

Aeronautics and Astronautics in the University of Southampton: Prof. G. M. Lilley

PROF. G. M. LILLEY, who has held the chair of experimental fluid mechanics in the Department of Aerodynamics at the College of Aeronautics, Cranfield, since January 1963, has been appointed to the chair of aeronautics and astronautics at the University of Southampton in succession to Prof. E. J. Richards, who has recently been appointed to the new chair of applied acoustics and to the directorship of the Institute of Sound and Vibration Research at Southampton. Prof. Lilley, a graduate of the University of London, has been a resident member of

the teaching staff at Cranfield since 1946. First appointed as a lecturer in the Department of Aerodynamics, he was promoted to senior lecturer in 1950 and to deputy head of the Department in 1955. He has played a major part in the development of the Aerodynamics Laboratory and its research activities. Prof. Lilley is a recognized authority on turbulent flow and its relationship to aerodynamic noise and is chairman of the Noise Research Committee of the Aeronautical Research Council. Other fields of research in which Prof. Lilley has made important contributions include hypersonic flow and its related problems, boundary layers, the design and operation of wind-tunnels and their associated instrumentation and problems of fluid motion. He is a Fellow of the Royal Aeronautical Society, and a member of the Society's Man-Powered Aircraft Group Committee.

Agricultural Research Council Food Research Institute, Norwich: Prof. S. R. Elsden

Prof. S. R. Elsden, professor of microbiology in the University of Sheffield and honorary director of the Agricultural Research Council's Unit of Microbiology, has been appointed the first director of the Agricultural Research Council's new Food Research Institute at Norwich; he will take up his appointment on July 1. Prof. Elsden graduated from Cambridge in 1936 with first-class honours in Part II of the Natural Sciences Tripos in biochemistry. In 1937 he was appointed assistant lecturer and, later, lecturer in physiology in the University of Edinburgh. In 1943 he returned to Cambridge as a member of the scientific staff of the Agricultural Research Council's Unit of Animal Physiology in the Department of Physiology. Prof. Elsden's early research dealt with bacterial fermentation, and he was much influenced by the work of the late Marjory Stephenson. He was the first worker to demonstrate that the formation of succinic acid in the fermentation of glucose by *Escherichia coli* is dependent on the presence of carbon dioxide. His research in the Unit of Animal Physiology was concerned mainly with rumen bacteria and he has retained an interest in these organisms, especially in their fatty acid metabolism. In 1946 he went to the United States for a year to work with Prof. C. B. van Niel and Prof. H. A. Barker of the University of California. This visit stimulated him to direct his attention to the metabolism of photosynthetic bacteria. In 1948, Prof. Elsden was appointed senior lecturer in microbiology in the Department of Bacteriology in the University of Sheffield, and in 1952 he was placed in charge of the new Department of Microbiology in the University and appointed honorary director of the Agricultural Research Council's Unit of Microbiology. He held an exchange professorship in the Department of Bacteriology of the University of Illinois for six months during 1956, and was appointed West Riding professor of microbiology in the University of Sheffield in 1959. At Sheffield Prof. Elsden has extended his researches into the physiology of microbial growth, and particularly into the relationship between growth and adenosine triphosphate production. He has built up a strong team of microbiologists some of whom will go with him to Norwich.

Science in Parliament: Cancer Research

In a written answer in the House of Commons on November 23, the Secretary of State for Education and Science, Mr. M. Stewart, said that the Medical Research Council expected to spend about £1,029,000 on cancer research, excluding capital expenditure. The Government supported cancer research by financial grants to universities and medical schools through the University Grants Committee and at hospitals within the National Health Service. Much basic research which could throw light on this disease was supported from public funds, while in 1963 the British Empire Cancer Campaign for Research approved expenditure of £1,314,787 on research and related activities, and the Imperial Cancer Research Fund in 1962–63 expended £1,232,000.

Under-water Research

In reply to a question in the House of Lords on November 26, Lord Shackleton stated that there was already very close co-operation between the Ministry of Defence, the Fisheries Departments and other agencies and other Government-supported bodies concerned with research under water. Pressed further for an assurance that an effective co-ordinating body would be set up, Lord Shackleton said that he assumed that Lord Wakefield was referring particularly to diving techniques, which could be used in many fields and especially for research purposes. He questioned whether this was a field in which the Government should attempt to co-ordinate all activities, but it would be interested to receive any representations from Lord Wakefield or from the British Sub-Aqua Club, to which the Department of Education and Science made a direct subvention of £1,500.

Overseas Research and Development

In a written answer in the House of Commons on November 25, the Minister of Overseas Development, Mrs. B. Castle, announced that the targets for sending volunteers overseas in 1965 would be increased from 1,000 to 1,200 for graduates and from 300 to 400 for school-leavers and apprentices. The Government would, as already arranged, pay 75 per cent of the British costs of this programme, which were estimated at £900,000.

Industrial Research and Development

In a written statement in the House of Commons on November 26, the Prime Minister stated that the Minister of Technology had the general responsibility of guiding and stimulating a major effort to bring advanced technology and new processes into British industry. The methods used would include an intensified use of the appropriate research stations and of the National Research Development Corporation, civil development contracts, and investigations to identify particular industries or parts of industries suitable for action. The Minister would consider what changes might be made in the arrangements for procurement financed by public funds to contribute to the promotion of technological advance. He would initiate investigations of the status of the engineering profession. All these tasks would be undertaken in close consultation with the other Departments concerned.

As already announced, the Minister would be responsible for the Atomic Energy Authority and the National Research Development Corporation and would take over elements of the Department of Scientific and Industrial Research headquarters which have been concerned with research and technical development in industry. The Ministry of Technology would in future be the sponsoring department for the machine tools, electronics, telecommunications and computer industries. Close relations would be established with the British Standards Institution and the Ministry would support its work in the engineering field. Mr. Wilson added that in the Science and Technology Bill now before Parliament it was proposed to extend the powers of the Atomic Energy Authority to permit the Authority to undertake research and development outside the nuclear field, and legislative action would also be taken to increase the powers and resources of the National Research Development Corporation.

Research Councils

The statement on November 26 was complemented by a written answer given on the same day by the Secretary of State for Education and Science outlining the arrangements that would be made following the enactment of the Science and Technology Bill. The Secretary of State

for Education and Science would be responsible for the Research Councils and for the support of research in the universities. The Research Councils would be financed by the Ministry, which would be advised on finance by the Council on Scientific Policy. The Natural Environment Research Council, which would be created, would support research in the earth sciences and ecology, taking over the Nature Conservancy, the Geological Survey and Museum, the Hydrology Research Unit, the National Institute of Oceanography, and the functions of the Development Commission in relation to marine and freshwater biology and fishery research. Recognizing that the Minister of Land and Natural Resources would be closely interested in some of the work of this Research Council, Mr. Stewart undertook to consult him and other Ministers interested on certain appointments in this organization.

A new Science Research Council would also be created, taking over the functions of the Department of Scientific and Industrial Research in respect of research grants and postgraduate training awards not within the fields of the other Councils. It would also take over the activities of the National Institute for Research in Nuclear Science and would also be responsible for the Radio Research Station, the Royal Observatories, and the scientific space research programme, and for advising on relations with the Centre for European Nuclear Research and with the European Space Research Organization. Except for the Road Research Laboratory, which would become a responsibility of the Minister of Transport, and the Tropical Products Institute, which would be the responsibility of the Minister of Overseas Development, the remaining stations of the Department of Scientific and Industrial Research would become a responsibility of the Minister of Technology. The other functions of the Department would be divided between the Ministry of Education and Science and the Ministry of Technology, which would take over information functions concerned with disseminating technical information in industry. The National Lending Library for Science and Technology, and the more general information work and the control of the Scientific Attachés, would come under the Ministry of Education and Science.

National Physical Laboratory and the National Chemical Laboratory

Mr. Stewart stated that the decision to place the National Physical Laboratory under the Ministry of Technology was reached after careful consideration and discussion with the President of the Royal Society. It is intended to combine the National Chemical Laboratory with the National Physical Laboratory under one director, and arrangements have been concluded with the Royal Society which will continue the long-standing close association between the Laboratory and the Royal Society.

Post-doctoral Research at the Smithsonian Institution, Washington

The U.S. National Academy of Sciences and the Smithsonian Institution have announced a joint programme to extend postdoctoral research opportunities to visiting investigators from other institutions in the United States and abroad. Under the programme, which begins in 1965, 12 visiting research appointments tenable at the Institution have been established in the following departments: *History of Science and Engineering*, including research in the history of science and technology within the new Museum of History and Technology; *Biological Sciences*, including research in anthropology, systematic biology, mineral sciences, and ecology within the Division of Radiation and Organisms, Museum of Natural History, National Zoological Park, and the Canal Zone Biological Area; *Physical Sciences*, including research in the Smithsonian Astrophysical Observatory at Cambridge, Mass. Applications for the programme at the Smithsonian Astrophysical Observatory, with tenure beginning in January 1965, are immediately available. Brochures describing the 12 separate associateships in detail and applications for the programme at the Smithsonian can be obtained from the Fellowship Office of the National Academy of Sciences–National Research Council, 2101 Constitution Avenue, N.W., Washington, D.C.

The Indian Brain Research Association

The Indian Brain Research Association, an organization of scientists actively associated with research and professions relating to the brain, has recently been formed. This Association, it is hoped, will meet the long-felt need for an organization aimed at unifying research and professional activities within their diverse fields. The Association is a non-profit, scientific and educational organization, and will serve as a platform for the interchange of ideas and results concerning brain research by means of discussions, symposia, meetings, publications, etc. All branches of neurosciences are open to membership of the Association. The headquarters is located at the Department of Applied Chemistry, University College of Science and Technology, Calcutta-9. At present a steering committee, headed by Dr. B. Mukerji, director, Chittaranjan National Cancer Research Centre, Calcutta, performs the executive functions. A bi-monthly bulletin of the Association, *Brain News*, will help to keep members abreast of recent discoveries and techniques in neurological fields, with emphasis on the latest developments in brain research, teaching and profession. Articles of special interest and papers presented in the Association's meetings and seminars will also be featured.

Wellcome Trust Grant for the Royal Society of Medicine Library

A grant of £50,000 has been made by the Wellcome Trust to the Royal Society of Medicine in order to enable the Society to provide new storage space for its Library—probably the most comprehensive medical collection in Europe. Space will be made available in the basement of Chandos House, an Adam mansion in Queen Anne Street, London, W.1, of which the Society has purchased a 99-year lease. The Society's Library is noted for its collection of medical journals, built up in the nineteenth century and now totalling more than 2,000 titles. The growth of the Library is such that about 150 yards of shelving are filled every year. About 50,000 volumes each year go out on loan from the Library, which is open 12 hours a day for reference by Fellows of the Society. During 1952–53 a grant of £125,000 from the Wellcome Trust enabled the Society to extend its present building and to provide an extra floor for library use.

Peaceful Uses of Outer Space

A review of national and co-operative international space activities has been issued by the Committee on the Peaceful Uses of Outer Space of the United Nations General Assembly (*Review of National and Co-operative International Space Activities*. Pp. 114. New York: United Nations, 1964). Such activities are listed for 37 countries, 17 of which are represented on the Committee itself, while 4 others have other international commitments in this field. Detailed information on outer-space research activities is not included, but fairly full information is given, particularly for the U.S.S.R., the United Kingdom and the United States.

Tropical Medicine

For many centuries the term 'tropical medicine' suggested something exotic: strange diseases caused by strange parasites producing strange clinical manifestations. During the past two decades this outlook has been entirely revolutionized, and it has now come to be realized

that tropical medicine is merely a facet of general medicine and that disease recognizes neither colour nor race. Geographical factors undoubtedly play a part, but, fundamentally, the sick African and the sick Asian respond to the vicissitudes of life in the same way as the sick Caucasian. Equally important in emphasizing the universality of disease, from the British practitioner's point of view, has been the increasing number of African, Asian and West Indian immigrants settling in Britain, and to-day practitioners are encountering diseases entirely new to them. It is mainly for these reasons that a symposium in *The Practitioner* has been devoted to "Tropical Medicine" (193, No. 1154; August 1964). The symposium presents an authoritative review of the more important aspects of the subject. As modern methods of transport speed up the comings and goings of the inhabitants of all five continents, it is incumbent on every practitioner to be aware of this world picture of medicine.

Training Courses in Concrete

THE programme of residential training courses offered by the Cement and Concrete Association in 1965 varies little from that of the previous year (*Training Courses in Concrete—Prospectus and Programme for 1965*. Pp. 15. London : Cement and Concrete Association, 1964). The *Prospectus and Programme for 1965* now available lists the various courses offered at different times spread throughout the year; it includes instruction on many aspects of concrete technology, among which may be mentioned: concrete roads and cement-stabilized bases for general foremen, clerks of works and highway superintendents; concrete for engineers; concrete mix design and quality control for engineers; ultimate load design; design of reinforced and pre-stressed concrete bridge decks (in two parts, including a combined course and overseas visit); concrete roads and cement-stabilized bases for engineers; concrete construction for local government engineers and surveyors; formwork for engineers; concrete for architects; concrete products; concrete technology for suppliers of ready-mixed concrete; and aggregates for concrete. The educational and technical value of these training courses, ever since their inception, has been widely appreciated, as is attested by the fact that the demand for places offered during 1964 was such that as many applications had to be refused as were accepted. This is one of the reasons why the programme for 1965 is following closely the 1964 pattern: to give those who could not be admitted in 1964 an opportunity to attend in 1965. With an eye to the future, the decision has been made to build a new Training Centre on a neighbouring estate recently acquired by the Association, capable of housing more than twice the number of applicants at present catered for. It is anticipated that the new premises, on which work has already begun, will be ready for use in 1966. As a result of this move, the residential facilities at the Centre will be greatly improved; two fully equipped lecture theatres and new laboratories will be built, with greatly increased facilities for carrying out the experimental work which is a vital part of most of the courses.

Crystallographic Course in Peking

A SCHOOL in "Experimental X-ray Crystallography" was held, under the auspices of the Scientific and Technical Association of the Peoples' Republic of China, in the recently built Conference House, Peking, during November 2–14. About 70 students attended the lectures and demonstrations and a further 60 attended the lectures only. The course was similar to that held in Cambridge during August 31–September 2 (*Nature*, 204, 731; 1964). The lectures, originally written in English by Dr. W. A. Wooster, had been translated and were delivered in Chinese by members of the staffs of various universities and academic institutes. The translated lectures were printed together with all diagrams, photographs and charts, and a complete set of this material was issued to each student before the course began. The lecturers amplified the original text, where they thought it would help the students, and the style of lecturing showed a good understanding of the students' needs. In the practical demonstrations, Dr. Nora Wooster and Mr. A. M. Wooster assisted, and, with the aid of interpreters, the language difficulty did not prove serious. The students came not only from Peking but also from distant parts of China. In addition to the 20 lectures and demonstrations there were seven sessions of questions put by the students—the day's work started at 8 a.m. and continued through to 6 p.m. The large Conference House, Peking, was completed in four months during this summer. It had a well-equipped lecture theatre, and numerous rooms were made available for the practical demonstrations. Apparatus for demonstration in the lectures was lent by many institutes, and the willing co-operation shown by so many professors and research workers was the basis of the successful organization of the course, under Prof. Wu Chien-chang (Institute of Physics, Peking).

The Jean Julliard Hæmostasis Prize

AN annual prize of 2,000 French francs is being offered by the Editor of the journal *Hémostase* in memory of one of its founders, the late Jean Julliard. The prize is intended to honour and to assist a young research worker who submits an original paper on an experimental or clinical subject relating to hæmostasis, coagulation, hæmorrhagic disease or thrombosis. In exceptional cases the award may be shared. Candidates, who may be of any nationality, must be less than 35 years old. Manuscripts may be written in French or English and the prizewinning entry will be published in the journal. Further information can be obtained from Editions S.P.E.I., Département Médical, 14, rue Drouot, Paris-9ème.

Announcements

DR. ROBERT GLEN, assistant deputy minister (research) of the Canada Department of Agriculture, has been awarded the 1964 Gold Medal of the Entomological Society of Canada, in recognition of his distinguished services to entomology, especially his outstanding research on elaterid larvæ.

THE Scientific Products Foundation Award for "Outstanding Contribution to the Advancement of Pathology" for 1964 has been conferred on Dr. Alan R. Moritz, vice-president of Western Reserve University, for his many contributions to the field of forensic pathology. Dr. Moritz was instrumental in founding the first academic department of forensic medicine in the United States, at Harvard University. The award is made by the College of American Pathology.

A SYMPOSIUM on "Modern Aspects of Stereochemistry", arranged by the Sheffield Local Section of the Chemical Society, will be held in Sheffield on December 18. Further information can be obtained from Dr. J. McKenna, Department of Chemistry, The University, Sheffield.

AN informal open meeting on "Electronic States of Biological Macromolecules" will be held by the Physical Biochemistry Group of the British Biophysical Society at King's College, London, on January 16. Further information can be obtained from Dr. A. Herzenberg, Department of Theoretical Physics, University of Manchester.

CORRIGENDUM. In the article "European Mid-Devonian Correlations", which appeared on p. 363 of the October 24 issue of *Nature*, the words "half-size" in the caption to Fig. 1 should read "twice natural size", and "*Desatrypta flabellata*" should read "*Desatrypa flabellata*". In the caption to Fig. 2, "half-size" should read "natural size".

UNITED STATES BUREAU OF STANDARDS

1963 Research Highlights is the title of the detailed annual report for the year ending December 31, 1963, of the activities of the National Bureau of Standards*. The director, A. V. Astin, is assisted by two deputy directors and three associate directors. One deputy director is responsible for basic standards and services, and the other for technological standards and services. New laboratories for the Bureau are under construction at Gaithersburg, Maryland, and one of the associate directors is primarily responsible for the transfer which will shortly take place. The Bureau consists of twenty-four scientific divisions, sixteen of which are at present located in Washington. The remainder constitute the Boulder Laboratories in Boulder, Colorado, which are under the management of R. B. Scott. The total staff of the Bureau numbers about 4,160, of whom some 1,450 are professional physicists, chemists, engineers and mathematicians, and 200 research associates and guest workers. The 1963 programme cost 59 million dollars, and was financed by grants from Congress, payments for specific research and development tasks, and payments by industrial concerns, universities, research institutions and Government agencies for calibrating and testing services.

The report consists of a general review of the Bureau's activities, followed by more detailed reports of the research programmes of the individual divisions arranged so as to bring together related types of activity. In the metrological section, it is reported that a new standard of irradiance has been developed. This consists of a 200-W quartz–iodine lamp with a coiled-coil tungsten filament operating at about 3,000° K, no auxiliary optics being required. In order to calibrate standards to 10^{-7} in. an ellipsometer was used to determine the thickness of the molecular film between gauge blocks when in contact as the film becomes an inherent part of the length standard. The lenses of the 26-in. refracting telescope at the University of Virginia were tested *in situ* by a wave-front shearing interferometer placed at the focus of the telescope, and by applying the 11,530 Å infra-red light produced by a helium–neon gas laser to a modified Michelson interferometer fringes were produced which when converted to visible wave-lengths were observed and photographed over a 200-m optical path. The freezing pressure of mercury at 0° C was determined, and the value obtained, 109,722 ± 30 lb./in.², indicates that this can serve as a calibration fixed point in high-pressure measurement. For the measurement of low pressures, a detailed investigation of capillarity effects on the McLeod gauge has been made, and a search is being undertaken for suitable force-per-unit area techniques to replace the more conventional indirect methods of measurement.

A number of advances in the field of electrical measurement are announced. To meet the need for greater speed and accuracy in the determination of a.c. voltages at audiofrequencies a new d.c.–a.c. comparator was developed and a usable precision of 10 parts per million attained. A permanent adjustable standard of magnetic susceptibility, formed by using the magnetic equivalence of a uniformly polarized volume of paramagnetic material and a solenoid carrying an electric current, was incorporated into a magnetic susceptibility bridge of high accuracy and sensitivity. A new cæsium beam frequency standard, 'NBS-3', was completed, its spectral line width being less than half that of 'NBS-2'. The 'NBS-1' cæsium beam was converted into a thallium beam and approximately the same precision as previously was obtained. In the field of quantum electronics, investigations included the measurement of the velocity of γ-rays by means of the Mössbauer effect, the determination of the fine structure constant using singly ionized helium, and the examination of the mechanisms by which blue fluorescence may be induced in anthracene using the red light of a pulsed ruby laser.

The comprehensive programme of research on the thermodynamic properties of simple light-element compounds important in rocket propulsion was continued. The low-temperature heat-capacity data of some 80 substances taken from the literature were analysed by means of a computer and the thermal functions determined. A computer programme which requires no 'manual' operation is now being developed. The Bureau has continued to act as the world centre for the collection and determination of most of the atomic physics data needed particularly by plasma and astrophysicists. Emphasis has centred on the observation and analysis of the spectra of the rare-earths. A new edition of the extensive solar spectrum is near completion. From an analysis under high resolution of the infra-red spectrum of carbon suboxide (C_3O_2) conclusive evidence has now been obtained that the molecule is a straight chain consisting of three carbon atoms with an oxygen atom at either end.

The solid-state physics programme of measurements of electronic transport, optical characteristics, magnetic resonance and dielectric properties has been supported by theoretical investigations and by crystal growth and structural investigations. The main emphasis was on Ti-O compounds, CdTe, PbF_2 and several azides. Measurements of the Seebeck coefficients of TiO_2 and $SrTiO_3$ down to 15° K have shown that the phonon-drag effect contributes most to the coefficient of rutile at low temperatures, but is almost absent in $SrTiO_3$. Good crystals of CdTe were grown by the Bridgman method. Argon and krypton crystals were grown at temperatures near their triple points, 84° K and 116° K, respectively, and the properties and growth characteristics of these gaseous crystals, which can be regarded as prototype molecular solids, were found to accord closely with those predicted by theory.

The 100-MeV linear accelerator (*Linac*) and the 1·5- and 4-MeV direct accelerators, which are to become operational during 1964 and are housed at Gaithersberg, will enable the Bureau to meet the increased demands for basic experimental data, improved standards and refined measurement techniques in radiation physics. A broad programme of basic research in physical chemistry is carried out by the Bureau. This covers the kinetics of very fast reactions at high temperatures, the photosynthesis of labelled organic molecules, the structure of reactive molecules, surface catalysis, and the interaction of high-energy radiation with organic molecules.

Metal fatigue in aluminium alloys, deformation of metals at low temperatures, anisotropic effects in a series of lead–iridium alloys, the kinetics of metal whisker growth from the vapour phase, electrocrystallization, and the Peltier effect at liquid junctions are some of the items discussed in the metallurgy section. Other sections deal with investigations carried out on natural and synthetic polymers, radio propagation including ionospheric, tropospheric and satellite communication, cryogenic engineering, building research, mathematical processes including machine translation, computer facilities and data processing systems.

* United States Department of Commerce: National Bureau of Standards, Miscellaneous Publication No. 255: *1963 Research Highlights of the National Bureau of Standards*, Annual Report, Fiscal Year, 1963. Pp. ix+246. (Washington, D.C.: Government Printing Office, 1963.) 1 dollar.

During the year under review 1,122 papers and documents were published and in addition some 420 classified and unclassified reports were issued to Government agencies. A comprehensive list of these is given in the appendix to the report. The periodicals published by the Bureau include *Journal of Research*, which is issued in four separate sections—A (Physics and Chemistry) bimonthly, B (Mathematics and Mathematical Physics) quarterly; C (Engineering and Instrumentation) quarterly; D (Radio Propagation) bimonthly—and *Technical News Bulletin*, which is a monthly in which present-day research, development and test activities of the Bureau are summarized. In addition, various non-periodical series publications, the Monograph, Handbook, Miscellaneous Publications, and Technical Notes series, are issued at frequent intervals. Monograph No. 56, *Systems of Electrical Units*, by F. B. Silsbee, is an excellent survey of the various systems of units used in electricity and traces briefly their historical development. *Tensile and Impact Properties of Selected Materials from* 20° *K to* 300° *K* (Monograph No. 63) is concerned with structural materials for use in low-temperature applications, and data relating to their tensile strength, yield strength, elongation, stress–strain curves, and impact properties are given. *Standard X-ray Diffraction Powder Patterns* (Monograph 25) is the twelfth in the series with this title and contains 37 standard patterns of value to chemists in the identification of unknown crystalline materials.

A 16-mm colour film, *Scatter Radar: Space Research from the Ground*, released during 1963, tells the story of the Bureau's new Jicmarca Observatory situated near Lima, Peru. The Observatory has a 22-acre radar aerial and is used to measure at 50 Mc/s the characteristics of ionization in the ionosphere and exosphere. It has made observations of the planet Venus, and will investigate the solar corona and solar gases. The Bureau participated in 18 scientific and technological exhibitions, and as part of the sixtieth anniversary celebrations of the Department of Commerce the Bureau's Washington laboratories were opened to the public for the first time for many years on May 11, 1963. Some 7,000 visitors toured the 100 laboratories and saw the special exhibits on display.

S. Weintroub

ROAD RESEARCH

In recommending that the Road Research Laboratory should be transferred to the proposed Industrial Research and Development Authority, the Trend Committee had reservations as to whether the arrangement should be permanent and suggested that the position should be reviewed after a reasonable interval, with the view of the possible transfer of the management of the Station to the Ministry of Transport. This would be in line with present-day opinion for the strengthening of the scientific resources within the several Departments, but in spite of this the Road Research Report Board in its annual report for 1963* expresses the emphatic view that the value of the Laboratory's research is enhanced by its independence and by the fact that it is seen to be independent by the public and the many authorities and other bodies who benefit from its work. It regards the Laboratory as carrying out its task with conspicuous success within the limits of its resources, but it should be noted that it does not consider that it is the function of a research organization, or within its competence, to apply the results itself, although it admits that a research organization must accept some responsibility for effectively disseminating the results of its researches. The Board reiterates a recommendation it made early in the year, as to the importance that plans should provide for the large staff that will be needed in the near future in view of the great increase forecast for road traffic and road building. The Board welcomes the Laboratory's concern with research on road casualties and urges that prompt action should be taken to apply such measures as research has shown to be appropriate to diminish this continuing loss of life and limb. On lighting problems, in view of the demonstration by the Laboratory's investigations that the accident rate is greater at night than by day and that effective street lighting appreciably reduces the night accident figures, it recommends that an investigation should be made of the effect on accidents and traffic flow of lighting other busy rural roads carrying fast traffic, including motorways. It also welcomes the arrangements now being made in Birmingham and other cities for further trial this winter of the use of dipped headlights in all streets. The large-scale road experiments, which are now an essential part of the Laboratory's research, throw light on many problems of design which face the road engineer and which can be solved only from an examination of results obtained in the prevailing conditions of traffic and climate. It is of the first importance that such experiments should be made and their performance recorded and assessed with a view to the earliest possible application of the results. A further investigation of the effect of frost on the performance of existing road pavements is to be made and a paper on the design of urban sewer systems was published, outlining the recommended procedures for the application of the new design method for surface water sewerage systems: more than a dozen applications have already been received from Local Authorities in England.

The Board notes that the Department of Technical Co-operation has now decided to devote almost one-tenth of its research allocation to provide for the continuation of the activities of the tropical section of the Laboratory. These activities are dealt with more fully in an appendix to the report. They include further investigation of road-making materials from tropical countries with the object of expanding knowledge of overseas soils and of determining the causes of unusual properties, as well as a review of pavement design and soil-moisture movement in tropical and sub-tropical countries. The Board emphasizes the continuing need to make the Laboratory's work better known overseas, and it considers that the primary need in developing countries is knowledge of their own soils and of the other road-making materials available to them. The Board's own report includes the report of the Director of Road Research and appendixes giving details of the committees, staff and publications of the Station during the year. The activities of the Research Station on road systems and layout and parking needs have provided much of the fundamental information required in planning for traffic in towns and elsewhere. Much attention is being given to safety matters and for the first time for 10 years 1962 showed a significant decrease in the proportion of skidding accidents on wet roads. Prevention of some 2,000 accidents is attributed largely to the use of 'dead' rubber for tyre treads and of road materials with a high resistance to polishing under the action of traffic. Research on safety belts and harnesses continued along four main lines, including assistance to the British Standards Institution Committee on seat belts in the specification of a dynamic form of test for safety belts, studies of design, and performance surveys of the usage of the belt, and analyses of

* Department of Scientific and Industrial Research, *Road Research 1963: The Report of the Road Research Board with the Report of the Director of Road Research*. Pp. vi+168+12 plates. (London: H.M. Stationery Office, 1964.) 11*s*. 6*d*.

accidents in which safety belts were worn. Analysis of 600 accidents in which 837 drivers or front-seat passengers were wearing safety belts at the time of the accidents indicated that serious injuries were reduced by about 80 per cent and overall reduction in injuries was about 50 per cent. Six accidents on motorways during the past 2½ years were attributed to spray obscuring a driver's vision and problems involving splashing and production of spray by commercial vehicles are being investigated. A preliminary study has shown that the front wheels are particularly important in producing spray because they generally run in a thicker film of water than the rear wheels, and that the pattern of distribution of spray behind the vehicle depends largely on its shape. Work on road pavements is mainly directed at the construction and observation of full-scale experimental roads, generally on routes carrying heavy traffic, and to theoretical and field studies of the stresses and deflexions in multi-layer pavements and the influence on them of repeated loadings. In the experimental section constructed in 1957 on the A1 at Alconbury Hill the best sections continue to be those with 4 in. asphalt surfaces on a 9-in. lean concrete base and on a 6-in. asphalt base. Studies are in progress on the amount of control testing that should be carried on large road construction projects, to assess the practical limits for the number of tests, the man-power required to carry out the testing, and the cost of testing in relation to the total cost of each project. The first study suggests that tentative proposals made by the Laboratory are likely to be suitable. To determine the effect of wet weather on the construction of earthworks and to provide some indication of the amount of working time likely to be lost through wet weather, records of the utilization of plant at major earthwork sites have been collected to compare the actual performance each day with the performance during dry weather. Weather at the site has been recorded or records are being obtained from nearby stations of the Meteorological Office. Computer programmes are now available for carrying out the calculations involved in the Road Research Laboratory's hydrographic method of sewer design. The Laboratory is also attempting to supply more precise information on the loads imposed on bridges by moving vehicles. This is required in estimating the fatigue life of the structure, and a short-term study is being made of the way in which traffic flow data can be converted into traffic loading data, and a long-term collation of information on vehicle axle weights, using electronic weighbridges at selected sites. The effects of temperature, creep and shrinkage on the performance of a structure are being investigated by measurements on a number of bridges, including the Hammersmith Flyover and the Medway Bridge, and the results have indicated how the behaviour of a structure can be affected by its form and environment.

USE OF WHOLE-BODY COUNTERS IN RADIOLOGICAL PROTECTION

"THE Use of Whole-body Counters in Radiological Protection" was the title of a symposium held at the Middlesex Hospital Medical School on April 7, 1964. It was the eighth in a series sponsored by the Joint Health Physics Committee* and on this occasion was organized for the Committee by the Radiological Protection Service. The proceedings were divided into two sessions, the first being devoted to a review of the development of whole-body counters and an account of experience in their use over a period of more than six years at two centres undertaking this work in the United Kingdom, and the second to reports of some special problems encountered in several laboratories. Mr. W. Binks (Radiological Protection Service) acted as chairman of the first session and Prof. F. W. Spiers (University of Leeds) of the second.

In his opening address to the first session Prof. Spiers (University of Leeds) traced the history of the development of whole-body counters from the early work by Schlundt, Barker and Flinn (1929), and Schlundt, Nerancy and Morris (1933), using ionization chambers, to the highly sophisticated apparatus of the present day utilizing scintillation detectors and multi-channel analysers (Table 1).

From this it could be seen that, after Schlundt *et al.* had made improvements in the ionization-chamber method, there was an additional improvement in sensitivity with the advent of high-pressure ionization chambers, bringing the sensitivity in the region necessary for the measurement of the natural γ-ray emission of the body. The use of large scintillation counters in 1956 brought about a further increase in the sensitivity and it was this order of sensitivity that had provided the health physicist with a powerful tool for measuring body radioactivity far below the maximum permissible values.

Prof. Spiers stressed that, for a high detection efficiency, both high counting rates and large signal-to-background ratio were important, as could be seen from the well-known formula:

$$S.E.\ (\text{as percentage of } S) = \frac{100}{S}\left(\frac{B}{t_B} + \frac{B+S}{t_S}\right)^{\frac{1}{2}} = \frac{100}{(aS)^{\frac{1}{2}}}\left(\frac{1}{t_B} + \frac{1+a}{t_S}\right)^{\frac{1}{2}}$$

(where $S.E.$ is the standard error as a percentage of S, the counts per sec due to the source, B is the counts per sec due to background, $a = S/B$, and background observations are made for t_B sec and source observations for t_S sec). In order to make the standard error small, S should be large, which meant a physically large detector should be used, and a should be increased as far as possible by reducing B.

Table 1. DEVELOPMENT OF WHOLE-BODY COUNTING SINCE 1929

Date	Experimenter and apparatus	Subject observation time	Approximate limit of detection (or standard error): As μg ^{226}Ra equivalent*	Approximate limit of detection (or standard error): As a percentage of total body potassium
1929	Schlundt *et al.*, Ion chamber at 1 atm.		~5	
1933	Schlundt *et al.*, Ion chamber at 1 atm.		~0·2	
1937	Evans, Geiger-Müller tube		0·1	
1947	Hess and McNiff, Ion chamber at 1 atm.		0·03	
1951	Sievert, High-pressure ion chamber	2 h	0·005 (*S.E.*)	~50 (*S.E.*)
1953	Burch and Spiers, Differential high-pressure ion chambers	2 h	0·003 (*S.E.*)	~30 (*S.E.*)
1956	Sievert, High-pressure ion chambers underground	3–4 h	0·001 (*S.E.*)	~10 (*S.E.*)
1956 to 1960	Los Alamos, 4π liquid scintillator (Anderson, 1956)	15 min	~0·0001 (*S.E.*)	1 (*S.E.*)
	Argonne National Laboratory NaI scintillator (Marinelli, 1956)	15 min	~0·0003$_5$ (*S.E.*)	3·5 (*S.E.*)
1960	Leeds, Plastic scintillator apparatus (Bird and Burch, 1958)	15 min	~0·0001$_5$ (*S.E.*)	1·5 (*S.E.*)

* By 'μg ^{226}Ra equivalent' is meant μg ^{226}Ra with all daughter products in equilibrium.

* The Joint Health Physics Committee consists of representatives of the British Institute of Radiology, the British Occupational Hygiene Society, the Central Electricity Generating Board, the Faculty of Radiologists, the Hospital Physicists' Association, the Institute of Physics and the Physical Society, the Radiological Protection Service, the Society for Radiological Protection and the United Kingdom Atomic Energy Authority.

Prof. Spiers referred to a number of different materials, from chalk to lead, which had been used to provide the shield against environmental radiation in order to reduce B (Trott, Parnell, Hodt and Entwistle, 1963, and Wilson, 1964). He considered that attention must also be directed to the problems of other sources of contamination both within the scintillator and its container, also in the bases and resistance chains of the photomultiplier tubes, and other miscellaneous parts (Marinelli, Miller, May and Rose, 1962, and Wilson, 1964). Given a sufficiently sensitive detector and adequate background shielding, statistical accuracy equivalent to about 1 or 2 per cent of the natural ^{40}K content of the body could be achieved. This same order of accuracy was also possible for repeated observations on one person, but there were limitations when determining absolute amounts of radioactivity in the body. Most of the difficulties arose because the activity was distributed about the body and offered a different geometry in each configuration of detector and subject. Moreover, this distribution frequently changed with time after the intake of radioactive material into the body. It was very difficult, therefore, to devise 'phantoms' containing calibrating solutions which approximated at all closely to the *in vivo* distribution, except with radionuclides which dispersed uniformly throughout the whole body. Exceptionally, it might be possible to carry out *in vivo* calibrations with isotopes of the radioactive material concerned, for example, ^{42}K for ^{40}K, and this could increase the accuracy very considerably.

Another important aspect of the accuracy of measurement concerned the overall stability of the apparatus. This became specially important for long observation times and when computer analysis of the results was to be used. One solution to this was to build some form of automatic stabilization into the equipment (Dudley, 1962). As to the choice of detector in any particular instance, this would depend very much on the circumstances and the problem in hand. For rapid screening of large numbers of individuals there was great merit in the use of the large plastic and liquid scintillators, though it must be remembered that time for changing and preparation of the individual was also required, and the actual observation time was only part of the whole operation.

Finally, Prof. Spiers commented that it must be generally conceded that in protection work the sodium iodide crystal detector offered most advantages with regard to higher energy resolution and, therefore, the identification of different radionuclides in the body. Where any large body of people was concerned, it would seem that a simple fast scheme could be worked out using large plastic or liquid scintillators for initial screening purposes. Persons with significant activities could then be passed on to a sodium iodide counter for detailed study.

In his paper entitled "Some Applications of Body Radioactivity Measurements to Radiological Protection at Harwell", Dr. J. Rundo (Atomic Energy Research Establishment, Harwell) stated that there were two applications for whole-body counters at Harwell, namely, the measurement of body radioactivity of persons who had been involved in accidents with radioactive materials and the undertaking of more fundamental studies on the distribution of radioactive material in body tissues and its elimination from the body. The apparatus used had been described in detail elsewhere (Rundo, 1958). During the past five years the number of suspected accident cases examined at Harwell had amounted on average to only 18 a year. By no means all these had been found to be contaminated and the main effort, therefore, had been devoted to careful investigations of a few cases. With regard to observations on the distribution of radioactive material in the body, this could be achieved in three ways, namely: (*a*) by scanning, that is, movement of the detector over the surface of the body; (*b*) by a single large crystal close to an organ of the body; and (*c*) by use of a slit collimator to give a profile curve.

Three cases which illustrated the types of problems encountered in radiological protection were described. All three cases concerned the accidental inhalation of radioactive materials, which Dr. Rundo believed was the predominant mode of entry into the body in most practical circumstances; a view which was later endorsed by others in the discussion. The first case involved ^{90}Sr carbonate (Rundo and Williams, 1961). Measurements were made of body radioactivity, and also of the activity in urine. The significant feature emerging from the results was that if the body radioactivity had been inferred from the initial urine measurements, using data given in the report of Committee II of the International Commission on Radiological Protection (I.C.R.P. II, 1959) for the biological half-life, the estimated body-burden would have been much larger than that actually measured in the whole-body counter. Therefore, it was clear that, wherever possible, it was necessary to have excretion data supplemented by measurements of total-body radioactivity by external counting. The second case (Rundo, 1964) concerned an individual who had inhaled dust generated while grinding and polishing irradiated uranium. The accident was detected when widespread contamination was found in the working area. It was demonstrated that a number of radionuclides had been inhaled, and later it was calculated that the initial dose-rate to the lungs was nearly twice the maximum permissible value of 0·3 rem/week recommended by I.C.R.P., but this decreased by about an order of magnitude in six days. The retention of radioisotopes of Cs, Ce, Ru, and Zr with Nb was followed with the whole-body counter over several hundred days. The whole-body scan showed that, with the exception of ^{137}Cs, all the radionuclides were located in the chest and their activities in that region over a period of time decreased in a manner which was identical with that expected by radioactive decay alone, suggesting that there was little, if any, elimination of these materials from the body. This fact is, of course, quite at variance with the views of I.C.R.P. II that the half-life of particulate material in the lungs is 120 days.

In the third case (Newton, 1963) the accident concerned a man who inhaled dust during maintenance operations on a reactor. The principal contaminants were ^{65}Zn and ^{110m}Ag, identified from examination of a handkerchief used by the man immediately after the accident. His radioactivity was followed by whole-body counting over a period of 2 yr. and profile curves were determined. The profile curves indicated that both ^{65}Zn and ^{110m}Ag were transferred almost completely from the lungs within a few days. A widespread distribution of ^{65}Zn in soft tissues was apparent from the early stages, with an appreciable concentration in the liver during the first 100 days. ^{110m}Ag was concentrated in the liver and was eliminated from that organ with a biological half-life of about 50 days. For both silver and zinc the excretion pattern from the whole body could be described by the sum of two exponential terms. In the case of zinc, 27 per cent was eliminated with a biological half-life of 18 days and 73 per cent with a half-life of 453 days. Most of the silver (about 85 per cent) disappeared very rapidly with a biological half-life of about one day and 15 per cent with a half-life of 52 days, that is, not very different from the rate of elimination from the liver.

In none of the cases described had the individual concerned received a significant radiation dose as a result of the incident. This could have been predicted early in the investigation and the prolonged studies had been made solely to determine the behaviour in the body of the various radionuclides concerned. In answer to a question, Dr. Rundo said that most of the silver and zinc were excreted in the fæces. There was no measurable excretion of silver in urine. He emphasized that excretion patterns varied considerably between individuals, as illustrated by measurements of Furchner, Richmond and Trafton (1962) on four people to whom ^{65}Zn was administered orally.

Table 2. RADIOACTIVITY OF VARIOUS MATERIALS

Material	Radioactivity: Uranium 10^{-12} c./g	Radioactivity: Thorium 10^{-12} c./g	Radioactivity: Potassium 10^{-6} g/g	Primary photon emission in photons/min.g: Uranium	Thorium	Potassium	Total
Brick	75	150	35,000	3·5	10·0	7·4	21·0
Cement	40	70	5,000	1·9	4·7	1·1	7·7
Soil (sample)	40	120	Not measured				
Chalk (North Downs)	10	3	20	0·5	0·2	Very small	0·7

Reviewing the use of whole-body counters at the Radiological Protection Service, Mr. J. Vennart considered that the most interesting feature of the equipment was the construction of the low-background laboratories. Early measurements of the local gamma-ray background in 1956 (Vennart, 1957) had indicated that the chalk underlying the site at Belmont, Surrey, where the Radiological Protection Service was situated, had a very low radioactivity. The reason for this could be seen from information he presented showing the concentrations of the principal naturally occurring radioactive materials, uranium, thorium and potassium (potassium due to the naturally occurring isotope ^{40}K), in a representative sample of brick, cement, soil and chalk and the primary photon emission in photons/min/g in the material from each radionuclide and for the mixture (Table 2). It showed that the total rate of primary photon emission in brick was 30 times greater than in chalk. As soon as this fact was appreciated, a temporary laboratory was built below ground in the chalk and this was still in use at the present time (Vennart, 1963). The detector consisted of four 4½-in. × 2-in. sodium iodide crystals placed in line above the body and in a second counter, recently completed, there were four crystals both above and below the body.

The largest group of people measured had been more than 500 people who had worked in the radium luminizing industry (Vennart, Maycock, Godfrey and Davies, 1964). Twenty of them had been found to have more than the maximum permissible body burden, namely, 0·1 μc. of ^{226}Ra (I.C.R.P. II). The majority contained quite trivial amounts of radium and the important feature was that no one who had luminized exclusively since 1941 contained more than half the maximum permissible body burden. The reason for this might be traced to the fact that it was realized in 1939 that there would be a large increase in the luminizing industry during war-time and, in view of the serious injuries known to have occurred among some of the early American workers concerned with luminizing, the occupation of luminizing in Britain had been brought under the medical supervision of the Ministry of Labour. This supervision, besides including regular medical examinations of the workers, had also resulted in the institution of very simple rules of hygiene in the industry. It was interesting, in view of the present-day efforts in the health and safety field, to see how effective these very simple rules had been. The other large series of measurements had been of fission products in members of the population, principally ^{137}Cs (Maycock, Terry, Vennart and Wise, 1960, and Maycock, McCansh and Vennart, 1962), but also during 1961 of ^{131}I (Vennart, 1962). It had also been possible to measure ^{131}I in the thyroid glands of people living in London following the Windscale Reactor accident in 1957 (Maycock and Vennart, 1958).

During the period that the counter had been in operation (1957–64), body radioactivity of only 85 people had been measured because of incidents involving the dispersal of radioactive materials, and only six of these had more than one-tenth of the maximum permissible body burden of the radionuclide concerned. The radioactivity of the majority of these people was measured because of uneasiness on the part either of the individual himself or of his employer. In other words, they came mainly to be reassured that there was no radioactivity in their bodies. There remained a doubt as to whether it was only the most apprehensive people, or those employed by the most careful employers, who were referred for measurement of radioactivity, and whether more severely contaminated people had escaped attention. Some justification for this assumption might be obtained from the results of measurements of radioactivity carried out on people at present working in the radium luminizing industry, in which they were selected for measurement more or less at random. In this study, 33 people out of a total of 82 measured had contained more than one-tenth of the maximum permissible body burden of radium, which was a much higher proportion than was found in the people referred to the Radiological Protection Service because of incidents involving radioactive material.

These facts suggested that there should be more routine measurements of radioactivity of people working with radioactive materials, but not necessarily with high sensitivity equipment. For the majority of radionuclides which could be measured by detectors placed outside the body, the activities which were of interest could be determined by using very simple apparatus. Significant activities were often several microcuries, not only because the maximum permissible body burdens were large, but also because the radionuclides were often eliminated rapidly from the body and, therefore, the intake of maximum permissible body burdens led to radiation doses which were quite trivial by our present standards (Vennart, 1963). In the discussion which followed about this latter point, it was pointed out that the prevention of intake of radioactive materials by adequate environmental control was also important.

The second session was opened by a paper entitled "The Problem of External Counting of Plutonium at Low Levels *in vivo*", in which Mr. B. Taylor (Atomic Energy Research Establishment, Harwell) described the difficulties of measuring ^{239}Pu *in vivo* by detection of the extremely low-intensity γ-rays emitted. Using a 9-in. × 6-in. sodium iodide crystal, the minimum detectable activity of ^{239}Pu in the lung was about 2–3 μc., while the maximum permissible amount in the lung, corresponding to a dose rate of 0·3 rem/week, was only 16 nc. An alternative method was to measure the *L* X-rays of uranium, which are emitted following the disintegration of ^{239}Pu, with an intensity of about 4×10^{-2} photons per α-particle. These X-rays, of energies 13·6 keV, 16·9 keV and 20·2 keV, had half-value layers in soft tissue of about 0·4, 0·7 and 1·0 cm respectively. The half-value layer in bone was only about 0·03 cm, and the determination of ^{239}Pu in bone at levels near the maximum permissible body burden of 40 nc., therefore, appeared unlikely.

Attempts to measure ^{239}Pu in lung had first been made by Roesch and Palmer (1962), using a thin NaI crystal. In these measurements, a large contribution to the background in the energy band of the *L* X-rays was due to photomultiplier dark noise. The lower background and superior energy resolution obtainable with gas-filled proportional counters had led to the adoption of this type of detector at Harwell. A 6-in.-diameter end-window flow counter constructed from 'Perspex' and containing argon with 10 per cent methane was used with a well-shaped plastic phosphor anti-coincidence shield. The counter background inside a small lead shield 4 in. thick was 1·5 c.p.m. in the energy band 10–22·5 keV, and the best energy resolution achieved at 13·4 keV was 11 per cent, which was close to the theoretical minimum value. Because of the high absorption of the X-rays in body tissues, the effects of variation in distribution of the inhaled ^{239}Pu in the lung, and differences in chest-wall thickness between individuals, had made calibration difficult. However, an estimate of the effective depth of the material might be possible by differential measurements of the three X-rays, since their absorption coefficients are markedly different.

Two further methods of calibration involving human subjects were discussed. The first concerned a case of accidental inhalation of ^{235}U. In this case the amount of the material in the lung could be estimated by measure-

ments of the 186-keV γ-ray it emitted, using a 9-in. × 6-in. sodium iodide crystal detector. Since ^{235}U also emits the characteristic *L* X-rays of thorium, the energies of which are close to those emitted following the disintegration of ^{239}Pu, it was possible to estimate the sensitivity of the proportional counter for ^{239}Pu in lung. Measurements made with the window of the proportional counter close to the chest of the subject had shown that the counting rates were significantly higher than those from two normal subjects with similar potassium and ^{137}Cs contents. The minimum detectable activity was estimated to be 55 nc. of ^{239}Pu for a 100-min measurement. An improvement in sensitivity was expected from the use of a larger multi-wire counter which had just been constructed. Xenon was proposed as the filling gas, though considerable purification from krypton impurity (containing ^{85}Kr) might be required to obtain a sufficiently low background. An alternative method of calibration proposed was the inhalation by volunteer subjects of a 'mock' ^{239}Pu. This would consist of an insoluble mixture of three radionuclides of medium atomic number, which decay by electron capture, giving *K* X-rays of the same energy as the uranium *L* X-rays emitted following the disintegration of ^{239}Pu. Radionuclides which were being considered were ^{85}Sr, ^{93}Mo, ^{103}Pd and ^{103}Ru. Both ^{85}Sr and ^{103}Ru also emit higher-energy γ-rays, thus enabling the lung content after inhalation to be determined by measurements with a sodium iodide crystal detector. An advantage of this method was that reasonably high counting rates might be obtained with the proportional counter, without large radiation doses being received by the volunteer's lungs, since the half-lives of the radionuclides proposed were relatively short.

In his paper entitled "Measurements of Some Long-lived Alpha-Gamma-Emitters in the Thorax" Mr. D. Newton (Harwell) directed attention to the fact that for many α-emitters the maximum permissible amounts in lung, corresponding to a dose rate of 0·3 rem/week, were only a few nc. The detection and estimation of such small amounts *in vivo* might, therefore, be difficult because the γ-radiation accompanying the α-emission is generally of low intensity and in the low-energy region, where the contributions from scattered photons arising from ^{40}K and ^{137}Cs in the body might be difficult to assess. By way of illustration, Mr. Newton described measurements of body radioactivity which were being made on two people who had accidentally inhaled radioactive materials.

The first case involved ^{227}Ac, the long-lived parent of a chain of α-emitters. The parent emitted no detectable γ-radiation itself, and its presence *in vivo* could only be inferred from the γ-rays emitted by its daughters, ^{227}Th (18 days) and ^{223}Ra (11·7 days) *et seq.* The activity in the chest was measured with a single 9-in. × 6-in. sodium iodide crystal placed behind the thorax. The difficulties of calibration were stressed, especially with regard to the excretion of daughter nuclides. For example, it appeared in this case that the ^{223}Ra was excreted more rapidly than the ^{227}Th, but estimation of the equilibrium amounts of these daughters was complicated by the similarity of their spectra and the possibility of their being distributed differently in the chest. Between 200 and 800 days after the incident the chest contents of ^{227}Th and ^{223}Ra were evidently constant at about 1 nc. and 0·6 nc., respectively, showing that there had been no loss of ^{227}Ac during this period, although a small reduction could have been masked by the subject's steeply rising content of ^{137}Cs resulting from nuclear weapons testing. However, measurements of the subject's whole-body radioactivity made during the first 200 days using the four-crystal spectrometer (Rundo, 1958) had indicated that some of the inhaled material had been eliminated more rapidly.

The second case involved a mixture of ^{231}Pa with about 7 per cent of its daughter ^{227}Ac. Measurements with the single large crystal showed that the chest content of ^{231}Pa was unchanged between 7 and 300 days after the incident, at about 10 nc. or two-thirds of the maximum permissible amount in lung. The ^{227}Ac content was apparently about 1 nc. or one-quarter of the maximum permissible amount in lung, but may have decreased very slightly during the period of observation. In conclusion, Mr. Newton said that the investigations had shown that it was possible to measure about a quarter of the maximum permissible amounts in the lung of ^{227}Ac and ^{231}Pa with sufficient accuracy for protection purposes. However, there remained a doubt about the exact location of this material within the chest. In neither case was it certain that the activity was deposited solely in the lungs. If it were instead in the pulmonary lymph nodes, then lower maximum permissible amounts would presumably apply because of the smaller tissue masses involved. In the discussion, attention was directed to the fact that the long biological half-lives of these materials in the lung were contrary to Baird's findings with dogs (Baird, Tombropoulos and Park, 1963), and unexpected from the physiology of the human lung. If the contaminants had been in a soluble form and deposited in bone, this might explain the different results. Was there any information on the chemical form of the materials, or the presence of activity in the fæces? Mr. Newton replied that nothing was known of the chemical form of the ^{227}Ac inhaled by the first subject and, in the second case, the chemical form of the ^{231}Pa was not known with certainty: possible compounds were Pa_2O_5 and $KPaO_3$. None of the parent nuclides had been found in the fæces of either subject, though in the first case ^{223}Ra had been detected after 100 days.

In a paper entitled "Measurement of Fall-out ^{137}Cs in Infants", by F. W. Spiers and A. R. Wilson (University of Leeds), Mr. Wilson described measurements of ^{137}Cs and ^{40}K using a detector consisting of four 6-in. × 6-in. sodium iodide crystals. The crystals were positioned above and below the infant in such a way that the sensitivity of the counter was almost independent of the position and attitude of the infant during the measurement. For a measurement time of 30 min, the standard errors of measuring 1 nc. ^{137}Cs and 8 g potassium in a new-born infant were respectively ±7 per cent and ±10 per cent. It had been possible to estimate the turnover of ^{137}Cs in two cases in which the babies had been fed on dried milk. In calculating the turnover time it had been assumed that the newly born baby had the same ^{137}Cs concentration per unit mass as the mother. The way in which the observed body content of ^{137}Cs changed with time could be accounted for by assuming a biological half-life of 8 days for one baby and 7 days for the other. Both babies had been measured up to about 30 days old. Mr. Wilson considered that it would have been of interest to study breast-fed babies and older children, but it was very difficult to determine the ^{137}Cs intake in such cases.

The way that the ratio $^{137}Cs/K$ changed with time in the two cases was conflicting. In one baby the ratio rose to a maximum in a few weeks, whereas in the other it fell to a minimum at about the same age. Commenting on this in the discussion, Mr. Wilson suggested that the reason for this discrepancy probably lay in calibration and that it was not easy to get a truly representative model when the potassium and ^{137}Cs were varying and also probably undergoing re-distribution in the body. In the discussion, Dr. Rundo briefly described some measurements of body radioactivity which he had made on two children. The ways in which the body content of ^{137}Cs changed with time were consistent with a biological half-life of 10 days for a child measured up to 30 days old and a half-life of 7 days for another measured up to 37 days old. Mr. G. Stott said that he had made measurements of body radioactivity on one child who was 90 days old which showed that the ratio $^{137}Cs/K$ was twice that of adults measured at the same time. The radiation dose received would also be about twice that received by adults.

Mr. C. O. Peabody considered the general philosophy of the use of whole-body counters in radiological protection

in his paper, "The Value of Routine Measurements at the Atomic Energy Establishment, Winfrith". He compared and contrasted the problems and methods of minimizing and measuring exposure to external radiation with those for the internal irradiation produced by radioactive contamination in the body. Where external radiation was concerned the exposure of individual workers was minimized by providing adequate shielding, by controlling working times and by frequent environmental surveys. Finally, the adequacy of protection was further checked by the wearing of personal dosemeters, such as film badges. In the case of internal radiation the risk was minimized by the containment of radioactive material, by adequate ventilation, and by environmental monitoring for contamination on surfaces and in the air. There was, however, no system of routine personal dosimetry for internal radiation which was at all comparable in its simplicity and accuracy with that of the film badge in monitoring external radiation. A case could be made for the need for such a routine system, but it was at present impracticable. The practice generally adopted, and implied in some codes of practice, was that measurements of body radioactivity need be made only when it seemed possible that the person had been exposed to an undue risk of taking radioactive material into the body.

Mr. Peabody went on to consider the problems involved in making routine measurements of body radioactivity. For example, it was necessary to make measurements at realistic intervals of time commensurate with the counting facilities available and with the time for which the subject could be taken away from his work. It was impossible to lay down a standard interval between measurements which would be applicable in all cases, because this depended on the effective half-life of the radionuclide in the body. The situation at Winfrith was that there were about 200 people who were exposed to a possible risk and only about 30 per week could be measured in the whole-body counter there. If all these people were measured continuously, therefore, the interval between measurements would be seven weeks and for many of the radionuclides concerned this would be far too long. Accordingly, a programme of continuous measurement of body radioactivity of all people had not been adopted and measurements had been limited to a representative sample of the people concerned. People were chosen for the sample according to their estimated risk of contamination. Unexposed staff such as office workers were used as controls. So far, no person had been found to contain more than a negligible fraction of the maximum permissible body burden of any radionuclide while, for the majority, the only radionuclides detected were ^{40}K and fall-out ^{137}Cs. The system had proved to be advantageous in that it had found a few cases of personnel contamination which had not been previously suspected and, equally important, it had served to reassure staff and supervisors. In the discussion that followed there were several comments about the time taken to prepare an individual for measurement in a whole-body counter. The need for the individual to take a shower bath depended on whether or not the person had been exposed recently to the risk of contamination. It was agreed that it was always necessary to change into clean and previously monitored clothing.

In dealing with "The Usefulness of Routine Measurements in a Whole-body Counter as a Check on the Effectiveness of Contamination Control", Mr. G. Stott (Dounreay Experimental Reactor Establishment) stated that the principal uses of the whole-body counter at Dounreay had been for routine measurement of body radioactivity of workers, experimental investigations of people who had accidentally acquired radioactive materials in their bodies, measurement of samples taken on environmental surveys and diagnostic tests of thyroid function as a service to ordinary members of the public in the vicinity of Dounreay. There were about 300 people who might be at risk at the Dounreay Experimental Reactor Establishment. These were mostly employed on fuel processing, so the radioactive materials concerned were chiefly fission products. During 1963, when there had been a large increase in fuel processing, extensive air sampling had been undertaken in order to ascertain whether there was any increase in environmental contamination. This showed that the concentration of radioactive materials in the atmosphere had rarely been excessive and the effectiveness of this environmental monitoring with regard to the protection of the workers had been checked by measurements of the body radioactivity in the whole-body counter.

Measurements of ratios $^{137}Cs/K$ (pc./g), taken in 1961 during a slack period, showed values of 81 for the workers and 91 for controls. The corresponding values for 1962–63 were 318 for workers and 293 for controls. There was, therefore, no difference between workers and controls in either period. However, the percentage of workers contaminated with more than 5 nc. of the fission products ^{95}Zn and its daughter, ^{95}Nb, increased from 20 per cent in 1961 (highest value 50 nc.) to 70 per cent in 1962–63 (highest value 40 nc.). The results of air sampling were approximately the same during both periods. Hence, although the usefulness of air sampling was confirmed, in that the body radioactivities due to ^{95}Zn and ^{95}Nb were negligible by comparison with the maximum permissible body burden of 20 μc., the results of air sampling were not sufficiently sensitive to direct attention to the fact that the intake of radioactive materials into the body had been increasing during the period of observations. This was only discovered by *in vivo* monitoring.

Mr. B. E. Godfrey (Radiological Protection Service) discussed the parameters required for estimation of radiation doses following single or protracted intake of radioactive material into the body. He pointed out that, when persons were measured for body radioactivity following accidents, it was seldom possible to determine all the parameters required. For example, the body radioactivity of the individual concerned was very frequently measured some time after the accident had occurred, so that the initial build-up of the radioactive material in the various organs and tissues was not observed. Very often it was not possible to determine the exact distribution of radioactive material in the body and impracticable to make repeated measurements on the individual to find the excretion pattern. In most cases, therefore, it was necessary to adopt some simple model for the distribution of the radioactive material in the body and its elimination. He referred to the model adopted by Vennart and Minski (1962) in which the pattern of elimination is described by a single exponential term and illustrated the use of such a model by describing some cases which had been measured in the whole-body counter at the Radiological Protection Service.

The first example concerned measurements of body radioactivity on three people who had accidentally taken in ^{137}Cs while at work. Measurements had been made over a prolonged period starting a few weeks after the accident and for each individual it was possible to fit the data to a single exponential function. The biological half-lives of ^{137}Cs were found to be 79 days, 118 days and 123 days (Taylor, Taylor and Vennart, 1962). The second example concerned measurements of ^{131}I in human thyroids following the Windscale Reactor accident of 1957, and also following the increase of nuclear weapon testing in 1961. In both cases it had been possible to fit a theoretical expression to the measured points on the assumption that radioactive iodine is eliminated from the thyroid according to a single exponential term (Maycock and Vennart, 1958, and Vennart, 1962). The third example concerned a patient injected with 25 μc. of ^{22}Na in a diagnostic test for exchangeable sodium, whose body radioactivity was later measured in the whole-body counter for a period extending from $2\frac{1}{2}$–$4\frac{1}{2}$ years after the test (Vennart, 1963). The measurements showed that about 0·35 per cent of the injected dose was eliminated from the body very slowly at

a rate which could be described by a single exponential term. The effective half-period of elimination was 513 days, corresponding to a biological half-life of about 1,000 days. The value for the effective half-life was in accord with that of 445 days reported by Richmond (1958) and 475 days reported by Jagger, Hine, Cardarelli and Burrows (1963). The final example concerned two women whose body radioactivity had been measured during the period 5–12 weeks following the administration of ^{131}I in tests for thyroid function. The elimination of ^{131}I from the thyroid could be explained by a single exponential term corresponding to an iodine turnover of about 1·5 per cent per day, a value which is in reasonable agreement with that of 2 per cent per day quoted by Ziliotto (1961), but about twice the values quoted by Rosenburg (1958), Burns, Fish, Hackett and Hickey (1951), and Maurer (1961). However, it was necessary to be cautious in the interpretation of the results because these patients had been diagnosed as having anxiety states following their presentation with symptoms common to thyrotoxicosis. There was a possibility, therefore, that they might have slightly over-active thyroids.

In the discussion on this paper, Dr. Rundo referred to the use of collimated counters to investigate the distribution of radioactive materials in the body. Mr. Vennart doubted whether the degree of collimation would ever be sufficient to determine the activity in different organs of the body, except in very special cases such as the thyroid.

In a general discussion at the end of the symposium the main topic of interest concerned the problems of estimating the activities of β-emitting radionuclides in the body by measurement of bremsstrahlung in whole-body counters. It was clear from this that one of the chief limitations on the estimation of small β-activities was the presence of ^{40}K and ^{137}Cs in the body. Degraded photons from both these radionuclides contribute to the bremsstrahlung spectrum of any β-emitter in the body and due allowance for this must be made when estimating the β-activity. The uncertainty of an estimation of β-activity must, therefore, include the uncertainties of estimating both ^{40}K and ^{137}Cs. Furthermore, estimations of the contributions from ^{40}K and ^{137}Cs involved considerations of several factors, including the weight of the subject and the effect of inhomogeneous distribution (Rundo, 1962), as well as the body content of the radionuclides. Lidén and McCall (1962) had made a careful study of the problem of measuring β-emitters in the body and had estimated the minimum detectable body activity of ^{90}Sr in their apparatus to be 20–25 nc., of which a considerable fraction was due to the uncertainty of estimating the contribution from ^{40}K and ^{137}Cs. It was clear that the error of estimating β-emitters in the body would increase as the ^{137}Cs from fall-out increased. Dr. Rundo said that at present body contents of ^{137}Cs from fall-out were as high as they had ever been and he had recently measured 70 nc. of ^{90}Sr in one person with an estimated standard error of ±30 per cent.

At the conclusion of the symposium Mr. Binks directed attention to a difficulty which faced all those who had to deal with assessments of internal radiation exposure. This was to decide in what circumstances and with what frequency measurements of body radioactivity should be attempted. Clearly measurements need not be carried out in those cases where the amount of radionuclides used was so small that inhalation or ingestion of the whole amount could not lead to significant amounts relative to the maximum permissible body burdens. At higher levels it was not possible to relate the internal hazard with the amount of radionuclide used since other factors, for example, the degree of containment, affected the situation.

It would seem that sample measurements of body radioactivity were necessary to establish the likely levels of uptake into the body. Where a need to carry out assessments of body radioactivity was established, the next question was the frequency of testing. Some of the papers presented during the symposium had indicated how some organizations had attempted to meet these difficulties. Any decisions reached about general policy would greatly affect the future national effort in assessing internal radiation exposures.

J. Vennart

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MELTING AND FREEZING*

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MANY present-day problems concerning melting and freezing can be regarded as structural and statistical developments of the Boltzmann expression for the entropy of fusion, S_f:

$$S_f = R \ln W_L / W_S$$

Some authors have postulated a critical melting limit, such that $S_f \rightarrow 0$, and there is then no discontinuous increase of entropy on passing from the solid to the liquid

* Substance of a lecture recently given before the Göttingen Akademie der Wissenschaften, Germany.

state. But notwithstanding theoretical considerations, S_f is, in fact, positive for all cases of melting so far investigated experimentally.

Statistical interpretation of this conclusion about a discontinuous increase of entropy on passing from solid to liquid needs to be linked with modern knowledge about the structure of the two phases. For crystalline states of matter, structural information is often detailed and extensive. On the other hand, our knowledge about the structure of melts tends to be much less specific. Indeed, one outcome of modern investigations of melting is to clarify concepts and conclusions about the structure of the liquid state, in any event near the freezing-point.

A particularly straightforward outcome of the Boltzmann equation is to recognize that the increase of entropy on melting involves some kind of increase of disorder. With crystals of inert gas atoms, the possibilities are reduced to their simplest form. Owing to the change in volume and other rearrangements of atomic positions on melting, we need to consider an increase of vibrational entropy associated with changes in vibrational frequencies. Added to this are the changes in entropy arising from positional disorder associated with movements of atoms to equilibrium positions not on the ideal lattice.

In this simplest case:

$$S_f = S_{\text{vib}} + S_{\text{pos}}$$

A well-known theory of melting due to Lennard Jones and Devonshire[1] postulates $S_{\text{vib}} = 0$ and calculates S_{pos} in terms of one simple type of interstitial positional defect. Aspects of this theory which are particularly suggestive for modern developments include the following:

(1) As the temperature of a crystal increases, melting is the culmination of a normal process of thermal positional disordering. A discontinuous jump of entropy and density on passing from crystal to melt arises because the energy ε required to create a thermal defect decreases as the fraction n/N of defect sites increases. This co-operative interaction between n/N and ε is allowed for in the Lennard Jones–Devonshire theory by assuming a Bragg Williams type of dependence. The melt is thus regarded as a highly defective version of the crystal lattice, which is conveniently termed 'quasi-crystalline'.

(2) At the melting-point, the solid already contains a finite concentration of the same type of defect, the multiplication of which results in the jump from crystal to melt. In the case of crystalline argon, for example, the particular parameters assumed by Lennard Jones and Devonshire lead to a fraction $n/N \sim 0{\cdot}05$ in the solid at T_f.

The general implication is that premonitory formation of thermal defects in the crystals preceding the jump in properties on passing from crystal to melt is a normal feature of positional disordering. The extent of such pre-melting proves to be highly sensitive to the values assumed for specific parameters such as repulsion potential energy. Pre-melting phenomena are thus likely to vary in prominence in different kinds of crystals.

(3) Models for positional disorder with co-operative inter-action between the energy of defect creation ε and the fraction of defect sites n/N can be formulated, which are less naïve than the Bragg Williams procedure. Dislocations can be treated as co-operative defects, such that at T_f the crystal contains only a small fraction, and the melt a fraction near to unity[2]. Mathematical aspects of such a model are less amenable to elegant formulation than the Bragg Williams co-operative interaction used by Lennard Jones and Devonshire. For example, in a model for dislocation melting of metals[3,4], the necessary approximations are considerably harsher. It is interesting to note that this particular model leads to the conclusion:

$$S_f = S_{\text{pos}}\ (1{\cdot}08\ \text{e.u.}) + S_{\text{vib}}\ (0{\cdot}69\ \text{e.u.})$$

Dislocation models for melting may become important for their thermodynamic consequences, such as the finite contribution from S_{vib}. They may also become important in pointing to possible ways of bringing together thermodynamic with mechanical theories about melting. Some of the transport properties of liquid crystals[5], for example, may require dislocation theory for their interpretation. The Lindemann theory of vibrational instability, and other theories of shear instability, must essentially be recognized as mechanical theories of melting. It seems likely, though I will not attempt to justify the statement here, that for most crystals:

$$T_f\ (\text{mech.}) > T_f\ (\text{thermodynamic})$$

On the other hand, crystals of certain globular molecules become plastic[6] below T_f; for these, possibly:

$$T_f\ (\text{mech.}) < T_f\ (\text{thermodynamic})$$

(4) Theories of the increase of positional disorder on melting are directly applicable when this is the only mechanism of disordering. Unfortunately, crystals of the inert gases which are the best examples constitute only a minute part of the enormous diversity of crystal structures. More complex mechanisms of disordering must be considered when the structure of the crystals is less simple than those of inert gases. One fairly simple extension is found with molecular crystals, in which the units of structure are quasi-spherical, as, for example, in the group CH_4, CF_4, CCl_4, SiH_4. In such crystals, the molecular axes are found to assume specific orientations at low temperatures. However, these orientations randomize in the crystals above a transition temperature T_c below the melting-point T_f. The disordering of molecular axes takes place with an uptake of entropy of orientational disorder S_{orient} frequently by way of a λ-transformation in the crystals. (Much interesting work on structural aspects of λ transformations is making use of investigations on single crystals. These emphasize the importance of domains in such co-operative changes[7a]; but since their connexion with melting is indirect they will not be detailed here). In the range between T_c and T_f crystals in which the units of structure have disordered molecular axes behave as though these units of structure are spherical or 'globular'. The Lennard Jones–Devonshire calculations about melting can be extended to cover such cases likewise[7b] so that a reasonable theoretical discussion can now be presented of mechanisms of melting represented by the possibilities:

$$S_f = S_{\text{vib}} + S_{\text{pos}} + S_{\text{orient}}$$

A great merit of models of disordering on melting, of the type discussed, is that they serve as a workable norm, which makes the discussion of even more complex mechanisms of melting much clearer. As a start, it may be noted that even with more complex mechanisms of melting, X-ray investigations show that the melts are always positionally disordered with respect to the crystals. Consideration of these more complex mechanisms can be based on the simplifying assumption that operations of disordering are separable, that is:

$$S_f = S_{\text{vib}} + S_{\text{pos}} + S_{\text{orient}} + S \ldots \text{additional mechanisms}$$

Additional Mechanisms of Melting

A characteristic feature of the theories just referred to is that the melt is treated as a highly defective crystal. The fact that the structure of the melt is referred to the crystal lattice warrants the description 'quasi crystalline'. In addition to their statistical theromodynamic significance, quasi crystalline models for melts are well known

and often used in their application to the calculation of transport properties of liquids. An increasing variety of melts is beginning to be recognized, however, for which a quasi-crystalline structure is grossly inadequate as a basis for calculation. Probably the longest known instance is the class of molecular crystals formed by flexible molecules, such as *n*-alkanes and their derivatives. When these flexible molecules are widely separated from one another, as in the dilute vapour phase, they assume various configurations of approximately equal energy, as a result of rotations about single C—C bonds. It used to be thought that stretched configurations were quite common even in dilute vapours, but actually collision processes in the gas suggest[8] that the molecules when widely separated from one another are predominantly crumpled, apparently so as to minimize Van der Waals potential energy terms. On passing from vapour to melt, the proportion of stretched configurations increases, but at all temperatures the liquid contains a mixture of forms. On passing from melt to crystal, in many cases a unique fully stretched configuration is selected for packing into the crystal lattice. The process of crystallization of the melt acts as a kind of molecular sieve. In such cases, an additional term appears in the summation of mechanisms of fusion which now reads:

$$S_f = S_{\text{vib}} + S_{\text{pos}} + S_{\text{orient}} + S_{\text{config}}$$

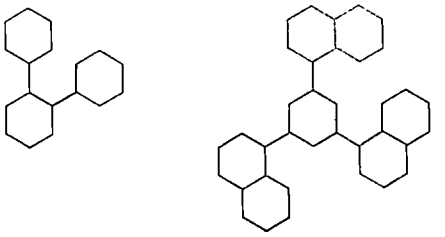
With large flexible molecules the number of configurational options can become very large, so that the increase of configurational entropy may swamp the more familiar mechanisms of fusion. This situation, which leads to convergence or limiting melting-points however large the molecule, is of great importance for the thermodynamic and mechanical properties of melts of thermo-plastic high polymers. Melting mechanisms of such solids will not, however, be detailed further here. From another aspect, these melts provide a clear instance of liquids the structure of which cannot even approximately be represented by reference to a crystal lattice. They are in fact anti-crystalline.

In discussing these and other types of melts that are definitely not quasi-crystalline it is sometimes convenient to regard them as generated from a hypothetical quasi-crystalline melt of the same molecules, which itself is formed from the crystals by one of the Lennard Jones operations already mentioned, that is:

$$\text{crystal} \xrightarrow{\text{(positional + orientational defects)}} \text{quasi-crystalline melt} \xrightarrow{\text{(configurational randomization of molecules)}} \text{Anti-crystalline melt}$$

Considerable thought and experimentation are being devoted at the present time to melts the structure of which departs progressively from a quasi-crystalline model, as they are cooled down to, and below, the freezing-point. Particular reference may be made to a number of different types of melts in which it seems likely that a fraction of the molecules pack into more or less transient clusters, of which the relaxation time for formation or dispersion is notably larger than the reciprocal of the hopping frequency for individual molecules in the quasi-crystalline melt. Reasons for such clustering can be quite diverse[9]. For example, in melts of H_2O hydrogen bonding appears to be the predominant cause. Melts of molecules with marked tendency to enmesh may form clusters even when only Van der Waals forces are operative. Particular attention to this simple geometrical possibility has been paid in some of our investigations on various polyphenyl molecules, including striking instances such as *o*-terphenyl (I) and *tri*-α-naphthylbenzene (II)[10].

Ionic melts with non-spherical anions such as silicates or nitrates provide further possibilities. In this very brief summary, I will only refer to some of the leading



I II

thermodynamic and structural considerations about consequences of cluster formation, regarded as a departure from quasi-crystalline structure of the melts. Essentially what is involved is the examination of the structure of melts in a region of temperatures on either side of and near the freezing-point T_f. As it happens, this is facilitated by the fact that when anti-crystalline features become prominent in the structure of a melt, it tends to supercool more readily. The group of problems to be discussed can conveniently be referred to as 'pre-freezing' effects.

Conventional X-ray diffraction investigations in their present stage are of little use for revealing marked fluctuations in packing density in a melt, since the data are computed in terms of a single smoothing parameter, the radial distance r starting from any one centre. On the other hand, in simple cases transport properties such as viscosity yield useful information. Experimentally, this property has the advantage that its measurement can often be extended into supercooled regions well below T_f. Theoretically, viscosity is a convenient property for investigating anomalies of liquid structures, since if the molecules aggregate in any special way in small regions in a melt the bulk of which is quasi-crystalline, these aggregates or clusters can be treated like colloidal particles. This is because they are compelled to move together in fluid-flow so long as the relaxation time for their formation or dispersion is large compared with the relaxation time for the bulk of the same melt which is effectively quasi-crystalline. Following a theorem originally due to Einstein, the presence of such particles blocks a fraction φ of the 'fluid' volume. Provided that $\varphi \leqslant 0{\cdot}3$ approximately:

$$\frac{\eta_{\text{actual}}}{\eta_{\text{ideal}}} = 1 + 2{\cdot}5\varphi + 7\varphi^2 \qquad (1)$$

when the particle shape is spherical. In applying this expression to investigate cluster formation in melts, the assumption made is to treat the fluid as quasi-crystalline, with values of η_{ideal} obeying an Arrhenius type of expression:

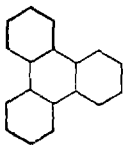
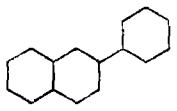
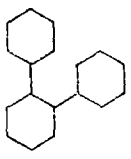
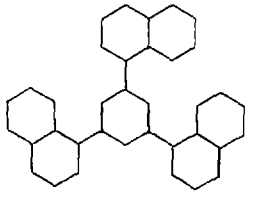
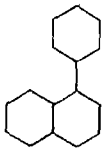
$$\eta_{\text{ideal}} = A + B \exp(-E\eta/T) \qquad (2)$$

which is in fact followed closely not too near T_f by very many melts.

This particular approach to the examination of clustering receives considerable empirical support from viscosity data for a wide range of polyphenyl liquids. For simple polyphenyls, such as benzene, diphenyl, *p*-terphenyl, triphenylene and fluorene, the straight-line Arrhenius expression is followed quite closely over an extensive range of temperatures. On the other hand, when the repulsion envelopes of polyphenyls have marked re-entrant portions, as in the case already qnoted, large excess viscosities become apparent as the melt is cooled down to T_f and below it. At T_f some typical values of φ_f in the melt at the freezing-point are given in Table 1.

Although viscosity is a property which can be widely applied in this way, other more discriminating methods for examining the marked fluctuations of molecular packing which may be present in a melt are obviously desirable to elucidate non-quasi-crystalline structure. In brief:

Table 1. VOLUME FRACTION φ_f BLOCKED FOR FLOW BY CLUSTERING AT T_f

Molecule	T_f° C	φ_f
Triphenylene 	198·1	Nil
2-phenyl-naphthalene 	—	0·038
o-terphenyl 	55·5	0·58
1-3-5 tri-*α*-naphthylbenzene 	197·3	0·40
1-phenyl naphthalene 	Glass	0·55 even at 25° C

(1) Other transport properties such as heat and mass diffusion have been much less investigated than viscosity, particularly in the pre-freezing region. Anomalies in thermal conductance have been reported for *n*-alkanes and also for *o*-terphenyl[11] near T_f.

(2) In favourable cases, optical methods can be used. For example, in melts of alkanes detailed investigation of the infra-red absorption spectra should reveal any trends in the proportions of various configurational isomers, on approaching and traversing T_f. Similarly for melts of pyridine and *o*-chlorophenol changes in the Rayleigh line 'wings' as the temperature approaches and traverses T_f (ref. 12) can probably be attributed to cluster formation. Again, the ultra-violet absorption band E_2 of melts of alkali nitrates normally shows a shift of the position of the band peak with volume, and thus with temperature, in a uniform linear trend. But in certain mixtures which remain molten down to sufficiently lower temperatures, accelerated shifts are found. The onset of these roughly coincides with the temperatures at which the viscosities of the melts begin to show marked enhancement above the linear dependence indicated by equation (2) and may be plausibly attributed to the formation of transient clusters of higher density. The effects are particularly striking in mixed melts of nitrates which behave as practically ideal binary systems with respect to molar volumes[13].

In conclusion, the role of pre-freezing anomalies in the statistical thermodynamics of melting may be briefly indicated. As is well known, packing of the molecular units of structure on a regular three-dimensional crystal lattice leads to a reduction in enthalpy of the magnitude H_f per mole. At or below T_f, this lowered enthalpy outweighs the higher entropy of the melt so that the free energy $G_S = H_S - TS_S$ of the crystal is lower than that $G_L = H_L - TS_L$ of the melt, and the crystal is the stable phase. Consideration of fluctuation theory leads to three refinements of this classical thermodynamic statement about melting.

(1) Even the crystal shows a proportion of defect sites in thermal equilibrium. The proportion n/N of such sites at T_f is large enough to become obvious as a pre-melting effect only for exceptional crystal structures.

(2) Micro-regions with crystalline arrangement can exist in equilibrium in the melt and constitute the so-called crystal nuclei. Their concentration and size are determined *inter alia* by the temperature and by the interface energy σ_{SL} between the looser packing of the quasi-crystalline melt, and the denser packing in the micro-crystalline region.

(3) Starting with any one unit of structure in the melt, and packing successive units as close to it as possible, will generally lead to reductions in enthalpy comparable with H_f in magnitude. But unless this packing is made on a regular crystal lattice as in (2), as the aggregate or cluster grows it soon becomes necessary to include clefts or voids. For this reason, normally the decrease in enthalpy falls off more or less steeply to zero as the size of an aggregate increases, except for crystalline lattice packing. The greater freedom of arrangement in a melt may, however, permit molecules of exceptional shape, or with exceptional types of intermolecular force fields to pack closely in a cluster that is definitely anti-crystalline. This may permit favourable reductions in enthalpy for aggregates considerably larger than, say, in melts of inert gas atoms. In such cases, fluctuation theory indicates that such clusters will be formed progressively as the melt is cooled. Formation of anti-crystalline clusters appears to be a common cause for marked pre-freezing anomalies in certain types of melts. Reference has already been made to favourable examples in the polyphenyls. Anomalous packings not represented in the crystal state are likewise encountered in ionic melts. A striking instance has recently been reported in mixed systems of thallium and potassium halides[14]. New methods of study are likely to reveal many other examples.

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CRYSTAL STRUCTURE OF HYDROXYAPATITE

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APATITES and related compounds are of fundamental importance in several areas, including the structural chemistry of hard tissue. Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) is the first compound to be examined in a programme which calls, in part, for detailed refinements of several of these materials.

The space group and unit cell dimensions are, respectively: $P6_3/m$ and $a = b = 9{\cdot}432$, $c = 6{\cdot}881$ as determined by Posner, Perloff and Diorio[1].

Neutron diffraction data were obtained at Oak Ridge National Laboratory on mineral hydroxyapatite mined in a Holly Springs, Georgia, foliated talc and serpentine quarry. The crystals were found on layers of foliated talc in serpentine as described by Mitchell, Faust, Hendricks and Reynolds[2]. Chemical analysis of a typical specimen gave the fluorine content as $0{\cdot}28 \pm 0{\cdot}01$ per cent by weight. The neutron data consist of 253 reflexions in the $hk0$ and hkl zones. The $|F|^2$ values are available on request. The X-ray data were those collected by Posner, Perloff and Diorio[1] on synthetic crystals with an integrating Weissenberg camera.

It was assumed that the previously determined structure[1] was correct except for the missing hydrogen atom. The overall structure of hydroxyapatite was discussed in ref. 1 and is indicated in Fig. 1 with the new results incorporated. Interest here centres on the OH's which occur in columns parallel to c. The main lines of the columns pass through the centres of Ca triangles which are on the mirror planes at $z = 1/4$ and $z = 3/4$. Successive triangles are rotated 60° about c. Fig. 2 illustrates both these features and some disorder features discussed later.

In a Fourier difference projection down the c axis the neutron data showed the hydrogen to be at $x = y = 0$. Line sections at $x = y = 0$ using X-ray and neutron data showed the oxygen to be displaced about 0·3 Å from the mirror plane at $z = 1/4$ (Fig. 1). This result leaves only 0·7 Å between half oxygens and implies a configuration in which only one of two possible positions is filled near each mirror plane. The close possible approach of the hydrogens ($< 0{\cdot}85$ Å) implies short-range ordering of the hydroxyls, a not unusual event for other groups (see, for example, the disordered structure of $NaNO_2$ (ref. 3)). Least-squares refinements on several models were carried out with the Busing, Martin and Levy programme[4]. Attempts to refine with no mirror plane ($P6_3$) ended in failure. By refinement on scale factors of hydrogen atoms it was shown that, within experimental error, none of the OH's straddles the mirror plane. The diffraction data thus lead to two models which are indistinguishable on the basis of these data alone: (1) in the 'disordered column'

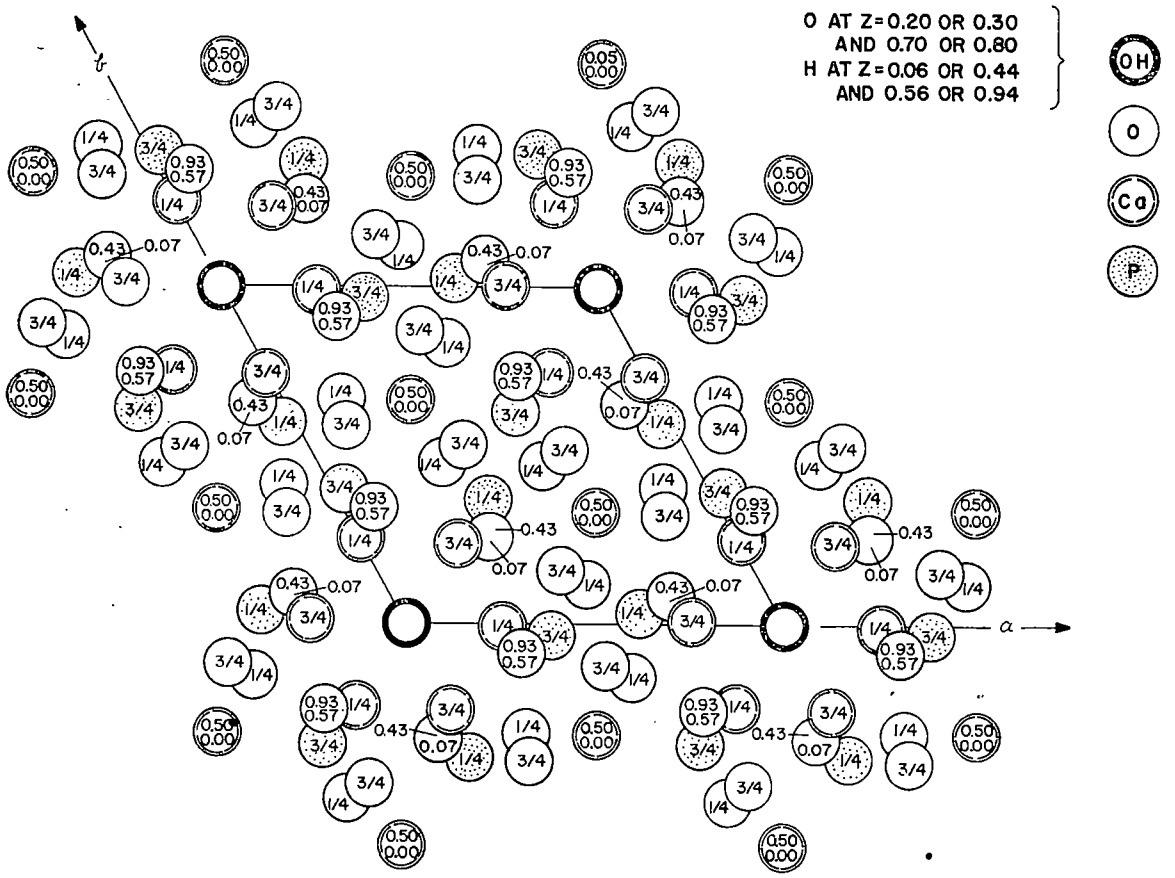


Fig. 1. Hydroxyapatite structure projected on x, y plane

model the orientation of the OH's is reversed at various places within a column; (2) in the 'ordered column' model all the OH's in a given column are oriented the same way, but the choice of orientation is random.

A refinement of the X-ray data gave results very similar to the neutron data. The old X-ray results (based on refinements with isotropic temperature factors), new X-ray results and neutron diffraction results are presented in Table 1. A diagram of the structure is presented in Fig. 1 and the close approaches are given in Table 2.

The final $R = \frac{\Sigma \, | \, |F_0| - |F_c| \, |}{\Sigma \, |F_0|} = 0{\cdot}075$ for the X-ray results and $R' = \frac{\Sigma \, | \, |F_0|^2 - |F_c|^2 \, |}{\Sigma \, |F_0|^2} = 0{\cdot}055$ for the neutron data after correction for extinction. The extinction correction used was the rough approximation:

$$|F_{corr}|^2 = |F_{obs}|^2 (1 - (5{\cdot}6 \times 10^{-6})I)$$

I is the number of counts in the peak. The correction was almost a factor of two for some reflexions. Thus there are some systematic errors in the neutron data. However, it is not expected that these errors affect positions by more than a few thousandths Å. The effects of extinction should be mitigated by the fact that, when the correction was large, the complete extinction correction was added to the standard deviation of the observed data in the least-squares refinement. Where the correction was moderate, one-half the correction was added to the standard deviation.

Correlations among parameters presented no difficulties; the largest correlation coefficient, that between β_{11} of the hydroxyl oxygen (O_H) and β_{11} of the hydrogen, was only 0·76.

It is interesting to note that, if refinement of the structure with an isotropic temperature factor and the X-ray data is carried out with the hydroxyl oxygen at 0·25 (that is, on the mirror plane), the ultimate agreement factor is the same as is obtained when the correct configuration is used; the positional error is suggested only by an unusually large β_{33} on the hydroxyl oxygen. The experimental evidence of the new, disordered positions for the hydroxyl oxygen, whether arising from ordered or disordered columns, is found partially in the X-ray difference map but more strongly in the neutron data. The latter give a hydrogen position that is consistent only with the new structure. This position of the hydrogen atom confirms Elliott's[6] polarized infra-red results indicating that the O—H bond direction is parallel to the c-axis.

The dimensions for the $PO_4^{\equiv}$ group are given in Table 2. Considering the standard deviations, the differences in P—O length are not considered significant, but the $PO_4^{\equiv}$ tetrahedron is probably slightly distorted.

In the discussion here, some conclusions depend on the thermal vibration parameters. While the value of these

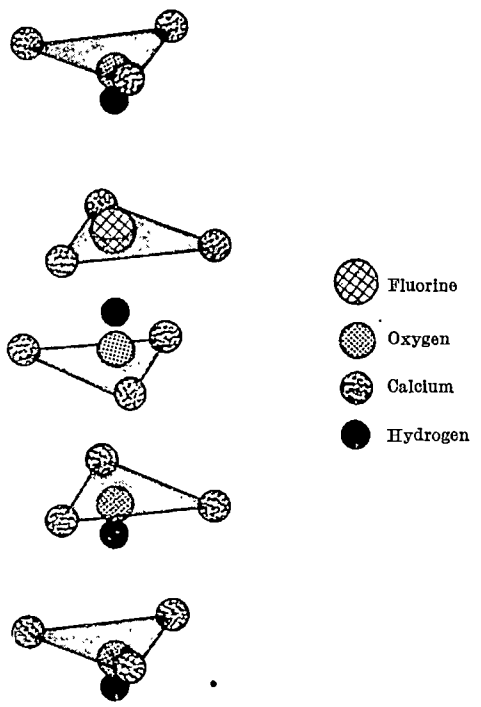


Fig. 2. Possible disordered arrangements

Table 1. Results of Refinements of X-ray and Neutron Diffraction Data*

Atom parameter	Old results (isotropic T. factors) value	New results (anisotropic T. factors) value	σ	Results with neutron data value	σ
Oxygen I					
x	0·329	0·3272	0·0012	0·3283	0·0002
y	0·484	0·4837	0·0011	0·4846	0·0002
z	0·250	1/4		1/4	
B	0·295				
β_{11}		0·0044	0·0012	0·0034	0·0002
β_{22}		0·0039	0·0012	0·0027	0·0002
β_{33}		0·0039	0·0020	0·0042	0·0002
β_{12}		0·0027	0·0010	0·0024	0·0001
Oxygen II					
x	0·589	0·5899	0·0012	0·5876	0·0002
y	0·466	0·4666	0·0012	0·4652	0·0002
z	0·250	1/4		1/4	
B	0·496				
β_{11}		0·0040	0·0013	0·0020	0·0002
β_{22}		0·0039	0·0012	0·0024	0·0002
β_{33}		0·0138	0·0029	0·0086	0·0003
β_{12}		0·0026	0·0011	0·0009	0·0001
Oxygen III					
x	0·348	0·3457	0·0009	0·3433	0·0001
y	0·259	0·2595	0·0008	0·2579	0·0001
z	0·073	0·0736	0·0013	0·0705	0·0002
B	0·632				
β_{11}		0·0107	0·0011	0·0082	0·0001
β_{22}		0·0051	0·0010	0·0038	0·0001
β_{33}		0·0063	0·0017	0·0042	0·0002
β_{12}		0·0051	0·0010	0·0042	0·0001
β_{13}		−0·0057	0·0012	−0·0032	0·0002
β_{23}		−0·0046	0·0011	−0·0020	0·0001
Phosphorus					
x	0·400	0·3999	0·0003	0·3982	0·0002
y	0·369	0·3698	0·0003	0·3682	0·0002
z	0·250	1/4		1/4	
B	0·192				
β_{11}		0·0021	0·0005	0·0017	0·0001
β_{22}		0·0012	0·0004	0·0020	0·0002
β_{33}		0·0020	0·0007	0·0024	0·0002
β_{12}		0·0006	0·0003	0·0009	0·0001
Calcium I					
x	0·333	1/3		1/3	
y	0·667	2/3		2/3	
z	0·001	0·0010	0·0005	0·0014	0·0004
B	0·666				
β_{11}		0·0038	0·0003	0·0036	0·0001
β_{22}		0·0038		0·0036	
β_{33}		0·0038	0·0007	0·0027	0·0002
β_{12}		0·0019		0·0018	
Calcium II					
x	0·246	0·2464	0·0003	0·2466	0·0002
y	0·993	0·9938	0·0003	0·9931	0·0002
z	0·250	1/4		1/4	
B	0·328				
β_{11}		0·0029	0·0004	0·0026	0·0002
β_{22}		0·0022	0·0004	0·0027	0·0002
β_{33}		0·0038	0·0006	0·0034	0·0002
β_{12}		0·0012	0·0003	0·0012	0·0001
Hydroxyl oxygen (O_H)					
x	0·000	0		0	
y	0·000	0		0	
z	0·250	0·1930	0·0046	0·2008	0·0007
B	0·875				
β_{11}		0·0019	0·0013	0·0028	0·0002
β_{22}		0·0019		0·0028	
β_{33}		0·0300	0·0125	0·0121	0·0008
β_{12}		0·0010		0·0014	
Hydrogen					
x				0	
y				0	
z				0·0617	0·0015
β_{11}				0·0147	0·0008
β_{22}				0·0147	
β_{33}				0·0110	0·0014
β_{13}				0·0074	

* The positions given are in space group $P6_3/m$, number 176 in the *International Tables*[5]. O_{III} is in the 12-fold general position (i); O_I, O_{II}, P, Ca_{II} are in the 6-fold special position (h); Ca_I is in the 4-fold special position (f); the hydroxyl group (O_H and H) is in the 4-fold special position (e) in 2-fold disorder as depicted in Fig. 1.

Table 2. Close Approaches and Angles with Standard Deviations* as calculated from Neutron Diffraction Results

Atoms involved		Distance or angle	σ
Phosphate tetrahedron			
P—O_I	(Note 1)	1·538 (1·542) Å	0·008 Å
P—O_{II}		1·547 (1·552)	0·008
P—O_{III}	(Note 2)	1·529 (1·534)	0·006
O_{III}—P—$O_{III'}$	(Note 3)	107·75°	0·5°
O_I—P—O_{II}		111·0°	0·15°
O_I-P-O_{III}		111·5°	0·3°
O_{II}—P—O_{III}		107·6°	0·25°
O—H Group			
O_{II}—H		0·957 (0·999) Å	0·013 Å
Ca_{II}—O_{II}		2·384	0·012
Ca_{II}—H		2·692	0·012
Other approaches			
Ca_I—O_I	(Note 2)	2·408	0·009
Ca_I—O_{II}	(Note 2)	2·454	0·011
Ca_I—O_{III}	(Note 2)	2·808	0·014
Ca_{II}—O_I		2·707	0·014
Ca_{II}—O_{II}		2·358	0·012
Ca_{II}—O_{III}	(Note 2)	2·345	0·010
Ca_{II}—O_{III}	(Note 2)	2·514	0·011

* A standard deviation of 0·5 per cent in cell edge was assumed and probably overstates the error.
Note 1—If the lighter atom 'rides' on the heavier, the result is given in parentheses.
Note 2—Lengths repeated by symmetry.
Note 3—O_{III} is related to $O_{III'}$ by a mirror plane at $z = 1/4$.

may be somewhat in doubt, because of the over-simplified extinction correction and other systematic errors, the general trend of them is probably correct and their expected errors should not alter the conclusions. As evidence, compare the X-ray and neutron thermal parameters in OH.

The O—H distance was corrected[7] for thermal vibration on the assumption that the H 'rides' on the O. The result of 0·999 Å may be slightly large, but it is barely one standard deviation larger than the 0·984 obtained in $Ca(OH)_2$ by Busing and Levy[8].

The most significant results are found in the geometry of the O—H group, determined by means of the neutron diffraction data, and in the relationship of that group to its surroundings. The O of the O—H groups is located about 0·3 Å (on the average) above or below the triangle of Ca_{II}^{++} on the mirror plane at $z = 1/4$ and again at $z = 3/4$. The geometry is approximately similar to that found in $Ca(OH)_2$, but each side of this Ca_{II}^{++} triangle is 4·09 Å as compared to 3·59 Å in $Ca(OH)_2$. The Ca_{II}—O_H distance of 2·38 Å is, however, quite comparable to the Ca—O distance, 2·37 Å, in $Ca(OH)_2$. In other words, compared with the similar configuration in $Ca(OH)_2$, the Ca triangle in hydroxyapatite is pulled apart and the O—H drops closer to the Ca plane while essentially the same Ca—O distance is maintained. It will be noticed from the root mean square thermal vibration amplitudes of the hydroxyl group in the c direction, 0·18 Å (0·17 for O and 0·19 for H), that the OH group is able to move rather freely along the c-axis. This freedom would be expected as, being nearly perpendicular to the Ca_{II}—O_H direction, such motion may become relatively large without appreciably changing the Ca_{II}—O_H length. Perpendicular to the c-axis, however, the root mean square amplitude of thermal motion is 0·10 Å for O_H but 0·22 Å for H. Large rocking motion of the H about the O or the centre of mass of the system is thus indicated. Such large librational motion is consistent with the generally open nature of the structure in the neighbourhood of the H.

In the disordered column model a special structural circumstance may occur at one-half of the points where the direction of the O—H group is reversed. Fig. 2 indicates some of the possibilities. If OH groups were to extend toward each other from the average structure mirror planes at $z = 1/4$ and 3/4, the H's would be too close together. Thus it would appear that an OH should be missing at each such point. Such a site could either be vacant or contain a fluorine ion, F. Some fluorine has always been found in mineral hydroxyapatite. An F at the centre of the Ca triangle might be expected to have at least a slight attraction for the H of neighbouring OH's. Thus the presence of the F impurity might even be expected to produce disorder within the column and would, at the least, accommodate it. Because this disordered column model seems to provide such a convenient structural location and function for the fluorine impurity, one is tempted to prefer the disordered column over the ordered column model. From similar reasoning for the case of the fluorine-free synthetic apatite (for which only X-ray data are now available), it would seem that either vacancies must exist at these reversal points or the ordered column model should apply. Present data are inadequate for the determination of structural detail as fine as a reversal mechanism which need operate only in a small percentage of the unit cells, not all. Indeed, probably undetectable with present data, and, therefore, not yet to be ruled out, are conceivable reversal mechanisms involving: (1) an occasional O—H placed across the pseudo-mirror plane, or (2) an occasional O—H pair pointing generally toward each other from adjacent pseudo-mirror planes, but making a substantial angle with the c-axis in order that the H's do not approach each other too closely. Further neutron diffraction investigations relative to this structural detail are to be undertaken. Infra-red investigations now being done by ourselves and others[9] on details of the O—H stretching region of the spectrum may also illuminate this point.

It is felt that, in contrast to disordered OH in hydroxyapatite, the F in fluorapatite is probably at $z = 1/4$, where present structure determinations[10] place it. (The crystal structure of fluorapatite is at present under investigation with precision X-ray techniques.) This structural difference may be the principal cause of the increased relative stability of fluorapatite, particularly if the disordered column, rather than the ordered column, model is the correct one. In biological apatites, significance of this stability difference has been shown recently by *in vivo* bone apatite growth-rate investigations[11,12]. There, fluoride-containing bone apatite grew more rapidly and to larger crystallite size than did the fluoride-free specimens.

In summary, the crystal structure of hydroxyapatite has been determined from X-ray and neutron diffraction data. The result agrees generally with the earlier determination by Posner, Perloff and Diorio[1]. However, the O—H groups are disordered about the mirror planes at $z = 1/4$ and 3/4 with the oxygens about 0·3 Å from the mirror planes. The O—H direction always points away from the mirror plane. It is assumed that this feature may be correlated with the relative instability of hydroxyapatite as compared to fluorapatite.

We thank Drs. H. A. Levy and W. R. Busing, who placed their equipment and time at our disposal for the collection and reduction of the neutron data, and the Oak Ridge National Laboratories. We also thank Ephraim Segerman for advice concerning the ordered column model, Mr. Alan Friedman for help in carrying out some of the calculations, Mrs. Charlene Lampkin of Lockheed-Georgia Corporation for running refinements on the IBM 7094 computer, and Prof. W. E. Moody for obtaining the crystal.

This work was supported in part by U.S. Public Health Service grant *DE*–01912–01 from the National Institute of Dental Research. The chemical analyses were provided through Public Health Service grant *DE*–01945–01.

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THE LIMESTONE ASSIMILATION HYPOTHESIS

IN a recent communication describing the synthesis of feldspathoids by feldspar-carbonate reactions, R. D. Schuiling[1] discussed the limestone-syntexis hypothesis, which proposes that rock-types under-saturated in silica can form from silica-over-saturated magmas by assimilation of limestone, and he stated that, from a physico-chemcial point of view, there was no essential difference between this process and one involving the assimilation of sialic material by a carbonatite magma. He remarked that: "Nobody, however, seems to have tried to verify experimentally such proposed processes". The purposes of this communication are: (1) to direct attention to an extensive series of experiments by Stansfield[2] which were aimed directly at testing assimilation hypotheses, and which included investigation of the extent and products of assimilation of limestone by various igneous melts; (2) to outline some of our own experiments[3,4] which are concerned with the origin of carbonatites and associated alkalic rocks and, inevitably therefore, with the limestone assimilation hypothesis as well; (3) to comment on some points of Schuiling's communication.

Stansfield[5] advanced field and chemical evidence to support his conclusion that the okaite at St. Joseph du Lac, Quebec, had originated by assimilation of Grenville limestone by a magma of unknown type (possibly peridotite), and he suggested that, in general, rock magmas which yield melilite and monticellite on crystallization have dissolved considerable amounts of limestone. He later[2] conducted a series of fusion experiments in which ten different igneous rocks were made to assimilate materials representing pure sediments. The igneous rocks varied in composition from granite to peridotite, and the sedimentary materials were calcite, kaolin, and glass sand. The igneous rocks and calcite were mixed in various proportions, and the mixtures were fused in 57 experiments at temperatures ranging from 1,270° C to 1,470° C. Most of the products of the experimental assimilation of calcite contained glass, and all were characterized by melilites. No feldspathoids were developed. The experiments thus support the hypothesis that melilitic rocks may be derived by limestone assimilation, but provide no evidence for the hypothesis that feldspathoidal magmas or rocks can be produced in the same way. Stansfield concluded tentatively that 10 or 15 per cent may be the limit of foreign rock matter which can be assimilated by natural magmas.

The frequent association of alkalic igneous rocks and carbonate rocks is thoroughly documented, but the present status of the limestone assimilation hypothesis is somewhat more uncertain than it was when Shand[6] stated the case in 1946. This is due in no small measure to the accumulation of evidence confirming that most carbonatites have characteristics distinguishing them from sedimentary, metamorphic, or xenolithic limestones[7-9]. The discovery of Wyllie and Tuttle[10] that relatively low temperature melts in the system CaO—CO_2—H_2O are capable of precipitating calcite through a wide pressure range has provided experimental verification for the magmatic origin of carbonatites where the field evidence is consistent with such an origin. Following these results, our experimental work has been extended into a number of carbonate–silicate systems aimed at elucidating the relationships between carbonate rocks and the associated alkalic igneous rocks. One approach adopted has been to add mineral molecules such as nepheline, albite, and orthoclase to the synthetic carbonatite magmas with compositions on the join $CaCO_3$—$Ca(OH)_2$. At the other end of the composition joins so obtained, we have information about the effect of adding $CaCO_3$, $Ca(OH)_2$, or mixtures of these components, on the melting relationships of nepheline, albite, and orthoclase, respectively, in the presence of water vapour under pressure.

These experiments confirm that less than 5 weight per cent of the silicate molecules dissolve in the synthetic carbonatite magma at 1 kilobar pressure, before liquidus temperatures rise steeply from the low-temperature part of the liquidus[3], with melilite being a prominent crystalline phase. The wide stability range of melilite, in terms of both compositions of mixtures and of temperature, is a notable feature of the systems[4]. At the other ends of the composition joins, the sequence of phase assemblages produced by adding CaO-rich material to the feldspathic melts indicates that desilication does occur. The assemblage albite + liquid is first replaced by wollastonite + plagioclase + liquid, and further addition of lime then stabilizes nepheline, but not in the presence of liquid. More CaO-rich subsolidus assemblages contain the silica-undersaturated phases melilite, rankinite and larnite. Phase assemblages involving orthoclase are replaced initially by assemblages with leucite and then kalsilite. Relationships in the nepheline join are complicated by the formation of phases with the cancrinite and nosclite structures.

The work completed in the feldspar composition joins shows that they are divided into three parts. At the feldspathic ends of the joins, solidus temperatures in the presence of water vapour under pressure are moderate, and at the synthetic carbonatite ends of the joins, melting temperatures are even lower. In the central portions of the joins, however, melting temperatures are higher than at either end of the join. This means that addition of CaO-rich material to a feldspathic liquid causes crystallization of the liquid before desilication proceeds very far, and development of feldspathoids results from sub-solidus reaction.

We are dealing with sections through a six-component system, and the actual phase assemblages encountered are complex. The phases present were identified by optical methods, and by X-ray powder diffraction patterns. The petrographic microscope is an indispensable instrument in phase equilibrium investigations, and we have found it unwise to rely on X-ray diffraction patterns for the recognition of phases.

Schuiling's experiments[1] were conducted at 1,000° C at 1 atmosphere, and ours were conducted at temperatures between 550° C and 950° C at 1 kilobar pressure, with 25 weight per cent water added to the mixtures[3,4]. Schuiling reported that his reactions "proceed rather rapidly", whereas our subsolidus reaction rates were slow, and we were not confident that equilibrium had been achieved in some mixtures even after runs of 3 weeks' duration. The difference may be due in part to our lower temperature range, but probably a more important factor is the difference between the two experimental approaches. $CaCO_3$ dissociates at 894° C at atmospheric pressure, and it is therefore likely that in Schuiling's experiments the calcite or dolomite dissociated while the mixtures were being heated to 1,000° C. The reactions which then occurred during the 25 h of the experiments were reactions between the feldspars and oxides rather than the listed reactions between feldspars and carbonates. In order to investigate the reaction between feldspar and calcite at 1 atmosphere pressure, the mixture should be heated at a temperature of less than 894° C. In our closed-system experiments, the carbon dioxide released during the reactions mixed with the water vapour and remained in the system, and the vapour, or gaseous phase, was an essential phase in each reaction. This is a significant

difference between the two experimental approaches, because we have found that in systems containing a vapour phase composed essentially of water and carbon dioxide, the nature of reactions may be controlled as much by variations in the composition of the vapour phase as by variations in temperature and pressure[4]. One example is relevant to the process of limestone assimilation.

Wyllie and Tuttle[11] have shown experimentally that carbon dioxide is only slightly soluble in feldspathic and granitic liquids. They showed further that the effect of adding carbon dioxide to hydrated feldspathic or granitic liquids is to extract the water from the liquid, thus raising its solidus temperature considerably and causing crystallization by a quenching process. The process of limestone assimilation necessarily involves the release of large quantities of carbon dioxide and the crystallization so induced in a granitic magma would offset the tendency of the assimilation process to yield a desilicated magma.

Thus, for two different reasons based on two different sets of experiments, it appears to us that assimilation of limestone by a granitic magma would lead to crystallization of the magma, with the concomitant development of some lime silicates (wollastonite in our experiments) and the formation of slightly undersilicated rocks only locally, in the vicinity of the limestone undergoing assimilation. There is petrological evidence that such processes do occur on a local scale. A third serious problem facing the syntexis hypothesis concerns the availability of heat for the assimilation process. This has been discussed at length by Bowen[13], and it appears that this heat must be supplied by crystallization of the magma.

In discussing the limestone assimilation hypothesis there is an important distinction to be drawn between the formation of an under-silicated, alkalic magma, and the formation of an under-silicated, alkalic rock from a magma which is forced to crystallize as syntexis proceeds. We agree with Schuiling's conclusions[1] that the production of leucite and nepheline from feldspars by reaction with carbonates is an experimentally proved possibility. The process of desilication of a magma, however, is limited by the three factors which have been discussed here, and we believe, therefore, that the development of an alkalic magma by limestone syntexis is unlikely. The formation of alkalic, silica-under-saturated rocks in reaction zones at limestone contacts with silica-saturated igneous rocks is to be expected. Continued sub-solidus reaction between contiguous limestone and igneous rock could produce more feldspathoids in the igneous rock, but there is no evidence known to us that sub-solidus reactions of this kind occur extensively in nature.

After a detailed review of the literature it appears to us that there is little convincing evidence to support the view that limestone assimilation has led to the formation of alkalic magmas, or even of alkalic igneous rocks except as contact phenomena. As Schuiling has remarked, the regional association of alkalic rock-types and carbonatites is indeed suggestive, but reasonable attempts have been made to explain this association by many processes other than that of assimilation of sialic material by carbonatite magma or that of assimilation of limestone by magmas[7–9,14]. In the limestone assimilation hypothesis attention is directed to addition of CaO-rich material and desilication of the magma. In the associations involving carbonatites, on the other hand, there is evidence suggesting that, for the development of some alkalic rocks, migration of alkalis may be as significant as desilication processes.

There are so many processes possible on paper which could conceivably account for the petrology and field relations of the associations of alkalic igneous rocks and carbonate rocks that the need for experimental testing of the various hypotheses is urgent. We conclude that evidence from our high-pressure phase equilibrium investigations weighs against the limestone assimilation hypothesis, but this conclusion may have to be modified in the light of future results, and by more detailed investigations by Schuiling on specific reactions performed at atmospheric pressure.

This work was supported by U.S. National Science Foundation grants *G* 19588 and *GP* 1870.

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Several years ago a young geologist in a lecture at a congress proposed a theory highly differing from earlier views expressed by Eskola. A member of the audience wished to defend these earlier views and tried to enlist the help of Prof. Eskola, who stoically remarked: "I proposed this theory fifty years ago, and it is about time to have a new theory instead". Watkinson and Wyllie, in their comments on one point of my communication[1], seem to feel the same with regard to the limestone assimilation hypothesis, which has reached about the same age, and which is, according to them, only "one of the many processes possible on paper which could conceivably account for the petrology and field relations of the associations of alkalic igneous rocks and carbonate rocks". They thereby reduce the only theory which really has been demonstrated in the field with a degree of certainty, scarcely ever achieved by petrological observation, to the common level of guesses, unsupported by facts.

I am grateful for their remarks about Stansfield's experiments, which had escaped my attention, and which on the whole seem to have received only scant or no attention at all by such proponents of the limestone–syntexis theory as Daly and Shand.

Stansfield's experiments[2] at temperatures between 1,270° and 1,470° C with mixtures of igneous rocks and limestone produced melilite-bearing assemblages and are accepted by Watkinson and Wyllie as evidence that melilitic rocks may be derived by limestone assimilation. It would seem logical then if they would accept my experiments at 1,000° C (geologically speaking, a more acceptable temperature) with mixtures of, admittedly simplified, igneous rocks and carbonates producing feldspathoid-bearing assemblages as supporting evidence that feldspathoidal rocks may also be derived by limestone assimilation. Instead, they try to show that, as the carbonates I used in my experiments are probably dissociated before any appreciable reaction occurs (the same applies *a fortiori* to Stansfield's experiments), there would be a significant difference in the reaction mechanism of the experiments of Watkinson and Wyllie and mine. As reaction mechanisms are not well understood anyhow, and as it is, also, not very clear in what way Nature works, a more fruitful approach is from the thermodynamical side. This has the additional advantage that we can state quantitatively what Watkinson and Wyllie state qualitatively by say-

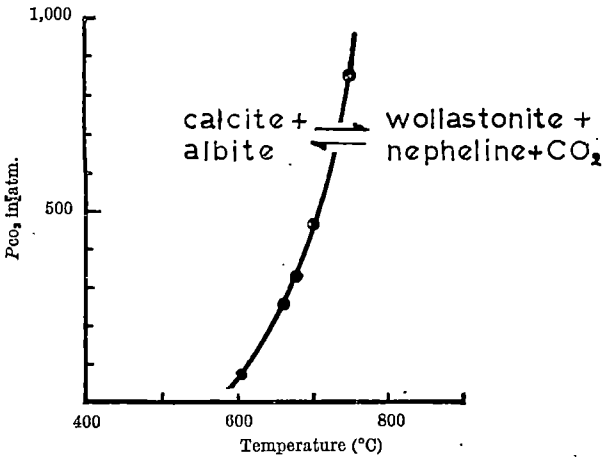


Fig. 1. Computed equilibrium curve for the reaction: calcite + albite ⇌ wollastonite + nepheline + CO_2 ↗

ing that "the nature of reactions may be controlled as much by variations in the composition of the vapour phase as by variations in temperature and pressure".

We have calculated the equilibrium curve of the reaction:

albite + calcite → nepheline + wollastonite + CO_2 ↗

by starting with the known curve for:

quartz + calcite → wollastonite + CO_2 ↗

and the value of $*\mu_d^5 = -2{,}500 - 0{\cdot}05\,T$ cal. for the reaction:

½ nepheline + quartz → ½ albite[3]

With the aid of these data and those for the fugacities of carbon dioxide at high pressures and temperatures[4], we can calculate the curve given in Fig. 1; any mixture of albite + calcite under conditions on the right-hand side of this curve will react to nepheline + wollastonite, independent of any supposed reaction mechanism and total composition of the vapour phase.

In fact, runs of three weeks produced the association:

nepheline + wollastonite

at 605° and 650° C at 1,000 atm. vapour pressure starting from mixtures of $Ca(OH)_2$ (portlandite) and albite; apparently the small, but not infinitely small, partial pressure of carbon dioxide (present in occluded air, solved in the water, and undoubtedly too in the $Ca(OH)_2$ at the moment the gold capsules were sealed) was not sufficiently high to prevent complete reaction. Analogous experiments with microcline + portlandite at 605° and 650° C at 1,000 atm. vapour pressure produced only small amounts of leucite + wollastonite at 605° C, but reaction was complete at 650° C. This is in agreement with the fact that $*\mu$ of the reaction:

quartz + leucite → potash feldspar

is significantly greater than the $*\mu$ of the corresponding reaction:

quartz + ½ nepheline → ½ albite,

which means that in carbonate–feldspar mixtures nepheline will form at lower temperatures than leucite, or at higher pressures of carbon dioxide at the same temperature. We may thus expect to find in Nature the association:

nepheline–sanidine–wollastonite

but not the chemically equivalent association:

leucite–albite–wollastonite[3]

The second line of reasoning of Watkinson and Wyllie against the concept of the limestone assimilation hypothesis consists in stating that the addition of carbon dioxide (which of necessity must be released on reaction with carbonates) results in the crystallization of the 'granitic' liquids by a quenching process. The word 'quenching' may be a somewhat unfortunate choice of term, as it is normally used only for rapid cooling; in their instance it is meant as "bringing about rapid crystallization by the addition of carbon dioxide", if I have understood these authors' use of the term correctly. Their argument gives rise to some speculation. From the pioneering experimental work of Wyllie and Tuttle[5–7] on the system $Ca(OH)_2$—$CaCO_3$—H_2O, petrologists have probably gained the impression that the composition of carbonatite magma would lie somewhere near the eutectic of portlandite and calcite, which is scarcely probable, as portlandite has never been found as a constituent of carbonatites; that it has never been found can be interpreted best by saying that carbonatites are probably high up on the calcite side of the join $Ca(OH)_2$—$CaCO_3$, which means that their melting temperatures are more probably of the order of 1,000° C instead of eutectic temperatures about 700° C, depending on the presence of impurities.

When such a carbonatite melt encounters water-rich sialic material, the addition of water will leave us with a magma which has acquired a large amount of superheat thanks to its new composition. We may thus say that Watkinson and Wyllie's argument works one way when applied to assimilation of limestone by sialic magma, and the other way when applied to Holmes's[8] hypothesis of assimilation of sialic material by carbonatite magma. If the system is open with respect to carbon dioxide, the argument becomes immaterial.

I see no reason why carbonatites and alkaline rocks should ever acquire their high explosiveness because of high pressure of carbon dioxide, unless such pressures are developed at the loci of magma-generation; it is not reasonable to assume that they should (against what is certainly a carbon dioxide pressure gradient), attract carbon dioxide from their surroundings. We see no other possibility but that they generate it themselves by the breakdown of carbonates; the equilibrium pressure of carbon dioxide for calcite at 1,000° C is only ~3 atm. (ref. 9); on the other hand, the equilibrium pressure of carbon dioxide for a reaction like calcite + albite → nepheline + wollastonite + carbon dioxide will be of the order of 10^4 atm. at the same temperature. Only by carbonate–silicate reactions (including the formation in melilite, monticellite, larnite, etc., mentioned by Watkinson and Wyllie) can we explain high pressures of carbon dioxide, and I am therefore still in favour of the limestone–syntexis theory, or its alternative, sial–syntexis by carbonatite magma.

This does not mean that I wish to exclude other petrogenetical processes, like alkali-transfer; I have repeatedly[10–12] invoked alkali-migration as a petrogenetical process.

I thank Mr. Vink for assistance in the computation of the equilibrium curve of Fig. 1.

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INACTIVATION OF CHYMOTRYPSIN IN AQUEOUS SOLUTION BY ATOMIC HYDROGEN

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A COMPARISON of the effect of heat, chemicals and ultra-violet light with that of ionizing radiations on the kinetic parameters of chymotrypsin indicated the varying mechanisms of different degrees of specificity by which these agents produce the inactivation observed[1]. Ionizing radiations act on solutes in dilute aqueous solution through labile reactive intermediates produced in the water and it is often difficult to determine which of the intermediates is responsible for a particular reaction. A recent extension[2] of the work of Butler and Robins[3] suggests that the mechanism of inactivation of trypsin in dilute solution by ionizing radiations is likely to include an effective process of reduction as well as oxidation. The lack or reversal of the oxygen effect on other enzyme systems[1,4–6] indicates that reductive processes may be involved in inactivation by ionizing radiations.

Recently a method was developed[7] for the quantitative study of the reactions of hydrogen atoms with acceptors in aqueous solution, using a high-frequency electrodeless discharge to dissociate pure hydrogen gas into hydrogen atoms and passing these, under carefully controlled conditions, into aqueous solution. It was shown that the reactive species is identical in its reactions to hydrogen atoms produced by ionizing radiations or by ultra-violet light. In this method, however, hydrogen atoms are the only reactive species present, so that their specific action may be examined separately[8].

The present work attempts to establish whether hydrogen atoms have a specific effect on chymotrypsin by examining the effects on the kinetic parameters of the enzyme. Data on spectroscopic changes were used to support the kinetic evidence.

α-Chymotrypsin, obtained from Worthington Biochemical Corporation, was a crystalline preparation from bovine pancreas, the substrate, *N*-acetyl-l-tyrosine ethyl ester (ATEE), was from British Drug Houses, Ltd., and all chemicals were of analytical reagent quality.

Solutions of the enzyme were prepared in triple distilled water and the *p*H adjusted to *p*H 3 with dilute hydrochloric acid and to *p*H 6 with sodium hydroxide. The protein concentration was determined on the basis of a molar extinction coefficient of $2{\cdot}07 \times 10^3$ at 280 mμ. The solutions were treated with atomic hydrogen by first passing hydrogen gas through the system to eliminate oxygen and then lighting the discharge when hydrogen atoms passed through the solution. Control solutions were treated similarly, but without lighting the discharge. The esterase activity of chymotrypsin was determined, using ATEE as substrate, by a continuous potentiometric titration method as described previously[1,9]. The Michaelis constant, *Km*, and the maximum velocity, *V*, were determined from plots of reciprocal reaction velocities against reciprocal substrate concentrations, *S*, giving a linear dependence: $\frac{1}{V} = \frac{Km}{V}\frac{1}{S} + \frac{1}{V}$ (ref. 10). The inter-

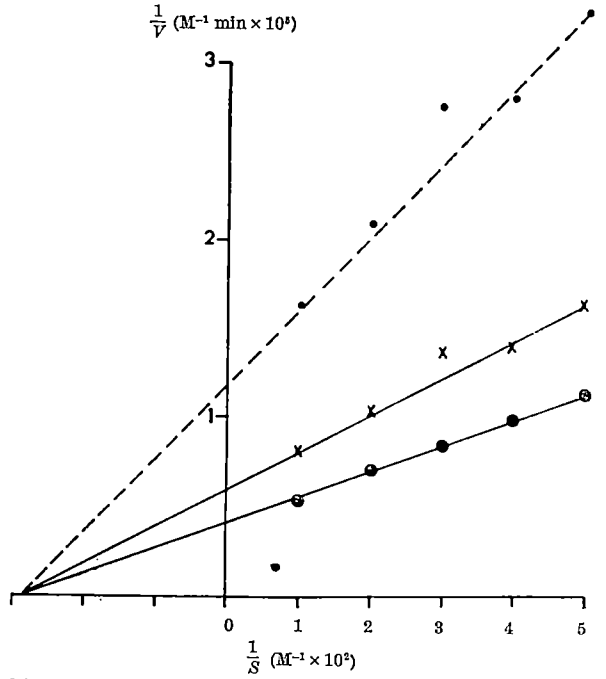


Fig. 1. Effect of hydrogen atom treatment of chymotrypsin in solution (2×10^{-5} M, *p*H 3) on the kinetic parameters, *Km* and *V*. Inactivation: 65 per cent. ● — ●, Control (enzyme diluted × 10 for assay); ×, hydrogen atom treated—dose 8×10^{-4} moles/l. (enzyme diluted × 5 for assay); ● - - - ●, hydrogen atom treated (corrected to enzyme dilution × 10)

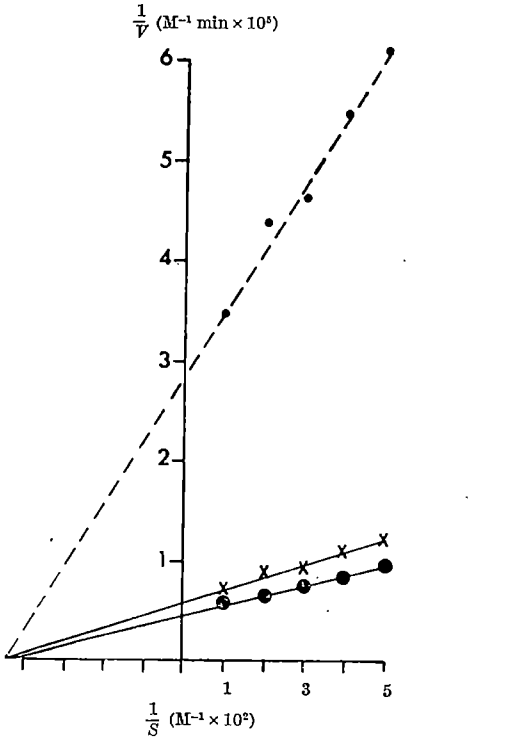


Fig. 2. Effect of hydrogen atom treatment of chymotrypsin in solution (2×10^{-5} M, *p*H 6) on the kinetic parameters, *Km* and *V*. Inactivation: 85 per cent. ● — ●, Control (enzyme diluted × 10 for assay); ×, hydrogen atom treated—dose 8×10^{-4} moles/l. (enzyme diluted × 2 for assay); ● - - - ●, hydrogen atom treated (corrected to enzyme dilution × 10)

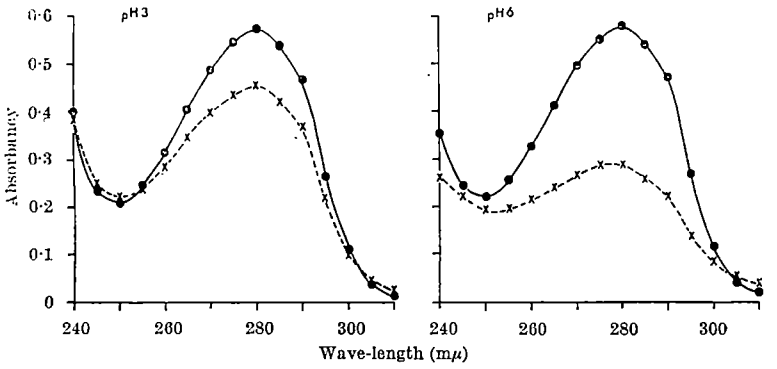


Fig. 3. Effect of hydrogen atom treatment of chymotrypsin in solution (2×10^{-5} M) on the absorption spectrum. ●, Control; ×, hydrogen atom treated—dose 8×10^{-4} moles/l.

cept on the vertical axis gives $1/V$ and on the horizontal axis $-\frac{1}{Km}$.

The Lineweaver–Burk[10] plots show that at *p*H 3 (Fig. 1) and *p*H 6 (Fig. 2) V is decreased considerably without change in Km, indicating that treatment of the enzyme with hydrogen atoms produces an all-or-none loss of activity. Fig. 3 shows the change in the absorption spectrum of chymotrypsin. The absorption peak at 280 mμ, which is contributed mainly by tryptophan residues in chymotrypsin, decreases considerably under the action of hydrogen atoms, but changes in other parts of the spectrum are relatively small. The decrease in activity and changes in the absorption spectrum are comparable with those obtained with trypsin at a similar concentration, *p*H and total dose of hydrogen atoms[11].

The inactivation of chymotrypsin by hydrogen atoms resembles the inactivation of the enzyme by heat, urea or 2537 Å ultra-violet light, which produce changes in V alone[1]. Ionizing radiations, longer wave-lengths of ultra-violet light[1] and Fenton's reagent[12] produce changes in Km, as well as in V, indicating the formation of partially damaged molecules, whereas the action of peroxides—peracetic acid[1] or hydrogen peroxide[12]—produces changes in Km without change in V. The radicals and molecular products resulting from the irradiation of water, which might be involved in the inactivation of chymotrypsin, include solvated electrons, e^-_{aq}, hydrogen atoms, hydrogen peroxide, as well as some molecular, non-reactive hydrogen. Initially, most of the reducing species are in the form of e^-_{aq}, which can be converted into hydrogen atoms at acid or neutral *p*H values; in the presence of oxygen, O_2^- and HO_2 radicals are formed from e^-_{aq} and hydrogen atoms depending on the *p*H. The reaction of e^-_{aq}, hydrogen and O_2^- with the enzyme may all result in reduction, HO_2, OH and H_2O_2 in oxidation. By virtue of their strong H—OH bond formation (~118 kcal/1 mol), OH radicals may attack and oxidize indiscriminately peripheral bonds, such as CH bonds, on the enzyme, modifying it without complete inactivation. Fenton's reagent, which produces OH radicals, acts similarly, only some of the reacting radicals producing complete inactivation: peroxides appear to cause mainly peripheral modification.

The results presented here are consistent with the assumption that hydrogen atoms, which are produced also in solutions irradiated with ionizing radiations, may specifically inactivate chymotrypsin, their reaction with it being more selective than that of the more reactive oxidizing OH radicals. Hydrogen atoms appear to attack specifically disulphide bonds in trypsin[11] and some evidence has been obtained for a similar effect in chymotrypsin.

The observations on the changes in the absorption spectrum of chymotrypsin support the conclusion of a selective attack by hydrogen atoms. The 280-mμ peak, which is contributed mainly by tryptophan residues in chymotrypsin, is decreased considerably, without significant changes in the absorption spectrum elsewhere. Compared with this selectivity, X-ray irradiation causes some decrease at 280 mμ, but increase at other wave-lengths[1], which may be accounted for by light scattering from aggregates formed[13] and result possibly from peripheral oxidative attack on other parts of the molecule.

For ionizing radiations, energy transfer within the protein molecule specifically to certain bonds, such as disulphide, has been postulated to account for efficient inactivation[14]. The present results and other recent work[15,16] indicate that this assumption may not be necessary and that the observed selectivity may be accounted for by specific reductive processes, mediated by radicals.

We thank Prof. J. S. Mitchell for having enabled us to carry out this co-operative work and for his advice. One of us (L. K. M.) was supported by a travel grant from the British Empire Cancer Campaign for Research, making it possible for her to accept the hospitality of the Department of Physical Chemistry of the Hebrew University of Jerusalem. This work was supported by the Biology Division, U.S. Atomic Energy Commission.

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RADIOACTIVE STRONTIUM CONTENT OF RAINFALL

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THE development of a simple procedure for the convenient determination of strontium in rainfall, the essence of which was the successful separation of strontium from barium by methods more simple than the usual ones, has already been reported[1].

The determination of radioactive barium is necessary for the estimation of the time of nuclear explosion, for directly after such an explosion the amount of barium-140 atoms present in the atmosphere is 1870 times that of strontium-90. This ratio changes constantly on account

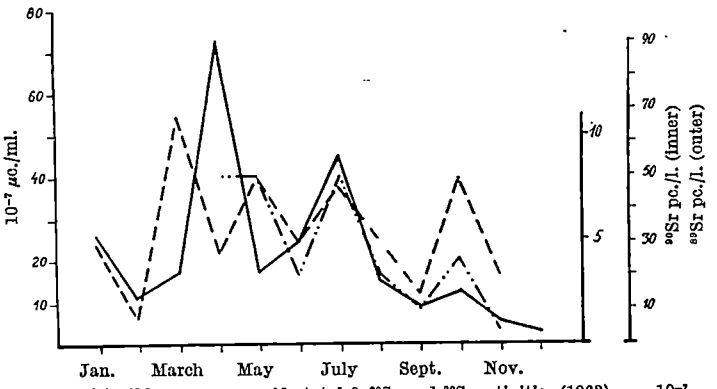


Fig. 1. Monthly average specific total-β, ^{89}Sr and ^{90}Sr activities (1963). —, 10^{-7} μc./ml.; — — —, ^{90}Sr pc./l.; — · · —, ^{89}Sr pc./l.

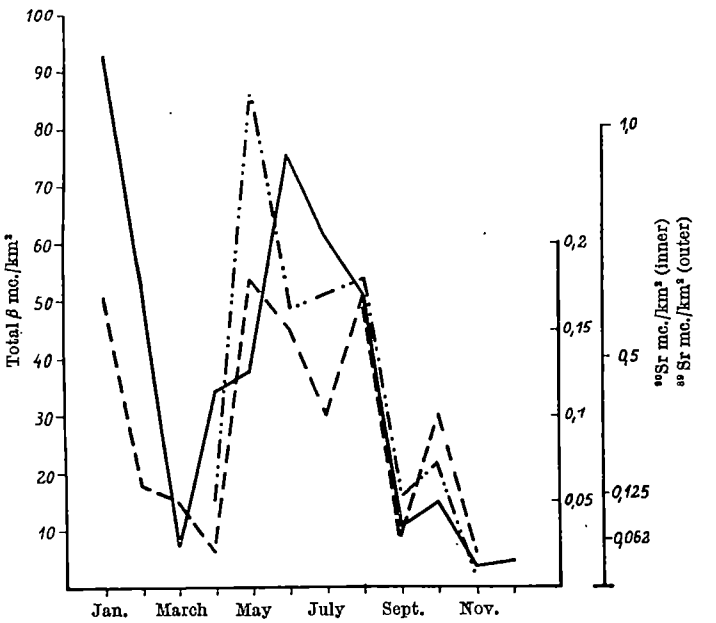


Fig. 2. Total-β, ^{89}Sr and ^{90}Sr activities, carried to the ground by rainfall (mc./km²) (1963). —, total β; — —, ^{90}Sr; — · · —, ^{89}Sr

of the different half-lives. The time of the explosion can be calculated from the ratio of both elements present in the atmosphere at the time of any measurement. The half-life of radioactive barium is 12·8 days; hence the determination of the quantity of this element would be of some value only soon after an explosion. However, if the aim of an investigation is to examine monthly average values—as it is in the case recorded here—then this possibility may be disregarded. So the course of separation by chemical means is substantially shortened by the omission of the separation of barium.

To 3 l. of filtered water is added some strontium carrier and evaporated to about 200 ml. For the elimination of foreign activities 5 mg Fe^{III} (as ferric chloride), and for the masking of calcium a calculated amount of ethylenediamine tetraacetic acid (EDTA), are added. The solution is made alkaline and the precipitated hydroxides are filtered off. Strontium is precipitated as carbonate. This precipitate is dissolved in hydrochloric acid, neutralized and precipitated in a weakly acetic acid medium as the oxalate. The precipitate is collected on a filter candle, washed and dried, and its weight and activity measured; the latter only after a period of about three weeks. This interval is necessary to make possible the recovery of radioactive equilibrium between strontium-90 and yttrium-90, which is, as a matter of course, disturbed by the process of chemical separation. For the improvement of radiochemical purity it is convenient to repeat the separation.

Since strontium-90 is always accompanied by some strontium-89 and yttrium-90, any measurements on strontium activity will give the sum of the three respective activities. For the individual measurement of the activities of single isotopes, yttrium should be separated from strontium to render possible determination of its activity. This activity represents also at the same time the activity of strontium-90, since with radioactive equilibria the activities of parent and daughter elements are equal. The activity of strontium-89 is calculated by difference.

The principle of the separation of yttrium is briefly as follows:

(1) Yttrium is separated from strontium most frequently by means of the differential solubility of the hydroxides of both elements. However, for this type of separation the oxalates must be decomposed first. Oxidation of the oxalates is effected by evaporation with concentrated nitric acid. It is convenient to repeat the separation via the hydroxides several times. Yttrium is separated at last from boiling N/10 nitric acid medium as the oxalate. The precipitates separating at different temperatures will contain different quantities of water of crystallization. The composition of the precipitate separating at 100° C corresponds to the formula: $Y_2(C_2O_4)_3 \cdot 2H_2O$ (ref. 2). In the given circumstances any traces of strontium present in the system do not precipitate.

(2) Attempts have been made to circumvent the evaporation of concentrated nitric acid by solving the task of separation on the basis of the differential solubilities of the oxalates, rather than that of hydroxides as is generally used. (Particulars of these experiments will be published elsewhere.) Yttrium oxalate separates from dilute nitric acid medium, whereas strontium remains dissolved. Separation was also found to be more conveniently achieved at 40° C, rather than at 100° C, since in the former case a precipitate composed of $Y_2(C_2O_4)_3 \cdot 10H_2O$ will separate.

Yttrium oxalate is collected as usual on a filter candle, washed with cold water, dried with alcohol and ether, and its activity measured. The activity values are plotted on semi-logarithmic paper as a function of time. The activity value extrapolated to zero supplies the activity at the time of separation. The activity values measured at different times will lie only on the same straight line, if the separated yttrium is radiochemically pure.

At the beginning of the year of these experiments procedure No. 1 was used; however, in the second half of the same year the more simple method No. 2 was applied for the separation of yttrium.

The results of measurements effected during January–December 1963 are summarized in Table 1. Besides the specific activities of strontium-89 and strontium-90, the

Table 1. Results of Measurements made during October 1962–December 1963

Time of sample collection	Monthly quantity of rainfall (l./m²)	^{89}Sr activity pc./l.	^{90}Sr	^{89}Sr/^{90}Sr	^{89}Sr activity mc./km²	^{90}Sr	Total β-activities 10^{-7}/ μc./ml.	mc./km²
Oct. 1962	5·00		4·2			0·02		
Nov. 1962	22·20		1·1			0·02		
Dec. 1962	9·30		3·2			0·03		
Jan. 1963	35·63		4·8			0·17	26	92·63
Feb. 1963	47·09		1·2			0·06	11	51·79
Mar. 1963	4·45		10·8			0·05	17	7·56
Apr. 1963	4·78	50	4·4	11·4	0·24	0·02	72	34·41
May 1963	22·02	50	8	6·2	1·10	0·18	17	37·44
June 1963	31·06	19·5	4·8	4·1	0·60	0·15	24	74·56
July 1963	13·53	48·4	7·6	6·4	0·65	0·10	45	60·88
Aug. 1963	33·87	20	5·2	4·0	0·68	0·17	15	50·80
Sept. 1963	12·54	9·6	2·4	4·0	0·12	0·03	8	10·03
Oct. 1963	12·08	24	8	3·0	0·29	0·10	12	14·50
Nov. 1963	6·22	3·9	3·2	1·2	0·02	0·02	5	3·11
Dec. 1963							2·5	3·89

quantity of monthly rainfall per square metre is given, as well as the amount of strontium-89 and strontium-90 expressed in mc./km². The ratio $^{89}Sr/^{90}Sr$ is also registered. This ratio can also be applied to some extent for the estimation of the time of an explosion; but it does not give such an exact result as does the ratio $^{140}Ba/^{90}Sr$, partly because the half-life of barium-140 is shorter ($T\frac{1}{2}$ = 12·8 days) than that of strontium-90 ($T\frac{1}{2}$ = 50·3 days), partly because the ratio $^{89}Sr/^{90}Sr$ amounts to 185 at the moment of an explosion; however, the ratio $^{140}Ba/^{90}Sr$ is as high as 1840. Activity of strontium-90 of stratospheric origin may also be misleading.

In Fig. 1 the monthly total specific β-activity values of atmospheric origin are summarized in units of 10^{-7} μc./ml. as well as the monthly specific activities of strontium-89 and strontium-90, respectively, in pc./l. From April onwards the curves follow similar courses, hence the strontium-89 content of the atmospheric rainfall changes in proportion with the total activities. It is striking that the strontium-89 and strontium-90 contents of rainfall in November are practically equal—a fact which can be attributed to the stopping, at that time, of nuclear explosions.

In Fig. 2 the sums of total specific β-activities as well as the monthly averages of strontium-89 and strontium-90 activities are compared. It may well be seen that the January rainfall practically washed out the atmosphere, and strontium-90 activity, as well as all other β-activities, which have been falling to the ground, are similarly high. The February rainfall brought scarcely any activity to the ground. The spring maximum presents itself in May, but for radioactive strontium it is far better expressed than for the total activities, and, in fact, the total β-activities carried to the ground with the rainfall in June are very much greater than those during the spring. The autumn minima present themselves markedly and can be attributed not only to the usual seasonal fluctuation, but also to the stopping of the nuclear weapon experiments. The relatively high October values must probably be regarded as a consequence of stratospheric penetration.

From November 1962 onwards, the radiostrontium content of atmospheric rainfall was checked with the method developed by us.

All investigations carried out by us so far show partly that only a very minute fraction of the activity of atmospheric rainfall is derived from both dangerous isotopes; partly it was generally experienced because in the first place the amount of strontium-89, but also a certain amount of strontium-90, changes proportionally to the total β-activity of the atmospheric rainfall. This partially supports the fact, well known from the literature, that the radioactive strontium content of fall-out is present chiefly in soluble form, namely, the measurement of total activity is carried out with shaken-up water samples rather than with samples which had been previously filtered, whereas strontium contents were determined out of filtered water. On the other hand, it is shown that, in the course of wide-range, present-day determinations, the estimation of total activities supplies practically reliable and satisfactory results.

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METABOLISM OF COUMARIN BY A MICRO-ORGANISM: O-COUMARIC ACID AS AN INTERMEDIATE BETWEEN COUMARIN AND MELILOTIC ACID

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IT has been shown in an earlier work[1] that a species of *Arthrobacter*, isolated from soil and utilizing coumarin as the sole carbon source, deposited l-tyrosine in the culture medium. In the course of an investigation attempting to find intermediates in this conversion, a highly specific enzyme, NADH : *o*-coumarate oxidoreductase, was partially purified from extracts of the organism[2]. This enzyme was found to catalyse the stoichiometric reduction of *o*-coumaric acid to melilotic acid with the concomitant oxidation of NADH[2]. The evidence linking both *o*-coumaric and melilotic acids to coumarin, however, was based primarily on the spectral changes coumarin underwent during enzymatic attack, and secondarily, on the high degree of specificity exhibited by NADH: *o*-coumarate oxidoreductase towards *o*-coumaric acid. This was so in spite of the fact that the organism from which the enzyme was obtained had been grown exclusively on coumarin. The purpose of the present work is to demonstrate the occurrence of both *o*-coumaric and melilotic acids in the culture medium of the organism, and by isotope dilution experiments to show the order of their appearance as actual degradation products of coumarin.

The conditions of culture and the means of gathering the organism have been described elsewhere[1,2]. Similarly, methods for the preparation of crude enzyme extracts, as well as the partial purification of NADH : *o*-coumarate oxidoreductase also have been described[2].

Two litres of culture medium taken from a 16-h old culture were reduced by vacuum distillation to 50 ml., filtered, and the filtrate acidified to *p*H 1. The phenolic constituents of the media were extracted 3 times with 50 ml. portions of diethyl ether, and the combined ether extracts brought to a small volume. This material was chromatographed overnight on Whatman 3 *MM* paper using 1-propanol–15 N NH_4OH (7:3) as developing solvent. When viewed under ultra-violet light (267 mμ), the dried chromatogram exhibited a number of fluorescent and quenching bands. Of particular interest were a yellow fluorescent band (R_F 0·42) and an absorbing or quenching one (R_F 0·70). Both bands were eluted from the paper with water and the fluorescent band was co-chromatographed with an authentic sample of *o*-coumaric acid in three different solvent systems, giving a single spot in each one (1-propanol–15 N NH_4OH (7 : 3), R_F 0·47; 2-propanol–15 N NH_4OH–water (8:1:1), R_F 0·35: methylethylketone-water–diethylamine (921:77:2), R_F 0·15). Similarly, the quenching band, when co-chromatographed in the above-mentioned solvent systems with authentic melilotic acid, gave a single spot in each case (1-propanol–15 N NH_4OH, R_F 0·70; 2-propanol–15 NH_4OH–water, R_F 0·60; methylethylketone–water–diethylamine, R_F 0·38).

The ultra-violet absorption spectra in acid and alkali of the yellow band were identical with those of *o*-coumaric acid in similar solvents. In a like manner, the ultra-violet spectra of the quenching band were identical with those of melilotic acid under acidic and alkaline conditions. From the foregoing, it is concluded that the yellow fluorescent band contained *o*-coumaric acid whereas the absorbing or quenching band contained melilotic acid.

Once having established that both *o*-coumaric and melilotic acids were intermediates in coumarin degradation, the sequence in which these compounds arose was determined by isotope dilution investigations. Crude

extracts of the organism were incubated with buffer and coumarin-^{3}H (Volk Radiochemical Co.) both in the presence and absence of NADH.

Both reaction mixtures were kept at 30° for 1 h, and at various time-intervals (see Tables 1 and 2). 1-ml. aliquots were withdrawn and placed in a 15-ml. centrifuge tube containing 0·2 ml. of 2 N HCl. The precipitated protein was removed by high-speed centrifugation and the clear supernatant solution extracted three times with equal volumes of diethyl ether. The combined ether extracts were evaporated to a small volume and applied on Whatman 3 *MM* paper. One μmole each of unlabelled *o*-coumaric and melilotic acids were applied over the ether extract and the mixture chromatographed overnight using 1-propanol–15 N NH_4OH as developing solvent. Both *o*-coumaric and melilotic acids were detected and eluted as described earlier. These compounds were then re-chromatographed in two additional solvents (2-propanol–15 N NH_4OH–H_2O and then methylethylketone–H_2O–diethylamine) to constant specific activity. The eluate removed from the last chromatogram of each compound was taken to dryness and the residue dissolved in 2 ml. of absolute methanol. An aliquot of this solution was counted in a Tri-Carb scintillation counter. In the absence of NADH, the activities found in each of the compounds (at the time intervals shown) are recorded in Table 1.

Table 1. Products of the Enzymatic Degradation of Coumarin-^{3}H in the Absence of NADH

Product	Incubation time (min)			
	10	20	30	60
	(c.p.m.)			
o-Coumaric acid	16,000	38,000	63,000	170,000
Melilotic acid	2,200	3,800	4,200	4,000

The reaction mixture consisted of 750 mμmoles of coumarin-^{3}H, 1·5 ml. of crude enzyme (15 mg protein) and 3·4 ml. of 0·05 M phosphate buffer, *p*H 7·3, in a total volume of 5·0 ml.

Approximately 12 per cent of the original activity administered as coumarin-^{3}H ($2·6 \times 10^6$ c.p.m.) was recovered in the combined activities of both acids. It is apparent, however, that only a small percentage of this was actually present in melilotic acid, the greatest amount being found in *o*-coumaric acid. Moreover, the data in Table 1 indicate that the reaction giving rise to melilotic acid was, in fact, finished at the end of 20 min, presumably because of the utilization of endogenous NADH. Undegraded coumarin accounted for some 40 per cent of the remainder of the original activity, and the rest was lost, probably by mechanical means, through three chromatographic runs. When NADH was included in the reaction mixture, the results (Table 2) were strikingly different.

Table 2. Products of the Enzymatic Degradation of Coumarin-^{3}H in the Presence of NADH

Product	Incubation time (min)			
	10	20	30	60
	(c.p.m.)			
o-Coumaric acid	46,000	90,000	140,000	250,000
Melilotic acid	50,000	150,000	320,000	580,000

The reaction mixture consisted of 750 mμmoles of coumarin-^{3}H, 1·5 μmoles of NADH, 1·5 ml. of crude enzyme (15 mg of protein) and 2·9 ml. of 0·05 M phosphate buffer, *p*H 7·3, in a total volume of 5·0 ml.

At the end of the first 10 min of incubation, both compounds were labelled to about the same extent, but 10 min later the activity in melilotic acid had surpassed that within *o*-coumaric acid. Finally, at the end of an hour, there was somewhat over twice the activity in the former compound as in the latter. It should be noted, moreover, that the counts within each of the acids was greater than in the previous experiment. In fact, the combined total activity within the two compounds accounted for about 60 per cent of the original activity. Another important difference between these two experiments is that with NADH added, coumarin had been completely utilized. This indicates that, with respect to the degradation of coumarin, the absence of NADH is rate-limiting. When boiled enzyme was used, either in the presence or absence of NADH, coumarin remained undegraded. The evidence presented above suggests that *o*-coumaric acid is one of the early products formed during coumarin degradation, and that it is the reduction of this compound in the presence of NADH which gives rise to melilotic acid. This view is further substantiated by the results of the next series of investigations summarized in Tables 3 and 4.

Table 3. Conversion of *o*-Coumaric-^{3}H Acid to Melilotic-^{3}H Acid

Enzyme	*o*-Coumaric-^{3}H acid (c.p.m./μmole)	Melilotic-^{3}H acid (c.p.m./μmole)
Crude extract	$2·8 \times 10^4$	$2·63 \times 10^4$
$NADH_2$–*o*-coumarate oxidoreductase (30-fold purified)	$2·8 \times 10^4$	$2·7 \times 10^4$

When crude extract was used, the reaction mixture consisted of the following; 2·1 μmoles of *o*-coumaric-^{3}H acid; 1·5 ml. (15 mg of enzyme protein) of enzyme; 4·0 μmoles of NADH and 5·5 ml. of 0·05 M phosphate buffer, *p*H 7·3. The entire mixture was contained in a total volume of 10 ml. In the second experiment (that is, with partially purified enzyme) all constituents of the reaction mixture were the same as in the first, except that 1·0 ml. of enzyme was used. Both reaction mixtures were incubated at 30° for 30 min and then the reactions stopped by the addition of 1 ml. of 6 N HCl. The protein was removed by centrifugation and the phenolic constituents of each reaction mixture were removed by ether extraction. The ether extracts were each chromatographed through 3 solvent systems (as described in the text) until melilotic acid reached constant specific activity.

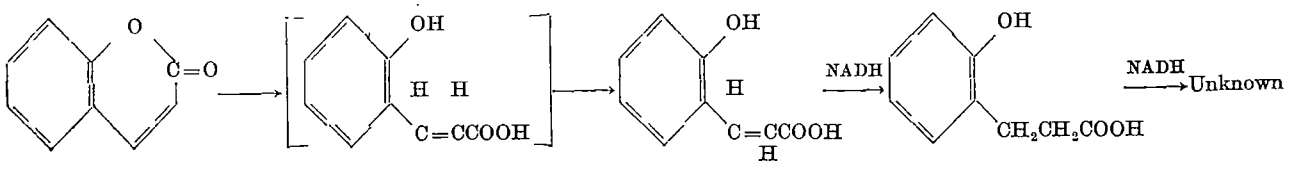
Table 4. Irreversibility of NADH–*o*-Coumarate Oxidoreductase with Melilotic Acid as Substrate

Enzyme	Melilotic acid (c.p.m./μmole)	*o*-Coumaric acid (c.p.m.)
Crude extract	$1·8 \times 10^4$	12
$NADH_2$–*o*-coumarate oxidoreductase (30-fold purified)	$1·8 \times 10^4$	55

The reaction mixture consisted of 1·5 μmoles of melilotic-^{3}H acid; 1·5 ml. of crude extract (15 mg of enzyme protein); 3·0 μmoles of NAD and 500 μmoles of phosphate buffer *p*H 7·3 in a total volume of 10 ml. In the partially purified system only 1·0 ml. of enzyme was used; all other constituents were the same. Both reaction vessels were incubated at 30° for 2 h and the reactions stopped by the addition of 1 ml. of 6 N HCl. The phenolic constituents of the reaction were extracted with ether. To each ether extract, 1 μmole of *o*-coumaric acid was added, and the mixtures chromatographed as described in the text. The *o*-coumaric acid was eluted from the last chromatogram with methanol and counted. The data in the table under *o*-coumaric acid represent counts above background.

In the first study (Table 3), *o*-coumaric acid of known specific activity was incubated either with crude extract or with thirty-fold purified NADH : *o*-coumarate oxidoreductase[2]. In both cases the specific activity of the melilotic acid recovered from the reaction mixture was the same as that of the substrate, *o*-coumaric acid. With both preparations, between 70 and 80 per cent of the *o*-coumaric acid present initially was utilized at the end of 30 min. Only with the crude preparation, however, were products (that is, quenching bands on the first chromatogram) other than melilotic acid seen. These were derived, presumably, from the further degradation of melilotic acid. When the reverse of this system was examined, that is, when melilotic acid of known specific activity was incubated with either crude enzyme or a partially purified preparation in the presence of NAD, 90 per cent of the melilotic acid could be recovered at the end of a 2-h incubation period. No trace of *o*-coumaric acid could be found. Nor on addition of unlabelled *o*-coumaric acid to ether extracts of the reaction mixture and chromatography afterwards could any significant counts above background be found in the *o*-coumaric acid present on the chromatogram (Table 4). The enzyme system, NADH : *o*-coumarate oxidoreductase, appears then to be an irreversible one, that is, melilotic acid in the presence of NAD and enzyme could not be oxidized to *o*-coumaric acid.

When, however, melilotic acid was incubated with crude extract and NADH, and the reaction followed spectrophotometrically at 340 mμ, NADH was found to be rapidly oxidized. Subsequent chromatography of the reaction mixture revealed that essentially all the melilotic acid had disappeared and that a new quenching or absorbing band was present. The compound within this band gave a positive test with Evans's reagent for dihydroxyphenols and had after elution about the same R_Fs in the solvents used by Coulson and Evans[3] reported for 2,3-dihydroxyphenylpropionic acid. The exact nature of this compound and the enzyme affecting its transformation from melilotic acid is presently now being investigated.



Coumarin *cis*-Coumarinic acid *o*-Coumaric acid Melilotic acid

Fig. 1. Scheme for degradation of coumarin

Melilotic acid, as a degradation product of coumarin, has been reported by a number of workers in a variety of biological systems[4–6]. It has been shown, for example, by Kosuge and Conn[4] that melilotic acid arose in the sweet clover as a result of the reduction of coumarin to dihydrocoumarin with the subsequent hydrolysis of this latter compound. In a microbial system, on the other hand, Fernley and Evans[5] felt that melilotic acid was derived from coumarin via *cis*-coumarinic acid although no enzymatic evidence for this transformation was presented. In the work recorded here, both *o*-coumaric and melilotic acids have been identified in the culture medium of an organism growing on coumarin. Further, by isotope dilution techniques, the labelling pattern of the two compounds obtained from enzymatic degradation of coumarin-^{3}H indicates quite clearly that *o*-coumaric acid is formed before melilotic acid and that, in fact, it is the reduction of the former compound which gives rise to melilotic acid. Earlier attempts to show the reverse of this reaction, that is the ability of the enzyme NADH–*o*-coumarate oxidoreductase to catalyse the oxidation of melilotic acid with the concomitant reduction of NAD, were unsuccessful[2]. These results were borne out when melilotic-^{3}H acid was used as a substrate for the enzyme. In the presence of NAD and enzyme, even after a 2-h incubation period, some 90 per cent of the melilotic-^{3}H acid could be recovered.

The results of these investigations suggest a tentative scheme (Fig. 1) for the degradation of coumarin. The first reaction would be, presumably, the hydrolytic cleavage between the oxygen and carbon atoms of the pyrone ring. Since coumarin is *cis*-coumarinic lactone, the product of this first reaction would be *cis*-coumarinic acid, a compound which, in fact, cannot exist as the free acid. Moreover, at neutrality, the partially purified NADH : *o*-coumarate oxidoreductase does not catalyse the reduction of coumarin[2], so that the ring opening reaction is probably accompanied by some kind of isomerization in which *cis*-coumarinic acid is transformed to its trans isomer, *o*-coumaric acid. This compound is reduced to melilotic acid in the presence of NADH: *o*-coumarate oxidoreductase and NADH. In turn, melilotic acid is converted by another enzyme, also utilizing NADH as a co-factor, to an as yet unknown compound.

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METABOLISM OF EHRLICH ASCITES CARCINOMA CELLS GROWN IN MICE ON A MEAT DIET

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IT has been reported[1] that mice kept on a diet of fresh or boiled meat eventually develop an anæmia. In young mice the anæmia is accompanied by depressed growth and osteoporosis[2]. The typical course of infection with *Plasmodium vinckei* is suppressed in mice kept on this diet[1]. In normal mice the cæcum is packed with *Trichomonas muris*, but in these anæmic animals the flagellates are scarce[3].

Our aim was to determine whether mice confined to a diet of meat for six weeks could support tumour growth. Eighteen such animals as well as eighteen controls from the same batch were injected intraperitoneally with Ehrlich ascites cells. Eight days later the mice were killed, the ascitic fluid collected and the volume of packed cells determined in each group. In the control mice the average volume of packed cells per mouse was 3·2 ml., whereas in mice on the meat diet the average volume was 1·7 ml.

In view of the foregoing finding, possible metabolic differences between Ehrlich ascites cells grown in mice fed meat, and cells derived from mice on a normal diet, were investigated. Respiration, glycolysis and fatty acid oxidation were not significantly different in the two types of Ehrlich ascites cells. The incorporation of compounds labelled with carbon-14 into proteins of the Ehrlich ascites cells was examined in the two types of cells (Table 1). The methods used for preparing and incubating the cells have been described previously[4]. The incorporations of carbon-14 labelled precursors into proteins and nucleic acids were made as described previously[5] using the extraction procedure of Schneider[6].

From the results in Table 1, it is apparent that there is a reduced incorporation of carbon-14 from formate into protein of cells grown in mice on a meat diet. This effect is peculiar to formate as it is not observed when the amino-acids glycine-1-^{14}C, methionine methyl-^{14}C or serine-U-^{14}C are added to the medium.

It is known that formate is incorporated into proteins as serine[7]. Since incorporation of carbon-14 from formate but not from serine is reduced in cells from mice on a meat diet, it seemed possible that serine synthesis was reduced in these cells. To measure serine synthesis the

Table 1. INCORPORATION OF ^{14}C FROM FORMATE-^{14}C, SERINE-U-^{14}C, METHIONINE-METHYL-^{14}C AND GLYCINE-1-^{14}C INTO PROTEIN

Radioactive precursors added	Cells from mice on normal diet. c.p.m./mg protein	Cells from mice on a meat diet. c.p.m./mg protein
Formate 1 mM 120,000 counts/min/μmole	641	388
Serine 2 mM 16,700 counts/min/μmole	142	193
Methionine 2 mM 41,700 counts/min/μmole	166	206
Glycine 2 mM 16,700 counts/min/μmole	210	209

0·1 ml. of packed cells was incubated for 2 h at 37° C in Krebs-Ringer bicarbonate buffer, *p*H 7·4, containing 10 mM glucose[5] and 1 mM glutamine, with a gas phase of O_2/CO_2 (95:5). Each figure given is the average of at least six separate determinations. For each experiment, cells from three to six mice of each type were pooled.

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acid-soluble fraction containing nucleotides and amino-acids was extracted from the cells with 6 per cent cold trichloroacetic acid after incubation with formate-^{14}C (ref. 5). To separate nucleotides from amino-acids the trichloroacetic acid extract was first extracted with ether to remove the acid and a portion of the latter was passed through a column (1 cm × 1 cm) of 'Dowex-50' in the H+ form (12 per cent cross-linked, 200–400 mesh). The resin was washed extensively with water to remove nucleotides and then washed with 4 ml. of 2 N ammonium hydroxide[5]. It has previously been shown that serine is the only amino-acid formed from formate-^{14}C under the present experimental conditions and is eluted from the resin with NH_4OH (ref. 5).

The results in Table 2 show that serine synthesis from formate-^{14}C is appreciably reduced in cells from mice on a meat diet.

Table 2. Incorporation of ^{14}C from Formate-^{14}C into Free Serine and Soluble Nucleotides

	Serine c.p.m./mg dry wt. of tissue	Nucleotides c.p.m./mg dry wt. of tissue	Nucleic acids c.p.m./mg dry wt. of tissue
Cells from mice on meat	125	348	349
Cells from control mice	226	327	366

Conditions as in Table 1. No. of determinations and pooling of cells as in Table 1.

Since formate is incorporated into nucleotides and nucleic acids as well, we examined the incorporation of formate into these substances in the two types of Ehrlich ascites cells. The results in Table 2 show that formate-^{14}C incorporation into nucleotides and nucleic acids is not reduced in cells from mice on a meat diet.

To ascertain whether the inhibition in the incorporation of formate-^{14}C into protein and serine was peculiar to ascites cells grown in animals on a meat diet or whether it occurred in other tissues as well, formate-^{14}C utilization by kidney cortex slices, liver slices and spleen slices were examined.

In the livers of mice on a meat diet incorporation of formate-^{14}C into all fractions was lower than in controls. This may be due to the fact that the livers were very fatty[8]. In Table 3 it is apparent that the incorporation into nucleic acids in kidney was appreciably lower in meat-fed animals. It seems that in contrast to tumour cells where biosynthesis of serine from C_1 units is most sensitive to the diet, nucleic acid biosynthesis is more vulnerable

Table 3. Incorporation of Formate-^{14}C into Protein, Serine, Nucleotides and Nucleic Acids of Mouse Spleen and Kidney Slices

		Protein c.p.m./mg protein	Serine c.p.m./mg dry wt. of tissue	Nucleotides c.p.m./mg dry wt. of tissue	Nucleic acids c.p.m./mg dry wt. of tissue
Normal mice	Spleen	98·0	28·3	22·8	50·6
	Kidney	53·6	37·6	79·2	36·8
Mice on meat	Spleen	100·0	38·5	29·5	46·1
	Kidney	40·8	44·3	85·0	24·6

Tissue slices were incubated for 2 h at 37° C in Krebs-Ringer phosphate buffer, *p*H 7·4, with a gas phase of pure oxygen. Other conditions were as in previous experiments. Each figure represents an average value of at least four determinations.

in kidney. In spleen, little alteration from normal utilization of formate-^{14}C is observed in mice on a meat diet.

It has been shown[8,9] that both the anæmia and osteoporosis are prevented and cured by the addition of calcium salts to the meat diet. The addition of copper, as the sulphate salt, both prevented and cured the anæmia and partially prevented the osteoporosis. Manganese partially prevented the osteoporosis. We therefore examined the effects of these ions on formate-^{14}C incorporation into the protein of these cells. The presence of the ions did not enhance formate incorporation into protein.

Serine synthesis is the only aspect of C_1 metabolism, so far examined, which is affected in the tumour cells by the dietary regimen of the host. Although formate utilization for serine and purine synthesis has a common pathway so far as the formation of $N^{5,10}$-methenyl-tetrahydrofolic acid (THFA.) is concerned, for serine synthesis this compound must be further reduced to methylene-THFA[10]. It may be that the requirements for this last step are different in tumours from those in normal tissue and that the dietary deficiency brings about a decreased availability of the factors involved in the reduction of the formyltetrahydrofolic acid derivative.

We thank Prof. J. H. Quastel for his advice and help. This work was supported by the National Cancer Institute of Canada.

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RECOMBINATION BETWEEN PROTAN AND DEUTAN GENES; DATA ON THEIR RELATIVE POSITIONS IN RESPECT OF THE G6PD LOCUS

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VAN DER DONCK and Verriest[1] have reported the occurrence of two normal sons in a sibship which included two deuteranopic and one protanomalous son. They concluded that this situation could be best explained by assuming that the mother of the propositi was a double heterozygous (in repulsion phase) at the deutan and protan loci, and that the two normal sons therefore had to be considered as recombinants.

Kalmus[2] emphasized the importance of such a finding in relation to the debated hypothesis[3] that the genes for protan and deutan colour blindness pertain to different loci, and, on the basis of the available data on the linkage relationship between either of these genes and that responsible for the G6PD-deficiency[4–6], he put forward the hypothesis that the sequence of the three corresponding loci along the X-chromosome should be:

Deutan.....G6PD.....Protan

During a large-scale collection of data in Sardinia for the mapping of the X-chromosome[7,8,10], we have recently observed an extremely interesting pedigree (Fig. 1) that

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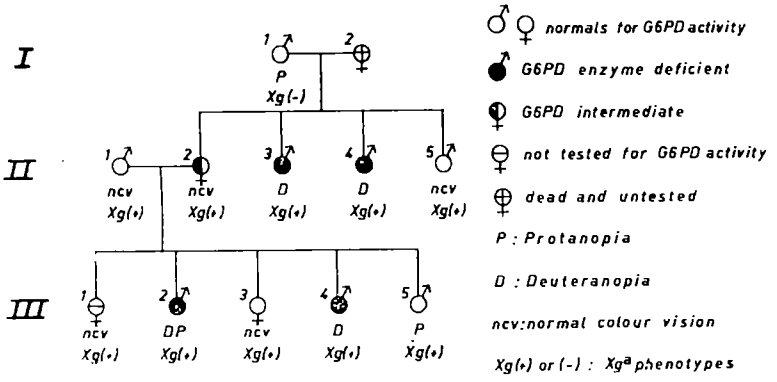


Fig. 1. Pedigree AH3–Irgoli (Sardinia)

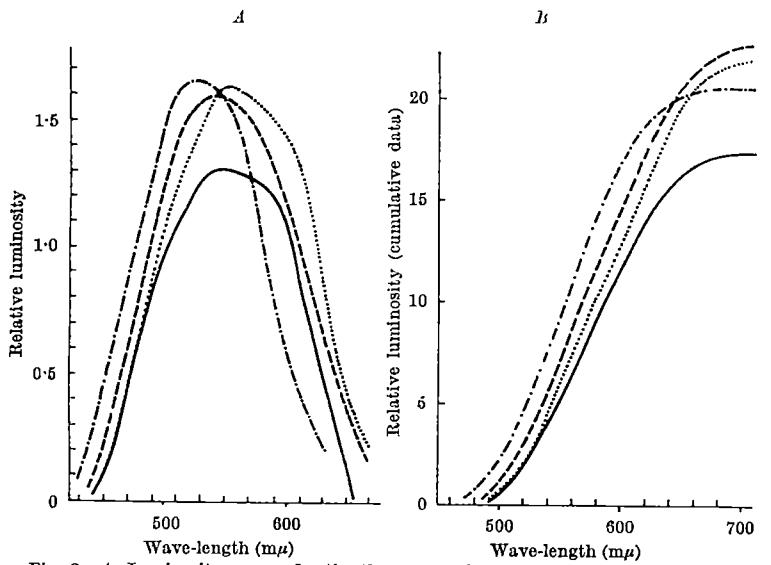


Fig. 2. *A*, Luminosity curves for the three colour-blind sibs and a normal control. Relative luminosity is expressed in logarithmic units. Each curve is the average of three separate sets of measurements; *B*, The data in *A* converted into cumulative curves. ·····, Normal control; —·—·—, III_5; – – – –, III_4; ——, III_2

throws light on this particular issue and strongly supports Dr. Kalmus's conclusions.

The propositus in our pedigree was a protanope child (III_5) with normal G6PD activity. The family was further investigated in the hope that it would yield data concerning the linkage comparison between protan and Xg^a loci.

To our surprise the next child examined in the same sibship (III_4) was found to be a definite deuteranope and to be G6PD-deficient. Among the maternal uncles two were deuteranope and enzyme deficient (II_3 and II_4) and one was normal for both traits. The maternal grandfather (I_1) was protanope and G6PD normal like his youngest grandson (the propositus III_5). Thus there was no doubt that the mother (II_2), who had normal colour vision and showed intermediate G6PD activity, must have carried on one of her X-chromosomes the genes for deuteranopia and G6PD-deficiency and, on the other, that for protanopia together with the normal G6PD allele.

However, when the eldest son (III_2) was examined, he was found to be enzyme deficient, like his deuteranopic uncles and younger brother, but showed colour vision defects of a peculiar nature[9]. He was repeatedly tested with the H.R.R. and the Ishihara plates as well as with the Farnsworth dychotomique test, and always gave ambiguous results, in the sense that each time he made mistakes typical for protanopia and/or for deuteranopia.

The same answer was obtained when later on he was tested with the 100 Hue–Farnsworth–Munsell test and with the Anomaloscope of Nagel (Ophthalmological Clinic of the University of Rome).

Finally, a scotopic luminosity curve was performed on each of the three sons, and on a normal control, at the National Institute of Optics at Arcetri (Florence).

As may be seen from Fig. 2, the curve for the protanope child (III_5) shows low red luminosity values and a shift of the maximum towards a shorter wave-length, whereas the curves of the deuteranope child (III_4) and of the normal control were very similar, as was expected. The curve for the eldest son (III_2) is peculiar in that it overlaps with that of the normal control in the green-blue area, it shows a markedly reduced luminosity in the green-yellow area, and it approaches the shape of the protanope curve in the red area. The differences and similarities in shape of these four curves may perhaps be better appreciated if they are transformed into cumulative curves (Fig. 2*B*).

The obvious conclusion from all this is that the eldest son is carrying the genes for both protan and deutan colour blindness, and this implies that his X-chromosome is the result of a recombination between deutan and G6PD genes on one side and the protan gene on the other.

The discovery of a second family showing recombination between deutan and protan genes therefore confirms the duality of the corresponding loci and makes very likely the conclusion that their degree of linkage is rather loose. If this is so it follows also that the locus for G6PD-deficiency must be located between them since it is known, from independent investigations, that the G6PD gene behaves as if very closely linked to both the colour-vision genes though seemingly somewhat closer to the deutan locus[4–7].

Table 1 summarizes our own data bearing on this particular point. The linkage between G6PD and deutan loci is very close indeed and that between G6PD and protan loci seems slightly looser, but certainly not so loose as one would expect if the G6PD locus were located on the other side of the deutan locus.

Table 1. Linkage Data for G6PD-Deficiency, Deutan and Protan Colour-blindness
(Sardinian data only)

Type of comparison	No. of families	Scorable nco	Sib. co	Log scores[13] $\text{Log}_{10}\frac{\text{Prob F/rec}}{\text{Prob F/(rec = 0·5)}}$ rec = 0·5	0·10	0·20	0·30	0·40
G6PD/Deutan	57	179	3	+33·5	+31·1	+23·6	+15·5	+6·6
G6PD/Protan	10	26	1	+4·8	+4·5	+3·5	+2·4	+1·2

Rec, recombination frequency; nco, non cross-over; co, cross-over
The estimate of recombination frequency between G6PD and deutan genes is less than 4 per cent, with very narrow confidence intervals; that between G6PD and protan genes is 5 per cent but with much broader confidence intervals. For a complete evaluation of these data see ref. 8.

Thus a tentative conclusion is that the sequence of the three loci in question may be:

Deutan.....G6PD.....Protan
c.o.v. < 0·04 • c.o.v. ≥ 0·05

It is interesting to note that the pedigree described here is informative also for the Xg^a locus[10], the maternal grandfather being Xg^a negative and an illegal paternity being obviously out of the question because of the segregation of protanopia down to the third generation. Thus it would appear that in this family the Xg^a locus, which has

recombined twice with protan and once with deutan, must be located nearer to the deutan locus than to protan, and yet far enough from both deutan and the G6PD loci, with which it is known to recombine quite freely in Sardinia[7,8]. The sequence of the four genes appears to be:

Xg^a.....Deutan.....G6PD.....Protan
c.o.v. ~ 0·40 c.o.v. < 0·04 c.o.v. ≥ 0·05

These conclusions, which are now supported by data based on 34 new pedigrees involving 3 generations, all with known phases, do not accord with those drawn by other workers from the data collected in Israel[12] and in Greece[12], where the sequence of the genes controlling the Xg^a blood group, G6PD-deficiency and deutan colour blindness has been given as

Xg^a.....G6PD.....Deutan
c.o.v. ~ 0·27 c.o.v. ~ 0·05

Whether this disagreement is due to chance, or to genetic heterogeneity, or to chromosomal rearrangements, remains to be seen. Alternative interpretations are to be discussed in detail elsewhere[7,10].

This work was supported by grants from the Rockefeller Foundation and the Italian National Research Council. We thank Prof. A. Wirth and Prof. F. Bozzoni for their advice, and for help in evaluating the colour vision data, and Prof. V. Ronchi for the luminosity measurements in Fig. 2.

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ABILITY OF TAMARINS TO PRODUCE AND RESPOND TO ERYTHROPOIETIN

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EVIDENCE for the existence of a humoral erythropoietic factor is ascribed to Carnot and Déflandré[1], who, in 1906, stated that the serum of rabbits which had been made anæmic by bleeding was capable of stimulating erythropoiesis when administered to normal rabbits. For several decades investigators attempted to confirm and elaborate on these findings with mixed success. Experiments performed by various authors (refs. 2–10) and others provided new interest in and strong support for the existence of a humoral erythropoietic factor produced in response to induced hypoxia. This humoral factor (or factors) controlling erythropoiesis, erythropoietin (EPF), can be recovered from body fluids, plasma, urine and milk of a number of animals (rabbits, dogs, monkeys, rats, sheep, guinea-pigs and mice) in which hypoxia has been produced by abrupt bleeding, by ropeated injections of phenylhydrazine, and by hypoxic hypoxia, or by the administration of excessive amounts of cobalt[1–10]. (*Addendum*. Recent data show that testosterone can stimulate and œstrogen can inhibit the production of erythropoietin in tamarins.)

Despite the work on EPF during the past decade, there was a paucity of investigations on primates[11–14]. It was thought worth while, therefore, to determine whether the small sub-primate, *Tamarinus nigricollis* (white-lipped tamarin), could produce detectable EPF with and without the presence of the kidneys, and respond to exogenous EPF from human and rabbit sources.

(*a*) Plasmas were obtained from non-nephrectomized *Tamarinus nigricollis* which had been bled to hæmatocrit-levels of 30, 25 and 20 per cent. Bilaterally nephrectomized tamarins were made anæmic by bleeding at several intervals after surgery. 24–30 h after surgery, when the hæmatocrits reached 25–20 per cent, the animals were bled. Plasmas from non-anæmic bilaterally nephrectomized tamarins were collected for controls at 24 and 30 h after surgery. These plasmas were assayed for EPF in polycythæmic mice.

(*b*) Intact and bilaterally nephrectomized tamarins were given 10 per cent oxygen for 8–120 h and 24–30 h, respectively. After each interval, tamarins were bled promptly after leaving the chamber. The plasma from these animals was stored at −20° C and assayed later in polycythæmic mice.

(*c*) Rabbit EPF prepared from the plasma of phenylhydrazine-treated rabbits by the method of Lowy *et al.*[15] was injected into tamarins. Each tamarin received subcutaneously 1–2 ml./100 g of rabbit EPF for 7 days.

(*d*) Human EPF extract was prepared from the urine of patients with hypoplastic anæmia. This extract was injected into tamarins in the same manner as rabbit EPF.

The criteria of erythropoietic response have included increased values of red blood cells, hæmoglobins, hæmatocrits, reticulocytes, increase in erythroid bone marrow elements, or measurement of the amount of iron-59 incorporated into circulating red blood cells in a given interval of time. To determine the erythropoietic response in groups (*a*) and (*b*), plasma was assayed in polycythæmic mice[16] and 48-h iron-59 uptake by the blood was determined[17]. Any mouse losing weight or having a hæmatocrit value less than 60 per cent on completion of the assay was discarded. The polycythæmic mouse iron-59 uptake assay method, though it did not allow absolute measurements of EPF concentrations, was sufficient to detect relative increases of plasma EPF levels. The erythropoietic response in tamarins receiving rabbit EPF or human EPF was determined by injecting 1 μc. of iron-59 intravenously into tamarins and measuring iron-59 uptake by the blood over 48 h after the last injection.

Jacobson *et al.*[5,18] reported the absence of EPF production in animals which had been bled after bilateral nephrectomy and proposed the kidney as the site of EPF formation. Mirand *et al.*[19], Naets[20], and Reissmann *et al.*[21] have confirmed and extended these findings in non-primate animals.

Table 1 demonstrates the effect of bleeding on the plasma EPF levels of non-nephrectomized tamarins. Note that the level of EPF was related to the severity of the anæmia as indicated by an 8·45 per cent 48-h uptake of iron-59 in the blood of polycythæmic mice. This was 70 times greater than the normal value, 0·12 per cent, obtained from plasma from non-anæmic, non-nephrectomized tamarins.

Table 1. Effect of Bleeding on Plasma Erythropoietin Production in Non-nephrectomized Tamarins

Material injected*	Assay in polycythæmic mice: No. of mice	48 h ^{59}Fe uptake by the blood	Ht.
(A)			
Plasma from anæmic tamarins (30 ht.)	5	4·07 ± 1·3	65
Plasma from anæmic tamarins (25 ht.)	5	6·57 ± 2·8	62
Plasma from anæmic tamarins (20 ht.)	5	8·45 ± 1·4	65
(B)			
Plasma from non-anæmic tamarins	5	0·12 ± 0·02	64

* Mice received a single injection of 0·5 c.c. intraperitoneally.

The results in Tables 1 and 2 indicate that the kidney of the tamarin played an integral part in EPF production. The 48-h uptakes of iron-59 in the blood of polycythæmic mice which received plasma from anæmic bilaterally nephrectomized tamarins bled to hæmatocrit levels of 25 and 20 per cent were comparable to the uptakes of iron-59 in non-anæmic, bilaterally nephrectomized and non-anæmic, non-nephrectomized tamarins.

Table 2. Effect of Bleeding on Plasma Erythropoietin Production in Bilaterally Nephrectomized Tamarins

Material injected*	Assay in polycythæmic mice: No. of mice	48 h ^{59}Fe uptake by the blood	Ht.
(A)			
Plasma from anæmic bilaterally nephrectomized tamarins (24 h, † ht. 25)	5	0·22 ± 0·05	67
Plasma from anæmic bilaterally nephrectomized tamarins (30 h, ht. 25)	5	0·16 ± 0·02	67
Plasma from anæmic bilaterally nephrectomized tamarins (30 h, ht. 20)	5	0·17 ± 0·06	65
(B)			
Plasma from non-anæmic bilaterally nephrectomized tamarins (24 h, ht. 41)	5	0·22 ± 0·03	70
Plasma from non-anæmic bilaterally nephrectomized tamarins (30 h, ht. 40)	5	0·12 ± 0·01	68
Plasma from non-anæmic non-nephrectomized tamarins (ht. 41)	5	0·12 ± 0·02	64

* Mice received single injection of 0·5 c.c. intraperitoneally.
† Time tamarins bled after bilateral nephrectomy.

Although the kidney of the tamarin played an integral part in EPF production, erythropoietic activity had not been detected in tissue extracts prepared from kidneys of normal or hypoxic tamarins. A survey of other tissues yielded negative results. Gordon *et al.*[7], Reissmann and Nomura[22], and Mirand[23] had observed this for other species. As in the case of other hormones, this may be due to lack of storage.

Osnes[24], from investigations with irradiated mouse kidneys, proposed that the juxtaglomerular apparatus was the source of EPF. Hirashima and Takaku[25] had also suggested this site in the kidney. Their conclusions were based on an increased granulation in the juxtaglomerular apparatus following bleeding or as a result of severe hæmolysis and a reduction in granulation in the juxtaglomerular apparatus during hypertransfusion.

The evidence of renal EPF production in experimental animals was reinforced by observations in man. Patients with hypernephroma or renal cysts had been shown to develop secondary polycythæmia[26–28].

Plasma from non-nephrectomized tamarins exposed to 10 per cent oxygen for 8–120 h was assayed for EPF in polycythæmic mice. Note that the findings in Table 3 demonstrated that the uptake of iron-59 increased steadily from the eighth hour and still persisted at high levels at 120 h. In some instances, the values obtained were 100 times greater than normal values. The persistence of high levels was in contrast to our findings in the rat[6,19,29] and rabbit[30], where we saw a drop in EPF levels after 48 and 72 h respectively.

Table 3. Erythropoietin Levels from Tamarins exposed to 10 per cent Oxygen for 8–120 h

Plasma from hypoxic tamarins, at hour*	Assay in polycythæmic mice: No. of mice	48 h ^{59}Fe uptake by the blood	Ht.
0	7	0·1† ± 0·66	71
8	7	6·0 ± 3·8	67
16	6	11·1 ± 4·1	70
24	6	12·2 ± 2·3	68
32	5	6·9 ± 1·9	70
48	6	8·4 ± 2·0	63
72	5	9·6 ± 2·3	67
96	5	11·7 ± 1·7	70
120	5	8·4 ± 1·4	71

* Mice received a single dose of 1 c.c. intraperitoneally of hypoxic plasma.
† Saline control and uninjected control, uptake of iron-59, 0·2 ± 0·1.

Jacobson *et al.*[5,18], using cobaltous chloride or bleeding as a stimulus, were not able to demonstrate significant levels of EPF in the plasma of bilaterally nephrectomized animals. As indicated previously, Jacobson postulated that the kidney was the sole source of EPF production. Mirand *et al.*[6,19,29] confirmed this finding in bilaterally nephrectomized rats, when cobaltous chloride or bleeding, but not when hypoxic hypoxia, was used as a stimulus. Since then, Jacobson[31] and others have confirmed the hypothesis that although the kidney appeared to be the principal site of production of EPF, there may be an extrarenal site(s) of such production as indicated by the ability of bilaterally nephrectomized animals to produce EPF to a hypoxic stimulus. Moreover, Fisher *et al.*[32] demonstrated a slight response of the bilaterally nephrectomized rat to cobalt, and felt that this was further evidence of the existence of an extrarenal site(s) of EPF production.

The data in Table 4 demonstrated the existence of an extrarenal site(s) for EPF production in bilaterally nephrectomized tamarins subjected to hypoxic hypoxia. The tamarin contained significant levels of plasma EPF in the absence of the kidneys but at a much lower level. It was interesting to note that the amount of plasma EPF produced in the rat in absence of the kidneys appeared to be greater than that in the tamarins[6].

Table 4. Effect of Hypoxic Hypoxia on Plasma Erythropoietin Production in Bilaterally Nephrectomized Tamarins

Plasma from hypoxic* bilaterally nephrectomized tamarins, at hour†	Assay in polycythæmic mice: No. of mice	48 h ^{59}Fe uptake by the blood	Ht.
(A)			
24	5	0·30 ± 0·14	66
24	5	0·70 ± 0·06	68
30	5	1·02 ± 0·08	69
(B)			
Plasma from non-hypoxic bilaterally nephrectomized tamarins, at hour†	No. of mice	48 h ^{59}Fe uptake by the blood	Ht.
24	4	0·10 ± 0·02	73·5
24	5	0·22 ± 0·03	70
30	5	0·12 ± 0·01	68

* Mice received single injection of 0·5 c.c. intraperitoneally.
† Length of time at 10 per cent oxygen level.

Although it has been shown that exogenous EPF obtained from the plasmas of anæmic hypoxic or hypoxic hypoxic tamarins will stimulate erythropoiesis in tamarins, findings from our laboratory also showed that tamarins can respond to human and rabbit EPF.

From Table 5 it can be seen that EPF from human urine stimulated erythropoiesis in tamarins. Tamarins received urine from hypoplastic anæmic patients and after the last injection a 48-h uptake of iron-59 by the blood was determined. In six different experiments, all blood uptakes of iron-59 exceeded normal values. Tamarins responded to plasma EPF from phenylhydrazine-treated rabbits. The uptakes of iron-59 were not as high as in those animals which received EPF from human urine. Regardless of the source of exogenous EPF injected, the increase in erythropoiesis as determined by uptake of iron-59 by the blood was maintained as long as EPF was given. Normal erythropoiesis was evident 7 days after administration of EPF was stopped.

These results demonstrated the effectiveness of exogenous EPF from non-primate and primate sources to stimu-

Table 5. Effect of an Active Standard Human Urinary Erythropoietin Extract on Tamarins

Exp. No.	Tamarins: Experimental No.	48 h ^{59}Fe uptake by the blood	Ht.	Control No.	48 h ^{59}Fe Uptake by the blood	Ht.
1	3	50·4 ± 12·9	45	2	24·6 ± 4·8	48
2	3	28·6 ± 5·4	40	2	15·3 ± 2·2	43
3	3	20·4 ± 11·8	49	2	14·1 ± 1·3	47
4	5	31·2 ± 3·5	44	5	15·8 ± 2·9	40
5	3	30·4 ± 4·6	42	3	15·6 ± 2·6	41
6	3	29·3 ± 3·0	43	3	17·6 ± 1·9	40

late erythropoiesis in the tamarin. Moreover, our findings concurred with Van Dyke's data indicating that EPF of primate origin was not required for stimulation of erythropoiesis in cynomolgus monkeys[14]. Osmond *et al.*[33] indicated some species differences in the response to EPF. This finding was not confirmed in these experiments.

This work was supported in part by the U.S. Public Health Service, Atomic Energy Commission, and the John A. Hartford Foundation.

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SYNERGISTIC ACTION OF PENICILLINS AND CEPHALOSPORINS AGAINST *Pseudomonas pyocyanea*

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MANY strains of *Pseudomonas pyocyanea* show an unusually high resistance to penicillins and cephalosporins, and also produce enzymes which inactivate these substances[1,2]. An inducible β-lactamase (*EC* 3.5.2.6) from one such strain of *Ps. pyocyanea* (NCTC 8203) has been examined in this laboratory[3]. The behaviour of this enzyme towards a number of derivatives of 6-aminopencillanic acid (6-APA) and 7-aminocephalosporanic acid (7-ACA) was found to differ greatly from that of penicillinases from *Staphylococcus aureus* and *Bacillus cereus*[4,5]. One example of this difference was the ability of methicillin ((6-[2 : 6]dimethoxybenzamido)penicillanic acid) and cloxacillin (6-(5-methyl-3-*o*-chlorophenylisoxazole-4-carboxamido)penicillanic acid) to act as powerful competitive inhibitors of the hydrolysis of susceptible derivatives of 6-APA and 7-ACA by the enzyme from *Ps. pyocyanea*, though they themselves were not hydrolysed at an appreciable rate. It thus seemed likely that methicillin and cloxacillin would delay the disappearance of hydrolysable derivatives of 6-APA or 7-ACA from whole cultures of this strain of *Ps. pyocyanea* in which the derivatives had induced β-lactamase formation.

Small inocula of penicillinase-producing staphylococci have been reported to be as sensitive to benzylpenicillin as penicillinase-negative strains[6], and the growth of heavy inocula of staphylococci in the presence of methicillin or oxacillin has been shown to occur only after the accelerated disappearance of the antibiotic[7]. In a serial dilution test with *Ps. pyocyanea* (NCTC 8203) the minimum inhibitory concentrations of benzylpenicillin and cephalosporin *C* were found to be reduced by 64-fold or more when the inoculum size was reduced 10^6-fold from a $0{\cdot}5 \times 10^{-2}$ dilution of an overnight culture[3].

Experiments were done with *Ps. pyocyanea* (NCTC 8203) to determine: (1) whether large inocula of this organism grow in the presence of concentrations of benzylpenicillin or cephalosporin *C* which are higher by several orders of magnitude than those required to suppress the growth of many other Gram-negative bacteria, or only after much of the antibiotic has been destroyed by enzymatic hydrolysis; (2) whether methicillin or cloxacillin in concentrations that are themselves antibiotically ineffective, can potentiate the effect of penicillins and

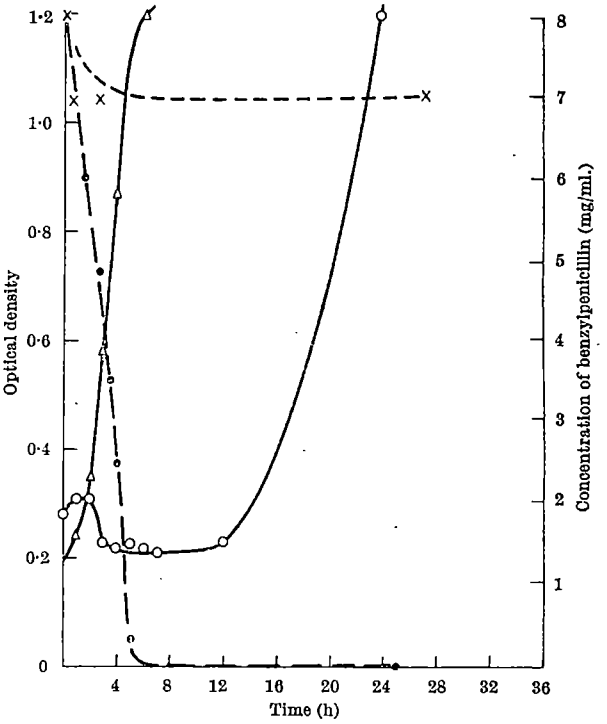


Fig. 1. Growth of *Ps. pyocyanea* and disappearance of benzylpenicillin from the culture. △, Control culture (no antibiotic); ○, growth after addition of benzylpenicillin (8 mg/ml.); ●, concentration of benzylpenicillin in the culture; ×, concentration of benzylpenicillin in sterile control

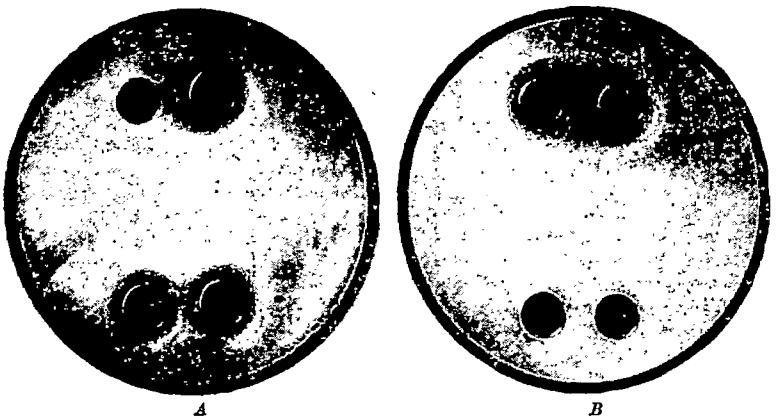


Fig. 2. Synergism on agar plates seeded with *Ps. pyocyanea*. Solutions of the substances studied were placed in holes cut in the agar. (*A*) Top pair: left, methicillin (1 mg/ml.); right, cephalosporin *C* (16 mg/ml.); bottom pair: each cephalosporin *C* (16 mg/ml.). With the top pair synergism is revealed by an eccentric zone of inhibition. The bottom pair show inhibition but no synergism. (*B*) Top pair: left, methicillin (4 mg/ml.); right, cephalosporin *C* (16 mg/ml.). Bottom pair: each cephalosporin *C* (4 mg/ml.). With the top pair synergism is revealed by eccentric and merging zones of inhibition. The bottom pair show no inhibition

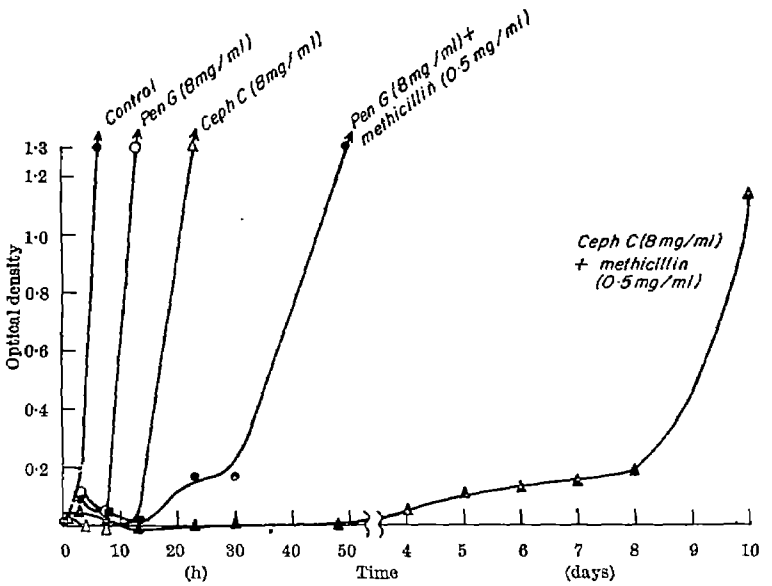


Fig. 3. Effect of benzylpenicillin (pen. *G*) alone, cephalosporin *C* alone and mixtures of these substances with methicillin on the growth of *Ps. pyocyanea* in inverted 'T' tubes

cephalosporins that are susceptible to hydrolysis by the β-lactamase (that is, act synergistically with them); (3) whether such synergism, should it occur, could be correlated with a decrease in the rates of destruction of the latter antibiotics.

A 10 per cent (v/v) inoculum of an overnight culture of *Ps. pyocyanea* was made in 'Oxoid' nutrient broth (10 ml. total volume) containing benzylpenicillin (8 mg/ml.) in inverted 'T' tubes (40 ml. capacity). The tubes were rocked 50 times/min at 35° C. Simultaneous measurements were made at intervals of bacterial growth (in terms of optical density, in a Spekker absorptiometer with a neutral grey filter, *H*508) and of the amount of antibiotic remaining. The latter was estimated enzymatically. Samples (0·5 ml.) of the whole culture were mixed with 0·5 ml. of a solution of crude β-lactamase from *B. cereus*[4] and 1 ml. of water in the reaction vessel (30° C) of a *p*H stat set to maintain the initial *p*H of the mixture (7·1 ± 0·2) by automatic addition of 0·1 N sodium hydroxide. The equivalents of alkali required to hold the *p*H constant during the reaction (which was always complete within 4 min) represented the equivalents of benzylpenicillin remaining in the sample. In a similar experiment with cephalosporin *C* (4 mg/ml.) a solution of preformed cephalosporinase[3] was used for estimation of the remaining antibiotic. In each experiment bacterial growth or antibiotic concentration in parallel control tubes, containing bacteria, benzylpenicillin, or cephalosporin *C* alone in broth, was estimated in a similar manner.

Fig. 1 shows that the rate of disappearance of benzylpenicillin in the presence of the inoculum of *Ps. pyocyanea* increased with time and became very much faster than that in the control; but that rapid bacterial growth did not begin until after the antibiotic level had fallen to a value below that at which it was detectable by the method used (50 μg/ml.). A single reading at 25 h would have suggested that this strain of *Ps. pyocyanea* was resistant to 8,000 μg/ml. of benzylpenicillin, although growth did not in fact occur at that concentration. When cephalosporin *C* was used in place of benzylpenicillin rapid growth was also delayed until the antibiotic concentration had fallen to less than 50 μg/ml.

Synergism was looked for in three systems involving: (1) diffusion on agar plates; (2) tube dilutions; (3) growth in inverted 'T' tubes.

(1) In the agar plate system Petri dishes were filled with 10 ml. of an 'Oxoid' nutrient agar seeded with 2 per cent (v/v) of an overnight culture of *Ps. pyocyanea* (NCTC 8203). Pairs of holes, each 1·5 cm (centre-to-centre) from its mate, were punched with a sterile cork borer of 7 mm diameter. One hole of each pair was filled with methicillin or cloxacillin (1 to 4 mg/ml.) and the other with cephalosporin *C* (1–16 mg/ml.). Synergism shown by two of a number of mixtures of cephalosporin *C* and methicillin is illustrated in Fig. 2.

(2) Tube dilution tests were performed by addition of 0·5 ml. of 'Oxoid' broth containing a 10^{-4} dilution of an overnight culture of *Ps. pyocyanea* (NCTC 8203) to 0·5 ml. of the same broth containing various concentrations (two-fold dilutions) of either a hydrolysable derivative of 6-APA or 7-ACA alone or of one of these derivatives together with an inhibitor (methicillin or cloxacillin).

Table 1. Activities against *Ps. pyocyanea* (NCTC 8203) of Mixtures of Hydrolysable Penicillins or Cephalosporins with β-Lactamase Inhibitors

Hydrolysable antibiotic	Inhibitor	Inhibitor concentration (μg/ml.)	M.I.C. of hydrolysable antibiotic (μg/ml.)
Benzylpenicillin	None	—	4,000–8,000
Benzylpenicillin	Cloxacillin	10	4,000
Benzylpenicillin	Cloxacillin	100	1,000
Benzylpenicillin	Cloxacillin	1,000	60
Cephalosporin *C*	None	—	1,000–4,000
Cephalosporin *C*	Cloxacillin	10	500
Cephalosporin *C*	Cloxacillin	100	500
Cephalosporin *C*	Cloxacillin	1,000	120
Cephalothin	None	—	4,000
Cephalothin	Methicillin	10	4,000
Cephalothin	Methicillin	100	250
Cephalothin	Cloxacillin	10	1,000
Cephalothin	Cloxacillin	100	500
Cephalothin	Cloxacillin	1,000	120
Cephaloridine	None	—	4,000
Cephaloridine	Methicillin	10	1,000
Cephaloridine	Methicillin	100	60
Cephaloridine	Cloxacillin	10	1,000
Cephaloridine	Cloxacillin	100	60
Cephaloridine	Cloxacillin	1,000	30

The tubes containing antibiotics and bacteria were read after 16–18 h. The lowest concentration of antibiotic to prevent visible growth completely, or permit only faint surface growth, was considered the minimum inhibitory concentration (M.I.C.). Controls (without antibiotic) showed marked turbidity and heavy surface growth. Neither of the inhibitors caused any detectable inhibition of bacterial growth when used alone in the concentrations listed. Under the conditions used the M.I.C. for methicillin alone was 4 mg/ml. and that for cloxacillin was 8 mg/ml.

The tubes were incubated at 37° C for 16–19 h before being read. Synergism was observed in these tests (Table 1). At concentrations in which they had no observable antibacterial effect alone, methicillin and cloxacillin increased the apparent antibacterial activity of benzylpenicillin, cephalosporin *C*, cephalothin (7-(2-thienylacetamido-cephalosporanic acid), or cephaloridine (the pyridinium (C_A) analogue of cephalothin) by as much as a factor of 100.

(3) The growth of *Ps. pyocyanea* (NCTC 8203) in inverted 'T' tubes containing 'Oxoid' broth and several derivatives of 6-APA and 7-ACA, alone and in pairs, was followed by measurements of optical density. The inoculum was 1 per cent (v/v) of an overnight culture in 'Oxoid' broth and the tubes were rocked at 35° C. The growth curve in the presence of methicillin (500 μg/ml.) was superimposable on that of the control shown (no antibiotic) while either benzylpenicillin (8,000 μg/ml.) or cephalosporin *C* (8,000 μg/ml.) caused some delay in growth (Fig. 3). However, addition of methicillin (500 μg) to a tube containing benzylpenicillin or cephalosporin *C* resulted in marked potentiation of the effect of either of the latter antibiotics alone. In all these cases the organisms which finally grew out were no more resistant than the parent strain to derivatives of 6-APA and 7-ACA when tested by the tube dilution method. The synergistic effect was also demonstrable at lower concentrations of cephalosporin *C* (800 μg/ml.) and methicillin (250 μg/ml.) (Fig. 4). Fig. 5 shows the relationship between bacterial growth (solid lines) and cephalosporin *C* levels (broken lines), the concentration of the latter in the medium being estimated from measurements of extinction at 260 mμ. In the presence of methicillin (500 μg/ml.) the concentration of cephalosporin *C* fell no more rapidly than in sterile control medium without bacteria and was still 3,000 μg/ml. after 7 days. No measurable growth had occurred by 7 days in the 'T' tube containing both cephalosporin *C* and methicillin.

A similar experiment was done in which cephalosporin *C* was replaced by benzylpenicillin. The growth curves

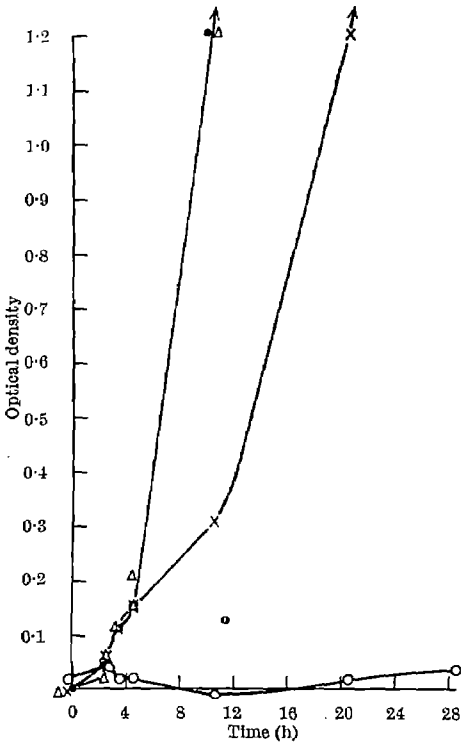


Fig. 4. Effect of: ×, cephalosporin *C* (800 μg/ml.) alone; △, methicillin (250 μg/ml.) alone; and ○, a mixture of the two antibiotics in these concentrations on the growth of *Ps. pyocyanea* in inverted 'T' tubes. ●, control culture growth without antibiotic

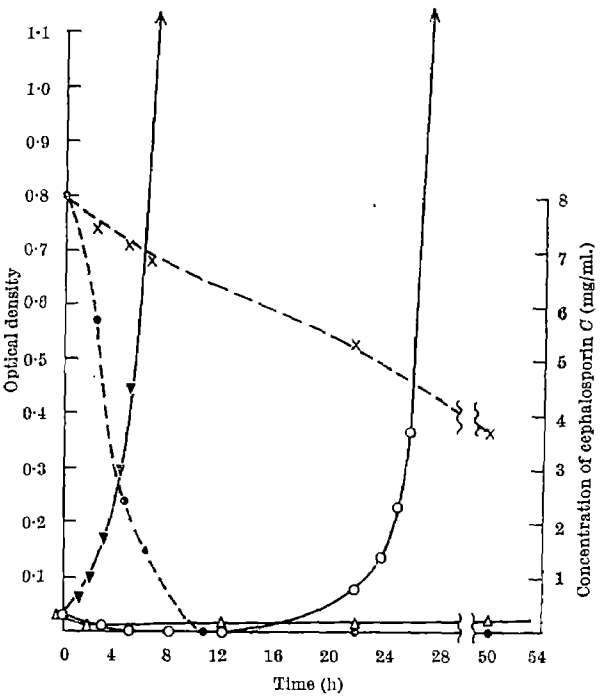


Fig. 5. Growth of *Ps. pyocyanea* and changes in the concentration of cephalosporin *C*. ▼, Growth in control culture; ○, growth after addition of cephalosporin *C* (8 mg/ml.); ●, concentration of cephalosporin *C* in the same culture; △, bacteria in the presence of cephalosporin *C* (8 mg/ml.) plus methicillin (0·5 mg/ml.), ×, concentration of cephalosporin *C* in the presence of bacteria and methicillin. In the presence of methicillin alone (0·5 mg/ml.) growth was similar to that in the control culture

were similar to those in Fig. 5 and the concentration of benzylpenicillin had fallen from 8,000 μg/ml. to less than 50 μg/ml. before heavy growth was detected after 10 h, in the tube without methicillin. In the presence of methicillin (500 μg/ml.) a detectable level of benzylpenicillin was maintained much longer than in the culture without methicillin. The delay in rapid growth produced by the mixture of benzylpenicillin and methicillin paralleled the delay in the disappearance of benzylpenicillin from the medium. In some cases relatively small increases in optical density occurred before rapid growth took place, but it is not known whether these changes were associated with cell multiplication.

These results indicate that synergistic antibacterial action against *Ps. pyocyanea* (NCTC 8203) can be obtained by use of a β-lactamase inhibitor (methicillin or cloxacillin) with any one of several derivatives of 6-APA or 7-ACA which are susceptible to enzymatic hydrolysis. The results of concurrent measurements of growth and antibiotic levels are consistent with the suggestion that there is synergism due to a delay in the destruction of the hydrolysable antibiotic. The biochemical basis for such an effect would reside in the existence of essentially non-hydrolysable competitive inhibitors (methicillin and cloxacillin) which have very much higher 'affinities' for the active centre of the β-lactamase from *Ps. pyocyanea* (NCTC 8203) than have the hydrolysable substrates and which do not themselves prevent the growth of *Ps. pyocyanea* in concentrations approaching those required to saturate the enzyme. The values of *Ki* (dissociation constant of the enzyme inhibitor complex) for methicillin and cloxacillin are 10^{-7} M and 10^{-8} M respectively, and the values of *Km* (Michaelis constant) for benzylpenicillin, cephalothin, cephaloridine and cephalosporin *C* are 10^{-5} M, 10^{-4} M 10^{-4} M and 10^{-3} M, respectively, while the minimum inhibitory concentration of methicillin and cloxacillin is about 10^{-2} M[3].

It is possible that the type of synergism found in the laboratory with this strain of *Ps. pyocyanea* will not be

demonstrable in patients even with strains which behave similarly *in vitro*[8]. The antibiotic levels at which synergism was shown to occur are still enormous compared with the levels at which these antibiotics are effective against 'susceptible' bacterial species. Such levels would not be reached systemically in man, except at unusual doses, and the antibiotics concerned would therefore not be expected to compete with polymyxin *B* and colistin in the chemotherapy of systemic infections. However, the concentrations used are easily achieved in the urine of patients receiving methicillin[9], cloxacillin[10], benzylpenicillin[11] and cephalosporin *C* (ref. 12) because of the active urinary secretion of these drugs. In the management of chronic pseudomonad infections of the urinary tract, or in patients with severely impaired renal function, for which the use of polymyxin *B* and colistin is not generally favoured, a synergistic combination of these non-toxic drugs could conceivably have application.

An earlier suggestion was made that synergism observed with cephalosporin *C* and benzylpenicillin against one penicillinase-producing strain of *Staph. aureus* might be due to competitive inhibition of the penicillinase by cephalosporin *C* (ref. 13), but this explanation of the phenomenon appeared to be unlikely after a study of the relative affinities of the antibiotics concerned for a staphylococcal penicillinase[4]. More recently, it has been reported that methicillin and cloxacillin are inhibitors of penicillinase produced not only by *Ps. pyocyanea*[2] but also by a number of other Gram-negative bacteria[14–16]. Synergism has been shown to occur with these substances and benzylpenicillin or ampicillin against some strains of *K. aerogenes*, *E. coli* and *Proteus* sp., but it was thought to be inadequate for clinical application[15,16].

The question arises whether different derivatives of 6-APA and 7-ACA will show differences in affinity for β-lactamases from other organisms, particularly those from Gram-negative bacteria, which are greater than those so far encountered. However, an affinity for an accessible enzyme which is high compared with that of an antibacterial substrate is only one of the properties which may be required of a competitive inhibitor in this context.

A second is an affinity for the enzyme which is high in relation to the antibacterial activity of the inhibitor. 'Cephaloram' (7-phenylacetamidocephalosporanic acid) has strong affinity for penicillinase from *Staph. aureus* (Ki $0{\cdot}43 \times 10^{-7}$ M) relative to benzylpenicillin ($Km = 2{\cdot}5 \times 10^{-6}$ M) and a high resistance to hydrolysis by it[4]. But its antistaphylococcal activity alone is high (minimum inhibitory concentration, 10^{-6} M) and in concentrations that are less than one-quarter of its minimum inhibitory concentration 10 per cent or more of the enzyme would be uncombined with this inhibitor, however low the concentration of substrate. In this respect the situation is therefore less favourable for synergism than that with methicillin or cloxacillin and the enzyme from *Ps. pyocyanea*, in which the corresponding values for uncombined enzyme would be about 0·004 and 0·0002 per cent respectively. A third property, the ability of the inhibitor to reach the enzyme, becomes significant when the enzyme is intracellular.

We thank the Lilly Research Laboratories for a sample of 'Cephalothin', the Glaxo Laboratories, Ltd., for a sample of 'Cephaloridine', Dr. G. G. F. Newton for helpful suggestions and Mr. J. O. Isaacs for his assistance.

This work was supported in part by a U.S. Public Health Service fellowship number 2–*F*3–*AI*–20,524–02 from the National Institute of Allergy and Infectious Diseases.

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SYNCHRONOUS AND DICHOTOMOUS REPLICATIONS OF THE *Bacillus subtilis* CHROMOSOME DURING SPORE GERMINATION

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GERMINATION of bacterial spores provides an unusual opportunity for analysing the process of development of a metabolically active state from an inert or dormant state[1]. The mode of chromosome replication during spore germination, therefore, should be of particular interest because of the possibility that during germination the sequence of transcription and translation of genes may reflect the sequence of gene replication. In this article, the process of gene replication during spore germination of *Bacillus subtilis* will be described. The main findings are as follows: (1) The chromosome in the spore is in completed form; (2) On germination, the genes replicate in definite sequential order from one end of the chromosome (origin) to the other (terminus), the same as in vegetative cells; (3) In an enriched medium, a dichotomous replication occurs, as in the exponential phase in the same medium.

Sequential replication of the bacterial chromosome in an exponentially growing population has been described in *Bacillus subtilis*[2] and in *Escherichia coli*[3,4]. In *Bacillus subtilis*, genetic markers on the chromosome have been shown to replicate in a definite order[5,6]. The result is most consistent with the replication model shown in Fig. 1. Thus, the replicating chromosome of *B. subtilis* has one replicating point when cells are grown in a standard medium (Table 1) with a generation time of approximately 40 min. The replication order of markers was followed by measuring the frequency of markers in the exponential growth phase by genetic transformation. Based on the replication order, a genetic map of *B. subtilis* was constructed[5] (Fig. 2). The theoretical basis for marker frequency analysis will be given elsewhere[8]. This single-fork replication is, however, not true when the cells are dividing rapidly in an enriched medium. An indication of multifork replication was first noted by an unusually high ratio, four, of *ade*/*met* in an enriched medium (Table 1) with a generation time of about 20 min (ref. 9), in contrast to the ratio of two in a standard medium[5,8]. A hypothetical

Table 1. Composition of Standard and Penassay Media

Standard medium* (1 l.)		Penassay medium (1 l.)	
K_2HPO_4	14·0 g	Bacto-beef extract	1·00 g
KH_2PO_4	6·0 g	Bacto-yeast extract	1·00 g
$(NH_4)_2SO_4$	2·0 g	Bacto-peptone	3·33 g
Na_3-citrate.$2H_2O$	1·0 g	Bacto-dextrose	0·67 g
$MgSO_4.7H_2O$	0·2 g	NaCl	2.33 g
Glucose	5·0 g	K_2HPO_4	2·46 g
Casamino-acids	0·05%	KH_2PO_4	0·88 g
L-Tryptophan	50 μg/ml.		
	pH 7·0		pH 7·0

* Similar to Anagnostopoulos and Spizizen (ref. 7), except 0·05 per cent casamino-acids instead of 0·02 per cent.

model of chromosome replication for this situation is shown in Fig. 3, and this special case of multifork replication is called 'dichotomous replication'[9]. Multifork replication has also been reported for *Escherichia coli* when cured from thymine deprivation[10]. Synchronous replication and an unequivocal demonstration of dichotomous replication of the *B. subtilis* chromosome are reported here for the case of spore germination in an enriched medium, and their implication for gene expression during spore germination and for the regulation of chromosome replication are discussed.

Experimentally, heavy spores of strain *W*168, which had been formed in a deuterium oxide medium, were germinated in a light-enriched medium (Penassay medium, Table 1) containing radioactive phosphorus. During the germination of spores, replication of deoxyribonucleic acid (DNA) was followed by cæsium chloride density gradient centrifugation in a swinging bucket rotor. The dichotomous replication gives a unique prediction of the distribution of markers in heavy, hybrid and light DNA peaks as a function of the stage of chromosome replication and the position of the marker on the chromosome (Fig. 4). The experimental results are in complete agreement with the prediction.

A wild-type strain *W*168 of *B. subtilis* was used as the DNA-donor strain. Two triple mutants, *leu-met-ade* (Mu8u5u16) and *leu-met-thr* (Mu8u5u5), were used as recipient strains. These markers were obtained by an ultra-violet–penicillin method. Markers *leu* and *met* in the two strains were identical. Heavy deuterated spores of strain *W*168 were produced by shaking the culture at 37° C. in the following medium (D_2O-spore medium). The mixture of 7·0 g K_2HPO_4, 3·0 g KH_2PO_4, 1·0 g $(NH_4)_2SO_4$, 0·1 g $MgSO_4.7H_2O$, 0·5 g Na_3-citrate.$2H_2O$, 2·5 g glucose and 2·5 g potato extract (Difco Lab., Detroit, Michigan) was dried *in vacuo*, dissolved in 500 ml. of D_2O (99·7 per cent, General Dynamics Corp., San Carlos, California) and sterilized by passing through a 'Millipore' membrane filter (pore size 0·45μ). Strain *W*168, first grown on a D_2O agar medium, was inoculated in 2 ml. of the above D_2O-spore medium. The overnight culture was transferred to 500 ml. of a fresh D_2O-spore medium and incubated at 37° C for 10 days with constant shaking. The spores formed were collected by centrifugation and washed once with water. The crude spores were then incubated with 25 ml. of sterile water containing 1 mg/ml. of egg white lysozyme (Worthington Biochemical Corp., Freehold, New Jersey) for 60 min at 37° C. 'Duponol' was added to the suspension (final concentration, 2 per cent), incubated for 30 min at 37° C, and washed four times with sterile water. The spores thus purified were suspended in 3·3 ml. of sterile water (3·01 × 10^9 spores/ml., optical density (*O.D.*) at 650 mμ, 17·55) and kept in a refrigerator. In a growth flask, 1·2 ml. of the spore suspension (3·61 × 10^9 spores) was added to 28·8 ml. of a 1·22 per cent Penassay medium (Difco antibiotic medium 3) containing 10 mc. of ^{32}P-phosphate (final 0·33 mc./ml.) and incubated at 37° C with constant shaking. The optical density of the culture was followed by Klett–Sommerson colorimeter with a red filter (No. 66).

Samples of 5–6 ml. were withdrawn from the culture at various times and immediately heated at 60° C for 10 min in a water bath to stop DNA synthesis. The samples were chilled, centrifuged and the cells resuspended in 0·9 ml. of a saline versene solution (0·15 M NaCl and 0·1 M versene, *p*H 8·0) and incubated with 1 mg/ml. of lysozyme at 37° C for 30 min. 'Duponol' (final concentration 2 per cent) was added and incubated further at 37° C for 30 min; 0·5 ml. of the lysate was mixed with 4·76 g CsCl and 3·24 ml. *tris*-versene buffer (0·01 M *tris*-hydroxymethylaminomethane and 0·001 M versene, *p*H 8·4). The mixture was centrifuged at 35,000 r.p.m. for 63–74 h at 25° C in a Spinco '*SW*39' rotor. Single drops were collected through the bottom of centrifuge tubes in sterilized tubes; the dripping rate was adjusted by hydropressure to about a drop each 2 sec. The total number of drops was 105–135 per centrifuge tube. Each fraction was diluted immediately with 1 ml. of sterile saline citrate solution (0·15 M NaCl and 0·015 M sodium citrate), and stored at 4° C.

Transforming activities were assayed by a modified method of Anagnostopoulos and Spizizen[7] using stored recipient cells. The stored competent recipient cells were prepared and used as follows: 2 ml. of cell suspension grown overnight in a standard medium (Table 1) with appropriate supplements (100 μg/ml. for amino-acids and 50 μg/ml. for adenine) were diluted in 40 ml. of the same medium and incubated at 37° C with shaking. When the growth approached the stationary phase, the total cell suspension was transferred to 360 ml. of a medium (Medium *T*) containing 0·01 per cent casamino acids, 5 μg/ml. of L-tryptophan and L-histidine, and 50 μg/ml. of adenine (otherwise same as standard medium). After 90 min incubation at 37° C with shaking, the cells were collected by centrifugation at room temperature and immediately suspended in 40 ml. of the same

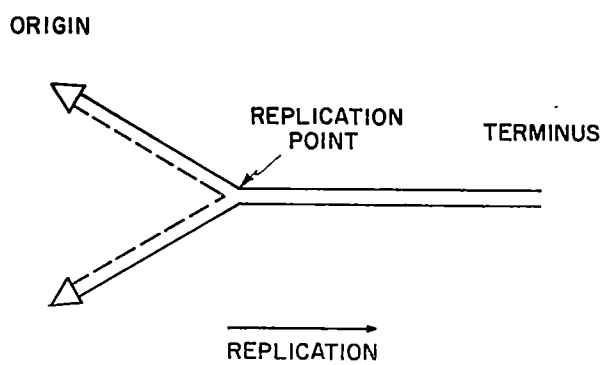


Fig. 1. A replication model of the chromosome. The solid line represents the old strand and the broken one the newly synthesized strand. This model does not differ, in principle, from a circular chromosome model in which replication stands at a fixed point and proceeds in one direction

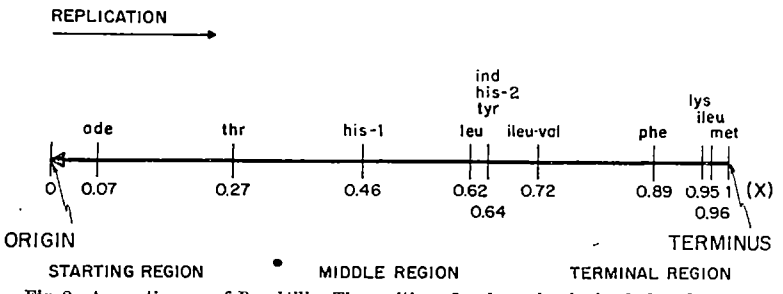


Fig. 2. A genetic map of *B. subtilis*. The position of each marker is simply based on the average of the results so far obtained by marker frequency analysis. Minor adjustments are expected in the future. For example, the *met* marker is assumed to have a position 1·00, which makes the *ade* position 0·07. Equally possible are the situations in which *ade* is located at 0·00 and *met* 0·93, or more generally, *ade* is in 0·00–0·07 and *met* in 0·93–1·00. The distance between *ileu* and *met* may be closer than indicated in the map because a linkage has been found for them by joint transformation (ref. 5). In our previous communication (ref. 5), the *his* 2-*ind*-*tyr* group was mislocated, for *his* 1 and *his* 2 were unjustifiably assumed to be identical. A detailed account of the map will be given elsewhere (ref. 17)

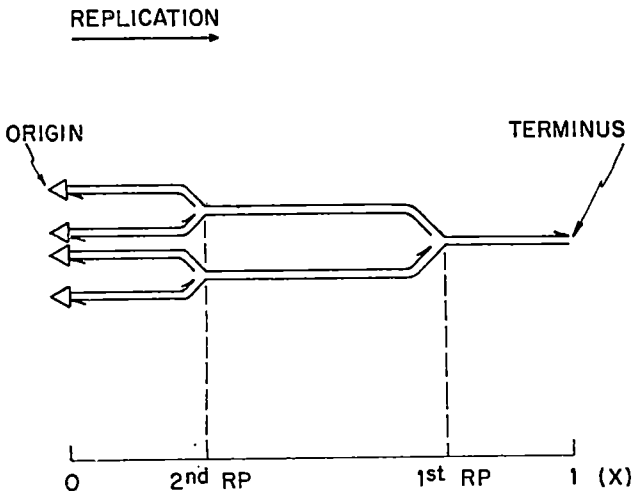


Fig. 3. A schematic representation of dichotomous replication of the chromosome. Before the first replication position (RP) reaches the terminus, the second RP starts from the origin. In an exponentially growing population, the simplest steady state can be set if the distance between the first and second RP's occupies half the chromosome and each replication point proceeds at a constant rate (ref. 8). In reality the two replication points at the second RP may not necessarily be at the same position

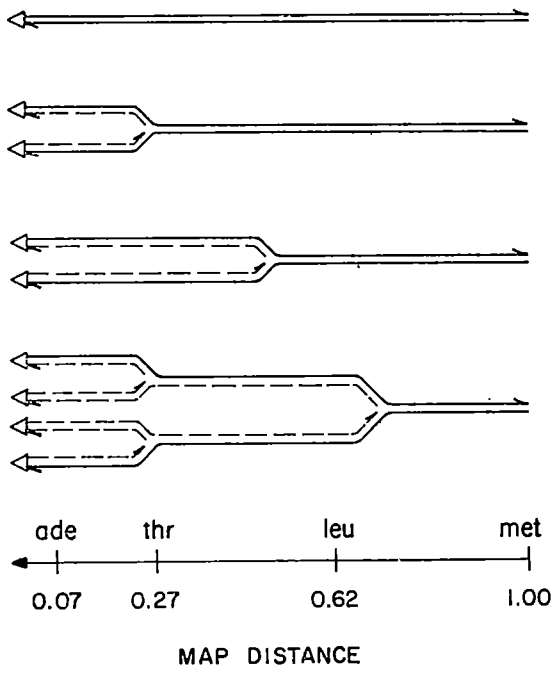


Fig. 4. A schematic presentation of successive stages of chromosome replication during germination of D_2O-spores in a ^{32}P-Penassay medium. The solid line represents the heavy non-radioactive strand and the broken one the newly synthesized strand (light and radioactive)

medium containing 4 per cent (w/v) glucose. 1-ml. aliquots of the thick cell suspension were poured into test-tubes, soaked in liquid nitrogen and kept frozen for at least 10 min. Then the tubes were transferred to a refrigerator adjusted at −80° C and stored at that temperature. For transformation, the tubes were transferred to a water bath (37° C) and thawed for 3 min with gentle shaking: 0·2 ml. of this cell suspension was diluted with 0·8 ml. of Medium *T* (minus glucose) containing DNA and incubated for transformation for 40 min at 37° C with constant shaking before the addition of DNase. Within our limited experience, no appreciable loss of transforming activities of competent recipient cells stored by this procedure was observed after one month's storage at −80° C.

In order to get radioactive profiles, 0·4 ml. aliquot of each fraction, 3 drops of a 0·5 per cent solution of salmon sperm DNA and 0·4 ml. of cold 20 per cent trichloroacetic acid were added. After keeping it in ice water for 30 min, the precipitate was collected on a membrane filter ('S and S', type *B*–6) and washed twice with about 30 ml. of 10 per cent cold trichloroacetic acid. The radioactivity was measured by a liquid scintillation technique.

The change in optical density of the spore suspension during germination and the schedule of sampling are shown in Fig. 5. The marker and phosphorus-32 radioactivity profiles of samples No. 1, No. 3, No. 4 and No. 5 are given in Fig. 6. Fig. 6*A* shows that the *ade* marker is mostly transferred to the hybrid peak while all the other markers (*thr*, *leu* and *met*) stay completely in the heavy peak. This is in perfect agreement with the replication order of markers which is shown in Fig. 2. Moreover, the results show that the chromosome in the spore has a complete non-replicating form and the synchrony of chromosome replication is excellent in this case. The result shown in Fig. 6*B* for sample No. 3 gives the most critical evidence for dichotomous replication of the chromosome. Thus the *ade* marker is distributed more or less equally in both hybrid and light peaks, while the *met* marker still remains totally in the heavy peak and the *leu* marker in the heavy and hybrid peaks.

On the basis of the dichotomous replication model (Figs. 3 and 4), the relative amount of phosphorus-32 radioactivity in the hybrid and light peaks allows us to calculate the position of replication points on the chromosome. In the case of sample No. 3, the calculated position of the first replication point on the chromosome is at 0·66 and the second replication points (or replication position) at 0·16. This predicts that the *leu* marker on the profile should be more in the hybrid than in the heavy peak (Fig. 2), which is exactly the picture. Data for later stages of germination are given in Figs. 6*C* and *D*. A sign of degeneration of synchrony is clear in the later stages.

The established order of chromosome replication during spore germination should provide a basis for our understanding of the developmental process. A basic issue here is whether or not the order of transcription and translation of genes during the germination process follows the order

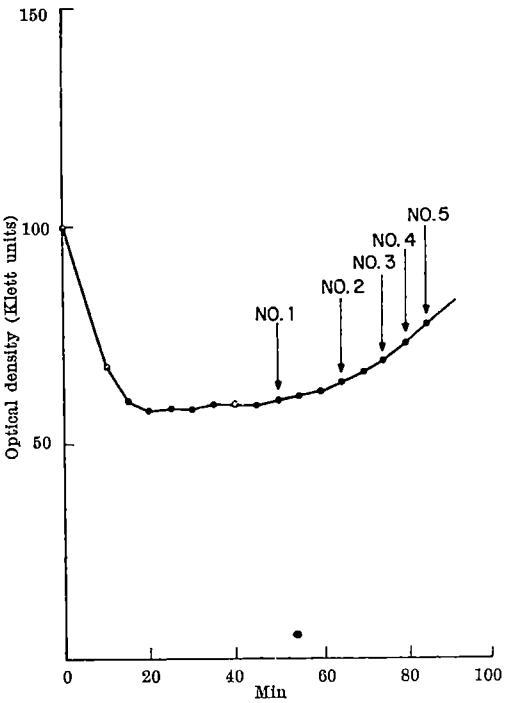


Fig. 5. Germination of *B. subtilis* D_2O spores. Spores were germinated in a Penassay medium at 37° C with constant shaking. Samples were taken at the time indicated and immediately heated at 60° C. for 10 min to stop further DNA replication. The volumes of samples taken were 6 ml. for No. 1, No. 2 and No. 3 samples, and 5 ml. for No. 4 and No. 5 samples. These samples were then lysed and analysed by CsCl density gradient centrifugation as described in the text

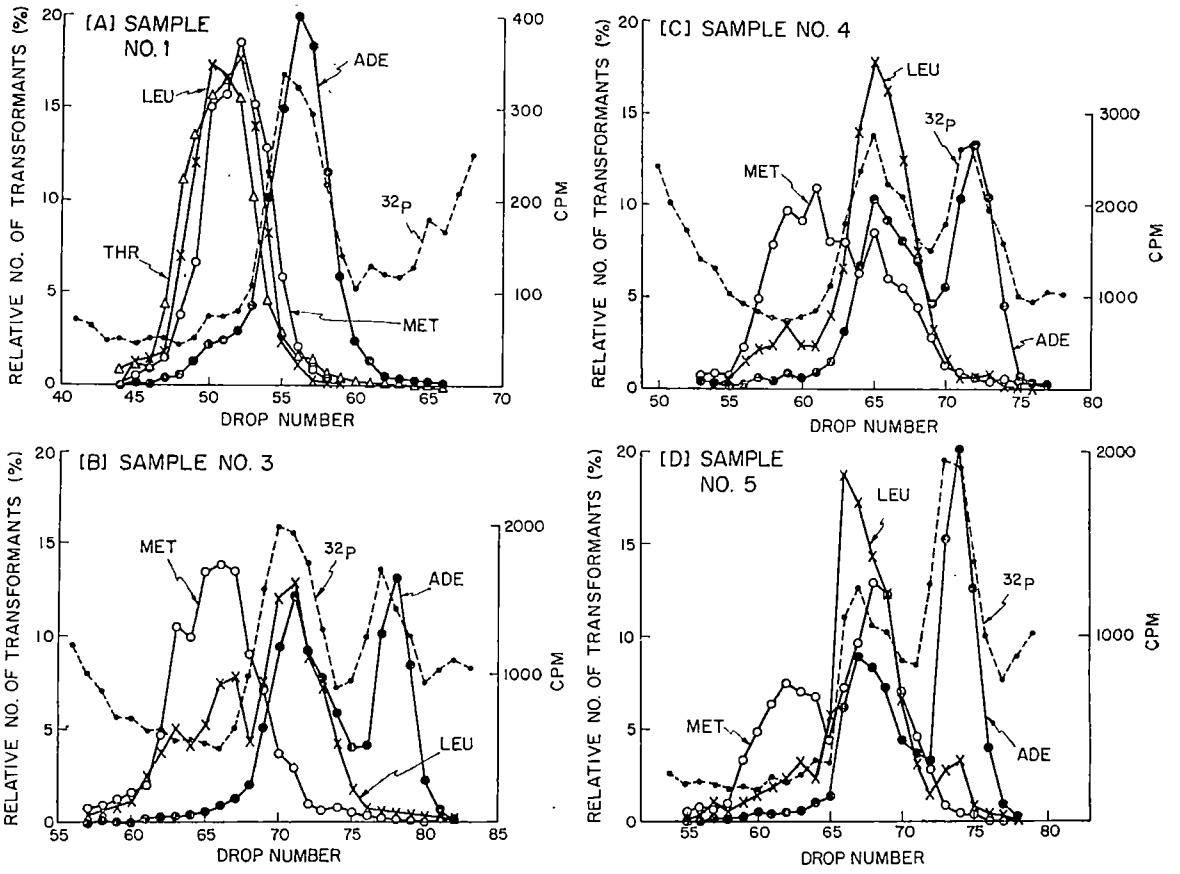


Fig. 6. Distribution of DNA from various samples indicated in Fig. 5 in CsCl density gradient centrifugation and analysis of transforming activities. Lysates of germinated spores prepared from samples in Fig. 5 were mixed with CsCl, centrifuged in a Spinco '*SW*39' rotor, and fractionated as described in the text. 40 per cent of each fractionation was used for assay of ^{32}P-radioactivity and the rest was analysed for transforming activity of various markers. The recipient strains used were two triple mutants: Mu8u5u16 (*leu-met-ade*) for No. 1, No. 3, No. 4 and No. 5 samples for leucine, methionine and adenine markers and Mu8u5u5 (*leu-met-thr*) for No. 1 samples for threonine marker. The relative number of transformants (ordinate) is the relative value (per cent) of the transformants of each fraction to the total number of transformants for each marker

of gene replication. If it does, the gene sequence on the chromosome should reflect the sequence of the function necessary for the reorganization of spores to become growing cells. In this respect, a positive effect of gene replication on the production of enzymes has been observed in *B. subtilis*[11], *Escherichia coli*[12], and yeast[14]. A unique messenger RNA produced at a stage of spore germination of *B. subtilis* also deserves attention[15]. The present system also allows us to separate physically DNA of the starting region of the chromosome from the rest, which should be useful for the structural examination of the replicating region of the chromosome.

The dichotomous replication of the bacterial chromosome demonstrated here should be operating also in the exponential growth phase in a Penassay medium[9], and presents several points of interest. An obvious advantage of the dichotomous replication model is that the generation time required for chromosome replication can be reduced to half, even if the rate of DNA replication at each replication point remains constant. In this connexion, the generation time in a Penassay medium (20 min) is approximately half that in a standard medium (35–40 min). Secondly, the initiation of chromosome replication does not necessarily require completion of previous chromosome replication. An attractive idea has been that in circular chromosomes the re-initiation of chromosome replication at the origin is triggered by the previous replication point reaching the terminus adjacent to the origin. The case of cure from a thymine-less condition[10] also indicates that such a requirement is not absolute. Thirdly, dichotomous replication is as well regulated a system as the ordinary single-fork replication. Apparently, re-initiation of the chromosome is repressed until the first replication point reaches the middle region of the chromosome. Fourthly, the germination process gives the best synchrony of chromosome replication so far reported and could be used in various studies. For example, the sequential increase of the enzyme level[11] in *B. subtilis* may gain better resolution. Finally, dichotomous replication should provide another convenient method for the mapping of markers on the chromosome.

Considering our previous work on marker frequency analyses[5], dichotomy is not expected during spore germination in an unenriched medium. During the progress of the present work, evidence for synchronous replication of tryptophan and methionine markers during spore germination in an unenriched medium was reported, where the ratio *tryp/met* did not indicate dichotomy[16].

As we have reported previously, unlike strain *W*23, strain *W*168 is an apparent non-polar strain which shows no difference in marker frequencies between DNA from exponential and stationary phase cells[2]. Recently, this apparent non-polar behaviour of strain *W*168 has been shown to arise from a less rigid regulation of chromosome replication rather than from a difference in the mode of replication (origin and direction of replication)[9]. Thus, when the spore DNA of *W*168 was used as the standard for marker frequency analysis, exactly the same polarity as in strain *W*23 was observed. The present results give direct evidence for the conclusion.

In summary, in *Bacillus subtilis* an excellent synchrony of chromosome replication has been observed during spore germination in an enriched medium. The system allows us to demonstrate the existence of dichotomous replication of the chromosome, in which three replication points per chromosome should exist.

This work was supported by U.S. National Science Foundation grant *GB*–1548 and U.S. Public Health Service grant *GM* 10923–02.

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PETIOLE BLEEDING SAP IN THE EXAMINATION OF THE CIRCULATION OF NITROGENOUS SUBSTANCES IN PLANTS

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THE sap which bleeds from the stump of a decapitated plant is usually regarded as arising from cut xylem vessels and has been used in studying export to the shoot of organic and inorganic compounds of nitrogen from the roots of plants[1–4].

These investigations have recently been extended by collecting bleeding sap simultaneously from the cut petioles of a plant. Such exudates are also considered to originate from the xylem, since they do not usually contain sugars, and in qualitative composition they resemble the bleeding sap collected from similar plants at ground-level. Analysis of petiole exudates may then be used to examine the composition of the xylem sap at different levels of the stem and to outline the part played by stem tissues in the initial assimilation of materials delivered from the root.

Four species have been investigated—tomato (*Lycopersicon esculentum* Mill.), balsam (*Impatiens glandulifera* Royle), field pea (*Pisum arvense* L.) and cocklebur (*Xanthium pennsylvanicum* Willr.). High root temperatures and short pre-treatments in a saturated atmosphere encourage an immediate exudation from cut petioles. The first drop of bleeding sap is discarded to minimize contamination from cut cells, and that collecting over only the following 0·5–1-h period is used. Sap analysis includes quantitative determinations of amino-acids and amides[5], measurements of conductivity and the concentrations of selected inorganic ions[2].

Tomato. In this species the level of organic nitrogen in the bleeding sap does not change significantly between lower petioles, but increases markedly from the middle to the upper petioles (Fig. 1). There is no parallel increase in the conductivity of the sap over the upper region of the stem. The amide glutamine accounts for 50–60 per cent of the organic nitrogen recovered in all exudates.

Balsam. This species also shows a substantial increase in levels of organic nitrogen in exudates from successive petioles up the stem, and a similar gradient is observed in exudates from the axillary shoots. More than 90 per cent of the organic nitrogen in petiole exudates is present as glutamine. Where free nitrate is recovered, its concentration tends to decrease towards the top of the stem, that is, a concentration gradient reverse to that of glutamine. On the other hand, phosphate-levels in petiole exudates show little variation along the length of the stem.

Field pea. The nitrogen recovered from the bleeding sap of nodulated plants raised in nitrogen-deficient media is entirely organic in form, and the amounts and relative proportions of the individual amides and amino-acids vary with the age and nutritional status of the host plant[1,5]. If petiole bleeding saps are studied at different stages in the life of the plant a progressive decrease in concentration of amino nitrogen is always observed from the oldest to the youngest petioles (compare tomato and balsam). In a typical analysis (Table 1) asparagine may show an eight- to ten-fold decrease in concentration from base to top of the stem, while much smaller concentration gradients are recorded for glutamine, homoserine, aspartic acid and the minor constituents of the bleeding sap.

Cocklebur. This species contrasts with the field pea in showing practically no organic nitrogen in petiole bleeding sap. Plants fed with varying amounts of nitrate all show free nitrate in their bleeding sap and tend to exhibit a decrease in concentration of nitrate from the lowest to the highest petioles.

All species mentioned here clearly exhibit gradients in the concentrations of the solutes present in exudates recovered from successive petioles up the stem. Since the volumes of bleeding saps are approximately proportional to the areas of xylem cut on the petiole surfaces, gradients in solute concentration must result from lateral exchange rather than from large differences in the rates of flow of water and solutes through different parts of the plant. The concentrations and gradients, of course, refer only to the defoliated plant, but there is evidence in at least two of the species (tomato[6] and field pea (Pate, unpublished

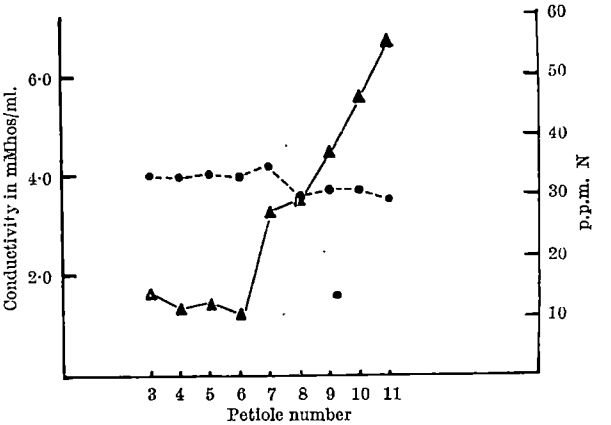


Fig. 1. Amino-acid content and conductivity of bleeding sap obtained from petioles of a 14-leaf tomato plant. Petioles are numbered from base to top of the stem

Table 1. Analysis of the concentrations of the common amino-acids and amides in bleeding sap collected from the 8 lower petioles of a 10-leaf nodulated plant of field pea grown in nitrogen-deficient medium

Petiole		Aspartic acid		Asparagine		Glutamine		Homoserine		Total N recovered in these four compounds μg N/ml.
(Lowest) oldest	1	101	12·2	425	51·3	249	30·0	54	6·5	829
	2	108	14·8	350	48·3	224	30·9	44	6·0	726
	3	108	17·4	265	42·9	210	33·9	36	5·8	619
	4	110	19·7	205	36·6	210	37·4	36	6·3	561
	5	112	26·0	115	26·8	175	40·7	28	6·5	430
	6	99	31·7	55	17·7	137	44·0	21	6·6	312
	7	88	33·9	35	13·5	119	45·9	18	6·7	260
(Highest) youngest	8	61	24·0	55	21·6	117	45·8	22	8·6	255

In each column the value on the left represents the concentration (μg N/ml.) of an amino compound, and that on the right the percentage that it represents of the total nitrogen recovered from the four common amino compounds (aspartic acid, asparagine, glutamine and homoserine).

results)) that a similar phenomenon may occur in the transpiring plant, despite the faster flow and lower solute concentrations to be expected in such a system.

It is interesting that several workers have described a lateral transfer of inorganic ions from wood to bark of various species of trees[7–9]. The investigations of Kursanov *et al.*[10] are also relevant in that they show a selective accumulation of organic substances by fibrovascular bundles. In the present work, lateral exchange with the transpiration stream is also envisaged as being selective, and may even involve a simultaneous accumulation of some solutes but release of others from living cells of the stem.

These exudate analyses, together with the results of other studies, reveal characteristic patterns of nitrogen assimilation in each of the species examined. The simplest situation is that encountered in *Xanthium*, in which the initial step in assimilation is the delivery of nitrate to centres of transpiration in the shoot. Practically no organic nitrogen moves in the xylem, and when nitrate is removed from the external medium it quickly disappears from the bleeding sap (Wallace, unpublished results). These observations suggest that roots do not function as major sites of nitrate reduction, and that there is no appreciable recycling of nitrate between the various pathways of translocation (compare studies on phosphate recirculation by Biddulph[11]). The absence of free nitrate in *Xanthium* leaves (Wallace, unpublished results) suggests that they are very active in reducing nitrate, and that the derived amino compounds are in part exported to other organs. In contrast, petioles and internodes are usually rich in stored nitrate, and these reserves are the last to be used when plants are removed to nitrogen-deficient media.

A slightly different type of circulatory system can be associated with nitrate-fed plants of balsam. Here, both organic and nitrate-nitrogen are regular constituents of the bleeding sap. The organic nitrogen leaving the root might result from a reduction of part of the incoming nitrate in the root, while that released from stem tissues might be derived from metabolism of nitrate accumulated in the stem axis. The marked upward concentration gradient in glutamine, and downward gradient in nitrate, would imply that older leaves receive greater amounts of nitrate via the transpiration stream, while younger leaves may utilize the amino group of amides as the major source of nitrogen. Of course, young leaves might also import other protein amino-acids through their phloem connexions with older organs.

Considerable information is available on the metabolism of nitrogen-containing substances in the tomato plant. Glutamine, γ-amino butyric acid and glutamic acid are the main components moving upwards from the root[2], and these have been shown to result primarily from the amination of α-ketoglutaric acid to glutamic acid[12]. The root system of tomato is relatively inactive in reducing incoming nitrate. In consequence, the level of organic nitrogen in bleeding sap is low (15–30 p.p.m. nitrogen) when nitrate is the sole source of nitrogen, but high (up to 200 p.p.m. nitrogen) when plants are fed with ammonium salts.

Nitrate reduction in the tomato has been shown to take place largely in photosynthesizing tissues[13], and the upward gradient in bleeding sap nitrogen probably results from an export of amino-acids recently synthesized in the mature leaves. Such an export may take place in both xylem and phloem elements (compare work on bean plants[14] showing a movement of photosynthetic products in elements of the secondary xylem).

Recent work on the tomato has shown that certain amino-acids may be progressively absorbed by surrounding tissues as they ascend the stem with the transpiration stream[15]. Selective absorption of this type may involve living cells in the immediate vicinity of the conducting vessels[6], and may well allow a direct exchange of solutes with the phloem and a rapid equilibration of solutes with the storage tissues of the stem.

The nodulated legume differs from the other species in that a substantial part of the carbohydrate produced in the shoot is immediately translocated to the root and utilized in the fixation of atmospheric nitrogen by root nodules[16]. A proportion of the derived amides and amino-acids is then exported in the transpiration stream[17]. Only a limited spectrum of amino compounds moves upwards in the xylem, and considerable metabolic rearrangement must occur in stem and leaves before a full complement of amino-acids is available for protein synthesis. The abundance of amides in bleeding sap suggests that they may be used in many of the synthetic and transamination activities in the shoot. Metabolism in the legume shoot is apparently complicated by an active and selective accumulation of incoming nitrogenous materials into stem tissues. Hence, younger leaves are supplied with xylem fluids with only a fraction of the concentration of amino nitrogen entering the lowest leaves. Transpirational delivery, in fact, favours older organs close to the root, and the deficiency of nitrogen going into younger organs and meristems may well be made up by redistribution through the phloem. Such an effect can, indeed, be demonstrated if amino compounds labelled with carbon-14 are fed to intact shoots via the transpiration stream. Accumulation of labelled material into the lower leaves and internodes is then found to be greatly in excess of present requirements in storage, and some 20–30 per cent of the label entering such tissues is released within 24 h and exported to meristems and actively expanding organs at the top of the shoot. Some of the materials sequestered by older parts of the plant are transferred direct and unchanged to the phloem, while others are metabolized and assimilated *in situ* into protein. This protein is then broken down and a proportion of the resulting amino-acids released for transport to other organs higher up the shoot.

We thank Prof. D. J. Carr, Botany Department, Queen's University, for his advice. Two of us (J. S. P. and W. W.) also acknowledge the support of the Agricultural Research Council in these investigations.

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LETTERS TO THE EDITOR

ASTRONOMY

Colour on the Moon

FOR more than half a century there have been reports of colour on the Moon. Few of these confirm one another in detail.

During the past three years, applied commercial photography techniques have been used in an attempt to establish what colours, if any, occur on the Moon, and in what regions.

Using 'Tripack' colour films, including 'Daylight' Kodachrome Types 1 and 2, high speed Ektachrome and *E*3 Ektachrome as well as colour negative materials, on a small telescope, no clearly defined colour was detected.

After fruitless tests by other methods, it was found that 'colour correcting masks'[1] (transparent film positives, having about 70 per cent of the contrast shown in the negatives) applied to high-contrast negatives made from suitable transparencies yielded, on colour paper, clear local variations in colour for different parts of the lunar surface. It was further found that the regional distribution of colour differences was reproducible from phase to phase, and month to month, as well as at different image scales on the telescope. It was later found that using high contrast black and white film on the telescope, and making exposures through tricolour filters on to separate frames, gave negatives which, when enlarged and masked, enabled a colour print to be made having substantially greater colour saturation than those made from 'Tripack' transparencies. This method confirmed the pattern of regional colour distribution

The two black and white prints (Figs. 1 and 2) are made from the red filter and green filter negatives, respectively, of the direct separation set described here. To deduce the colours present, one may say that, where both prints are light in tone, then the lunar feature is light; and where both are dark, the Moon is dark. But, where the red filter print is light and the green filter print dark, this indicates a red region (yellow greatly amplified becomes reddish in this process). From this we see that M. Serenetatis is redder (yellower) than M. Tranquillitatis with a clear-cut boundary; other clearly red regions are M. Frigoris, the north-west corner of M. Imbrium, the north-west corner of Sinus Iridum, the outer rays and interspersed material of Copernicus, the northern rays of Kepler, the floor of Plato, as well as the floors of craters for about 300 miles around Alphonsus. The three dark spots in Alphonsus are also brownish. The four very dark patches lying between the Hyginus cleft and Copernicus are blue.

It is interesting to compare these prints, using visible wave-lengths, with the two black and white prints made by W. H. Wright[2] in 1926, using invisible ultra-violet and infra-red. The colour distribution is virtually identical. Furthermore, Miethe and Seegert[3], in 1911, using ultra-violet and orange light, also obtained essentially the same regional distribution of colour.

A photoelectric photometer was used with tricolour filters to read those areas on the Moon having the most sharply defined colour differences. These readings gave a value of approximately 1·0 per cent blue/yellow colour difference. This was confirmed by tricolour densitometer readings from actual colour transparencies, which, allowing for the slight increase in colour contrast of these materials, gave values of the same order. It was then found that these slight colour differences could just be seen on the transparencies.

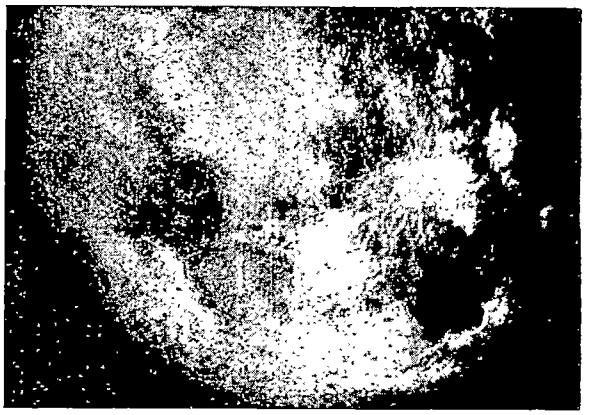


Fig. 1. Red filter

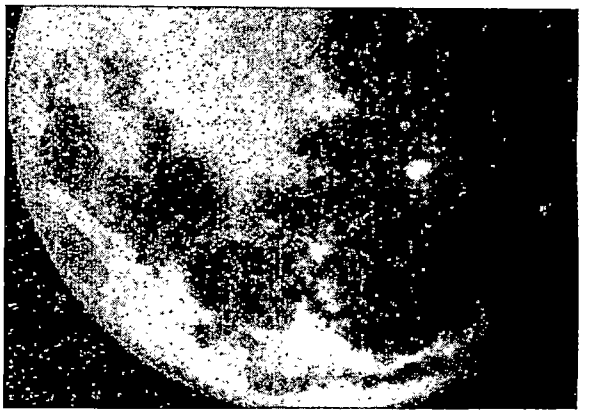


Fig. 2. Green filter

Tricolour reflexion densitometer readings from colour prints derived from the 'masked' separation negatives showed a gain in colour contrast of the order of 50–100 times. Full colour prints describe and define the distribution of colour far more completely than separate black and white prints or a verbal description.

During the course of this work it was observed that considerable colour variations in *integrated* light from the Moon occurred, having no relation to phase or zenith distance. Frequently the Moon was recorded on colour film as a sandy yellow, but even 24 h later, on the same film, might be recorded as a silvery blue. This has been confirmed on the tricolour photometer. Under 'blue Moon' conditions there appears to be an excess of blue light which swamps the basic yellow surface. The ultra-violet reflexion is also enhanced. The increase in blue content may be as much as four times. Under 'blue Moon' conditions the terminator remains relatively yellow.

Efforts have been made to correlate these blue/yellow variations in integrated lunar colour with terrestrial meteorological conditions, but without success. It has been observed that colour transparencies exposed in clear sunlight never show variations of this order for a given filtration suitable for the emulsion batch in use, provided exposures are not made within about 2 h of sunset or sunrise. The observed night-time lunar effect has been shown by experiment to be equivalent to exposing film to a sunlit subject with, and without, a Wratten *CC*50 yellow filter.

These variations of the order of four times for the blue content are absolute variations, and not shown as a result of the fifty times amplification derived from the masking procedure. Tricolour photometer readings obtained early on any particular night's observation, for a Moon not less than 30° above the horizon, have so far shown no detectable hue drift over a period of up to 4 h.

It appears that it is only possible to obtain clear colour prints from transparencies (or separation negatives) exposed under relatively 'blue Moon' conditions.

Over the past three years some 30 per cent of observations showed 'blue Moon' conditions, which do not appear to be statistically less frequent with the approach of the year of the Quiet Sun; for four nights in January 1964 the following were observed, photographed and recorded photoelectrically: January 21 and 26, yellow Moons; January 24 and 28, blue Moons. The photographic record was obtained on a single length of 35 mm Kodachrome II film, processed in one piece by Kodak, Ltd. Tests on standard test objects, including grey scales, for the 'Tripack' colour films used show negligible hue shifts for the full range of exposures used in the telescope.

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On a Possibility of measuring the Einstein-shift Photoelectrically

Willmore's[1] method of tracking artificial Earth satellites optically by photoelectric observations seems to be suitable for providing methods of measurement in astronomy with new objectivity and convenience.

The method sets one or more slits in the focal plane of a telescope instead of the reticle, which, as soon as an object appears on them, emit, by a multiplier, an electric signal. Such a system could be appropriate for substituting Repsold's impersonal micrometer; I am now carrying out experiments along these lines. The main problem with this effort is the improvement of accuracy. Fea and Newton[2] have also adopted the method with regard to satellite observations. The accuracy of their device is better than 2 sec of arc. Some of the points of view which led the authors to this figure are discarded when using a star instead of the fast-moving satellite, and the others, partially, referring to the equipment, are worth an attempt towards improvement.

Having made the photoelectric methods an equal partner of the conservative astrometric methods one could make further attempts to examine the effect of the deflexion of starlight by the Sun's mass, resulting from the theory of relativity; but in the past observations made by several astronomers did not accord with theory. Recently, Schmeidler[3] published a short summary of those efforts.

The use of photoelectric methods should result in faster and surer measuring methods, and also very faint stars might be recorded because of the sensitivity of the multiplier tube being greater than that of the photographic layer.

In principle the suggested method would measure the places of the shifted stars directly during the eclipse and not thereafter on a photographic plate. Nevertheless one should determine the places and proper motions of the unshifted stars some months before or (and) later, in the same manner.

For measuring the R.A. of a star it would be sufficient to allow the star to trail across a slit and to record the time of crossing. However, the time available is too short to measure the R.A. of all stars of the field, but one might use several slits and multipliers. The positions of the slits have to be calibrated by crossings of fundamental stars. The time and the star signals would be registered on a multichannel recorder.

More difficulties are met in measuring the declinations. One or more slits should be provided parallel to the equator. A slow motion of the slits through the focal plane, that is, an adequate slow motion of the entire instrument in declination, could replace the motion in hour angle of the device above. This motion having no natural correlation to time we meet the necessity of recording the momentary position of the slits or the instrument, too. We might do this by recording the readings of a level, but since a level should be subject to very slow motions and there is no instrument good enough to make such registration, we should use, for example, some kind of pendulum device with an electronic transducer, such devices being capable of measuring fractions of seconds of arc. While measuring the declination in this manner the instrument should follow the apparent movement of the firmament.

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PHYSICS

Electron Paramagnetic Resonance in Spinach Chloroplasts at Low Temperature

Photo-induced electron paramagnetic resonance signals in green plant material have been investigated for many years[1–4]. The description of the parts played by these unpaired electrons in the energy conversion processes of photosynthesis still remains a challenge. This communication describes the initial results of an approach based on the examination of the electron paramagnetic resonance signals at low temperature. It is hoped that the interpretation of the results is simplified in frozen systems by the possibility of excluding mechanisms that proceed with negligible rate in this state, for example, enzymatically catalysed reactions. Thus the observed changes in electron paramagnetic resonance signal intensity are expected to occur near the beginning of the energy conversion sequence in photosynthesis.

The electron paramagnetic resonance spectrometer used in these measurements utilizes a cylindrical TE_{011} mode transmission cavity with balanced barretter mixer detection[5] and operates at a frequency of about 11 gc/s. Magnetic modulation at a few hundred c/s is used for first or second derivative display. The cavity is surrounded by a glass Dewar and its top is cooled with a suitable liquid. Two separate 500-W tungsten projection lamps with water filters, interference filters, and coupled shutters are used to illuminate the sample simultaneously from front and back.

The 'whole' spinach chloroplasts were prepared by the method described by Pon[2]. The samples were run in 3 mm internal diameter quartz tubes after dilution with buffered sucrose solution to a suitable optical density. The optical transmission coefficients were measured in a flat 3-mm cell at a wave-length of 666 mμ with 20 mμ band-width. The light hitting the surface of the sample in the cavity has a wave-length of 700 mμ with about 20 mμ band-width and an intensity of 10^{16} quanta/cm²/sec. The chloroplast concentrations used corresponded to 1 per cent transmission at 666 mμ and 10 times this concentration. Neglecting scattering and assuming that the intensity decreases exponentially with sample penetration the 1 per cent transmission results in a maximum light intensity variation of 5 : 1 with the 2-light system. The

Table 1. Characteristics of the Three Electron Paramagnetic Resonance Signals in Spinach Chloroplasts at −40° C

Signal	g-value	Width (max. slope)	Rise time	Decay time in dark
S	2·004 ± 0·001	20 ± 2 œ	—	—
R_1	2·003 ± 0·001	9 ± 1 œ	<1 sec	sec
R_2	2·003 ± 0·001	8·5 ± 1 œ	min	h

low concentration proved useful for verifying kinetic studies while the high concentration was used for line shape and g-value measurements. The peak of the second derivative is monitored for kinetic measurements and the first derivative is used for the other studies. Potassium nitrosyl disulphonate crystals were prepared[6] and the magnetic field was calibrated at −40° C with the nitrosyl disulphonate ion in a water–methanol mixture using the room temperature g-value and hfs splitting[7]. Sample temperature is read by thermocouples attached to the top and bottom of the cavity.

At temperatures in the range −35° C to −50° C we found three electron paramagnetic resonance signals that can be distinguished by a combination of g-values, line shape and kinetic behaviour. We label the signals R_1, R_2 and S. The composite and individual signals are shown in Fig. 1, and Table 1 lists their characteristics. In some samples the S signal displayed partially resolved fine structure. No fine structure is observed with the R signals. Rough microwave saturation measurements at −40° C indicate that R_2 and S are inhomogeneously broadened lines with half maximum cavity input powers of about 10 mW and less than 1 mW respectively. We used typically about 6 mW input, about 3 œ modulation amplitude and time constant of 1 sec.

The broad signal S arises from the chloroplast sample and is present when the sample has been cooled in the dark from 0° C to −40° C. Although partially obscured by the overlapping R signals, no change could be detected in the relatively clear low field peak of S after many minutes of illumination. Our experimental uncertainty is too large to distinguish the R_1 and R_2 signals by g-value

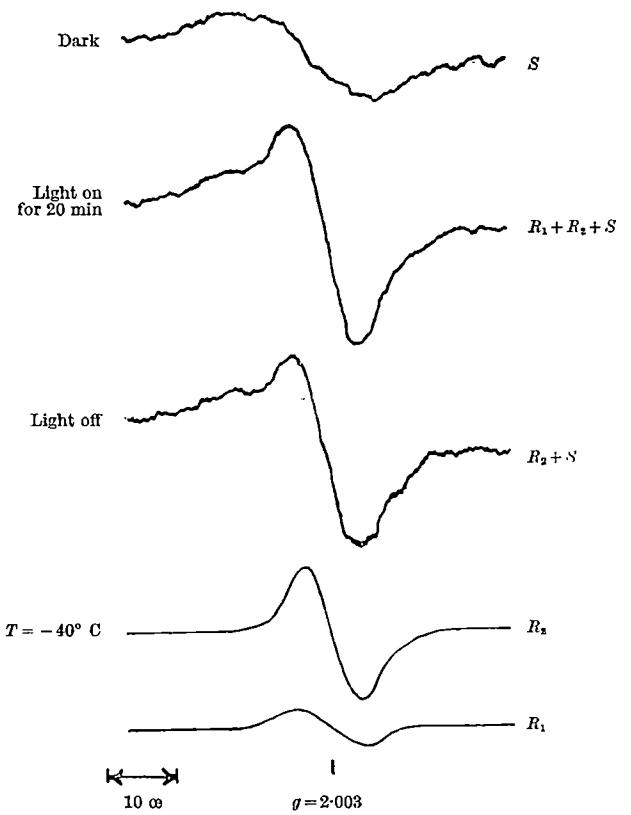


Fig. 1. Electron paramagnetic resonance signals in spinach chloroplasts at −40° C. R_1 and R_2 are obtained by subtractions of smoothed curves

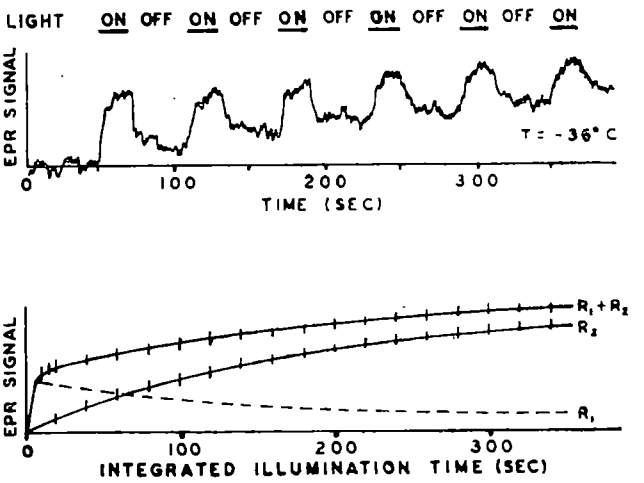


Fig. 2. Electron paramagnetic resonance signal rise and decay in spinach chloroplasts at −36° C with intermittent light and radical concentrations as a function of total illumination time

or line shape differences. That there are two R signals is made evident by kinetic data as shown in Fig. 2. The results are obtained with intermittent light having a typical 'on' time of 20 sec and 'off' time of 40 sec, while the peak height of the second derivative is continuously recorded. With a constant S signal and equal linewidths for R_1 and R_2, this method measures the relative concentration of the R_1 and R_2 electrons. When the light is turned on a composite R signal appears in a time that is instrument limited. When the light is turned off this R signal decays in several seconds to a value that we call R_2. The difference between the R signal and R_2 is designated the R_1 signal. The R_2 signal persists for many hours at low temperature but disappears in several seconds when the sample is warmed to 0° C or higher. The R_1 signal still exhibits a fast decay at −196° C. When intermittent light is incident on the sample kept at a temperature of about −40° C a slow build-up of the R_2 signal over a period of several minutes is observed while the R_1 signal becomes progressively smaller. The data also consistently show a slow rise in amplitude of the total R signal following the initial fast rise.

The interpretation of our results at this stage must be tentative. The radical S appears to be completely uncoupled from the light reaction in spinach chloroplasts at low temperature. The radicals R_1 and R_2 are intimately associated with the chlorophyll light receptors. R_1 reaches its maximum concentration very rapidly (less than 1 sec) after the light hits the chloroplasts and disappears in a few seconds when the light is turned off. It faces two modes of disappearance. It may: (*a*) return to its original 'non-electron paramagnetic resonance' state in which case it can return to its radical form when the light is turned on, or (*b*) it is converted or trapped into an R_2 radical and 'used up' so long as the low temperature is maintained. The behaviour of the R_2 radical is most interesting. A portion of the R_2 concentration comes from the slow rise of the total R signal and the remainder may be ascribed to the conversion or trapping of R_1 radicals. In the 10 different spinach chloroplast samples that have been examined these two proportions appear to be equal. At 10-min integrated illumination time the average ratio of the slow rise contribution to the amount of R_1 used up is 1·08 with a mean deviation of 0·15 and extreme values of 0·8 and 1·3. Thus the conversion of a single R_1 radical seems to give rise to 2 R_2 radicals. It is possible that R_1 initiates a reaction leading to 2 R_2 or perhaps the R_2 radicals are formed in pairs by two different, but coupled, light reactions; one proceeding via R_1 and one by a different route. Experiments at a set of temperatures down to −196° C are being undertaken with

the expectation that changes in relative values of the rate constants will define the mechanism more clearly.

This investigation was aided by contract *Nonr*–4028(00), *NR* 304–473, between the Office of Naval Research and Pomona College, and by a National Institutes of Health grant 2*G*–945.

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Measurement of the Emission of Low-energy Electrons in an Irradiated Vacuum Cavity Chamber

The residual current in an ionization chamber, when the pressure is reduced to very low values, has been attributed by Greening[1] to the transfer of slow electrons between the electrodes. He correlated several experimental studies[2–9] in order to estimate the number of low-energy electrons emitted from unit area of the wall of a vacuum chamber under irradiation. For graphite the emission was about 5×10^5 electrons/cm²/rad ($2{\cdot}5 \times 10^{-4}$ e.s.u./cm²/rad) and for elektron metal about $1{\cdot}0 \times 10^6$ electrons/cm²/rad (5×10^{-4} e.s.u./cm²/rad), when irradiated by radium γ-rays. From some measurements at reduced pressure in ionization chambers Burlin[10] also made an approximate estimate of the slow electron emission and obtained a value of 5×10^{-4} e.s.u./cm²/rad from graphite.

The magnitude of the slow electron emission from a copper surface irradiated by γ-rays has been measured directly. A spherical copper vacuum chamber, with the radii of inner and outer electrodes being 3·13 cm and 5·08 cm respectively, was irradiated in the γ-ray field from a 2,000-curie telecobalt 60 unit. The wall thickness of the outer electrode, 2·64 mm of copper, was sufficient to establish electron equilibrium.

The saturation characteristics of this vacuum chamber have already been reported[11] and are represented diagrammatically in Fig. 1. The plateau value of the current when the outer electrode is positive represents the case where all the electrons emitted from the central electrode reach to the outer electrode but all the electrons emitted from the outer electrode are repelled back to the outer electrode, that is, it represents the slow electron emission from an area $4\pi a^2$, where a is the radius of the central electrode. Similarly the plateau value of the current when the outer electrode is negative represents the emission from an area $4\pi b^2$, where b is the radius of outer electrode. Hence the difference between the two current plateaux represents the low-energy electron emission from an area $4\pi(a^2+b^2)$, and this is true even if the plateaux are displaced due to radiation attenuation or other effects. The current was measured on a vibrating reed electrometer. The input resistor was calibrated by means of a standard capacitor using a capacitor leak method and was $(0{\cdot}95 \times 10^{10}) \pm 1$ per cent ohms. The electrometer was calibrated to ± 1 per cent by means of a Weston standard cell and a potential divider. The difference between the current plateaux was found to be $0{\cdot}155 \pm 3$ per cent e.s.u./sec, which corresponds to an average slow electron emission of $(6{\cdot}96 \times 10^{-4}) \pm 3$ per cent e.s.u./sec/cm² from the chamber walls.

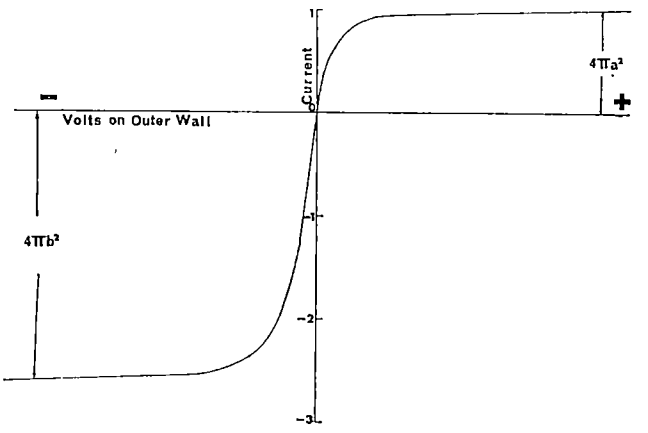


Fig. 1

There are two possible methods of determining the absorbed dose rate in the chamber walls. First, it can be calculated from the known exposure rate of the telecobalt 60 unit. This would involve a detailed knowledge of the radiation field and extremely complex corrections for the absorption and scattering in the chamber walls. In fact, these corrections would render this measurement of the slow electron emission rather inaccurate, and therefore this method was rejected. The second method is to fill the chamber with air at atmospheric pressure and use it as a cavity ionization chamber. The absorbed dose rate may then be determined using the Bragg–Gray[12] equation:

$$E_m = \frac{1}{f_m} W J_m$$

where E_m is the average absorbed dose rate in the chamber walls; f_m is the mass stopping power ratio of the gas to the solid for electrons; W is the average energy associated with the production of one ion pair in the gas; J_m is the ionization current per unit mass of gas.

The values for the average energy associated with the production of one ion pair, W, recommended by the International Commission on Radiological Units and Measurement[13] has been adopted (namely, 33·7 eV) and is assumed to be accurate to ± 1 per cent. To calculate the mass stopping power ratio for the high-energy electrons crossing the air cavity by the theory of Spencer and Attix[14] it is necessary to know the energy of an electron which will, on average, just span the cavity. The average path-length across a cavity formed by two concentric spheres can be shown to be:

$$\frac{4}{3}\frac{(b^3 - a^3)}{(b^2 + a^2)}$$

which in this case yields a value 3·76 cm. When allowance is made for the foreshortening of the electron range due to elastic scattering, an electron of 55·5 keV would traverse this distance[15]. The stopping power ratio, f_m, was then calculated as 1·32 from the Spencer–Attix theory, which recent experiments[15–17] have verified as accurate to at least ± 3 per cent in this particular case. The input resistor of the electrometer, measured on a Tinsley Wheatstone bridge, was $(1{\cdot}008 \times 10^6) \pm 0{\cdot}1$ per cent, giving a value of the ionization current of 497 $\pm$ 2 per cent e.s.u./sec corrected to N.T.P. The density of air was taken as 0·001293 gm/cm³ at N.T.P. Substitution in the Bragg–Gray equation then yielded an average absorbed dose rate in the chamber walls of $1{\cdot}56 \pm 6$ per cent rads/sec.

Thus dividing the average slow electron emission by the average absorbed dose rate yields a value of $4{\cdot}46 \times 10^{-4}$ e.s.u./cm²/rad or $0{\cdot}93 \times 10^6$ electrons/cm²/rad for the emission of low-energy electrons from copper, the surface of which was clean but oxidized, irradiated by 60 cobalt γ-rays. Allowing a maximum error of ± 5 per cent in

the volume to surface ratio, the maximum error in the value is ± 14 per cent and the most probable error ± 5 per cent. This result supports Greening's interpretation of the residual current in ionization chambers at very low pressures. Although Greening did not claim a high degree of accuracy for his estimates, this measurement of the low-energy electrons emitted from the walls of an irradiated vacuum cavity chamber is in remarkably close agreement with his estimates.

We thank Mr. D. E. A. Jones for allowing us to use the radiation facilities at Mount Vernon Hospital, Northwood, and Prof. J. F. Fowler and Mr. J. Yarwood for their advice. This work was supported by the Department of Scientific and Industrial Research.

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Tests with Solid Brushes on the Canberra Homopolar Generator

Early in 1963 a programme was begun with the view of establishing whether or not solid brushes could be used instead of liquid metal jets to collect the required current (1·6 million amp) from the rotors of the Canberra homopolar generator. Within the first few weeks of investigation it became clear that there were no insuperable problems. Many pilot tests were conducted and details of the design will be published later. Since then, brush gear has been designed, manufactured and installed, and large-scale tests have been made with this gear in the homopolar generator. The behaviour of these brushes has been very satisfactory.

The homopolar generator was designed to produce a roughly sinusoidal current pulse lasting for nearly 2 sec with a peak of 1·6 million amp. In the initial tests[1], this current was drawn from the rotors by means of sheet jets of sodium–potassium alloy. The brushes have been designed for the same duty as the NaK jets, namely, to carry 1·6 million amp from the rims (outer brush units) of the mild steel rotors, at a rubbing speed of up to 33,000 ft./min. The inner brush units were designed to take the same current from an auxiliary copper slip ring clamped to the rotor, with a rubbing speed of 11,000 ft./min. Both sets of brushes were required to carry current for about 2 sec.

The arrangement of the generator for these tests was different from that used for the initial tests. The lower rotor was used, the upper rotor being clamped. The inner brush units were bolted to contact blocks which, in turn, were bolted to copper segments clamped to the bottom pole face. The outer brush units were bolted to an annular copper ring around the rotor and were staggered to run on a band 2·5 in. wide on the rotor rim. Both sets of brushes were brought into contact with the rotor for the duration of the pulse only, this being done by means of compressed air. A complete outer brush set consists of 864 brushes of 1 cm² arranged in 72 units. Thus the peak current per brush is 1,850 amp. The inner set consists of 576 brushes, 2 cm thick by 0·5 cm wide, arranged in 36 units, the peak current in each brush being 2,780 amp. The brush material is a standard high copper grade (Morganite *CM1S*). The brush wear-rate appears to be about 0·0001 in. per pulse for the outer brushes and about one-twentieth of that for the inner brushes. The load holding each brush on to the rotor rim and slip ring was 8 lb. The contact voltage drop between the outer brushes and rotor was about 0·75 V at peak current, the inner ones being half this.

In order to facilitate tests, the decision was made to install only one-half on each set of brush units and proportionately to decrease peak current to 0·8 million amp. Maximum surface speed of the rotors used was 22,000 ft./min.

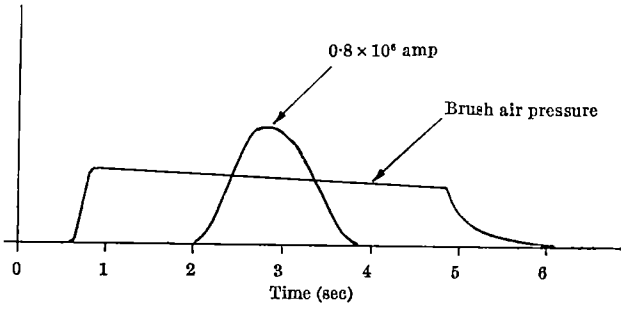


Fig. 1

During the tests, 137 current pulses were taken, 30 of which had 0·8 million amp peaks. Further, of these 30, four were taken from an initial rotor surface speed of 22,000 ft./min, and another 21 taken from an initial speed of 17,000 ft./min. The shape of the current pulses and the corresponding outer brush actuating air pressures are shown in Fig. 1. During these tests the machine and all its ancillary equipment functioned well for a total running time of more than 50 h. There has been no sign of damage to the brush track on the rotor rim so far.

The appearance and performance of the homopolar generator with brushes is clean, neat and impressive. These tests have demonstrated that large energy stores of this type are a safe practical engineering proposition, at least up to the conditions of the tests and probably up to the full design conditions. Life tests on the generator are continuing.

I thank Sir Mark Oliphant and my colleagues and the technical staff of the Brush Group for their help and advice.

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GEOLOGY

Some Radiometric Rock Ages and the Problem of the Southward Continuation of the East Greenland Caledonian Orogeny

The work of Lauge Koch and others has established that a powerful Caledonian orogeny occurred in what is now the coastal region of East Greenland, north of Scoresby Sound. Whether this orogenic belt continues down the east coast of Greenland and joins the Caledonian mountain

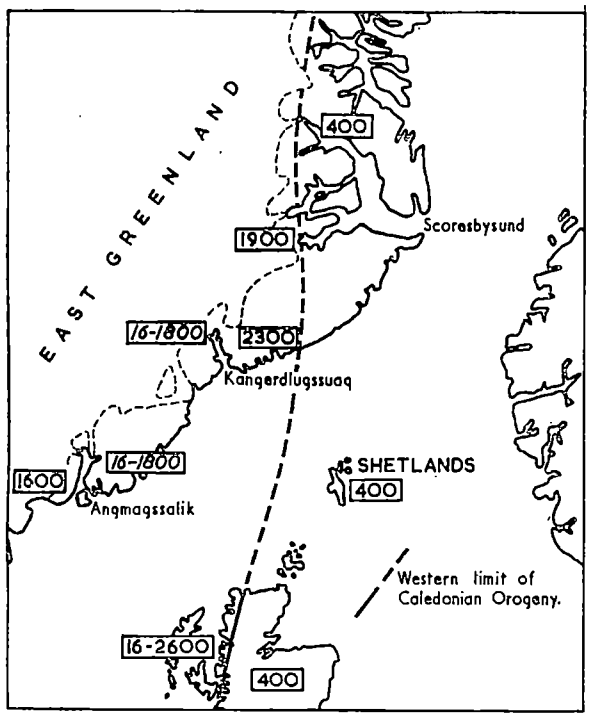


Fig. 1. Radiometric ages from East Greenland and Scotland in millions of years. The relative positions of the two sides of the Atlantic are those suggested by Bullard (Royal Society Symposium on Continental Drift, 1964). For Greenland, figures in ordinary type are from potassium/argon data and those in italics from rubidium/strontium

belt of eastern North America has been an open question but can now be answered by means of radiometric age determinations. The metamorphic basement rocks between Kangerdlugssuaq and Angmagssalik, which have a dominantly east–west structure[1], do not seem to fit into the idea of their having been involved in a north–south Caledonian orogenic belt; but if the effects of a Caledonian orogeny did not obliterate the earlier structures, this hypothesis would have to be accepted as a possibility.

With the view of deciding whether or not the metamorphic rocks south of Scoresby Sound have suffered from a Caledonian orogenic event ending about 400 m.y. ago, we have investigated the radiometric ages of rocks from certain critical areas. For some 200 miles south of Scoresby Sound, the coastal mountains (including the highest within the Arctic Circle) are cut from Tertiary basalt lavas which largely hide the basement complex. The most north-easterly exposures of the basement rocks in this area are in Nansen Fjord, 50 miles east-north-east of Kangerdlugssuaq (Fig. 1). These rocks are the most critical for the problem of the position of the Caledonian orogeny, and a granulitized biotite-gneiss collected from this point in 1935 has been investigated. The potassium/argon age of biotite from this rock proves to be 2,360 m.y. There is only a very small amount of rubidium in the biotite and a rubidium/strontium age could not be obtained. A hornblende from the basement complex at Kangerdlugssuaq has also been investigated and gives an age of 2,720 m.y. by potassium/argon, while biotites from the same rocks give 1,600–1,800 m.y. by rubidium/strontium. The ages of the rocks in this region are clearly high, but further work is required to understand the precise meaning of the divergent results.

The first exposed basement rocks south of Scoresby Sound apparently have ages comparable with the Scourian and Laxfordian rocks of Scotland. The ages of rocks from some parts of the Angmagssalik district (Fig. 1) also prove to be around 1,600 m.y., that is, comparable with the age of the Laxfordian, while in other parts the rubidium/strontium ages ranged from 1,600 to 1,800 m.y. Some of the Angmagssalik rocks are post orogenic from their textures and were thought to be perhaps Caledonian in age[2], but these also give ages around 1,800 m.y. For rocks from Gaase Fjord, a branch of Scoresby Sound, Kulp and Haller[3–5] have obtained rubidium/strontium ages of 2,300 m.y., while the potassium/argon age was found to be 1,900 m.y. These rocks, like those at Kangerdlugssuaq and Angmagssalik, lie to the west of the Caledonian orogenic belt (Fig. 1).

The chief ages determined so far from central East Greenland are shown on the map (Fig. 1). It is clear that the Caledonian orogenic belt must pass out to sea south of Scoresby Sound and north of Kangerdlugssuaq. If we accept the relative positions of Greenland and Europe, before the formation of the Atlantic, which have recently been obtained by Bullard[6] from estimates of the best fit of the 100 fathom line off the Greenland and European coasts (Fig. 1), it is seen that the East Greenland Caledonian orogenic belt passes, without change in direction, into the British Caledonian belt. The East Greenland metamorphic rocks between Kangerdlugssuaq and Angmagssalik, which lie west of the Caledonian belt, prove to be comparable in age to the Lewisian rocks of north-west Scotland, which similarly lie to the west of the Scottish Caledonian belt, and which they also resemble in general petrological character.

We thank Mr. D. C. Rex for carrying out the analytical work for the potassium/argon age determinations. A more detailed account of the ages obtained for these and other rocks from the East Greenland area will be given in the near future.

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Grain Growth Fabrics in the Cambrian Dolomites of Skye

Grain growth, as defined by metallurgists, is a process of migration of grain boundaries by which the grain mosaic adjusts itself to the requirements of minimum surface tension. Harker and Parker[1], Smith[2–4], and others, have demonstrated that the occurrence of grain growth in metals may be detected by the study of the intergranular relationships within the metal mosaic. It has also been shown that such a study may be made on any random section through the granular medium.

Bathurst[5,6] suggested that grain growth may be of a considerable importance in the diagenesis of calcareous rocks, but no quantitative study of this problem has been made.

In the course of investigation of the Cambrian dolomites of Sleat (Isle of Skye, Scotland), I conducted a series of observations on the intergranular relationships shown by these rocks in thin sections and photomicrographs. The average grain size of the mosaics examined varied from less than 20μ to 350μ, the grains did not show any preferred orientation. Following the procedure used in the metallurgical research, I recorded the frequency and shape of the triple junctions between the grains and measured the interfacial angles of these junctions: 200–300 triple junctions were analysed in each of the 32 sections investigated. The results obtained indicated the existence of two main types of textures: (1) those in which features attributable to grain growth were recognized; (2) those in which the

geometry of the mosaic suggests that no adjustment to the requirements of minimum surface tension has occurred.

The grain mosaics of the first type yield an interfacial-angle frequency distribution with a maximum in the range of 100°–140° (Fig. 1*a* and *b*). The proportion of triple junctions with three obtuse angles may reach 50–60 per cent of the total. Junctions with two straight angles are uncommon, usually less than 10 per cent of the total. The boundaries at the junctions are usually concave away from the smallest angle. The occurrence of three straight boundaries at junctions with three unequal angles is rare and so are boundaries which are concave towards the smallest angle. All these features correspond closely to those reported from metals in which grain growth was induced experimentally.

Mosaics of the second type yield an interfacial-angle frequency distribution with two maxima: one in the *r* range of 160°–180° and the other between 80° and 100° (Fig. 1*c* and *d*). Triple junctions with three obtuse angles are uncommon and rarely exceed 10 per cent of the total. At 40–50 per cent of the junctions having one acute angle the boundaries are either concave towards the smallest angle (that is, in the sense opposite to that expected to result from the surface tension adjustment), or are perfectly straight.

These mosaics, which obviously are not controlled by grain growth, fall into two main sub-groups.

The first one includes mosaics or parts of mosaics which originated as 'drusy growth', 'sparry calcite' or 'granular cement' in the sense implied by Sander[7] and Bathurst[5,6]. These mosaics are medium-to-coarse grained (100–350μ average grain size) and display a very high proportion of straight boundaries. Straight boundaries are thermodynamically more stable than the curved ones and thus the 'energy barrier' which must be overcome to start the migration of the boundaries is high. It is possible also that the migration was further hindered because of the relatively large original size of the grains (that is, low surface energy).

The other sub-group includes fine- and very fine-grained mosaics in which the straight boundaries are less frequent, whereas the junctions with three obtuse angles appear more frequently (at times up to 20 per cent of the total) than in the first sub-group. This seems to suggest that, though some grain growth may have taken place, the process of adjustment to the requirements of minimum surface tension has not proceeded to completion. One of the reasons for this 'locking' of the boundaries may lie in the presence of impurities. Indeed, in most of these samples the amount of insoluble residuum exceeded 15 per cent.

It has been found that, near planes of dislocation, the fine-grained rocks of this sub-group often pass into a rock with patchy texture. The coarse-grained patches which appear within the fine-grained matrix display all the features indicative of grain growth (Fig. 1*e*). It appears, therefore, that the tectonic stress may have supplied the energy necessary for the 'unlocking' of the boundaries.

The results outlined here indicate that grain growth (in the strict metallurgical sense) occurs in calcareous rocks. The occurrence or absence of a grain-growth stage in diagenesis may be established by quantitative study of the grain mosaic geometry—based on the metallurgical methods.

I thank Prof. C. S. Smith (Massachusetts Institute of Technology) and Prof. J. Sutton (Imperial College of Science and Technology) for their advice.

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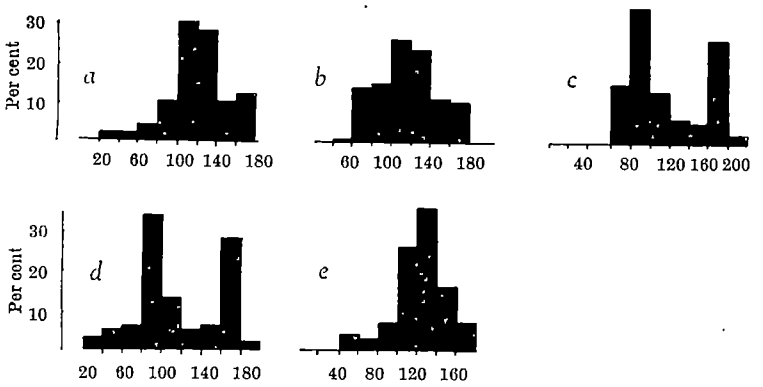


Fig. 1. Interfacial angle frequency distribution in the Cambrian dolomites of Skye. (*a*) Results from a thin section through a mosaic affected by grain growth; (*b*) cumulative results for all the grain growth mosaics,; (*c*) results from a thin section through granular cement; (*d*) cumulative results for all the granular cement mosaics; (*e*) results from patches in a thin section through a patchy mosaic

Graptolites from the Kashmir Himalayas, also a Note on the Discovery of Fossils in the Muth Quartzite

In continuation of our note[1] "Graptolites in the Indian sub-continent", field work was recommenced by one of us (V. J. G.) earlier in 1964 and resulted in further graptolite discoveries. The doubt conveyed in our previous note in referring the graptolites we illustrated to *Didymograptus* (?) was well founded because examination of these by Dr. C. J. Stubblefield revealed sicular structures of Monograptid type and he refers the figured specimens to *Monograptus* cf. *vulgaris* Wood and to *M.* cf. *colonus* (Barrande) of Lower Ludlow (Silurian) age. Additional graptolites collected this year from 3 ft. higher in the succession he refers to *Monograptus* cf. *dubius* (Suess), a species which, in Europe, ranges from Wenlock to Lower Ludlow in age. Further Monograptids were found associated with an internal mould of *Cardiola* cf. *interrupta* Broderip 30 ft. below *M.* cf. *dubius*; in Europe this lamellibranch species ranges from Wenlock to Ludlow. Thus we surmise that these 30 ft. of mudstones are of Lower Ludlow age, but possibly include some Wenlock.

These Silurian fossils are all preserved in weathered buff mudstones. They occur in the area mapped by Middlemiss[2] as Older Silurian and (?) Cambrian lying below those rocks in which he had found Upper Silurian shelly fossils.

The previously recorded occurrences of *Monograptus* nearest to Kashmir are in the Northern and Southern Shan States of Burma and, according to species determinations made by the late Miss G. L. Elles[3], they indicated a Lower Llandovery age.

The Muth Quartzite of Kashmir has been considered to be unfossiliferous since its first description by Stoliczka[4] more than a century ago. It overlies the fossiliferous division called by Middlemiss (ref. 2, p. 213) Upper Silurian rocks and underlies the Lower Carboniferous *Syringothyris* Limestone Series. Stoliczka[4] assigned an Upper Silurian age to it whereas Griesbach[5] regarded it as Carboniferous; Hayden[6] and Burrard and Hayden[7] considered it to be probably partly Silurian and partly Devonian. According to Middlemiss (ref. 2, p. 217), "No fossils

have been found it it; so that as in the case of Spiti and other areas its exact age cannot be known; the only limits being Upper Silurian below and Lower Carboniferous above. It may therefore be of either of these ages, or it may be Devonian, or represent any two or all three of them".

The fossils now found are well preserved and occur in profusion though extraction is difficult. We have identified the trilobite *Phacops rana* Green; the brachiopods *Athyris spiriferoides* (Eaton), *Meristella atoka* Girty, and *Spirifer* sp.; a gastropod as *Diaphorostoma* sp. and a lamellibranch as *Modiolomorpha* sp. This fauna indicates a Devonian age—thus proving the age of a part at least of the Quartzite.

Further work is in progress on both the new Silurian and Devonian faunas. *Note added in proof.* We have now learned that I. Strachan, J. W. A. Bodenhausen, T. de Booy and C. G. Egeler have announced (*Geol. en Mijnb.*, 43, 380; 1964) the finding of early Silurian graptolites in Nepal.

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CRYSTALLOGRAPHY

Electron Diffraction Study of Evaporated Beryllia

When beryllia flakes, supported on thin carbon films to form electron microscope specimens, are strongly irradiated with an electron beam, they disintegrate and small crystals condense on the surrounding carbon film. Willis, Austerman and Dearborn[1] reported that the condensed material gives electron diffraction ring patterns which they identify as being those of the high-temperature β-BeO phase examined by Smith, Cline and Frechette[2]. These latter authors suggest that the β-phase is cubic, $a_0 = 4{\cdot}76$ Å, with a non-trivial structure. According to Austerman[3], more recent work has suggested that the structure may be hexagonal or tetragonal.

The ring patterns of Willis *et al.* contain rings attributable to both the β-phase and the low-temperature α-phase. The absence of the (002) ring on α-BeO could be assumed to result from preferred orientations of the crystals.

In the course of an investigation of the disintegration of BeO crystals under electron irradiation in our *JEM-6A* electron microscope we have made observations on the crystals condensed on the supporting film which parallel those of Willis *et al.* However, we have found that under certain ill-defined conditions of electron irradiation, the condensed crystals take the form of comparatively large, thin crystal plates (linear dimensions of a few thousand Å) from which we have obtained fragmentary single-crystal patterns by selected-area diffraction methods.

From some areas we have obtained almost complete rotation patterns, given by a crystal or a set of parallel crystals bent around one axis.

Interpretation of these patterns shows clearly that the predominating ring pattern comes from a single substance rather than from a mixture of the α and β phases. The rotation pattern is readily interpreted in terms of a hexagonal unit cell with $a_0 = 5{\cdot}40$ Å and $c_0 = 4{\cdot}62$ Å, the accuracy of determination being in each case about 0·5 per cent. The rotation is about the c-axis. On the zero layer line, $l = 0$, the $hk0$ arcs are present only for both h and k even. The same may apply for $l = 2n$ because weak spots with h or k odd on layer lines with $l = \pm 2$ may be due to secondary scattering. Because the a dimension is very close on twice that for α-BeO (2·70 Å) and the c-dimension is very nearly equal to the (100) spacing (4·68 Å) the principal α-BeO rings appear to be present in the ring patterns (apart from the 002), and the rings not attributable to α-BeO have radii showing simple arithmetic relationships which could suggest a cubic structure.

The relationship between this hexagonal phase and the high-temperature β-BeO phase of Smith *et al.* remains obscure. The strong preferred orientation thought to be present in the high-temperature phase could possibly limit the pattern of the hexagonal phase to lines which could be indexed as cubic, and the report that the high-temperature phase could be hexagonal is suggestive. An identification of this sort would remove doubts that the observed hexagonal phase is actually BeO, since the possibility must be considered that it results from reaction of the BeO with the gases present or the carbon supporting film.

The existence of a structure derived from α-BeO with commensurate unit cell dimensions (except for a 6 per cent increase in c-axis) is of particular interest in relation to the anisotropic expansion of the lattice of BeO irradiated in a nuclear reactor at moderate temperature[4]. The relatively great expansion in the c-direction commonly observed could be explained if a primary effect of irradiation was the formation of small nuclei of this hexagonal phase.

This work was supported by a research contract from the Australian Atomic Energy Commission.

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METALLURGY

Effect of Grain Boundaries on the Diffusional Growth of Oxides on Metals

Several mechanisms[1-3] for the growth of thin films of oxides on metals have yielded equations of the form $y^3 = kt$, where y is the film thickness, k a rate constant, and t, time. However, none of these mechanisms can be satisfactorily extended to the case of thick films formed on such metals as titanium, zirconium and tantalum[4]. It is proposed that this so-called 'cubic' oxidation can result from a combination of diffusion along grain boundaries and normal bulk diffusion in relatively thick oxide films.

The usual high-temperature protective oxidation of metals obeys a parabolic kinetic law, $y^2 = k't$, and the mechanism universally accepted for this law is that proposed by Wagner[5]. The oxide is assumed to grow by the diffusion of defects across the film. Constant defect concentrations at the metal–oxide and oxide–gas interfaces provide a gradient of chemical potential inversely proportional to film thickness. It is assumed in this mechanism that diffusion occurs across the bulk of the oxide film and that grain boundaries in the oxide film can be safely ignored.

In laboratory determinations of diffusion coefficients the possibility of grain boundary effects, especially at moderate temperatures, is generally appreciated[6]. The significance of grain boundary effects can be examined by

two methods derived from the mathematical solutions of the equations appropriate to diffusion into a semi-infinite body from a planar source. The more usual method is to plot the logarithm of the concentration of the diffusing species as a function of the distance from the source. However, since growing oxide films have a constant concentration difference across them, this method is not applicable. The alternative is to determine the distance from the source at which a given concentration-level is found at successive times. For bulk diffusion the distance is proportional to $t^{1/2}$, but for grain boundary diffusion the function is $t^{1/4}$ (refs. 7 and 8). It has been generally appreciated that these two laws ($t^{1/2}$ and $t^{1/4}$) are limiting cases. Hitherto it has not been possible to appreciate the intermediate kinetic behaviour when grain boundary diffusion is playing some part but is not completely dominant.

Two papers have recently been published[9,10] presenting results of the numerical integration of the differential equation governing diffusion from a constant planar source into a semi-infinite solid containing a grain boundary perpendicular to the surface. These permit a closer examination of this intermediate behaviour, which can conveniently be discussed in terms of the parameter $\beta = \frac{D'}{D}\frac{\delta}{2}\frac{1}{\sqrt{Dt}}$, where D' is the diffusion coefficient in the grain boundary of width δ, D the coefficient of bulk diffusion, and t, time. This parameter can be regarded as a measure of the relative significance of grain boundary to bulk diffusion. If $\beta < 0{\cdot}1$ grain boundary effects are negligible and the $t^{1/2}$ law applies. When $\beta > 10$ grain boundary effects are dominant and the law becomes $t^{1/4}$. By using the published results for $\beta = 1$ and $\beta = 5$ (ref. 9) it has been demonstrated that the exponent of t decreases smoothly, as might be expected from $\frac{1}{4}$ to $\frac{1}{2}$ as β increases from approximately 0·1 to 10 and that values close to $\frac{1}{3}$ correspond to values of β between about 1 and 5, that is, when the effects of grain boundaries are comparable to those of bulk diffusion.

Since β is itself a function of time ($\propto t^{-1/2}$) the value of the exponent of t for $0{\cdot}1 < \beta < 10$ is not strictly a constant. However, the exponent increases from $\frac{1}{4}$ to $\frac{1}{2}$ as β is varied by two orders of magnitude, and this corresponds to a variation of four orders of magnitude of time. Hence, in practice, where time-intervals rarely cover even two orders of magnitude, the variation will be small and could easily be obscured by the scatter of experimental results.

To apply the above kinetic behaviour to the growth of oxides on metals with the usual assumption of the Wagner oxidation mechanism (constant concentrations of the diffusing defects at the metal–oxide and the oxide–gas interfaces) the oxide thickness should be equated to the penetration from the surface to a constant concentration contour. The published figures[9,10] relate solely to the penetration at the grain boundary, whereas oxidation measurements, as usually determined by weight gain or volumetric methods, provide a mean thickness of oxide film. The difference between the mean thickness and maximum grain boundary penetration is seen[9,10] to depend on both β and the concentration difference. For fairly small values of β and for the small concentration differences associated with defects in oxides this discrepancy between penetration and mean thickness is not expected to be large. It should also be noted that the model for the mathematical analysis involves only a single grain boundary, and hence is strictly only applicable if the boundaries are sufficiently far apart to exert independent influence. Grain size in oxide layers is typically of the same order of magnitude as the thickness and therefore this condition does not apply. However, one effect of multiple boundaries will be to make the mean thickness closer to the grain boundary penetration. Mathematical analysis of the multiple grain boundary model has not been evaluated but to a first approximation the effect of multiple boundaries will be to increase the concentration within the grains and that other boundaries will have relatively little effect on the penetration at a boundary itself. This lends some support to the application of the kinetics of grain boundary penetration to oxidation.

Since grain boundary diffusion kinetics arise from a combination of penetration along a boundary and lateral spreading away from the boundary some modification due to multiple boundaries is to be expected. In general terms it can be seen that the effect of material reaching the grains from more than one boundary will be to reduce the tendency of material to diffuse laterally away from grain boundaries. The magnitude of this effect is expected to increase as grain size is reduced, and in the limit of infinitely small grains diffusion would be characterized by the grain boundary diffusion coefficient alone. At this limit a parabolic law would again apply. The distance from a grain boundary enters the calculations as a reduced dimensionless parameter which incorporates $t^{-\frac{1}{2}}$ and therefore the effect of the grain boundary spacing in the multiple grain boundary model is also expected to be time-dependent. In the early stages with thin oxide films the boundaries are comparatively widely spaced and act independently with the kinetics of the mathematical model. However, the mean thickness will be comparatively less like the grain boundary penetration than in the later stages where the oxide thickness is similar to the grain size. At this stage, however, the mathematical model is inadequate. These two effects, the initial difference between mean thickness and grain boundary penetration and the later significance of grain boundary interaction, both act in the same sense to make oxidation behaviour somewhat closer to parabolic than the mathematical model of grain boundary diffusion indicates.

The boundary conditions used for the numerical integration correspond to a uniform concentration of diffusant over a planar source surface. For gaseous oxidation with growth proceeding by anion diffusion this should be appropriate provided the outer interface remains reasonably planar. For growth by cation movement this condition requires that the metal–oxide interface is planar and has high surface diffusion rates (with little surface diffusion at the gas–oxide interface). The boundary conditions would have to be modified if these assumptions do not apply.

To take account of grain boundaries the kinetic equation for the diffusional protective growth of oxides on metals has to be generalized to $y^n = kt$, where n takes the value 2 for negligible effect of boundaries but may be as high as 4 for complete dominance of grain boundary diffusion. The effect of grain boundaries may not be constant during oxide growth and the value of n may similarly change.

Not only does a consideration of grain boundaries in an oxide film provide a satisfactory mechanism for cubic and similar growth laws but it also suggests new possible mechanisms for the effect of alloy additions on oxidation characteristics and such phenomena as breakaway oxidation. It is hoped to explore these and give a more thorough analysis of the effect of grain boundary diffusion at a later date.

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CHEMISTRY

Vapour Pressures of Xenon (77°–180° K) and Krypton (77°–130° K)

For the evaluation of recently measured low-temperature adsorption data we required the vapour pressure curves of krypton and xenon from 77° K to about 20° K above their respective triple points. We therefore undertook a critical review of the literature in order to try to define for each gas the vapour pressure curve that is best substantiated by the reported values. We found that the vapour pressure data for krypton published by various authors[1–6] are generally in good agreement with each other. Most reported values lie within ± 1 per cent of the curve given by the vapour pressure equations of Freeman and Halsey[6], which can therefore be used with some confidence.

On the other hand, the vapour pressure data for xenon[3,6–9] show more serious inconsistencies, and the use of the equations recommended for solid xenon in different temperature regions results in a sharp change in the slope of the curve of $\ln p$ against $1/T$ when changing over from one equation to the next, although it is clear that no corresponding phase change of the condensed xenon occurs.

We have therefore examined the available vapour pressure data for xenon and compared them with the vapour pressure curve, which can be calculated from calorimetric data[10], in an attempt to find a satisfactory smooth curve from 77° K to 180° K. The vapour pressure equations considered are given in Table 1.

The *FH* equation for liquid xenon deviates quite considerably, especially towards 180° K, from those of *HO* and *MW*, which agree with each other within 0·5 per cent. For solid xenon, the temperature regions of the two available vapour pressure equations (*FH* and *PD*) do not overlap ; extrapolation of the *PD* equation yields an intersection point at 111·5° K, where, however, solid xenon exhibits no phase change.

A first indication of the reliability of the slopes of the vapour pressure equations near the triple point is obtained from Table 2 in which the Clapeyron melting enthalpies calculated from the difference in slopes of the vapour pressure equations at the triple point are compared to the calorimetrically measured values[10,11]. It is inferred that it is probable that the slopes of *HO* (and therefore of *MW*) and of *FH* for the solid are close to the true ones, whereas the slope of the *FH* liquid equation is erroneous. For krypton the two *FH* equations yield a value which is in reasonable agreement with the calorimetric one.

Table 1. Vapour Pressure Equations for Xenon

Vapour pressure equation (p) (mm Hg)	Stated temperature range in which equation valid (°K)	Authors and abbreviations used in text
$\log_{10} p = 7{\cdot}2488 - \frac{720{\cdot}7}{T}$	160·56–166·2	Freeman and Halsey[6] (*FH*)
*$\log_{10} p = 6{\cdot}9903 - \frac{678{\cdot}49}{T}$	160·8–166·0	Heuse and Otto[7] (*HO*)
$\log_{10} p = 26{\cdot}08413 - \frac{1{,}040{\cdot}76}{T} - 8{\cdot}25369 \log_{10} T + 0{\cdot}0085216\, T$	161·7–289·2	Michels and Wassenaar[8] (*MW*)
$\log_{10} p = 7{\cdot}7371 - \frac{799{\cdot}1}{T}$	110·0–160·56	Freeman and Halsey[6] (*FH*)
$\log_{10} p = 8{\cdot}044 - \frac{833{\cdot}33}{T}$	70 – 90	Podgurski and Davis[9] (*PD*)

* Equation obtained from their data with the method of least squares.

Table 2. Comparison of Calorimetric and Clapeyron Melting Enthalpies

	Calorimetric (cal/mole)	Clapeyron from *FH* equations (cal/mole)	Clapeyron from *FH* and *HO* equations (cal/mole)
Kr	390·7 (ref. 11)	401	—
Xe	548·5 (ref. 10)	359	552

The calorimetric data of Clusius and Riccoboni[10] are extensive, and have been used to calculate a calorimetric vapour pressure curve with the equation:

$$\log_{10} p = 4{\cdot}464 + 2{\cdot}50 \log_{10} T - \frac{L_0}{4{\cdot}576T} - \frac{\int_0^T \frac{c_p}{T}\,dT}{4{\cdot}576} + \frac{\int_0^T c_p\,dT}{4{\cdot}576T} - \frac{Bp}{4{\cdot}576T}$$

The last term is only significant above 120° K. It is a correction term for the non-ideal behaviour of the saturated vapour which is assumed to obey the equation of state, $pV = RT + Bp$, where:

$$B = \frac{9}{128}\frac{RT_c}{p_c}\left(1 - 6\frac{T_c^2}{T^2}\right)$$

from the Berthelot equation. In Fig. 1 it is shown how, using a value for the latent heat of sublimation of xenon at 0° K (L_0) of 3,780 cal/mole, which is also the value recommended by Hollis Hallett[12], the calorimetric vapour

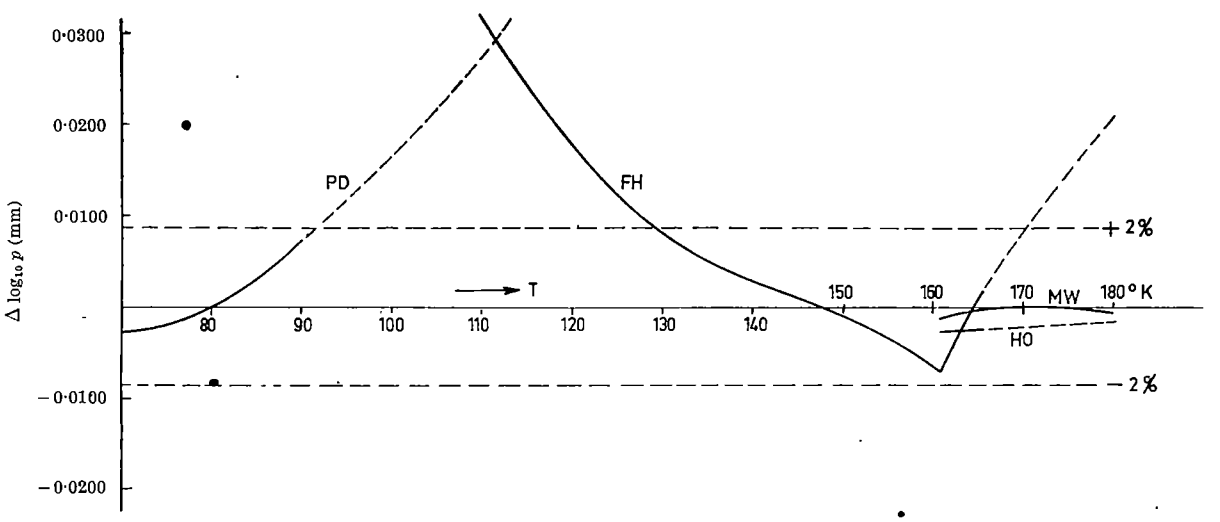


Fig. 1. Deviation of experimental xenon vapour pressure equations from calorimetric vapour pressure curve. ●, Liang (ref. 3); – – –, equation extrapolated outside experimental temperature region or stated region of validity

pressure curve deviates from the vapour pressure data available between 77° and 180° K.

It can be seen that for liquid xenon the *MW* and *HO* vapour pressure equations agree well with the calorimetric curve. For solid xenon the *PD* equation is in agreement within its stated experimental accuracy in the experimental temperature region but when extrapolated to higher temperatures it begins to deviate seriously. The *FH* equation agrees (within 2 per cent) with the calorimetric equation only at temperatures above 130° K. The value given by Liang[3] falls off by 5 per cent. It is possible that this deviation is due to uncertainty in the thermal transpiration correction as has been discussed by Podgurski and Davis[9].

We therefore recommend that, until more precise vapour pressure measurements are available, the calorimetric vapour pressure curve should be used for the vapour pressure of solid xenon. The estimated error is ± 2 per cent at any temperature between 70° and 161° K. The vapour pressure is most easily calculated from the values given by the *PD* or *FH* equation and by the application of the correction curve given by Table 3. For the vapour pressure of liquid xenon either the *MW* or, below 180° K, the *HO* equation can be used.

Table 3. CORRECTIONS TO BE APPLIED TO THE *PD* OR *FH* EQUATIONS TO OBTAIN THE VAPOUR PRESSURE OF SOLID XENON

T (°K)	*p* (mm Hg)	$\text{Log}_{10}(p)$
70	$1{\cdot}39 \times 10^{-4}$	*PD* + 0·0028
80	$4{\cdot}24 \times 10^{-3}$	*PD* + 0·0000
90	$5{\cdot}99 \times 10^{-2}$	*PD* − 0·0072
100	$4{\cdot}95 \times 10^{-1}$	*PD* − 0·0164
110	2·76	*PD* − 0·0275 *FH* − 0·0320
120	11·5	*FH* − 0·0173
130	38·2	*FH* − 0·0085
140	106	*FH* − 0·0030
150	258	*FH* + 0·0010
160	562	*FH* + 0·0067

We thank the O.E.C.D. High Temperature Reactor Project *Dragon*, the sponsors of our low-temperature adsorption investigations, for permission to publish this communication.

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Water Content of Black Soap Films

IN a recent investigation of the thickness of black soap films, Corkill *et al.*[1] determined the water content of the films by measuring the absorption of radiation at 2·93μ by placing a number of black films in an infra-red beam. In order to calculate the amount of water from the infra-red absorption, it is necessary to know the molar extinction coefficient at 2·93μ. Since the absorption is so strong, Corkill *et al.* measured the extinction coefficient with very thin cells (using a gold leaf spacer) and claim that the value of 133 obtained by this method was identical with the extinction coefficient obtained by measuring the optical density of water in deuterium oxide. There is no reason to suppose that the extinction coefficient should be identical for pure water and dilute water in deuterium oxide, and a re-determination was considered desirable, particularly as the value of 133 quoted by Corkill *et al.* is very different from the earlier value of 55 obtained by Fox and Martin[2].

The full experimental details for the determination of the molar extinction coefficients will be given elsewhere[3]; but it is found that the value for water in D_2O is 131, in good agreement with Corkill *et al.*, but that the value for pure water in thin films is 81. This difference between pure water and water in D_2O is reasonable and will be discussed later[3]. Taking the value of 81, the amount of water in the black soap films examined by Corkill *et al.* will be larger by a factor of 1·62 than the values quoted by these authors. Thus for a film drawn from an aqueous solution of $C_{10}H_{21}N(CH_3)_3C_{10}H_{21}SO_4$ (8×10^{-4} M) + NaBr (0·5 M) said to be 72 Å thick with a water core of 29·4 Å, the corrected water core thickness would be 47·6 Å. However, this calculation is open to objections. The extinction coefficient is certainly a function of the composition of the water phase, in the same way as the Raman intensities for the water vibration band are a function of the solute concentration and type in aqueous solutions[4,5].

We thank Dr. W. A. Senior for his advice.

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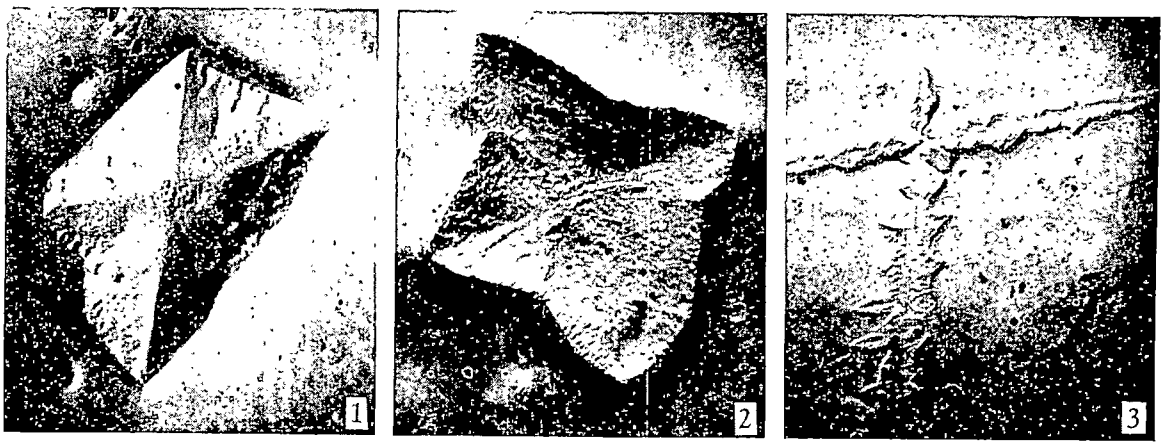
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Nucleation and Morphology of Chymotrypsinogen Crystals

IT has been suggested recently[1] that the morphology of enzyme crystals grown from solutions at various supersaturations[2] may be interpreted in terms of the basic mechanisms of nucleation. Little is known of either the nucleation mechanism or the solid-state properties of enzymes, since most are notoriously unstable even under mild conditions. The work presented here is a preliminary examination of the nucleation of bovine α-chymotrypsinogen from solution. This enzyme has been selected because it is available commercially in a particularly pure form and something is known of its solid-state structure. From X-ray diffraction data[3] the molecule is said to be spherical with a radius of 21 Å. It consists of approximately 246 amino-acid units[4] and has a molecular volume of 17,100 ml./mole[5].

If an aqueous solution of chymotrypsinogen is allowed to evaporate on a glass slide at a temperature of about 20° C very small crystallites are produced. Using carbon films and platinum shadowing it is possible to examine the morphology of replicas by low-magnification electron microscopy, some typical examples being shown in Figs. 1, 2 and 3. The crystals are generally in the size-range 1–10μ and the morphology shows several apparently familiar features with some unusual details. Fig. 1 indicates a collapsed pyramid structure similar to that known for several synthetic polymers in which chain folding is the dominant growth mechanism, and Fig. 3 shows dendritic growth with a marked central groove. Despite a search of many such crystals with high-resolution electron microscopy we have been unable to detect screw dislocations or regular growth steps. Although the absence of these features seems to indicate that chain folding is not the normal growth mechanism it could also



Figs. 1, 2 and 3. Electron micrographs of bovine α-chymotrypsinogen crystals grown from aqueous solution (platinum shadowed replicas. × c, 8,600)

have been caused by collapse of the crystals during the shadowing procedure.

Slow evaporation of a solution of the enzyme in the hemispherical well of a microscope slide has, however, given further evidence of the growth mechanism. Around the edges of the well clearly defined whiskers were formed (Fig. 4). The thickening of the whiskers at the base seems similar to the phenomenon observed by DeVries and Sears[6] for growth of Al_2O_3 whiskers from the vapour phase. These authors observed that the whiskers grew from a region of low super-saturation at the tip to a region of high super-saturation at the base where rapid growth ceased. The thickening in that case was postulated as occurring when the super-saturation was critical with respect to two-dimensional surface nucleation.



Fig. 4. Optical micrograph of the edge sector of evaporated enzyme solution (Reichert microscope. × c. 330)

In the present case it seemed that the whiskers might have grown with a central screw dislocation possibly instigated by heterogeneous nucleation. Closer inspection of Fig. 4 does, however, indicate that the whiskers are, in fact, hollow (at least in some places) and do not grow by a screw mechanism. Side branches apparently occur at massive dislocation junctions.

Since the relation between heterogeneous and homogeneous nucleation of the enzyme is reminiscent of features observable for less-complicated crystals, a more quantitative approach can be formulated. If it is assumed that at low super-saturations the enzyme undergoes heterogeneous nucleation and whisker growth, and at higher super-saturations generates a vast number of crystallites by homogeneous nucleation, then the critical super-saturation for the latter process can be calculated (approximately) from the geometry of the crystallization pattern on the microscope slide and the maximum metastable concentration. In solubility investigations of chymotrypsinogen we find that the solubility decreases with increase in temperature. The metastable limit from the temperature solubility studies occurs at a super-saturation ratio of about 1·15. The critical (homogeneous) super-saturation is consequently approximately 1·6. Applying classical concepts to the homogeneous process the interfacial energy is 0·4 erg/cm^2 and the critical radius 123 Å.

From the foregoing calculation, though crude, it would appear that the interfacial energy is considerably lower than that of chain-folded synthetic polymers[7] and this may indicate a more random configuration of the chymotrypsinogen molecule. The morphology and nucleation study are, therefore, in accord with the view that bovine α-chymotrypsinogen crystals probably grow by accretion of globular material which is randomly oriented (but specifically located) within the crystal. Such observations are in agreement with the available X-ray results.

This work was supported in part by the U.S. National Institutes of Health.

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Polarography of Quinazoline

THE polarographic behaviour of quinazoline displays special features which are not shown by the related compounds quinoxaline, phthalazine and 1,5-naphthyridine.

The anomaly is found in the *p*H-dependence of the limiting current. In neutral and alkaline solution the wave has the height of a normal two-electron wave. Between *p*H 6 and *p*H 1·5 the wave splits into two and the sum of the height of these two waves diminishes to less than 10 per cent of the height in neutral solution. In more acid solution the height of the waves increases and in 40 per cent perchloric acid ($H_0 \sim -2{\cdot}5$) it reaches approximately the height found in alkaline solution.

Whereas the wave-height of quinazoline at *p*H 6 is proportional to the square-root of the height of the mercury reservoir (diffusion controlled wave), the wave-height at *p*H 1·5 is independent of the height of the mercury reservoir, indicating that the wave-height is mainly governed by the rate of a chemical reaction.

4-Methylquinazoline and 2,4-dimethylquinazoline do not show this dependence of wave-height on *p*H; nor do 4-methoxy-, 4-mercapto-, 4-methylmercapto-, 4-chloroquinazoline, and 3,4-dimethylquinazolinium iodide. 3-Methylquinazolinium iodide, however, shows a similar feature in all solutions where the polarograms can be measured before hydrolysis of the ring.

Quinazoline also displays anomalies in other respects. The base strength ($pK_a = 3{\cdot}5$) is higher than expected (1·5, ref. 1) and the ultra-violet spectrum of the cation shows a hypsochromic shift compared with that of quinazoline. These anomalies have been explained by assuming that quinazoline in acid solution adds water with formation of an abnormal cation, 4-hydroxy-3,4-dihydroquinazolinium ion.

A dependence of wave-height on *p*H, similar to that of quinazoline, is found in the polarography of pyridine aldehydes[2,3]. This is attributed to the fact that the aldehyde groups in the protonated pyridine aldehydes at *p*H near unity are mainly in the hydrated state, which is not reducible.

The polarographic behaviour of quinazoline is thus explained by assuming that the reducible species in acid solution is the normal quinazolinium ion, whereas the abnormal cation, 4-hydroxy-3,4-dihydroquinazolinium ion, is not reducible. According to this the polarography of quinazoline (Q) is governed by the following equilibria and the rate of these reactions:

$$\begin{array}{ccc} Q + H_2O & \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} & Q,H_2O \\ \Downarrow + H+ & & \Downarrow + H+ \\ H^+Q + H_2O & \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} & H^+Q,H_2O \end{array}$$

with the four equilibrium constants:

$$K_1 = (Q,H_2O)/(Q)(H_2O) \quad (1)$$

$$K_2 = (H^+Q,H_2O)/(H^+Q)(H_2O) \quad (2)$$

$$K_3 = (Q)(H^+)/(H^+Q) \quad (3)$$

$$K_4 = (Q,H_2O)(H^+)/(H^+Q,H_2O) \quad (4)$$

The wave-height at *p*H > 2·5 is chiefly determined by the equilibrium concentration of the reducible, unprotonated quinazoline. This is shown by the fact that a similar curve with inflexion at the same *p*H (3·6) is found, when the extinction at 310 mμ of quinazoline is plotted against *p*H. The absorption at 310 mμ is mainly caused by quinazoline, whereas the abnormal cation has very little absorption at 310 mμ.

At *p*H 1 the wave-height is essentially governed by the rate of the dehydration of the irreducible, abnormal cation to the reducible, normal cation. The rate is the sum of an uncatalysed and an acid-catalysed reaction rate. The acid-catalysed dehydration is mainly responsible for the increase in wave-height between *p*H 0 and H_0 about − 2. In more acid solution the equilibrium concentration of the normal cation increases, and this, together with the presence of some unhydrated, doubly-protonated cation, is also of importance for the wave-height.

The assumption of the irreducibility of the abnormal cation is substantiated by the fact that 3-methyl-4-hydroxy-3,4-dihydroquinazoline is polarographically irreducible and only yields an anodic wave.

4-Chloroquinazoline is polarographically reducible and yields two two-electron waves in neutral solution. By controlled potential reduction the first wave was shown to be caused by a reduction to quinazoline. In acid solution at 25° C the compound is rapidly hydrolysed to the irreducible 4-quinazolone, but at − 5° to − 10° C the compound is stable enough for polarographic investigation in 40 per cent alcohol. At *p*H 1, 4-chloroquinazoline yields a two-electron wave followed by two one-electron waves; the sum of the heights of the one-electron waves is equal to the height of the two-electron wave. The two one-electron waves are found at the same potentials as those of quinazoline, but the wave-height of quinazoline under the same conditions is very much smaller. This indicates that the quinazolinium ion formed in the reduction of 4-chloroquinazoline is reduced before it has time to be hydrated.

From (3) and (4) it follows that:

$$pH = pK_3 + \log (Q)/(H^+Q) = pK_4 + \log (Q,H_2O)/(H^+Q,H_2O) \quad (5)$$

The experimental results show qualitatively that $(Q) \gg (Q,H_2O)$ and $(H^+Q) \ll (H^+Q,H_2O)$, and thus at the experimentally determined:

$$pK_A(3{\cdot}6)\ (Q) \simeq (H^+Q,H_2O) \quad (6)$$

Introducing (1), (2) and (6) into (5) one gets:

$$pK_A = pK_3 + \log (H_2O) + \log K_2 = pK_4 + \log (H_2O) + \log K_1 \quad (7)$$

pK_3 has been estimated to be 1·5 (ref. 1) and pK_4 has been measured at 7·77 (ref. 4). From these the values $K_1 \simeq 1{\cdot}5 \times 10^{-6}$ and $K_2 \simeq 2$ are obtained. According to this estimate about 99 per cent of the protonated quinazoline is present as the abnormal cation. From this value and the ratio of the limiting current at *p*H 1·3 and the diffusion current at *p*H 7 (and assuming the same diffusion coefficients for the different forms of quinazoline) one can calculate[5] the rate of the dehydration at *p*H 1·3; $k_{-2} = 0{\cdot}2\ sec^{-1}$. The value of k_{-1} at *p*H 10 has been found[4] by a method of flow technique to be $0{\cdot}077\ sec^{-1}$.

The uncertainty of the estimate of k_{-2} is mainly due to the difference between pK_2 and the estimated value 1·5. pK_2 is probably equal to or greater than pK of pyrimidine (1·3); spectroscopic data (concentration of normal cation ~ 0 per cent)[4] seem to exclude more than 2 per cent of the normal cation in the equilibrium at *p*H about 1. This means that k_{-2} with a high probability has a value between 0·1 and 0·4 sec^{-1} at *p*H 1·3.

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BIOCHEMISTRY

Crystalline β-Nigerose

Since its isolation from the partial hydrolysate of nigeran by Barker *et al.*[1], nigerose (3-O-α-D-glucopyranosyl-D-glucose) has been obtained from various sources. In each case, octa-acetate of the sugar was readily obtained in crystalline form, but the free sugar was highly hygroscopic and had hitherto not been successfully crystallized. We were able to prepare this sugar with a fairly good yield by the acetolysis of dextran[2] and we have now crystallized the free sugar.

When 390 g of the dextran from *Leuconostoc mesenteroides* B[3] (in our earlier report[2] the name of the strain was incorrectly given as *L.m.* NRRL, *B*–421) was acetolysed, fractionation on a charcoal column yielded 41 g of crude nigerose. When this amorphous nigerose was dissolved in 80 per cent methanol and allowed to stand at room temperature for about ten months, during which the solvent was evaporated, a part of the syrup showed crystalline appearance. A small portion of the crystallized part was added, as a nucleus, to a solution of 10 g of nigerose in 80 per cent methanol. Crystallization occurred very slowly and, after a week, 3·5 g of fine crystalline product was obtained (Fig. 1). The yield of the crystal was not satisfactory as compared with other common sugars, and crystallization took at least several days and sometimes several months.

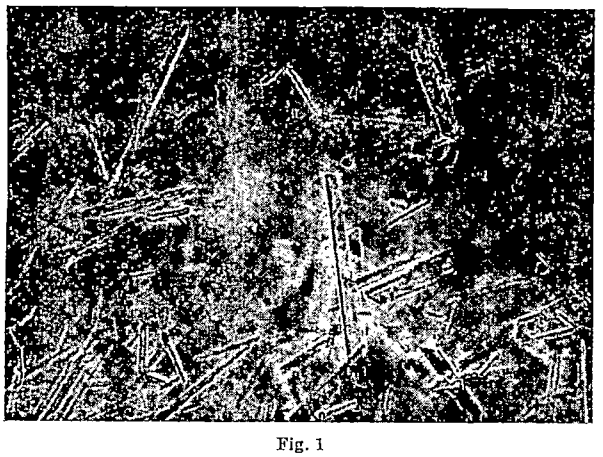


Fig. 1

On drying *in vacuo* at room temperature or at 100° C in atmospheric pressure, the crystal was less hygroscopic. Recrystallized nigerose had a melting point of 156° C (uncorrected) and $[\alpha]_D^{19} = +125° \rightarrow 138°$ (17 h) (approximately 2·0 in water). (Observed values were: C, 42·32; H, 6·43 per cent; values calculated for $C_{12}H_{22}O_{11}$: C, 42·11; H, 6·48 per cent). The upward mutarotation indicated that this crystalline nigerose was the β-anomer. Complete acid hydrolysis of this crystalline nigerose gave D-glucose equivalent to 99·8 per cent of the theoretical value. Acetylation with pyridine as a catalyst produced only β-nigerose octa-acetate, which showed no depression of melting point on admixture with the known specimen; m.p. 150° C, $[\alpha]_D^{2} = +83°$ (approximately 2·1 in chloroform). The infra-red spectrum (KBr disk) of this crystalline nigerose showed the absorption peak at 842 cm^{-1} (α-linkage)[4] and also at 892 cm^{-1} (β-anomer)[4]; the latter was not observed in amorphous nigerose[4,5]. Furthermore, no absorption peak around 1,650 cm^{-1} (free water or water of crystallization)[4] was observed in the above spectrum. The X-ray diffraction data (interplanar spacing (Å), CuK_α radiation: relative intensity as percentage of strongest line, estimated visually) were: 7·34 : 30, 7·08 : 20, 4·46 : 50, 4·29 : 20, 4·18 : 100, 3·81 : 20, 3·53 : 10, 3·34 : 10, 3·12 : 10.

From the above properties, it is now confirmed that this crystalline product is anhydrous β-nigerose.

We thank the Department of Pharmacology, of this University, for microanalysis, and Dr. J. Masui for X-ray diffraction measurements.

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Alpha-Amylase Activity of Varieties of English Wheat

One criticism of English wheat is its often very high and variable α-amylase activity. It is well known that as the wheat grain begins to germinate its α-amylase activity increases very rapidly. Conditions during a wet harvest are such that many samples of English wheat yield flours which are unsuitable for bread production by reason of this high activity.

In a recent examination of 109 flours milled from wheats supplied by the National Institute of Agricultural Botany grown at its headquarters and sub-stations, the α-amylase activity ranged from 1–141 Farrand units[1] with a mean of 7. Some samples of flour gave activities which one would normally associate with wheat containing weathered and prematurely sprouted grain, although based on their visual appearance they were bright and free from sprouted grain.

In particular, one variety of winter wheat, 'Professeur Marchal', gave flours the activity of which ranged from 15–141 units, with a mean of 72.

Flour from 'Cappelle Desprez', grown and harvested under the same conditions as the former variety, had activities ranging from 6 to 12 units, with a mean of 8.

Since much English wheat is bought and sold on its appearance, it is important to wheat buyers to know which varieties are likely to produce samples of unusually high α-amylase activity and yet appear to be bright and attractive. It would therefore be worth while to make a detailed examination of present and future varieties of wheat from this angle.

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Chemical Analysis of Proteins Low in Aromatic Amino-acids

Methods for protein estimation in common use such as ultra-violet absorption and the method of Lowry *et al.*[1] rely on the presence in the molecule of the aromatic amino-acids tyrosine and tryptophan, and if these are present in low concentrations, estimates of protein will be subject to error. Our *in vivo* investigations into blood coagulation[2] have revealed that the active moiety, serum thrombotic accelerator[3], which plays an important part in this mechanism, is low in aromatic groups. This deficiency was first indicated from the ultra-violet absorp-

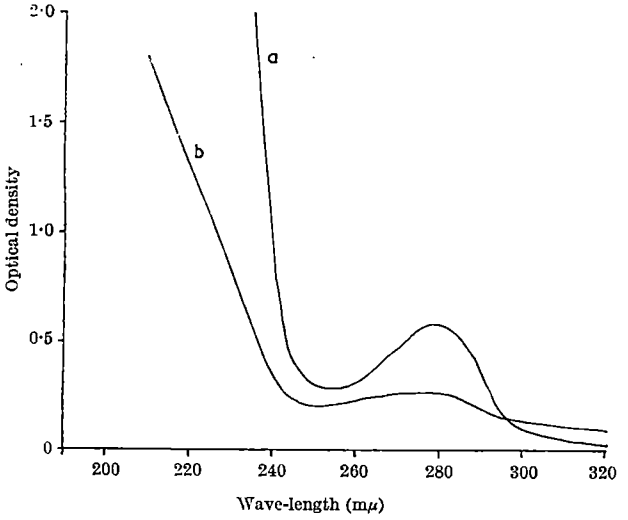


Fig. 1. Ultra-violet absorption spectra of (*a*) bovine serum albumin (1·3 mg/ml.) and (*b*) serum thrombotic accelerator (1 mg/ml.). Optical density was measured in a Unicam *SP* 500 spectrophotometer using 1 cm silica cells

tion spectrum of the serum thrombotic accelerator, which revealed a depressed peak at 280 mμ when compared with the corresponding spectrum of serum albumin (Fig. 1).

From the divergence between these spectra it was concluded that the error, using the method of Lowry *et al.*, would be large and this was confirmed as the isolation of serum thrombotic accelerator proceeded, since variable results were obtained with this method. The micro-biuret technique of Goa[4] was therefore substituted since the colour production depends only on the presence of peptide bonds in the protein molecule, except that the formula for the quantitative 'Benedict' reagent of Varley[5] and not that of Goa[4] was used in our estimations because of the greater stability of the former over a period of time. One disadvantage of the method is that the quantity of protein required (300 μg/ml.) is greater than in the method of Lowry *et al.* (30 μg/ml.).

Our investigations also required the estimation of the bound carbohydrate in the serum thrombotic accelerator for which we used the anthrone procedure of Goa[6] with some modifications. The low tryptophan content of the serum thrombotic accelerator proved to be advantageous, since this amino-acid is known to interfere with the anthrone reaction[7], and application of this latter procedure to serum proteins has therefore produced values for hexose which have varied, for example, serum albumin[8,9]. The colour production between anthrone and carbohydrate is normally measured at its maximum absorption peak of 630 mμ; however, the additional reaction due to the presence of tryptophan produces a second maximum at 530 mμ and interference at 630 mμ, resulting in a lower reading at the latter wave-length.

The absorption curve produced by the anthrone reagent and galactose is compared in Fig. 2 with those produced by the reaction of anthrone with albumin, fibrinogen and the serum thrombotic accelerator, respectively. It will be seen that no correction for tryptophan interference was necessary with the serum thrombotic accelerator since the colour production followed closely that of a pure sugar, whereas an additional absorption at 530 mμ with a concomitant reduction at 630 mμ occurred with fibrinogen and albumin.

Björnesjö[10] attempted to correct this interference by the use of a nomogram incorporating readings at 530 mμ and 630 mμ, and concluded that the error produced by tryptophan could reach 15–20 per cent. This author also emphasized the importance of standardizing the conditions of the reaction, and his observation that the optimum colour development occurred at 90° C over a period of 16 min was confirmed by us. We adopted the practice, originally described by Scott and Melvin[11], of layering the protein solution over the cooled anthrone reagent and then mixing very rapidly so as to dissipate heat quickly. We confirmed that this order of addition, as opposed to that of reagent to the protein solution, was important for maintaining the yellow colour of the blank solution as well as preventing precipitation of the anthrone.

The final method used incorporating these modifications is as follows: The proteins are precipitated with 0·2 ml. phosphotungstic acid (5 per cent in 2 N HCl) per ml. of solution, followed by centrifugation at 1,000 r.p.m. for 2 min. The supernatant is poured off, and after washing the precipitate with 95 per cent ethanol it is dissolved in one drop of 5 N NaOH and made up to 2 ml. Boiling-tubes containing 4 ml. freshly-prepared anthrone reagent (British Drug Houses, 0·2 per cent in concentrated sulphuric acid) are cooled in an ice bath, and the test samples carefully layered on top of the anthrone by running down the side of the tube which is immersed at an angle of 45° in the bath. This position is held for a few moments so that any heat produced at the interface is dissipated, and then the tube is rapidly swirled while still immersed so as to mix the reagent and sample, the swirling being continued until the contents of the tube are cooled. The tubes are kept in the ice bath until all have been similarly treated, and after being placed in a water bath at 90° C for 16 min are transferred back to the ice bath in order to cool rapidly. A period of 10 min is allowed for the tube contents to warm up to room temperature and the colour is then read in the spectrophotometer at 630 mμ against the blank, which consists of 2 ml. distilled water treated in the same manner as the samples. The blank should be clear and yellow after heating while the test solutions turn green. Standards should be run every time using an equimolecular mixture of galactose and mannose at 25 μg/ml. and 50 μg/ml. respectively.

It is felt that this method incorporates the most important practical details from a number of different anthrone procedures, and we feel that these together with our experiences of the various methods for protein estimation mentioned here will be useful to others working with proteins low in aromatic amino-acids. We would further emphasize the importance of obtaining an ultra-violet

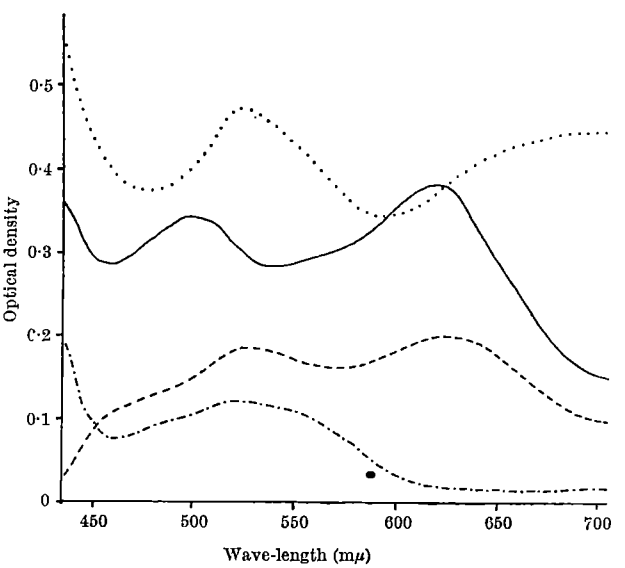


Fig. 2. Absorption spectra in the visible region of the colour produced between anthrone reagent and various plasma proteins, compared with that produced between anthrone and galactose. . . . ., Fibrinogen (4 mg): — — — —, serum thrombotic accelerator (1 mg); — . — . — . bovine albumin (6 mg); ——, galactose (50 μg)

absorption spectrum on the protein before a method for its estimation is chosen, and an absorption curve in the visible region on the anthrone colour, so that if necessary a correction can be made for tryptophan interference.

This work was supported by grant *HE*–06805 from the National Heart Institute, U.S. National Institutes of Health.

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Influence of Excreted Substance from Leaves on Decomposition of Zineb, a Dithiocarbamate Fungicide

A variety of substances are excreted by leaves[1], and have a direct influence on the germination of fungus spores[2] and on the activity of copper fungicides[3]. Since the fungicidal activity of the dithiocarbamate fungicides depends largely on their mode of decomposition and this in turn depends on environmental conditions[4], we examined the influence of the foliar excreta.

Leaves of grape (var. 'Trebbiamo'), potato (var. 'Majestic'), sugar beet (var. 'Maribo P.'), and apple (var. 'Golden Delicious') were collected. Four samples, each of 100 cm² surface, of each species were prepared by cleaning with a dry cotton plug and immersing for 3 h in 100 ml. of distilled water. Care was taken to exclude petioles. After 3 h one part of each liquid sample was mixed with a previously prepared 4 per cent suspension of zineb (zinc ethylene-*bis*-dithiocarbamate), in a ratio of one part of zineb to nine parts of wash-water, yielding 0·4 per cent zineb suspension. These operations were repeated on three consecutive days with the only difference that samples of the first day were chromatographed immediately after mixing wash-water and zineb; samples of the second day were mixed with zineb and maintained for 3 h at 26° C and those of the third day were mixed and maintained for 5 h before being chromatographed. As a control zineb suspension was mixed in every case with distilled water (one part of 4 per cent zineb with nine parts of distilled water).

Descending chromatograms were prepared on Whatman No. 1 paper with a mixture of distilled water, ethanol and butanol (1 : 1 : 1 mixture for 24 h at laboratory temperature). After drying, the chromatograms were developed with a spore suspension of *Aspergillus niger* in diluted peach juice ($7{\cdot}5 \times 10^5$ spores/ml.). 1·34 ml. of this suspension was distributed on 100 cm² paper. After 72 h incubation at 27° C the zones of inhibition were evident on the paper as white spots.

Neither of the wash-waters without zineb gave inhibition spots, whereas all those suspensions with zineb did so at $R_F = 0$, that is, at the start, corresponding to zinc-ethylene-*bis*-dithiocarbamate.

A second inhibition spot with $R_F = 0{\cdot}86 - 0{\cdot}89$ was evident with the following suspensions: (*a*) Chromatograms prepared immediately after mixing zineb and wash-water: zineb in distilled water, light spots in all four replications; in grape wash-water, light spots in two replications; in potato, in sugar beet, and in apple wash-water, no spots. (*b*) Chromatograms prepared 3 h after mixing zineb and wash-water: distinct spots with distilled water and with all wash-waters in the four replications. (*c*) Chromatograms prepared 5 h after mixing zineb and wash-water: only zineb in potato wash-water gave inhibition spots in all four replications. There were two distinct spots at $R_F = 0{\cdot}86 - 0{\cdot}88$ and at $R_F = 0{\cdot}70 - 0{\cdot}72$.

Table 1. *p*H Values of the Wash-waters obtained using Different Kinds of Leaves, with and without Zineb

	Mean	*S.E.*
Sugar beet wash-water	6·79	0·07
Sugar beet wash-water + zineb	7·11	0·24
Potato wash-water	6·94	0·05
Potato wash-water + zineb	7·76	0·03
Grape wash-water	7·27	0·09
Grape wash-water + zineb	7·51	0·04
Apple wash-water	7·38	0·02
Apple wash-water + zineb	7·56	0·01
Zineb alone	7·26	0·01
Distilled water	6·10	0·72

It is evident that the substances excreted from the leaves affect the decomposition and activity of the zineb sprayed on the leaf-surface; as a rule, the activity is enhanced. The small differences in the R_F-values suggest that the decomposition products are similar, with the exception of the product of the potato wash-water.

Comparison of the chromatographic results with the *p*H values (Table 1) suggests that the more distinct inhibition spots of the potato wash-water are due to its higher *p*H value, and this agrees with Thorn's suggestion[4] that decomposition of zineb is enhanced by an alkaline environment, where it produces ethylenethiurame-monosulphide. In fact, in other trials carried out by us (unpublished) the R_F values of the ethylenethiurame-monosulphide were 0·84–0·88.

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Chloroplast Ribosomes

Previously[1] we have found ribosomes in chloroplasts of *Chenopodium album* and *Clivia miniata*. As shown by electron microscopy and biochemical methods, ribosomes located in the matrix of chloroplasts are 180–200 Å in diameter and possess a sedimentation coefficient of about 70 *S* in the presence of 10^{-2} M Mg^{2+}. The work described here is concerned with the isolation of chloroplast ribosomes from a number of higher plants and investigation of some of their chemical and physico-chemical properties.

Chloroplasts were isolated from leaves of eight species of higher plants (before flowering) belonging to six families: *Clivia miniata* (Amaryllidaceae), *Beta vulgaris* and *Chenopodium album* (Chenopodiaceae), *Galinsoga parviflora* (Compositae), *Triticum vulgare* (Gramineae), *Lamium album* (Labiateae), *Phaseolus vulgaris* and *Pisum sativum* (Leguminosae). Wheat and pea seedlings, 7–10 days old, were also used.

The isolation of chloroplasts was carried out by differential centrifugation, the rate of precipitation of nuclear and chloroplast fractions being particular for each plant. The details of the procedure were described previously[1].

Chloroplast and cytoplasmic ribosomes were isolated as described previously[1] with the only modification that the former were purified not only by reprecipitation but

also by 0·5 per cent sodium deoxycholate treatment to remove chloroplast material rich in lipids which are present in the ribosome fraction.

The amount of protein was estimated by Lowry's method[2]; RNA was determined spectrophotometrically[3]. The nucleotide composition of RNA was estimated according to Spirin and Belozersky[4]. Sedimentation was carried out in a Spinco model *E* ultracentrifuge at 37,020 r.p.m. RNA ultra-violet absorption spectrum was measured in 1-cm quartz cells in an *SF*-4 model spectrophotometer.

An investigation of the chloroplast fraction in light and phase microscopes showed that 'intact' particles (chloroplasts with undamaged membrane) comprised 60–80 per cent of the fraction[5]. RNP particles isolated from chloroplasts of all the plants examined displayed an absorption spectrum with λ_{max} at 260 mμ and λ_{min} at 240 mμ. The $\lambda_{max}/\lambda_{min}$ ratio was 1·40–1·65. The ultra-violet spectrum of chloroplast ribosomes was similar to that of cytoplasmic ribosomes. The protein and RNA content of the chloroplast RNP particles was 60–40 per cent of protein and 40–60 per cent of RNA. With regard to its nucleotide composition, RNA of chloroplast ribosomes was of the so-called *GC*-type, the coefficient of specificity being 1·15–1·21 and purines prevailing over pyrimidines. The nucleotide composition of the chloroplast ribosomal RNA in various plant species was the same, differing insignificantly from that of cytoplasmic ribosomes (Table 1). Similar results concerning the nucleotide composition of the spinach chloroplast RNA were obtained by Lyttleton[6]; the coefficient of specificity amounted to 1·17–1·19. Only RNA of ribosomes isolated from *Euglena gracilis* chromatophores belongs to the *AU*-type, the specificity coefficient being 0·88–0·89 (ref. 6). In the analytical ultracentrifuge in the presence of 10^{-2} M Mg^{2+}, all preparations of chloroplast ribosomes investigated gave a single main peak with a sedimentation coefficient of about 70 *S*. All preparations also displayed a 17–20 *S* component and in some experiments very small peaks of dimers and sub-units were also discovered. Under these conditions the main components of the cytoplasmic ribosome fraction were also particles with a sedimentation coefficient of about 70 *S*. Together with the main component a small peak of dimers was always observed in this fraction. The slow component in the fraction of cytoplasmic ribosomes was absent in most experiments (Table 2). In experiments on spinach chloroplast ribosomes Lyttleton[6] and App and Jagendorf[7] found sedimentation coefficients to be close to our values. According to Lyttleton[8] the slow component in the chloroplast ribosome fraction is a water-soluble protein devoid of RNA which is related to the chloroplast photosynthetic function. In a magnesium-free medium chloroplast ribosomes dissociate into sub-units similar to those of cytoplasmic ribosomes (Table 3). This process is reversible, and the addition of Mg^{2+} to the medium results in the re-synthesis of initial ribosomal particles. If the concentration of Mg^{2+} ions increases to 3×10^{-2} M, particles with a sedimentation coefficient of about 70 *S* remain the main structural component (Table 4). Extrapolation of sedimentation coefficients obtained for chloroplast and cytoplasmic ribosomes of pea and bean to infinite dilution gives 79–81 *S*. Thus, chloroplast ribosomes do not differ from cytoplasmic ribosomes isolated from the same plants and belong to the type of 80 *S* particles common to all higher plants and animals.

Table 1. Nucleotide Composition of RNA of Chloroplast and Cytoplasmic Ribosomes

Ribosomes	G	U	C	A	Pur/Pyr	G+C/A+U	G±U/A+C
	(mole per cent)						
Chenopodium album							
Chloroplast	28·5	21·3	25·3	24·7	1·14	1·17	0·99
Cytoplasmic	30·3	20·4	23·9	25·3	1·25	1·18	1·03
Lamium album							
Chloroplast	28·2	20·7	25·3	24·8	1·15	1·17	0·96
Cytoplasmic	28·9	22·0	24·5	24·5	1·15	1·15	1·04
Phaseolus vulgaris							
Chloroplast	29·2	20·8	24·3	25·6	1·21	1·15	1·00
Cytoplasmic	29·4	21·2	24·4	25·1	1·19	1·16	1·02
Pisum sativum							
Chloroplast	29·8	21·2	24·8	24·2	1·17	1·20	1·04
Cytoplasmic	30·1	20·8	24·8	24·3	1·19	1·21	1·03

Mean values are given calculated from 8–10 individual estimations in each of 2–3 parallel hydrolysates of ribosomal preparations. Pur, Purine bases; Pyr, pyrimidine bases; G, guanylic acid; U, uridylic acid; C, cytidylic acid; A, adenylic acid.

Table 2. Sedimentation Coefficients ($S_{20,w}$) of Chloroplast and Cytoplasmic Ribosomes (Mg^{2+}, 10^{-2} M)

Source of ribosomes	Chloroplast ribosomes				Cytoplasmic ribosomes			
	Slow comp.	Main comp.	Additional components		Slow comp.	Main comp.	Additional components	
Beta vulgaris	17·5	71·1	—	—				
Chenopodium album	18·3	67·0		—	18·6	68·7	102·5	48·9
Clivia miniata	—	73·1	109·9	—	—	67·3	—	—
Galinsoga parviflora	18·9	70·6	—	—				
Lamium album	18·2	74·2	67·9	41·7	—	73·8	107·3	—
Phaseolus vulgaris	17·2	68·3	—	—	—	68·3	99·7	—
Pisum sativum	17·9	67·1	112·3	50·3	17·0	67·9	92·4	—
Triticum vulgare	18·2	71·9	105·1	—	—	72·6	108·9	—

The values of sedimentation coefficients result from individual experiments and are not extrapolated to infinite dilution.

Table 3. Dissociation of Chloroplast and Cytoplasmic Ribosomes in a Magnesium-free Medium

Source of ribosomes	Components of the ribosomal fraction, $S_{20,w}$						
	Chloroplast ribosomes				Cytoplasmic ribosomes		
Chenopodium album	18·3	67·0	48·7	30·5			
Lamium album	20·1	—	53·6	35·6	−72·5	34·1	49·4

Table 4. Sedimentation Coefficients ($S_{20,w}$) of *Chenopodium album* Chloroplast Ribosomes in the Presence of 3×10^{-2} M Mg^{2+}

Exp. No.	Slow component	Main component	Additional components		
1	—	66·3	96·5	38·6	53·5
2	18·6	72·2	—	42·3	52·9

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Modified Phycoerythrin from *Porphyridium cruentum* treated with *p*-Chloro-mercuribenzoate

Phycoerythrin, one of the biliproteins in red algae, which plays a part in photosynthesis, exhibits various absorption bands in the visible absorption spectrum[1,2]. Recent investigations have shown that these bands are independent of each other. Among three absorption bands at 500, 545 and 565 mμ of phycoerythrin from *Ceramium rubrum*, the two bands at 500 and 545 mμ disappear on reduction with sodium hydrosulphite[3]. O'hEocha and O'Carra presented evidence that the 500 mμ band behaves independently of the other two[4]. Furthermore, the 565 mμ band disappears on the addition of *p*-chloro-mercuribenzoate (PCMB) to phycoerythrin isolated from *Ceramium rubrum*[3] and *Porphyridium cruentum*[5]. These results indicate the existence of different chromophores absorbing at different wave-lengths.

Further investigation in this laboratory has revealed that phycoerythrin could be split into two units, each of

which contains different chromophores. This communication is concerned with the separation of a modified component of phycoerythrin, which has a single absorption band at 545 mμ, from PCMB-treated *Porphyridium cruentum*. An interaction of this component with glutathione will also be reported.

During the course of investigation on the effect of SH-blocking agent PCMB on energy transfer in *Porphyridium cruentum*, a gradual increase of an orange fluorescence has been observed. Since a decrease of the fluorescence by PCMB is accompanied by the disappearance of the 565 mμ absorption band in phycoerythrin free from the cells either in ruptured cells or in isolated form[5], this observation is an unexpected result. In spite of the reverse change of the fluorescence, the same loss of the 565 mμ absorption band occurs in PCMB-treated cells. This is shown in Fig. 1 by absorption spectra of cell suspension before and after treatment with PCMB (Fig. 1, *A* and *B*). In this measurement, the cells of *Porphyridium cruentum* separated by centrifugation from culture medium and suspended in 0·1 M phosphate buffer at *p*H 7·0 were saturated with PCMB (California Corp. Biochem. Research) and left standing overnight before measuring the absorption spectrum. Opal glass was used in a Cary spectrophotometer model 14.

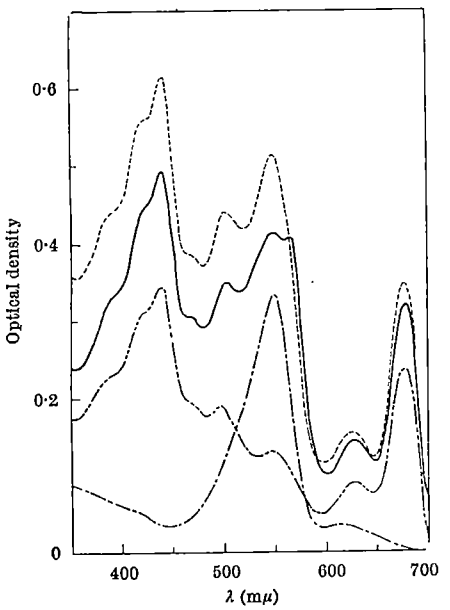


Fig. 1. Absorption spectra of (*A*) intact cells (——), (*B*) cells saturated with PCMB (- - - - - - -), (*C*) modified phycoerythrin (— - — - —) separated from the cells, and (*D*) cell residue (— - - — - - —)

The above suspension with PCMB was centrifuged at 10,000*g* for 10 min. A resulting fluorescent supernatant exhibited a single absorption band at 545 mμ, a small absorption at 615 mμ probably being due to phycocyanin (Fig. 1, *C*). The absorption spectrum of a greenish-brown cell residue re-suspended in the same buffer solution revealed that a component absorbing at 500 mμ remains in the cells (Fig. 1, *D*). This result indicates that the modified fluorescent component of phycoerythrin having a single band at 545 mμ was released from the cells and was responsible for the increase of fluorescence in the cells treated with PCMB.

It is known that phycoerythrin splits into smaller units in more alkaline media[6–8]; but the spectral characteristics of these units have not been examined. Very recent work by Vanghan[9] also shows that phycoerythrin is composed of sub-units. Previous investigations have shown that a change in protein structure can be induced by the blocking of protein SH-groups[10,11]. The dissociation of the phycoerythrin molecule into units in the cell and the release of the one unit from the cell is probably the result of PCMB changing the intermolecular protein structure and the permeability of cell membrane.

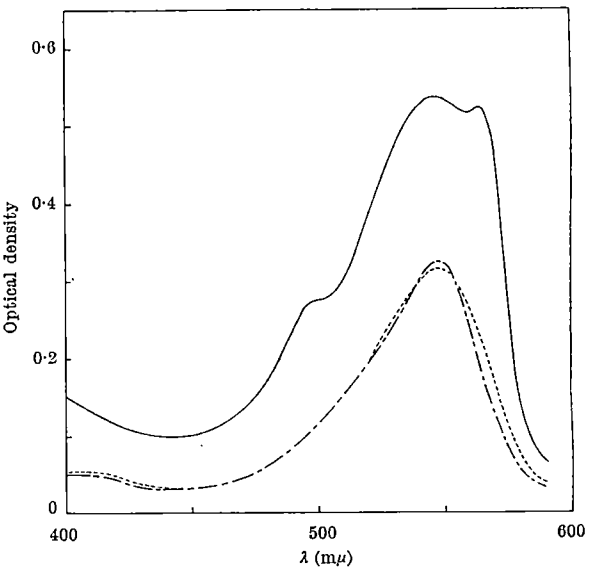


Fig. 2. Absorption spectra of native phycoerythrin (——) and modified phycoerythrin without (— - — - —) and with (- - - - -) glutathione (5×10^{-3}M) in 0·1 M phosphate buffer at *p*H 7

The absorption spectrum of the modified fluorescent segment of phycoerythrin is compared in Fig. 2 with that of native phycoerythrin which has three absorption bands at 500, 545 and 565 mμ. Native phycoerythrin was isolated from *Porphyridium cruentum* in the same method as reported previously[3], except that the cells were sonicated by means of a Raytheon sonic oscillation, model *DF*-101 (200 W, 10 kc/s), instead of freezing and thawing. Since the 565 mμ band disappears on blocking SH-groups of native phycoerythrin with PCMB, the possible presence of the inactive 565 mμ chromophore in this modified unit was examined. Treatment with glutathione (C. F. Boehringer and Soehne *G.m.b.H.*, Mannheim, Germany) did increase the absorption at 565 mμ region, reducing the absorption at 545 mμ, to some extent (Fig. 2). A striking change occurred in the fluorescence. As shown in Fig. 3, action spectra of the fluorescence at 575 mμ of the same solution as in Fig. 2 show a considerable increase of the fluorescence in the presence of glutathione. The action spectra were measured on an Aminco-Keirs spectrophosphorimeter equipped with an RCA *IP*21 photomultiplier. This shows that the 545 mμ chromophore becomes efficient in producing the fluorescence though its absorption does not increase. According to the previous investigation[5], the 565 mμ chromophore is highly fluorescent in native phycoerythrin, accepting an energy absorbed by the 500 and

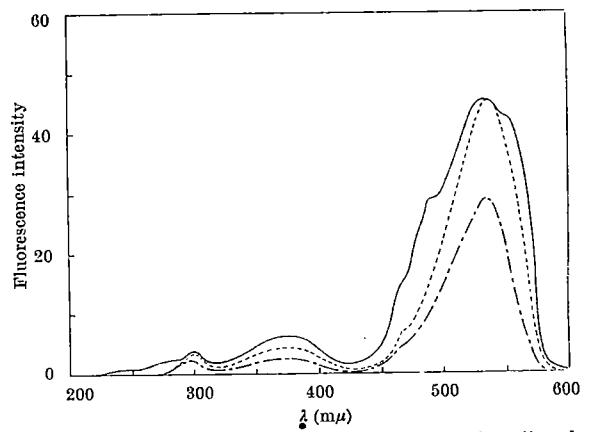


Fig. 3. Action spectra of the fluorescence at 575 mμ in native phycoerythrin (——) and modified phycoerythrin without (— - — - —) and with (- - - - - -) glutathione (5×10^{-3} M)

545 mμ chromophores. Therefore, in the glutathione-treated modified component the highly fluorescent 565 mμ chromophore regenerated in a small amount possibly contributes to the high yield of fluorescence through energy transfer from the 545 mμ chromophore.

This investigation indicates the existence of at least two main units in phycoerythrin from *Porphyridium cruentum* : one contains the 500 mμ chromophore and the other contains the 545 and 565 mμ chromophores. In the latter, SH-groups of protein interact with the 565 mμ chromophore. It would be possible that these units are composed of smaller sub-units. Further investigations on sub-unit structure of phycoerythrin are at present in progress.

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PHYSIOLOGY

Uptake of Labelled Precursors of Melatonin by the Epiphysis of *Xenopus laevis*

THE hormone, melatonin, was first isolated from bovine pineal glands by Lerner *et al.* in 1958 (ref. 1). Its only biological activity appeared to be to cause contraction of the melanocytes in Amphibia. Since that time melatonin has been found in pineal tissue of other mammals[2–4] and birds[5]; but there has been, as yet, no demonstration of its presence in amphibian pineal glands.

Axelrod and Weissbach[6] have shown that melatonin is synthesized from 5-hydroxytryptamine (5HT) by *N*-acetylation followed by *O*-methylation. The enzyme which catalyses this final step, 5-hydroxyindole *O*-methyl transferase, has been found only in pineal tissue. The donor for the methyl group is *S*-adenosyl methionine.

If the epiphysis of *Xenopus* is producing melatonin, then it is predictable that the cells of this organ should incorporate labelled 5HT and also methionine labelled in the methyl group. In the experiments reported here young adult *Xenopus*, 3–4 months after metamorphosis, were used. The wet weight of each animal was approximately 5 g. The labelled precursors used were 5-hydroxytryptamine-3′-^{14}C creatinine sulphate and ^{14}C-methyl methionine (supplied by the Radiochemical Centre, Amersham).

The first experiment was undertaken to see if labelled 5HT was incorporated into cells of the epiphysis. 5 μc. of the precursor was injected into the dorsal lymph sac of each of six frogs, which were placed in black painted plastic containers measuring 17·5 cm long × 11·5 cm wide × 5 cm deep, with transparent plastic lids. All animals were kept in constant light provided by a 60-W pearl bulb placed 1 m above the containers.

Table 1. Comparison of the grain counts made in areas of 10μ² over the epiphysis with those over the optic lobes in 5 animals injected with ^{14}C-methyl methionine and killed at different periods after injection. These results were subjected to a *t*-test and the difference between the mean count over the epiphysis and that over the optic lobe was in each case highly significant (P, 0·0001)

Time after injection (h)	Mean grain count over epiphysis	Mean grain count over optic lobe
2	27·8	15·6
6	56·7	33·1
24	75·7	38·2
72	81·4	40·9
168	55·0	31·9

Animals were decapitated 5 min, 6 h, 12 h, 24 h, 3 days and 1 week after injection. The brains were quickly dissected out, fixed, and prepared for autoradiography which was carried out using the stripping film technique with Kodak '*AR* 10' films. A similar series of experiments was performed using ^{14}C-methyl methionine.

In both cases incorporation into epiphysial cells of labelled precursors was observable. Fig. 1 shows a radioautograph over the epiphysis of an animal injected 3 days previously with ^{14}C-5HT that clearly demonstrates the uptake of the compound by the cells of the organ. Grain counts over the epiphysis and over the adjacent areas of the brain are given in Table 1, and again uptake of labelled precursor, this time ^{14}C-methyl methionine, is demonstrated.

The fact that there is a blood-brain barrier in higher vertebrates, which does not operate in the pineal region[7], suggests that the greater uptake of ^{14}C-5HT in the epiphysis of *Xenopus* as compared with other parts of the brain may not be in itself significant. However, what is significant is that 5HT is taken up at all, and in such large quantities.

The following experiments give a strong indication that both methionine and 5HT are incorporated into the same compound. It would be supposed that if the epiphysis of *Xenopus* is producing melatonin, stores of this hormone would become depleted in animals kept on a white background. There is a synchronous decrease in the amounts of both radioactive precursors detectable in the pineals of animals kept in white containers as compared with those kept in black ones. These results will be fully discussed elsewhere.

It has been demonstrated by these experiments that both ^{14}C-5HT creatinine sulphate and ^{14}C-methyl methionine are taken up by cells of the epiphysis of *Xenopus laevis*. The experiments do not prove that melatonin is made by the organ, but this would certainly seem probable.

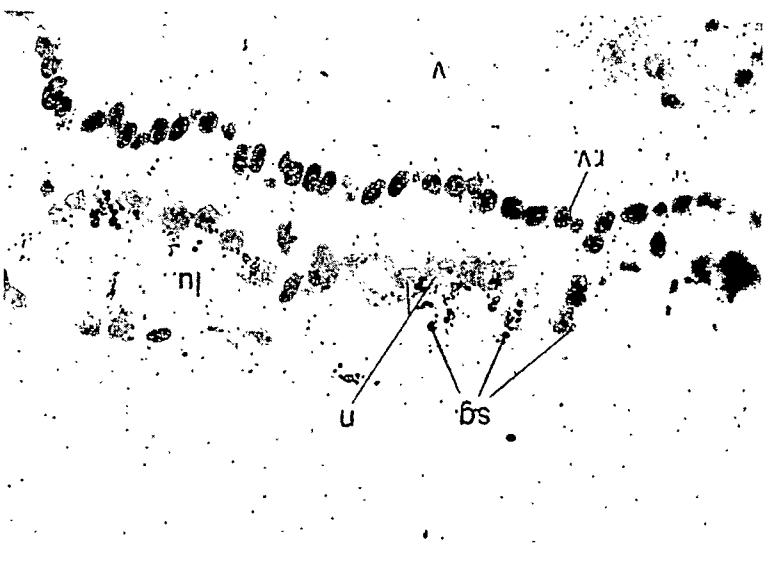


Fig. 1. Autoradiograph of the epiphysis of a young adult frog 3 days after injection of 5-hydroxytryptamine-3′-^{14}C creatinine sulphate. *lu*, Lumen of epiphysis; *n*, nuclei of epiphysial cells; *rv*, roof of third ventricle; *sg*, silver grains; *v*, third ventricle

This work was performed during the tenure of a research studentship from the Department of Scientific and Industrial Research.

Grants from the Gilbert Foyle Educational Trust and the Haberdashers' Co. were used towards the cost of radioactive chemicals. I thank Dr. P. C. J. Brunet for his advice.

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Marked Decrease of DNA in the Nuclei of the Adrenal Medulla of Rats exposed to Cold

Exceptions have been made to the concept of constancy of DNA per nucleus, established by Boivin[1] on the basis of biochemical determinations and nuclear counts, and confirmed by others[2] on the basis of histophotometric measurements.

Many of them have been explained as being caused by instrumental errors, polyploidy, polyteny or synthesis of DNA in preparation for mitosis (see Bonucci[3] and Hale[4]). Others so far resist critical scrutiny. The great majority of the variations reported are small (around 10–15 per cent). This work is concerned with determinations of the DNA content of the nuclei of the adrenal medulla of rats exposed to cold. A decrease of DNA, of the order of 40 per cent, could be demonstrated by three different methods.

Adult male albino rats, 'Italico' strain, bred in our department, weighing 250–330 g, were kept at +4° C for 15 h/day for a total period of 100 or 300 h.

A second group of rats, after exposure to cold for 300 h, was kept at room temperature (15°–20° C) for 10 days. Another group of animals was used as control. Suprarenal glands were fixed in neutral formalin and embedded in paraffin. Sections 15μ thick were stained by the Feulgen method.

The absorption of the DNA-Feulgen complex was measured at 546 mμ on whole nuclei by means of an integrator 'scanning' histophotometer, designed on the basis of Jansen's[5] model. The nuclei of the normal adrenal medulla showed a DNA content slightly higher than that of the diploid nuclei of the kidney. After exposure to cold, the DNA content of the nuclei of the adrenal medulla showed an average diminution (with respect to the controls) of 22·2 per cent after 100 h, and 39·6 per cent after 300 h. In the animals which had been exposed to cold and then kept again at room temperature for 10 days, the nuclear DNA content was found to return to the normal and in some nuclei was significantly above the normal values.

The variations in the DNA content of nuclei obtained by cytochemical methods, in order to be considered as the expression of physiological changes of the cells, must satisfy the following criteria[6,7]: (1) that DNA has not been synthesized in preparation for a mitotic division; (2) that the number of chromosomes be known; (3) that no errors have been made in the measurements.

As regards the first criterion, mitoses are not seen in the cells of the adrenal medulla after the 30th day of life (Mitchell)[8]. Synthesis of DNA *de novo* in our adult animals can, therefore, be ruled out.

As to the second criterion, it is evident for the above reason that a direct chromosomal count is not feasible.

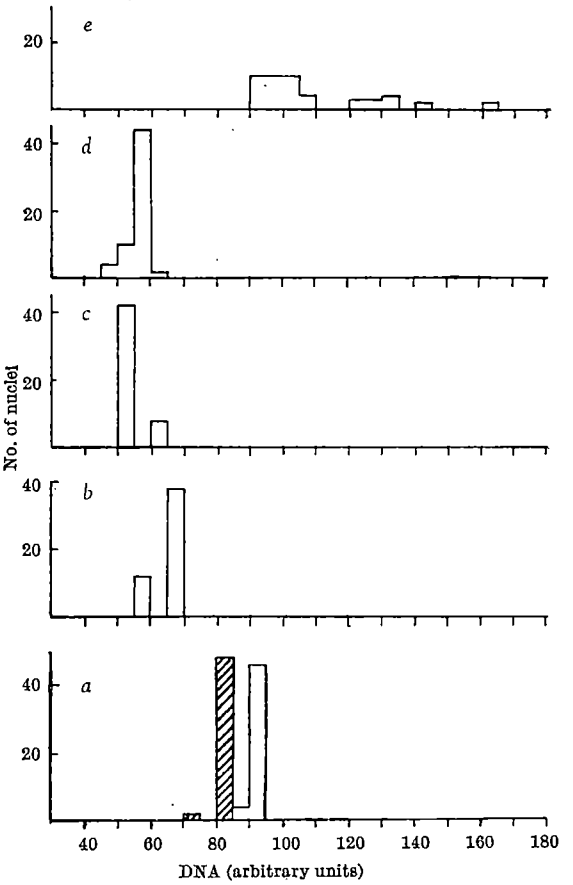


Fig. 1. Histograms of DNA content obtained by histophotometry in arbitrary units. *a*, From the nuclei of renal tubules (cross-hatched) and of the adrenal medulla in normal conditions; *b*, from the nuclei of the adrenal medulla after exposure to cold for 100 h; *c*, from the nuclei of the adrenal medulla after exposure to cold for 300 h; *d*, as *c*, smears of nuclei; *e*, from the nuclei of the adrenal medulla of rats kept for 10 days at room temperature, after exposure to cold for 300 h

However, if the observed decrease in DNA is due to a reduced chromosome number, it implies that 40 per cent of the chromosomes disappear during exposure to cold and are formed again (without mitosis) during the period in which the DNA content is restored to the normal value; an unlikely hypothesis.

As to the third criterion, the measurements made with our instrument are free from errors arising from the distribution of the DNA-Feulgen complex and from the shape of the nuclei. It is also possible to exclude errors arising: (*a*) from being 'out of focus', since the differences observed using smeared nuclei are coincident with those observed using sections; (*b*) from possible discrepancies in the absorption curve of the DNA-Feulgen complex (on the basis of tests made); (*c*) from variations in the time of hydrolysis or from changes in *p*H and SO_2 content of the Schiff reagent (since sections of control suprarenal gland and normal kidney and of experimental adrenal gland were stained in the same way on the same slide).

In order to confirm the data obtained by means of the histophotometric method, two other methods have been used: (1) Measurements of the dry mass by means of the interference (Baker-Smith) microscope on isolated adrenal nuclei, suspended in saline. After treatment with DNase, the loss in dry mass, considered as equivalent to the nuclear content of DNA, in rats exposed to cold is on the average 37·5 per cent less than in control animals. (2) Chemical determinations of DNA on medulla homogenates, by the micromethod of Steele *et al.*[9], based on the diphenylamine reaction of Dische[10] and the count of the nuclei. The results show that the nuclei of the adrenal medulla of rats exposed to cold have less DNA (on average

25 per cent less after 100 h; 34 per cent after 300 h) than those of control rats.

In either case, the results are in agreement, qualitatively and quantitatively, with those obtained by histophotometry. This agreement, together with the DNA diminution, clearly shows that the latter is not due to errors in measurement, but is real. On the other hand, the reaction of Dische on the acid-soluble fraction of the homogenates of adrenal medulla does not give rise to difference in colour development between control extracts and those from cold-exposed animals. This fact eliminates the possibility that the observed difference in DNA content may be due to a loss of DNA present in a depolymerized form in the nucleus *in vivo*.

Since the DNA content is restored to normal or supranormal values after withdrawal of the stimulus, it is presumably a reaction of the nucleus to a functional stimulus of exceptional intensity.

I thank Prof. E. Puccinelli and Prof. C. Pellegrino for advice. This work was supported by a grant from Consiglio Nazionale delle Ricerche.

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Increase in Length of Skeletal Muscle during Normal Growth

RECENTLY I attempted to answer the question of how mammalian striated muscle fibres increase in length during normal post-natal growth. Aronson[1] has shown that the length of the sarcomeres of the muscles of the tarsonemid mite increase during development. He also suggests that the exceptionally long sarcomeres in this mite result from an early determination of the number of sarcomeres in a given fibre.

The investigation described here was carried out on the biceps brachii muscle of the mouse. This muscle is ideal for this purpose because its fibres run from tendon to tendon and are all approximately the same length. It is now well established that the length of the sarcomeres of striated muscle fibre decreases as the muscle shortens during contraction[3,4]. Therefore, in order to fix the muscle at a standard length the animals were killed and pinned to a cork board with the forelegs stretched out horizontally. They were then kept for 48 h at 4° C to allow the muscles to go into rigor mortis in this outstetched position. The purpose of maintaining the mouse carcasses at this low temperature was three-fold: to cause the muscle to go into rigor very slowly and avoid any violent contractions of the fibres that sometimes occur when muscle goes rapidly into rigor[5]; to reduce any decomposition; and to prevent the precipitation of the sarcoplasmic proteins[6]. After 48 h the muscles were excised and their length from tendon to tendon was measured with an ocular micrometer using transmitted light. These muscles were then fixed in Flemming's solution without acetic acid[7], sectioned longitudinally and stained with Mallory's triple stain. The length of the muscle was measured after sectioning to ascertain whether any contraction in muscle length had occurred due to the histological procedure used. No change in length was in fact noted in any of the muscles examined.

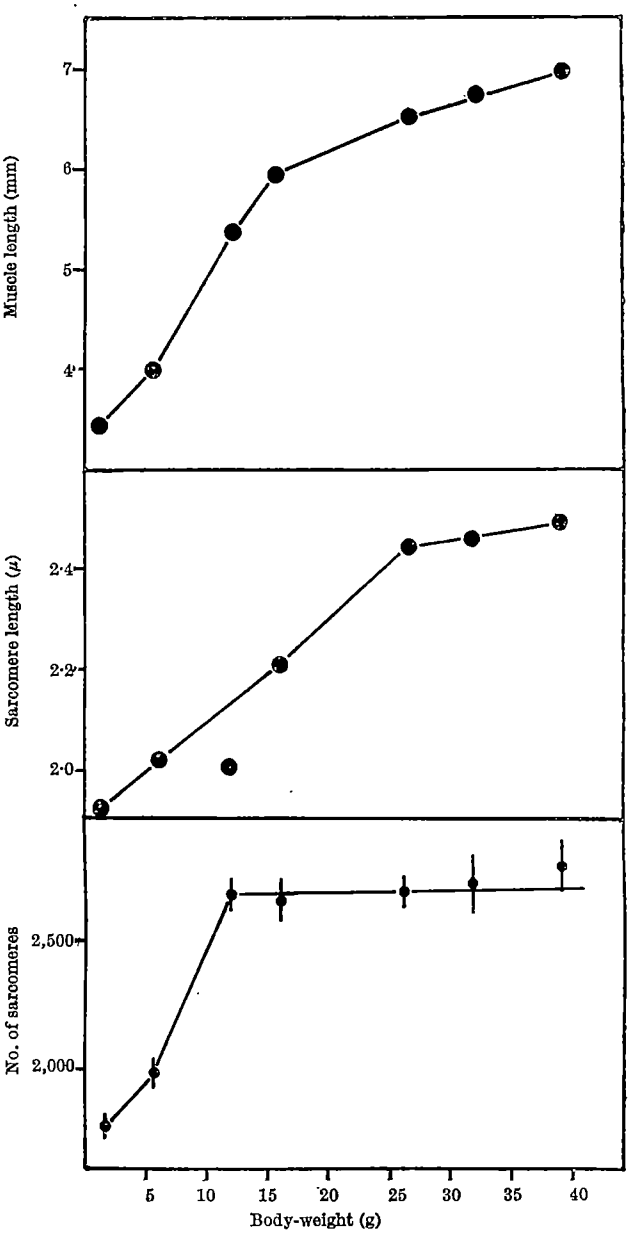


Fig. 1

The sections were then projected using a microscope fitted with a ground glass projection screen which was calibrated with an ocular micrometer. The number of *A* bands per 20μ length of fibre was then determined. These measurements were made on 6 fibres for each muscle and the average sarcomere length was calculated. An estimate of the total number of sarcomeres along the length of the muscle was then obtained from the average sarcomere length and the muscle length. This procedure was carried out on the biceps of mice ranging in size from newly born animals to adults.

The results of these measurements plotted against body weight are given in the three graphs of Fig. 1. It can be seen in Fig. 1 that the length of the muscle (top graph) increases with increase in body weight in a way that one might expect; increasing rapidly initially until the animal weighs approximately 15 g. After 15 g is exceeded the muscle lengthens at a slower rate. However, the average sarcomere length (middle plot) increases steadily until the animal weighs approximately 25 g. The lower plot shows the estimates of the number of sarcomeres along the length of the muscle (2 × the standard error is

given with each point). From this graph it is concluded that the number of sarcomeres along the long axis of this muscle increases only initially, that is, until the animal attains a body-weight of approximately 10 g. I have shown in earlier investigations[2,6] that embryonic differentiation of the biceps branchii of the mouse is not complete until the animal is approximately 8–10 g. Therefore this initial period of increase in the number of sarcomeres along the length of the muscle represents the completion of differentiation. After differentiation is complete then it would seem that the number of sarcomeres along the long axis of the muscle is fixed and no further increase takes place. My findings do in fact agree with Aronson's, who found that the post-embryonic elongation of striated muscle is due to the increase in length of each sarcomere and not due to the increase in the number of sarcomeres along the length of the muscle.

I thank Prof. J. N. R. Grainger for his interest in this work, and also the Agricultural Institute of the Republic of Ireland for the research fellowship they made available.

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HÆMATOLOGY

Plasma Angiotensinase Activity in Human Hypertension

Angiotensin is the pressure-active component of the renal pressor system and is suspected of playing a part in cases of human hypertension accompanied by primary or secondary renal lesions. The angiotensin-level results on one hand from its rate of synthesis by renin, on the other from its uptake by tissues and its inactivation by 'angiotensinase'. Angiotensinase activity results from a peptidasic enzyme system which has not yet been shown to be specific.

Blood or plasma angiotensinase activity has been measured with methods varying widely in principle; the level in hypertensive patients has been reported to be decreased[1], normal[2], and elevated[3–5]. These contradictory results led us to apply to 52 hypertensive patients a previously described method[6] based on: (*a*) the incubation of angiotensin with untreated plasma under standard conditions of time, temperature, *p*H, buffer and substrate concentration; (*b*) the assay of residual pressor activity in a rat preparation. Since the principle was strictly physiological, the results may have more significance than those obtained by measuring the degradation of a chemical or radioactive element of the angiotensin molecule. The degree of inaccuracy inherent to bioassays was reduced to 15 per cent by duplicating all assays, multiplying injections to each rat, using normal plasma as an extra control, and discarding less-than-ideal animal preparations.

Table 1. Mean Plasma Angiotensinase of 25 Normals and 52 Hypertensives. Normal Range is 80–120 per cent

Diagnosis	No. of patients	Mean ± *S.E.M.*	*P*
Normal	25	100 ± 2	
Essential hypertension	27	105 ± 4	> 0·05
Renal artery stenosis	14	109 ± 6	> 0·05
Malignant hypertension	11	115 ± 8	> 0·05

From the results shown in Table 1, it appears that the increase observed in the three hypertensive groups was not statistically significant according to the '*t*' test. No correlation was found between the angiotensinase-level and the blood-pressure reading at the time of sampling. Blood was obtained from one essential hypertensive patient every 3 h for 30 consecutive h to learn about the stability of the plasma enzyme-level; for an average value of 105 per cent, the standard deviation of the 11 samples was ± 7; considering the error due to technique, these fluctuations were of a minor degree. Two patients with renal artery stenosis in whom renal surgery was beneficial were examined before and after the operation; the post-operative plasma angiotensinase of one remained elevated, whereas it reverted to normal in the other.

These results confirm those of Klaus *et al.*[2], who observed a plasma angiotensinase increase of only 6 per cent in a group of 65 essential hypertensives. Those and the present results are not compatible with the production of a specific angiotensinase as an adaptive mechanism to prevent chronic hyper-angiotensinæmia.

We gratefully acknowledge the encouragement of Prof. R. M. Archibald, the technical aid of Mrs. Judith Lawson, the referral of patients by Dr. J. M. Bryant and Dr. A. I. Vagnucci, and the gifts of 'Hypertensin' by Dr. R. Gaunt, Ciba, Summit. The work was supported in part by U.S. Public Health Service grant *HE* 07473.

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Solubility of Fibrin Clots in Solutions of Heparin

Heparin has been shown to retard both the beginning and the general course of polymerization of purified fibrinogen[1]. Evidence will be presented that this anticoagulant, in solutions of low ionic strength, in addition to inhibiting the polymerization of fibrinogen, is able to depolymerize and thus dissolve preformed clots.

(*A*) *Effect of heparin on the polymerization of activated fibrinogen.* Saline solutions of four different preparations of bovine fibrinogen (34–54 mg/ml.) isolated by the method of Laki[2] and of two preparations of human fibrinogen (64 and 46 mg/ml.) obtained by ammonium sulphate precipitation of fraction I0 of Blombäck and Blombäck[3] (kindly supplied by Dr. A. M. Fisher of the Connaught Laboratories of Toronto) were mixed 1 : 1 with 2 M sodium bromide, *p*H 5·3. To these mixtures a thrombin solution of 100 N.I.H. units/ml. was added to a final concentration of 10 units/ml. and the samples kept at room temperature (24° C) for 3 h. One-tenth ml. from each mixture was then gently added to 9·9 ml. of each of several heparin solutions varying in concentration from 10–100 for human or 100–500 μg/ml. for bovine fibrinogen samples in 15 mm × 100 mm tubes. A small clot-like structure appeared immediately after the addition. The tubes were then stoppered and attached horizontally on a Kahn shaker with their long axis parallel to the motion of the machine. They were

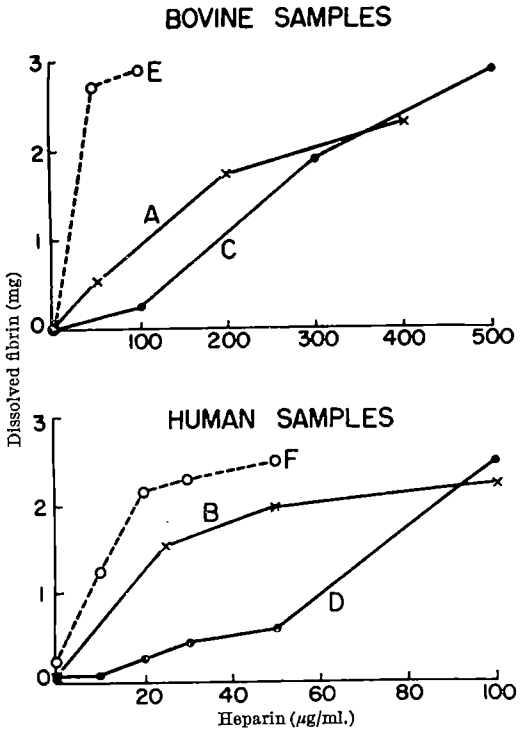


Fig. 1. Prevention of clotting of activated fibrinogen and dissolution of preformed fibrin by heparin. All graphs represent amounts of protein dissolved by shaking fibrin in aqueous solutions of heparin at the concentrations indicated on the two abscissæ, for 10 min. In *A* and *B* the parent bovine and human fibrinogens (2·6 mg) were subjected to the action of thrombin in 1 M sodium bromide of *p*H 5·3 for 3 h before being diluted 1 : 100 with the heparin solutions. In *C*, *D* and *E*, *F* the parent fibrinogens (3, 2·6 and 3, 2·6 mg, respectively) were clotted in distilled water left undisturbed for 10 min and then diluted with the heparin solutions. Thrombin was added at 10 N.I.H. units/ml. in the experiments of lines *C* and *D* but only at 1 and 0·5 units in those of *E* and *F*, respectively

shaken for 10 min after which they were centrifuged at a relative centrifugal force of 3,020*g* (5,000 r.p.m.) for another 15 min in order to separate the supernatant from the remaining undissolved clot, if any. The protein content of the supernatants was then determined by reading the absorption at 280 mμ in a Beckman *DB* spectrophotometer against a blank of 0·01 M solution of sodium bromide containing the same amount of heparin as each sample being tested. The protein concentration was read from a fibrinogen dilution curve which was prepared from a fibrinogen solution of known concentration. Lines *A* and *B* in Fig. 1 indicate that heparin solutions of low ionic strength ($\Gamma/2 = 0{\cdot}01$) are able to keep in solution smaller or greater amounts of fibrin depending on the heparin concentration. Comparison of the concentrations of heparin needed to keep in solution the same amount of activated fibrinogen shows that human samples are considerably more sensitive to the antipolymerizing action of heparin than are bovine preparations. Concentrations of heparin above 500 μg/ml. are even able to prevent the initial formation of a clot.

(*B*) *Effect of heparin on preformed fibrin clots.* Saline solutions of five preparations of bovine fibrinogen and two preparations of human fibrinogen containing 12·5–15 mg protein/ml. were buffered with 0·01 M imidazole at *p*H 7·3. 0·2 ml. volumes of these solutions were added into 15 × 100 mm tubes containing 9·0–9·8 ml. of distilled water and thrombin at a final concentration of 10, 1 or 0·5 N.I.H. unit/ml. Ten min after the formation of a clot 0·1–0·5 ml. of a heparin solution containing either 1 or 10 mg/ml. was added so that the final volume in each tube was 10 ml., while the final heparin concentration varied from either 10–100 or from 100–500 μg/ml. for human and bovine fibrinogen samples respectively. Immediately after heparinization the tubes were shaken on the Kahn shaker, as previously described, for 10 min and the amount of clot dissolved was determined by reading the absorption of the supernatant at 280 mμ after centrifugation of the mixtures. The blanks in each case were identical to the test samples except that they lacked fibrinogen. Curves *C* and *D* were obtained when the concentration used to clot the fibrinogen was 10 N.I.H. units/ml., while only 1 or 0·5 unit thrombin/ml. was added to the samples of curves *E* and *F* respectively. It is apparent that heparin is capable of dissolving preformed clots and that the amount of clot dissolved is proportional to the heparin concentration. It is also obvious that the clots produced with less thrombin, although of about the same protein content as those formed with higher concentrations of the enzyme, require considerably less heparin to be dissolved. Comparison of curve *C* versus *D* indicates that clots formed from human fibrinogen are more easily dissolved than clots from bovine fibrinogen.

Dissolution of the clots did not occur when (*a*) the clot was first wrapped on a glass rod and then shaken in the aqueous heparin solutions; (*b*) heparin was dissolved in saline instead of in distilled water—in such instances even concentrations of 1·2 mg heparin/ml. were ineffective; (*c*) fibrinogen was clotted in physiological saline containing 10 mM calcium chloride instead of in distilled water. Removal of the calcium-containing saline by repeated careful washings of the clot with distilled water, taking care not to increase its compactness, did not make the clot more soluble. On the other hand, clots formed in saline but in the absence of calcium chloride dissolved in heparin almost entirely, after washing.

Heparin has been claimed to activate the fibrinolytic system[4]. For this reason, 3,000 anti-Schwert-Takenaka units/ml. of pancreatic trypsin inhibitor[5] ('Iniprol', Choay Laboratories, Paris, France) were added to the aqueous solution of thrombin before the addition of fibrinogen. The presence of this excessive amount of fibrinolytic inhibitor did not affect the dissolving action of heparin, thus excluding the possibility that the lytic action was due to activation of the fibrinolytic system. Further proof against the idea of fibrinolytic action is the fact that addition of sodium chloride to a concentration of 1·7 per cent (double the physiological), or calcium chloride to 10 mM or neutralization of heparin by hexadimethrine bromide ('Polybrene'), resulted in the appearance of a new clot.

(*C*) *Effect of heparin on the solubility of fibrinogen.* Addition of 100 μg heparin/ml. to cloudy 1 : 9 dilutions of citrated human or bovine fibrinogen with distilled water resulted in redissolution of the fibrinogen and clearing of the turbidity of the mixture. Addition of 10 mM calcium chloride to the clear solution made it more cloudy than it was prior to the addition of heparin. Addition of sodium chloride to this suspension to a concentration of 0·9 per cent redissolved the floccules.

The evidence presented indicates that heparin in solutions of low ionic strength breaks the bonds holding the fibrin monomers together in clots. A solubilizing effect is also exerted on suspensions of fibrinogen of very low salt concentration. Calcium and hexadimethrine bromide reverse the solubilizing effect of heparin on both fibrinogen and fibrin. Sodium chloride neutralizes the effect of heparin on fibrin. However, due to the fact that saline itself solubilizes fibrinogen the effect of sodium chloride on the solubilization of fibrinogen by heparin is not clear. The fact that heparin is unable to dissolve clots formed in the presence of calcium indicates that the bonds broken by heparin are not covalent. This is similar to the effect of numerous other substances (like sodium bromide, urea, monochloroacetic acid, etc.) which at high concentrations are able to break hydrogen bonds and reverse the polymerization process which has taken place in the absence of the activated fibrin stabilizing factor or calcium[6]. The great difference in solubility between clots formed with 10 and clots formed with 1 or 0·5 N.I.H. units of thrombin activity/ml. indicates that the resistance of a clot to

heparin depends among other parameters on the amount of thrombin that generated the fibrin.

This work was supported by grants from the Medical Research Council of Canada, the Alberta Heart Foundation and the U.S. National Institutes of Health (*HE*–0714901).

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Hæmoglobin : Reversal of Oxidation and Polymerization in Turtle Red Cells

Hæmoglobins from the turtle, *Chelydra serpentina*, the frog, *Rana catesbeiana*, and certain strains of mice have been shown to polymerize *in vitro*[1]. Polymerization to an octamer began after hæmolysis and was completely inhibited by either *N*-ethylmaleimide or iodoacetamide. Experiments to be described here indicate that oxidized and polymerized hæmoglobin appears to be a frequent constituent in high concentrations in the red cells of some species of turtles. This polymerization and oxidation of hæmoglobin can be reversed by incubation of the cells with plasma, and appears to involve the formation of double molecules linked by one or more disulphide bridges. Disulphide bonds are suggested both by the finding that very low concentrations of mercaptoethanol dissociate already formed polymer and by the inhibition of polymerization by −SH reagents.

Freshwater turtles, *Pseudemys scripta elegans*, were purchased from Lemberger and Co., Oshkosh, Wisconsin, or captured in July in Bastrop County, Texas. Blood was collected and prepared as previously described for turtles[1]. Methæmoglobin was determined as described by Dubowski[2]. The following procedure was used to determine the presence of polymer because iodoacetamide inhibits polymerization but has no effect on polymer already formed[1]. Hæmoglobin (3·0 ml. of a 1·25 per cent solution in ~ 0·1 per cent NaCl), freshly prepared at 4° C, was reacted with 34·2 mg iodoacetamide and incubated at 25° C for 1 h. This solution was dialysed against 0·1 M phosphate buffer, *p*H 6·9, and run on a Spinco model *E* analytical ultracentrifuge as described elsewhere[1]. Red cells were incubated with glucose as described by Beutler and Baluda[3]. ACD–*A* solutions were used in the acid–citrate–dextrose incubations[4].

Six specimens from Texas were bled on the third day after their capture. Each possessed methæmoglobin, the values of which ranged from 5·2 to 60·3 per cent with an average of 30·1 per cent. Methæmoglobin levels in other turtles ranged from 50 to 90 per cent after 3 or more months in captivity. Polymerized hæmoglobin was present in every sample in which the methæmoglobin-level was more than 20 per cent. A typical sedimentation pattern is shown in Fig. 1. Small amounts of polymerized hæmoglobin may have been present in solutions containing less methæmoglobin, but are difficult to detect by ultracentrifugation.

Table 1. Incubation of Red Cells from a Single Turtle, *Pseudemys scripta elegans*, with Various Media at 4° C for Ten Days

Sample	1	2	3
		Vol. (ml.)	
Red blood cells	1·25	1·25	1·25
Plasma	3·00	3·00	—
0·8 per cent NaCl + heparin	0·75	0·75	—
ACD–*A* solution	—	0·75	—
0·15 M phosphate buffer *p*H 7·52	—	—	2·25
10^{-4} M methylene blue*	—	—	0·60
0·17 M glucose*	—	—	0·90
	Methæmoglobin values		
Initial methæmoglobin (per cent)	50·2	50·2	50·2
Final methæmoglobin (per cent)	6·1	34·4	10·1

* In 0·15 M phosphate buffer, *p*H 7·52.

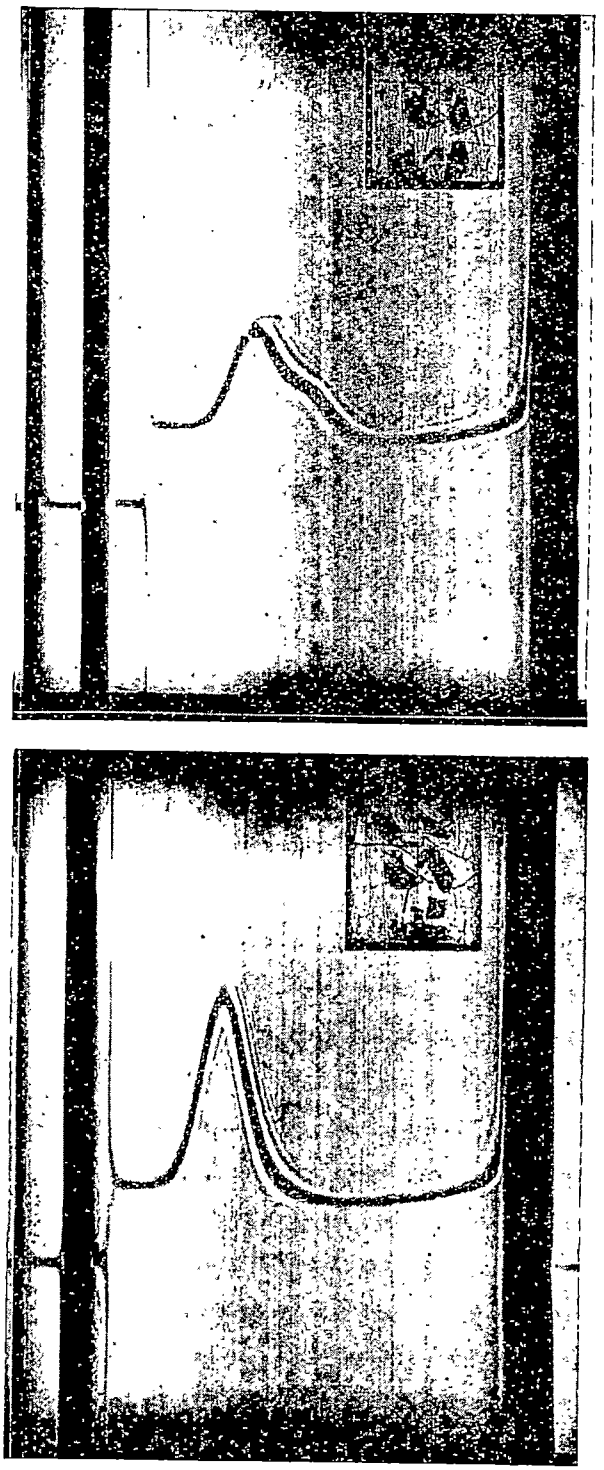


Fig. 1. Sedimentation patterns of freshly-prepared hæmolysates of the turtle, *Pseudemys scripta elegans*, before and after plasma incubation at 4° C for ten days. 64 min after reaching speed of 52,640 r.p.m.; hæmoglobin concentration is 1·25 per cent in 0·1 M phosphate buffer, *p*H 6·92. The sedimentation constants are corrected to water at 20° C. Above: before plasma incubation, showing the presence of two components with sedimentation constants of 4·1 and 6·6. The methæmoglobin concentration is 50·2 per cent. Below, after plasma incubation, showing the presence of a single component with sedimentation constant of 4·1. Methæmoglobin concentration is 6·1 per cent

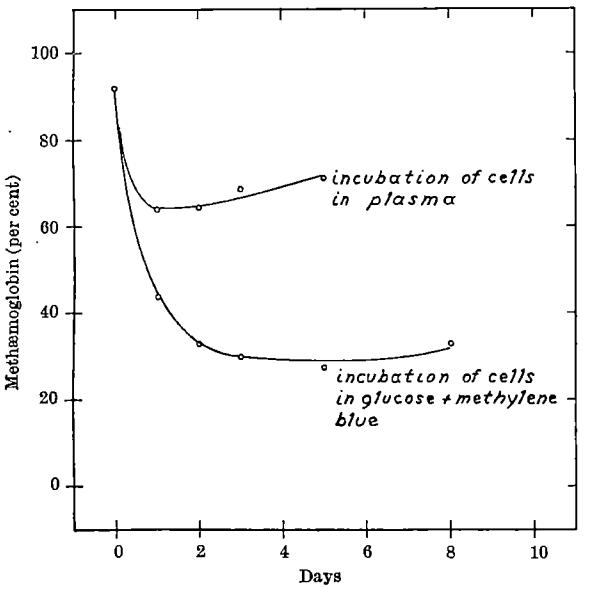


Fig. 2. Reduction of methæmoglobin by incubation of red cells of the turtle, *Pseudemys scripta elegans*, in plasma and in glucose–methylene blue medium at 4° C

Samples of freshly-obtained red cells containing 50·2 per cent methæmoglobin were incubated for 10 days in various media at 4° C (Table 1). Sedimentation patterns (Fig. 1) show that incubation of the cells with plasma resulted in the almost complete disappearance of the 7*S* component. The methæmoglobin content was reduced to 6·1 per cent. In other experiments plasma incubation completely reduced all methæmoglobin. Similar results were obtained with glucose–methylene blue incubations. Incubation in 0·8 per cent NaCl reduced the methæmoglobin and polymer content only slightly. Reduction of methæmoglobin by plasma incubation has been reported previously[5]. Fig. 2 shows that this reduction reaches a steady state in 1–2 days. Daily disturbance of the solution lowers the degree of reduction, presumably by increasing oxygen consumption. Glucose–methylene blue incubation appears to be more effective than incubation in plasma when the solution is disturbed. Although methæmoglobin formation is accompanied by polymerization of a portion of the hæmoglobin, the separation of the heavy 7*S* component from the 4*S* component by filtration through 'Sephadex *G*-100' equilibrated with 0·2 M NaCl showed that methæmoglobin formation occurs in both the polymerized and unpolymerized fractions.

The double molecule has been shown to dissociate on treatment with 0·5 M mercaptoethanol[1]. This might have been produced either by the reagent acting as a reducing agent to break disulphide bonds or as a dissociating organic solvent. We have now found that 0·01 M mercaptoethanol is sufficient to bring about the complete dissociation of the 7*S* component in a solution which initially contained more than 50 per cent double molecules. This finding supports the conclusion that —S—S— bonds are probably involved.

Methæmoglobin-levels in animals other than man have received little attention. Human red cells normally contain less than 1 per cent methæmoglobin[6]; the lethal level is probably near 70 per cent[7]. We have found more than 90 per cent methæmoglobin in a freshly-captured specimen of the box turtle, *Terrapene ornata ornata*. Otherwise, the animal appeared healthy. In some species of turtles polymerization accompanies methæmoglobin formation. Nutrition and red cell ageing are factors known to affect methæmoglobin-levels in other animals. We have observed that methæmoglobin-levels in turtles are higher in summer than in spring. The mean life-span of turtle erythrocytes from *Terrapene carolina* has been reported to be 600–800 days[8]. Examination of red cell synthesis in turtles indicates that their systems are different from those found in mammals[9]. Turtles can, under anoxic conditions, shunt blood by the lungs via the ventricular septum and are able to live for long periods of time on energy derived only from anaerobic glycolysis[10]. This may explain in part the tolerance of turtles to high levels of methæmoglobin.

The effect of polymerization on the oxygen affinity of the hæmoglobin is difficult to evaluate because we have not yet been able to isolate as a monomer that fraction which polymerizes without reacting the freshly-prepared hæmoglobin with iodoacetamide or *N*-ethylmaleimide. Each of these reagents is likely to affect the oxygen equilibrium.

This work was supported by grants *GM*–05818–01*A*2, *GM*–*K*3–3086, and *GM*–09326–03 from the U.S. National Institutes of Health. One of us (B. S.) was supported by a training grant 5*T*1 *GM*–337–04 from the National Institutes of Health.

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Hæmoglobin Types of the Wild House Mouse (*Mus musculus domesticus* Rutty 1772)

Ranney and Gluecksohn-Waelsch[1] described in laboratory strains of the house mouse the hæmoglobin (Hb) types *s* = single and *d* = diffuse which differ in their electrophoretic behaviour. Of 20 inbred strains which were investigated six belonged to the 'single' (Hb^s) and 14 to the 'diffuse' (Hb^d) type[2]. In crosses Hb^d is dominant over Hb^s (ref. 3). Popp[4,5] was able to localize the Hb-gene on chromosome I by discovering its linkage to the genes *p*, *c* and *sh*–1. In addition, there are differences in the solubility and peptide structure of the hæmoglobins within the Hb^s and Hb^d types which, as such, are differentiated by their electrophoretic patterns only[4–7].

From the existence of the two Hb types in inbred laboratory strains of mice, Russell and Gerald[2] concluded that they must also occur in Nature and that Hb^d is probably the commoner of the two; however, the existence of both alleles in wild mice was regarded as necessary to explain their actual distribution in the inbred strains examined.

Popp[5] has indeed found the Hb^s type in 6 from 18 wild mice. We have investigated the hæmoglobin pattern of wild house mice by means of the agar electrophoresis technique[8]. The animals are derived from five fairly closed populations; there is one additional mouse from a different locality (Table 1). The ratio of Hb^s to Hb^d found in our material is about 1 : 1·6, in agreement with Russell and

Table 1. Distribution of Hæmoglobin Types in Five Populations of Wild Mice

Population	Number of mice	Hb^s type	Hb^d type
T I	6	2	4
T II	7	6	1
T III	15	1	14
H I	6	0	6
H II	7	6	1
Additional	1	1	0
Total	42	16	26

Gerald's[2] suggestion that Hb^d may be more widespread in Nature. However, individual populations differed widely from one to another.

Furthermore, studies of mutants[1,5] show that animals with the Hb^s type can arise as mutants from Hb^d lines and possibly vice versa. Mutant strains can also be found with some animals of the single and some of the diffuse type[1,9]. We therefore do not always know for certain that the wild ancestors of laboratory strains already possessed the Hb^s allele. In the strain '*CE*', which originated from a wild mutant (Knight[10]), we found the Hb^s type in animals of the line *CE/J* Han Jena (F_{25}).

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Effects of Erythromycin and Tetracycline in Human Bone-marrow Cultures

In a previous publication[1] we have reported that chloramphenicol inhibits hæmoglobin synthesis in human bone-marrow culture. Erslev and Iossifides[2] found that not only chloramphenicol but also tetracycline and erythromycin had similar effects on immature erythrocytes *in vitro*. Because tetracycline and erythromycin do not cause toxic hæmatological reactions in man, this finding would seriously limit the validity of applying this *in vitro* method to the study of the effect of chloramphenicol on erythroid cells *in vivo*. It seemed important, therefore, to extend our experiments and to study the effect of tetracycline and erythromycin on hæmoglobin synthesis in human bone-marrow cultures, using the same methods as were used to study the effect of chloramphenicol.

The detailed method has been described previously[1]. Bone marrow was obtained by aspiration from the sternum or iliac crest. Cell suspension cultures were prepared under aseptic conditions, using Hanks's solution and 40 per cent autologous serum as medium. ^{59}Fe in the form of $^{59}FeCl_3$ (Abbott Laboratories, Ltd., Montreal) (specific activity, 4·4–11 μc./μg) was added to the cultures to a final concentration of 0·01 μg/ml. The amount of iron added never exceeded the iron-binding capacity of the serum. Crystalline chloramphenicol (Parke Davis, Ltd., Montreal), erythromycin (Abbott Laboratories, Montreal) and tetracycline (Pfizer Canada Chemicals, Montreal) were dissolved in Hanks's solution and added, in various amounts, to the cell suspensions. The maximum concentration of the three antibiotics in the culture was 500 μg/ml., 500 μg/ml. and 100 μg/ml., respectively. The low solubility of the pure crystalline tetracycline did not allow the use of higher concentration. The volume of the cultures was 2 ml. in each tube and the total nucleated cell count varied from 2–4 × 10^6 per ml. The cultures were rotated at 12 r.p.h. for 24 h at 37° C. At the end of the incubation period, they were centrifuged, the cells washed three times with physiological saline and hæmolysed with distilled water at 4° C.

The radioactivity of the total hæmolysate was then measured in a well-type scintillation counter. From this, the total Fe uptake was calculated and expressed in μg Fe/erythroblast/h.

After centrifuging the tubes containing the hæmolysates at 3,000*g* for 15 min, the supernatant was removed and its radioactivity determined. From this, the amount of Fe incorporated into hæm was calculated and also expressed in μg Fe/erythroblast/h.

Cultures containing no added antibiotics, but prepared from the same marrow specimen, served as controls. In each experiment, cultures were prepared in duplicate.

As noted in our previous communication, when chloramphenicol was added to the cultures, iron uptake by the erythroblasts was slightly decreased, whereas iron incorporation into hæm was markedly decreased. Therefore, iron utilization, that is, the fraction of iron utilized for hæmoglobin synthesis in relation to the amount taken up by the cells, was significantly lower in the presence of chloramphenicol. This effect was proportional to the concentration of chloramphenicol in the culture, and the lowest concentration which gave consistent results was 500 μg/ml.

The addition of erythromycin to the cultures had a different effect. This was consistent in cultures of five different bone marrows, and Table 1 shows one representative experiment. The amount of iron taken up by the erythroblasts was strikingly reduced in the presence of erythromycin. Consequently, less iron was found in the hæmolysate. However, in contrast to chloramphenicol, in the erythromycin-treated cultures the percentage of the iron taken up by the cells which was incorporated into hæm was the same as in the controls. Thus, iron utilization was not altered in the presence of erythromycin. The effect of erythromycin depended on its concentration in the culture and 250 μg/ml. was found to be the smallest effective dose. 500 μg/ml. further reduced iron uptake, but had no influence on iron utilization.

Table 1. Effects of Erythromycin and Chloramphenicol on Iron Uptake and Iron Incorporation by Erythroblasts in Bone Marrow Suspension Culture

Incubation time 24 h. Values of a representative experiment	Control	Chloramphenicol 500 μg/ml.	Erythromycin 250 μg/ml.
Fe uptake, × 10^{-3} μg Fe/cell/h	1·59	1·12	0·61
Fe incorporation	0·79	0·21	0·39
Percentage of iron uptake incorporated into hæm	49·7%	18·8%	63·9%

The effect of tetracycline on erythroblasts was studied in 14 bone-marrow cultures. When 50 or 75 μg/ml. was present in the medium, the iron uptake and incorporation by the erythroblasts were not altered as compared with the controls. When 100 μg/ml. tetracycline was used, the uptake of iron by the erythroblasts was decreased in four of seven cultures but, as with erythromycin, the percentage of iron which was incorporated into hæm was the same as in the controls. Hence, both erythromycin and tetracycline decreased iron uptake but had no effect on iron utilization by erythroblasts in these bone-marrow cultures. No relationship could be found between the susceptibility of the cultures to the toxic effect of these agents and the hæmatological status of the patients from whom the bone marrows were obtained.

These observations indicate that chloramphenicol, erythromycin and tetracycline do not have identical effects on erythroblasts cultured *in vitro*. Chloramphenicol

slightly diminished iron uptake by the erythroblasts and interfered markedly with iron incorporation into hæm. On the other hand, both erythromycin and tetracycline depressed iron uptake by the cells. The mechanism of this effect is not clear, but, as tetracycline is known to be a chelating agent[3], a possible explanation is that it binds iron, thus making it less available for the cells. Of the iron taken up by the erythroblasts, the same percentage was incorporated into hæm as in the control cultures. Since this step is related to hæm synthesis, it seems that, in contrast to chloramphenicol, neither erythromycin nor tetracycline interferes with this synthesis in the erythroblasts. Erslev and Iossifides[2] did not find this different pattern of behaviour *in vitro* and, therefore, concluded that there was a discrepancy between the *in vivo* and *in vitro* observations. In contrast, the difference in the action of these drugs *in vitro* in the experiments reported here may explain their different behaviour *in vivo* in relation to the hæmic cells. Also, in our experiments, tetracycline affected erythroblasts to a lesser degree than did erythromycin or chloramphenicol, which may be related to its lower concentration in the cultures.

The effective *in vitro* concentration of the three drugs in relation to their *in vivo* therapeutic blood level was also different. The 500 μg/ml. concentration of chloramphenicol is 10–15 times[4], the 100 μg/ml. tetracycline is 20–40 times[5,6] and the 250 μg/ml. erythromycin is 50–100 times[7,8] the average effective therapeutic blood level. Lower concentrations of erythromycin and tetracycline, corresponding to 10–15 times their effective therapeutic blood level, had no measurable effect in the cultures. The fact that relatively higher concentrations of tetracycline and erythromycin were necessary to cause an adverse effect *in vitro* may also reflect that the hæmic cells are less sensitive to these drugs.

Finally, the fact that the effects of the three antibiotics on iron metabolism *in vitro* were different suggests that their antimicrobial action may also be different. This is in agreement with Brook[9], who suggested that, although their action on bacteria is assumed to be similar, based on the inhibition of protein synthesis, this cannot be more than a superficial similarity, in view of their completely different chemical structure. It must be recognized, however, that the action of a drug on a bacterium, and its action on the human cells, may be quite unrelated.

In summary: (*a*) Both erythromycin and tetracycline decreased the iron uptake by the erythroblasts, but had no effect on the utilization of iron for hæm synthesis. This indicates that, in contrast to chloramphenicol, these antibiotics did not interfere with hæm synthesis under the same experimental conditions. (*b*) The difference between the effects of these antibiotics *in vitro* may be related to their different behaviour *in vivo* on the hæmic cells.

This work was supported by research grant *MA*–1411 of the Medical Research Council.

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IMMUNOLOGY

Suppression of Experimental Allergic Encephalomyelitis in Rats and Chickens treated with Reserpine

Previous investigations have shown marked suppression of delayed hypersensitivity reactions, prolongation of skin homograft survival, and impairment of antibody production in rats treated with reserpine[1]. Moreover, this *Rauwolfia* alkaloid was regularly found to produce thymic involution. This communication is an extension of these investigations, and deals with the effect of reserpine on the development of experimental allergic encephalomyelitis in the rat and chicken.

Adult male albino rats weighing 220–260 g from a randomly bred colony were treated with intraperitoneal 0·5 mg/kg injections of reserpine ('Serpasil', Ciba, Basle) for 17 consecutive days. Three days after the beginning of treatment all rats were injected with an emulsion containing fresh normal rat spinal cord and complete Freund's adjuvant[2]. Each rat received a single inoculation of 0·1 ml. of this antigen–adjuvant mixture in the left hind foot-pad. Control animals were treated with 17 injections of saline, and immunized with spinal cord adjuvant mixture as described for reserpine-treated rats. Animals were observed daily for the onset of clinical signs of disease. Those rats which showed paralysis or ataxia were killed on the day of onset of symptoms; the remaining rats were killed 19 days after immunization. Paraffin sections of cerebrum, cerebellum, medulla and three levels of spinal cord were prepared, and stained with hæmatoxylin and eosin. Histological changes were graded from 0 to +++[2]. Rats from both experimental and control groups were weighed every three days. At autopsy the thymus was also weighed.

Rhode Island Red chickens were treated with 2 mg/kg reserpine intraperitoneally every three days, beginning one day after hatching. At eight weeks, each chicken received foot-pad injections of homologous spinal cord adjuvant mixture as described elsewhere[3]. Control chickens of exactly the same age were immunized in an identical manner. Reserpine-treated and control birds were skin-tested 14 days following immunization; for this purpose 0·1 ml. containing 50 μg of chicken spinal cord lipid, obtained by petroleum-ether extraction, was injected into the left wattle. The right wattle was injected with 0·1 ml. of a 1 : 5 dilution of old tuberculin. The reactions were read at 24 h, and graded from 0 to +++ on the basis of œdema, induration and discoloration of the wattle. Sections of cerebrum, cerebellum, medullary bulb and spinal cord at three levels were stained with hæmatoxylin and eosin, and lesions were classified from 0 to +++[3]. Thymic lobes, spleen and bursa of Fabricius of all chickens were weighed at the end of the experiment.

The ability of reserpine-treated rats to develop experimental allergic encephalomyelitis was strikingly suppressed (Table 1). Eight of 10 rats injected with 0·5 mg/kg of reserpine showed no histological changes in the central nervous system, whereas 8 of 12 controls exhibited definite lesions characteristic of disease, and two rats of the latter group developed paralysis.

Table 1. Suppression of Experimental Allergic Encephalomyelitis in Rats treated with Reserpine

Group	No. of rats	No. of rats with encephalomyelitis* 0	+	++	+++	Average weight Thymus (mg)	Body (g)
Reserpine-treated (0·5 mg/kg)	10	8	1	1	0	90·0	129·7
Control	12	4	3	3	2	217·3	160·0

* Average severity of lesions observed in spinal cord, medulla, cerebellum and cerebrum.

Table 2. Suppression of Experimental Allergic Encephalomyelitis in Chickens treated with Reserpine

Group	No. of chickens	No. of chickens with encephalomyelitis*				Average weight (g)			
		0	+	+ +	+ + +	Thymus	Bursa	Spleen	Body
Reserpine-treated (2 mg/kg)	9	2	4	3	0	1·49	1·07	1·71	642·8
Control	12	0	2	1	9	3·30	2·60	2·58	1,156·0

* Average severity lesions observed in spinal cord, medullary bulb, cerebellum and cerebrum.

Table 3. Delayed Hypersensitive Reactions to Chicken Spinal Cord and Old Tuberculin in Reserpine-treated Chickens

Group	No. of chickens	No. of chickens with reaction at 24 h							
		Spinal cord lipid				Old tuberculin			
		0	+	+ +	+ + +	0	+	+ +	+ + +
Reserpine-treated (2 mg/kg)	9	8	1	0	0	5	4	0	0
Control	12	4	5	3	0	0	5	4	3

Repeated injections of reserpine regularly produced a marked involution of the thymus. The architecture of the thymic tissue was altered: cortical areas showed pronounced atrophy, and their cellular make-up, which normally consisted of densely packed thymocytes, was largely replaced by reticulum and epithelial cells. The average body-weight of reserpine-treated animals was less than that of controls; but treated animals were all in good condition and did not require artificial feeding. There were no signs of alimentary tract disturbances in these rats.

Only partial suppression of experimental allergic encephalomyelitis was observed in chickens injected with reserpine and killed 19 days after immunization (Table 2). However, histological quantitation of lesions found in various parts of the central nervous system revealed that the disease was more severe in controls than in chickens treated with reserpine. Three control chickens developed paralysis, whereas the clinical signs of disease were not observed in reserpine-treated birds. There was an involution (or slower development) of thymus, spleen and bursa of Fabricius in chickens injected with reserpine. Although there was a loss in body-weight in those birds, they did not show any obvious physiological and behavioural abnormalities. Chickens treated with reserpine and tested for delayed hypersensitivity 14 days following immunization with allogeneic spinal cord showed markedly impaired delayed wattle reactivity to chicken cord lipid and old tuberculin (Table 3).

The development of experimental allergic encephalomyelitis can be affected by a variety of factors including salicylates[4], cortisone and ACTH[5], alkylating agents[6], 6-mercaptopurine[7] and radiation[8]. The results presented here introduce a new member to the group of agents which suppress immunological potential or reactivity. Thus, reserpine is capable of impairing the evolution of an auto-allergic disease in rats and chickens. However, these findings do not imply that the mode of action of reserpine on the immunological capability of the animal's body is similar to that exerted by various antimetabolites, and that its effect should include mechanisms proposed for different types of drug-induced tolerance. Differences in responsiveness of different species to reserpine[9] may be responsible for less-striking suppression of experimental allergic encephalomyelitis in chickens when compared with the suppression obtained in rats.

Chronic treatment with reserpine of animals and chickens regularly results in involution of lymphoid organs such as thymus, lymph nodes, spleen and bursa of Fabricius, and causes profound cellular changes in those tissues (unpublished results). These findings may, therefore, provide a cellular basis for the impaired immunological reactivity of reserpine-treated animals. Bruscha[10] found in patients injected over a long period with a large dose of reserpine that a temporary drop in the eosinophil and monocyte count occurs. In addition, reserpine causes marked inhibition of the growth of a lymphoid tumour in mice[11], although this effect appears to be mostly non-specific[12].

Whether reserpine diminishes immunological capacity directly, or indirectly through its action on lymphoid tissues, or whether this alkaloid acts by releasing active substances such as 5-hydroxytryptamine and catecholamines from tissues is not known. Alternatively, these mediators may be necessary for the expression of auto-allergic lesions and other immune manifestations. Since one of the well-studied actions of reserpine is the depletion of brain serotonin stores, as well as the action on those brain regions known to control ACTH secretion[13], it is pertinent to consider whether this central effect is in any way related to the findings reported herein. It may be assumed that reserpine is interfering with the 'central control of immune response' by acting either on the brain, or on neuronal and endocrine pathways that monitor the immunologically competent tissues. The participation of the central nervous system in the expression and maintenance of immunological potential has not been investigated so far.

This work was supported by grants from the Yugoslav Foundation for Scientific Research.

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HISTOLOGY

Birefringence of the Spermatozoan Tail

THE motility of the spermatozoan tail depends on the contractility of its protein fibrils, which resemble myosin threads[1]. It is not surprising, therefore, to find that the tail is birefringent. This birefringence is difficult to demonstrate by the usual means, but is easily shown by special staining, combined with the use of elliptically polarized light (EPL). The stain is chromosome red ('Michrome No. 1091'). This is haplofavioxanthic acid, a special compound of acid fuchsin, sun yellow and violamine 3*B*. It has the effect of intensifying the anisotropy of already birefringent substances, particularly with EPL[2,3].

I first saw this phenomenon in paraffin sections of rat testis, fixed in formalin and cut at 8 or 10μ. These sections were stained for 5 min in a solution of chromosome red (0·8 g), acetic acid (2 ml.) and water to 100 ml. The birefringence of the sperm tails was seen both in the seminiferous and in the epididymal tubules. In the latter it was in strong contrast to the non-birefringence of the epididymal ciliariform processes or other cilia, prepared and examined in the same way. Since all anisotropic elements appear coloured in EPL, the colour depending on their orientation in the field, the helical form of the spermatozoan tail is easily demonstrated by the colour change as the helix changes direction in its course from the neck onwards. It is more readily seen with an oil-immersion than with a dry objective, although the birefringence of groups of spermatozoa is easily perceived with a low-power objective.

The combination of myosin content and birefringence thus makes the spermatozoan tail the equivalent of a fibril of smooth muscle. The intensity of colour seen is just that of smooth muscle examined under similar conditions. An investigation into the stage of spermatozoan development at which the phenomenon is first observable is in progress.

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HISTOCHEMISTRY

Uptake of Tetracycline by Human Bone *in vitro*

SINCE the demonstration by Milch *et al.*[1-3] that tetracycline is taken up in newly-formed bone after injection into the living organism, the tetracyclines have been used widely in the study of bone formation, especially by the technique of double labelling[4]. It has lately been shown by Harris *et al.*[5], comparing the uptake of tetracycline *in vivo* with the uptake of radioactive calcium, that, besides the distinct uptake in the calcification front, there is also an uptake in other parts of the bone, not related to new bone formation. In a communication to *Nature*[6], one of us showed that a diffuse uptake of tetracycline can be demonstrated in bone grafts, with the histological appearance of dead bone. We have, therefore, investigated the uptake of tetracycline in bone devitalized by different methods.

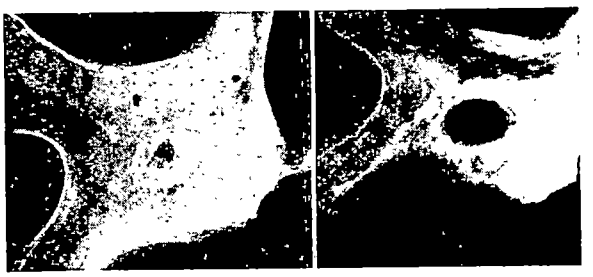


Fig. 1. Cancellous bone treated by alcohol during two weeks storage. Undecalcified specimen examined under ultra-violet light on the right and the corresponding radio-micrograph. Note the uptake on surfaces, in the demarcation zone and inside the bone in inverse relation to calcium density

Human bone, cortical or cancellous, was treated by freezing at −20° C for 2 weeks, boiling or storing in alcohol, or was kept as fresh bone. To each specimen was added a solution of 10 mg of 'Ledermycine' in 100-ml. citrated human plasma for 24 h. The bone was then rinsed in running tap water for a period of 48 h. The specimens were then fixed in formalin, dehydrated in alcohol and embedded in methyl-methacrylate. After cutting and grinding the bone specimens to a thickness of about 50μ, radio-micrographic pictures were taken, and fluorescence was observed in ultra-violet light, using a 'Reichert' filter *GG* 9/1 mm + *OG* 1/1·5 mm.

From the photographs (Figs. 1 and 2), showing the fluorescent picture and the corresponding radio-micrograph of cancellous and cortical bone, it is seen that mineralized dead bone takes up tetracycline on every surface. As in living bone, there is a greater uptake on the growing part, in the zone of demarcation, within the osteoid seam. In non-growing surfaces, like osteons which show edge-sclerosis on corresponding radio-micrographs, or in resorption cavities, there is a limited uptake. Besides this surface incorporation, there is also a diffuse uptake, which is more prominent in cancellous than in cortical bone: this is demonstrated by comparing Fig. 1 with Fig. 2. In all cancellous specimens, there is an inverse relationship between the mineralization of the bone and the degree of labelling with tetracycline. The results are not affected by the method of devitalization of the specimens.

Thus, the uptake of tetracycline by bone is evidently not dependent on the activity of living bone cells, or on the integrity of the physical properties of the intercellular components, which are greatly modified by boiling and storing in alcohol[7]. Tetracycline incorporation cannot be directly related to calcium uptake, because there were no free calcium ions in the citrated plasma used (citrate would be more likely to liberate calcium from the bone[8]). The localization of tetracycline inside the bone supports the conclusion that tetracycline uptake is not confined to growing surfaces. Its peculiar distribution might be explained by a diffusion phenomenon[5,6], but tetracycline uptake in low-density areas is less in cortical than in cancellous bone. Tetracycline staining is present whenever

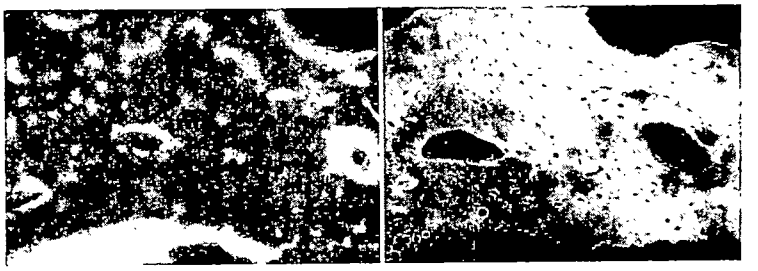


Fig. 2. Cortical bone refrigerated at 20° C and thawed after two weeks. Note the surface uptake, including the resting osteon, with the so-called edge-sclerosis and the fluorescent band corresponding to the growing osteon's calcification front. The fluorescence inside the bone is fainter and uniformly distributed. Ultra-violet light photograph and radio-micrograph as in Fig. 1

calcification is imminent, and its intensity is perhaps related to the rapidity of the calcification that follows.

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Histotopochemistry of Ascorbic Acid in Tendon Fibres

From investigations with the electron microscope, it has been reported that the precursors of collagen fibrils are formed within the cytoplasm of fibrocytes[1]. It follows that the sites of formation of the fibrils and those of the synthesis of collagen are identical. Hydroxyproline, which is a characteristic acid of collagen protein, originates from proline under the action of L-ascorbic acid[2].

For this reason we believe that determination of the distribution and concentration of ascorbic acid in the connective tissue, for example in the tendon, would suggest where hydroxylation of proline takes place and where the tropocollagenous cells, which are the basic elements of the fibrils, are formed.

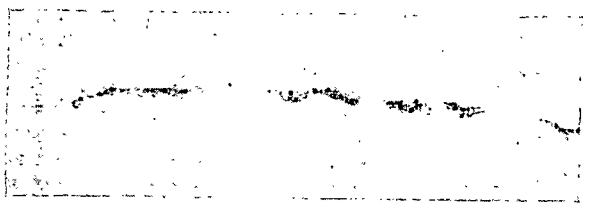


Fig. 1. Black granules of metallic silver in the tendon cells indicate L-ascorbic acid, which takes part in the conversion of proline to hydroxyproline

In this work L-ascorbic acid was determined in isolated fibres from the tail tendon of male albino Wistar rats, 4 months or 28 months old. The method of Giroud and Leblond, applying silver nitrate to metallic silver, was used. The granules occurring in the positive reaction are not granules of vitamin C but granules of metallic silver[3].

It has been found that in isolated tendon fibres of both the younger and the older rats the granules of silver are deposited only in the cytoplasm of fibrocytes; the remaining fibrillar structure is negative.

The occurrence of L-ascorbic acid in the tendon cells which intensively produce collagen supports the suggestion that conversion of proline to hydroxyproline occurs within the cytoplasm of the cells, and that a collagen molecule may there be built up from the individual amino-acids.

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PATHOLOGY

An Unidentified Virus which causes the Rapid Production of Tumours in Mice

In the course of routine passage of Moloney's leukæmogenic virus (MLV) plasma was collected from a leukæmic rat (Chester Beatty Institute outbred albino strain) which had been inoculated with MLV-containing mouse plasma when new-born. After storage at −70°C for three months the rat plasma was diluted 1 in 30 with Hanks's saline and passed through a 'Selas 02' filter, tested and found impervious to *Esch. coli*. The filtrate was injected into 15 new-born *BALB/c* mice, as a test of potency. Only 6 survived to weaning. On the 32nd day 5 had tumours at or near the injection site, and all had grossly enlarged spleens. Usually mice inoculated with MLV remain symptomless until they show the characteristic signs of leukæmia after 8 weeks.

Further samples of the same stored plasma filtrate were injected into new-born *BALB/c* mice, and Chester Beatty albino (outbred) and hooded (inbred) rats. Twenty-eight of 35 mice, and 1 of 13 rats, killed and examined *post mortem* had tumours, and all had splenomegaly.

The pathology of the disease is being examined: the result of early observations are as follows. The animals commonly die of splenic rupture, as in Friend or Rauscher disease. The histology of the spleen and the blood smears are similar to those of Friend disease. There is gross proliferation of reticulum cells in the spleen, and reticulum cells and erythroblasts appear in the blood. The tumours are of two main types, either solid and firm, or cystic and filled with blood. The former arise in the subcutaneous tissue and peritoneum, sometimes attached to the muscle of the abdominal wall, thorax, or diaphragm; their position suggests development at or near the site of injection or along the needle track. They are anaplastic sarcomata consisting of pleomorphic and spindle cells, some very large, invading and disrupting adjacent tissues. The latter may occur anywhere in the subcutaneous space or peritoneal cavity, and often in relation to lymph nodes. They are angiomatous tumours consisting of multiple dilated sinuses which are filled with blood and debris, and lined with cuboidal cells which often form ingrowths into the lumen.

Recent experiments have shown that of a number of animals injected with sarcoma virus (SV) when new-born 30/46 *CB* hooded rats developed gross splenomegaly, and 16 had sarcomas of the diaphragm or other sites. 4/9 cream and 5/19 golden hamsters have developed sarcomas, also mainly situated on or near the diaphragm. Many of the rats and hamsters had large thin-walled cysts at lymph node sites containing either clear or blood-stained fluid.

Two of 5 attempts to transplant sarcomas in *BALB/c* mice were successful, and the cell line is now in its fifth passage. All recipients have developed splenomegaly.

Tissues of tumour-bearing mice and plasma of injected rats were tested by inoculation into *BALB/c* mice for the presence of tumour-producing virus (Table 1).

'Selas 02' filtrates of tumour homogenates or plasma induced rapid development of tumours and splenomegaly in mice inoculated when 1–7 days old, in some cases as early as 12 days after injection. As the age of injection increased, the incidence of tumours declined, but that of splenomegaly remained high. Fifteen mice inoculated at 112 days all developed splenomegaly, but only two developed tumours.

One to 2 day-old Chester Beatty albino rats were injected with the original plasma filtrate. Their plasma was collected at 8, 30, and 54 days, and injected into 1–3-day-old *BALB/c* mice. Mice which received the 30- and 54-day samples developed tumours and splenomegaly in 12–15 days. Those which received the 8-day sample

Table 1

Inoculum	Age injected (days)	No. recorded *post mortem*	Mice with tumours	Mice with splenomegaly	Latent period to death (days)
(I) Plasma from tumour-bearing mice	1–3	12	10	10	28–66 (av. 38)
(II) As (I) 'Selas 02' filtered	1–3	20	11	20	24–42 (av. 30)
	11½	15	2	15	35–116 (av. 68)
(III) 'Selas 02' filtrate of tumour tissue	1–3	18	17	18	12–17 (av. 13)
	5–7	19	17	19	12–30 (av. 16)
(IV) 'Selas 02' filtrate of pooled infected rat plasma	1–3	13	13	13	18–20
Rat plasma 8 days after injection	1–3	6* (5 survive)	0	2	70–100
Rat plasma 30 days after injection	1–3	7	7	7	12–15
Rat plasma 54 days after injection	1–3	6 (2 survive)	6	3	13–?

* These mice died with generalized lymphatic leukæmia.

remained healthy for 70 days, when they developed the typical signs of MLV-induced leukæmia—an indication that Moloney virus was still present in the virus preparation (Table 1).

The possibility that the tumours produced might be due to polyoma virus was considered. Certain differences were already apparent: for example, no parotid or kidney tumours were seen in the injected mice, and polyoma tumours have never been reported as early as two weeks after inoculation. Certain specific tests were performed. (1) Active preparations of SV produced no cytopathic effect in mouse embryo tissue cultures, and did not agglutinate guinea-pig red cells in the cold. (2) The sera of mice bearing sarcomata did not inhibit hæmagglutination by polyoma virus. (3) In two separate tests samples of SV were found to be ether-sensitive. These results make it very unlikely that the tumours produced are due to polyoma, or any closely related virus.

The presence of other viruses in the SV preparations is being considered. As indicated above, MLV is almost certainly still present. The type of splenomegaly, and its short latent period, suggests this may be due to Friend, or possibly Rauscher, virus. In this connexion it is noteworthy that *BALB/c* mice may carry the Rauscher virus in a latent form[1]. Since none of these leukæmogenic viruses has been shown to produce sarcomata of the type reported here it appears likely that they are due to a hitherto unidentified virus. The Schmidt–Ruppin derivative of Rous sarcoma virus rapidly produces tumours in small mammals, and is ether-sensitive—in these respects it resembles SV. This virus is being tested for. Another possibility is that the observed effect may be the result of some kind of synergism between two or more viruses (as, for example, between the Moloney or Friend virus and mouse hepatitis virus[2], and this possibility is also being investigated.

I thank Dr. M. H. Salaman and Dr. K. E. K. Rowson of this Department, and Dr. R. J. C. Harris of the Imperial Cancer Research Fund Laboratories, for their advice. I also thank Dr. F. C. Chesterman (also of the Imperial Cancer Research Fund) for his advice concerning the histological preparations. Mr. R. Evans helped with the tissue culture and Miss J. M. Burke with histology and hæmatology.

This work was partially supported by the British Empire Cancer Campaign for Research.

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Ink Blue as a *p*H Indicator for Certain Biological Systems

The observation that commercial blue-black ink poured on to the uncolonized agar of a Petri dish culture of *Trichophyton mentagrophytes* became discoloured in the region of the dermatophyte, but not of a contaminating mould, led to further investigation of this phenomenon. A solution of one of the major constituents of blue-black ink—ink blue (supplied by Imperial Chemical Industries as 'Ink Blue AS' crystals)—behaved similarly, and it seemed probable that the clearing was caused by either *p*H or *Eh* conditions around the colony. The response of Ink Blue to changes of this kind was initially examined in aqueous solution.

The absorption maximum of a 0·005 per cent solution of ink blue in water, measured on a Unicam '*SP*600' spectrophotometer, was 610 mμ. It was found that a standing period of at least 5 min was required before the solution attained stability of optical density, and 25 min was necessary when 1 ml. of a 0·05 per cent solution was added to 10-ml. buffer at *p*H 7·8. Thereafter the absorption remained constant.

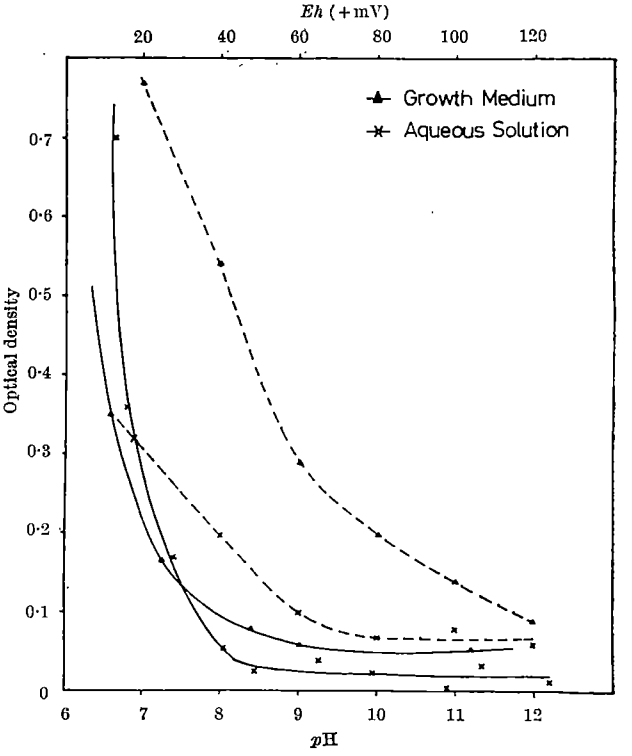


Fig. 1. Discoloration of ink blue solutions with increasing *p*H, ——, and *Eh*, - - - -

The behaviour of ink blue solution over the *p*H range 3·5–12·21 was investigated by adding 1 ml. of a 0·05 per cent solution to 5 ml. of an appropriate buffer. After it had stood for 30 min the optical density at 610 mμ was measured against a distilled water blank. The *p*H values were checked on a direct-reading *p*H meter (E.I.L., model '23*A*') using reference electrode '*RJ*23' and glass electrode '*GG*23'. The change from blue to colourless occurred between *p*H 7·38 and 8·04.

The influence of *Eh* on the discoloration of ink blue was examined by adding 1-ml. quantities of a 0·05 per cent solution to 5 ml. of appropriate dilutions (0·01–0·08 M) of molar sodium thioglycollate solution, giving a range of *Eh* of −100 mV to +120 mV, and the optical density of each sample was measured at 610 mμ against a distilled water blank (Fig. 1). *Eh* was measured on the E.I.L. *p*H meter using the platinum metal electrode '*EPT*23' and reference electrode '*RJ*23'. There was a progressive clear-

Table 1. Correlation of Ink Blue Discoloration by Culture Filtrates with Actual *p*H and *Eh*

	Uninoculated	*Trichophyton mentagrophytes*	*Microsporum canis*	*Epidermophyton floccosum*	*Aspergillus terreus*
Optical density	∞	0·14	0·47	1·5	∞
Eh (mV)	−160	−100	−100	−130	−130
*p*H (direct)	5·2	7·4	6·3	5·6	4·2
*p*H equivalent to optical density	< 5·5	7·45	6·32	5·6	< 5·5
Visual colour	Blue	Clear	Pale blue	Blue	Blue

ing of the solution from deep blue at the initial value of −100 mV (with no reducing agent), to complete discoloration at +60 mV.

The behaviour of ink blue was found to be modified when dissolved in a liquid growth medium. Batches of medium, containing 1 per cent mycological peptone ('Oxoid') and 4 per cent glucose in the appropriate buffer, were prepared in 5-ml. quantities and the optical density of each sample after the addition of 1 ml. of 0·05 per cent ink blue was determined as in aqueous solution. There was a general shift of the *p*H curve caused by the buffering action of the peptone, and discoloration occurred at a lower *p*H than in aqueous solution. The medium was pale blue at *p*H 6·5 and complete discoloration had occurred at *p*H 7·2 and above.

The influence of *Eh* changes in liquid growth medium was investigated in a similar manner to aqueous solutions. Unlike the *p*H curve, *Eh* was not modified by the constituents of the medium, but the optical density of each sample was higher (Fig. 1). The initial sample, with an *Eh* of −80 mV, was deep blue, and progressive clearing occurred until, at +100 mV, discoloration was complete. A change of 180 mV was thus necessary before complete clearing could be recorded.

These characteristics of uninoculated ink blue liquid medium were then correlated with the discoloration phenomenon caused by fungal growth. Samples were removed from liquid cultures of four species of fungi after 11 days' culture on unbuffered glucose–peptone medium. Each sample was centrifuged to remove any mycelium and the *p*H and *Eh* of 5 ml. of the supernatant were recorded. To each sample was added 1 ml. of a 0·05 per cent aqueous solution of ink blue, and, after standing 30 min, the optical density at 610 mμ was measured against a water blank (Table 1).

The measurements of optical density, when compared to the *p*H curve, showed that the *p*H equivalent to each optical density corresponded well with the actual *p*H. The change in *Eh* due to the growth of the fungus was in no case sufficient to cause discoloration, none of the fungi causing a difference of more than 60 mV when compared to uninoculated medium.

It was, therefore, concluded that the discoloration of ink blue solution by the three dermatophytic fungi tested was a reflexion of the release into the medium of alkaline metabolic by-products. A variety of dermatophytes have been shown to liberate alkali into their culture medium[1] but no evidence has been obtained for the presence of any strongly reducing conditions[2]. As non-keratinophilic fungi tend to turn media acid, these facts suggest that ink blue might be of value in the differentiation of keratinophilic and non-keratinophilic fungi. It would also appear that ink blue could be a useful non-toxic *p*H indicator in biological systems where *p*H changes in aqueous solutions occur in the region of 7·4–8·0

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BIOLOGY

Maintenance of Oyster Cells *in vitro*

The examination and control of oyster (*Crassostrea virginica* Gmelin) pathogens have received much impetus in recent years with the recognition of *Haplosporidium costale* Wood and Andrews, '*MSX*' an undescribed haplosporidian, and *Dermocystidium marinum* Mackin, Owen and Collier along the Atlantic and Gulf coasts of the United States. Research in the problems of isolation, identification and control of oyster pathogens could be facilitated if techniques for the culture of oyster cells were available.

Comparatively little work has been done with molluscan cell and organ maintenance. Probably no true molluscan tissue cultures have been established, if one considers a tissue culture to be an *in vitro* system where the cells undergo mitosis and from which sub-cultures may be formed. Maintenance investigations of molluscan tissues have been conducted by Gresson[1], Gatenby *et al.*[2] and Cameron[3].

In the work recorded here it has been found that oyster cells can be maintained for as long as 42 days in hanging-drop slide cultures. 1-mm² pieces of mantle margin were used as explants. Each piece was washed through six changes of sea-water containing 0·5 mg of streptomycin/ml. and 0·3 mg penicillin *G* (sodium salt)/ml. As determined by Zobell's marine bacterial agar medium[4], this technique was 80 per cent effective in obtaining sterile explants.

Crab (*Callinectes sapidus* Rathbun) plasma was used as a substrate for attachment of the explants and cells. The plasma forms a clear, firm and sterile coagulum when filtered through a 0·45μ 'Millipore' filter. 'Millipore' Filter Corporation's Swinny hypodermic adapter with filter and prefilter was used. No anticoagulant was used; all manipulations were conducted at 0° C in silicone-coated instruments to prevent premature coagulation. It was found that the cells and explants attached more readily and for longer periods of time to the coagulum than to the cover slip itself. The cultures were maintained between 23° and 25° C.

The medium which proved to be the most successful is given in Table 1.

Table 1

NaCl	$46{\cdot}66 \times 10^{-3}$ moles
KCl	$1{\cdot}02 \times 10^{-3}$ moles
$CaCl_2$	$1{\cdot}10 \times 10^{-3}$ moles
$MgCl_2{\cdot}6H_2O$	$1{\cdot}16 \times 10^{-3}$ moles
$MgSO_4$	$2{\cdot}86 \times 10^{-3}$ moles
$NaHCO_3$	$0{\cdot}25 \times 10^{-3}$ moles
'T.C. Yeastolate' (Difco)	0·100 g
Glass-distilled water	100·0 ml.

Eighty per cent of the above solution, 10 per cent oyster blood, 5 per cent female gonad extract, and 5 per cent adductor muscle extract—all by volume. 0·1 mg streptomycin and 0·06 mg penicillin/ml.

The physiological saline portion of the medium is after Cameron[5]. A hydrogen ion concentration of *p*H 7·2–7·4 was used. The osmotic pressure was adjusted by dilution with glass-distilled water or evaporation to within 2–3 atmospheres of the blood osmotic pressure in the tissue donor. The blood was found to range between 22 and 25 atmospheres; osmotic pressures were determined by freezing-point depression.

The extracts were prepared by grinding the selected organs with a volume of Cameron's sea-water which equalled three times the weight of the organ. The homogenizer consisted of a test tube with ground-glass interior and a close-fitting ground glass plunger. The homogenate was centrifuged at 5,000*g* for 20 min and the supernatant was utilized as the extract. Only female oysters which exhibited opaque gonadal tissue were used as a source of gonadal extract. No attempt was made to measure the condition of the gonads. The work was conducted during April–August 1962, which encompasses the major spawning period.

In the 40 cultures established, using the foregoing medium, the patterns of cell behaviour were consistent. Amœbocytes migrated out from the explant 30–40 min after establishing the culture. The cells were apparently identical with the lymphocytes and leucocytes found in oyster hæmolymph[6]. The predominant cell type was the leucocyte by a factor of about 100. They were greatly variable in size and form, measuring at the longest axis 9·0–26μ. Spherical cells were found suspended in the drop of medium and were probably dead or inactive leucocytes. They outnumbered the amœboid cells about 5 : 1 in most cultures.

A peak of migratory activity was reached at the end of the first 24 h, but cells continued to migrate out from the explant at a lesser rate during the entire culture period, as could be seen from the fact that the inactive spherical cells were replenished after each renewal of the medium. Fresh medium was added every third day. As early as 5 h after establishment of the culture, pseudopodia of adjacent amœbocytes came in contact, forming monolayer networks of cells. After 48 h an area of about 40–60 mm² around the explant was usually covered with the loose network of cells. Thereafter the monolayer remained about the same in terms of area covered and density of cells. There appeared to be no cellular connexions between the explant and the monolayer.

No evidence of mitosis was found using Delafield's hæmatoxylin and Feulgen's fuchsin. Probably all the cells found in monolayers had their origin in the explant. Histological sections of the mantle reveal connective tissue cells which resemble the amœbocytes of the monolayers in terms of cellular form, size, granules and reaction to the two stains mentioned above. However, whether mitosis occurred in the explant to a significant degree was not determined. Although large numbers of unattached, inactive leucocytes were washed away with each change of the medium, the explant did not seem to decrease in volume, as would be expected.

After a maximum of 42 days, the network was seen to break down into individual cells which became spherical and lost their attachment to the substrate. Many of the cells lysed several hours after assuming a spherical shape. The degeneration of the cultures was sometimes very gradual, beginning after the culture was 30–40 days old and lasting for 3–5 days thereafter. The cultures were destroyed after there ceased to be any amœboid cells. Ninety per cent of the explants were still alive at the end of the experiments, as evidenced by the ciliary action of the epithelial cells.

In 5 cultures out of the 40 which were established, leucocytes migrated close to the periphery of the drop and attached to cover slip. There they formed a dense, round group of spherical, inactive cells from which radiated a network of cells and their protoplasmic processes. The network was more filamentous in appearance than the network which formed around the explant. There appeared to be no difference between the two networks except that, in the former case, the cells originated from the periphery of the clump of leucocytes while, in the latter case, the network had no direct connexion with the explant.

A similar pattern of cell behaviour could be obtained by simply placing an explant in a drop of sea-water in a hanging-drop culture system. However, the monolayers which were formed were no more than 20 per cent as large as those mentioned here and they did not last longer than 4–5 days as monolayers.

Grace's medium[7] has been shown to provide a satisfactory environment for growth of insect tissues *in vitro*. In attempts to use a modified form of the medium to culture oyster mantle, the medium was found to be no better than sea-water controls. The only modification in the medium was to substitute Cameron's medium, twice as concentrated, for the physiological saline suggested by Grace.

This work was supported by U.S. Public Health Service research grant *EF* 00122 from the Division of Environmental Engineering and Food Protection.

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Influence of Fungicides on the Tomato and Growth of *Botrytis cinerea* Pers

SPRAYS of maneb (manganese ethylene *bis*dithiocarbamate) or zineb (zinc ethylene *bis*dithiocarbamate) increased the incidence[1] of grey mould of tomatoes, caused by *Botrytis cinerea* Pers., but adding thiram (tetramethylthiuram disulphide) or 'Dyrene' (2,4-dichloro-6-*o*-chloroanilino-*s*-triazine) to either of the above sprays prevented infection[2]. Apparently maneb and zineb either stimulate the growth of *B. cinerea* or induce a change in the tomato favouring growth of the fungus. To investigate this phenomenon the effect of these fungicides and other chemicals on the germination and growth of *B. cinerea* was examined, and tests were made for total-ninhydrin-staining compounds and carbohydrates in exudates washed from the surface of treated tomato leaves and fruit.

Fungicidal activity was determined by the slide germination method[3] using spores of *B. cinerea*. Assays were also made with mixtures of the juice of ripe tomatoes and fungicides. Standard and tomato juice controls were run with each test. The lengths of 20 germ tubes were recorded at each dilution where germination occurred. The results given in Table 1 show the average of 2 or more dilution tests. Additional assays were conducted using manganese and zinc salts at concentrations of Mn^{++} or Zn^{++} equivalent to that contained in maneb and zineb test solutions. The effect of casamino-acids, dextrose, lævulose and sucrose on length of germ tube was also determined.

Table 1. EFFECT OF FUNGICIDES ON THE GROWTH OF GERM TUBES OF *B. cinerea*

Fungicides	ED_{50} (p.p.m.)	Concentration showing stimulation (p.p.m.)	Average percentage increase in length of germ tubes over control
Zineb	46·0	0·9–125·0	83
Maneb	7·4	0·7–3·2	44
Thiram	1·4	Negative	Nil
'Dyrene'	0·8	Negative	Nil
Zineb + tomato juice	324·0	0·9–125·0	26
Maneb + tomato juice	> 250·0	0·9–31·2	43
Thiram + tomato juice	14·9	Negative	Nil
'Dyrene' + tomato juice	1·7	Negative	Nil

Table 2. INFLUENCE OF METAL SALTS, SUGARS AND CASAMINO-ACIDS ON GROWTH OF GERM TUBES OF *B. cinerea*

Material	Concentration showing stimulation (p.p.m.)	Average percentage increase in length of germ tubes as compared with controls
$MnCl_2.4H_2O$	0·2–12·9	15
$MnSO_4.H_2O$	0·2–12·9	23
$Mn(NO_3)_2$	0·4–12·9	12
$ZnCl_2$	0·4	3
$ZnSO_4.7H_2O$	0·4–0·8	11
$Zn(NO_3)_2.6H_2O$	0·4–0·8	11
Dextrose	39–1,560	13
Lævulose	19–10,000	102
Sucrose	19–10,000	78
Casamino-acids	19–10,000	112

Table 3. Total Amino-acids and Carbohydrates in Exudates of Tomato Leaves sprayed with Fungicides

Concentration (p.p.m.)	Exposure time (h)	Amino-acids* (μg/5 ml. of concentrate)					Soluble carbohydrates† (μg/5 ml. of concentrate)				
		D‡	M	T	Z	C	D	M	T	Z	C
1,600	36	19·4	13·1	67·5	14·4	17·5	157·5	105·0	177·5	177·5	117·5
8,000	36	8·4	8·7	6·5	13·3	15·0	200·7	297·5	209·2	310·0	287·5
16,000	36	5·1	85·0	25·8	103·0	64·5	208·0	209·5	250·0	189·5	200·5
1,600	120	36·6	39·3	55·0	17·7	24·3	306·0	196·0	196·0	163·5	241·0
8,000	120	10·2	22·6	24·9	26·0	17·7	292·5	488·7	388·7	370·0	333·3
16,000	120	76·6	298·0	90·0	96·6	43·3	94·0	295·5	83·0	112·0	90·0

* Calculated as glycine. † Calculated as glucose. ‡ *D*, 'Dyrene'; *M*, maneb; *T*, thiram; *Z*, zineb; *C*, control.

Three 5–6-week-old tomato plants, variety Stokesdale, were sprayed with either 'Dyrene', maneb, thiram or zineb at 1,600, 8,000 and 16,000 p.p.m. of active ingredient. After 36 h the fourth and after 120 h the fifth leaves distal from the apical meristem of the 3 plants at each dose were washed 3 times in 200 ml. of distilled water to collect released nutrients. Ten harvested ripe tomatoes, variety 'Michigan-Ohio', sprayed at 1,600 or 8,000 p.p.m. fungicide were similarly washed after 36 h. Plants and tomatoes sprayed with water served as controls. The solutions were then centrifuged at 9,000 r.p.m. for 10 min and the supernatant concentrated to 5 ml. at 45° C. The concentrated solutions were tested for total-ninhydrin-staining compounds[4] and total carbohydrates[5]. Similar foliage tests were conducted with exudates collected from plants 36 h after spraying with manganese and zinc salts. Following vacuum concentration of these manganese and zinc salt exudates, they were passed through 'Dowex 50' *W–X8* (ref. 6) cationic exchange resin columns, to remove Mn^{++} and Zn^{++} which interfere with the ninhydrin test. The amino-compounds were eluted with ammonium hydroxide, reconcentrated and tested for amino-acids. The solutions tested for carbohydrates passed through the 'Dowex 50'. It was not necessary to remove the cations in exudates from the fungicide treatments. All tests were performed at least twice.

Maneb and zineb stimulated the growth of germ tubes of *B. cinerea* whereas 'Dyrene' and thiram did not have this effect (Table 1). The addition of juice of ripe tomatoes reduced the toxicity of the four fungicides. The greatest reduction in fungitoxicity occurred with the metal-containing fungicides. This suggests a possible chelation of the metal ions by a component in the tomato juice, since Ludwig *et al.*[7] found that the metal ion promotes the formation of isothiocyanate (to which the *bis*dithiocarbamates may owe their fungitoxicity). Some stimulation of the growth of *B. cinerea* can be attributed to the cations Mn^{++} and Zn^{++} (Table 2). Casamino-acids and sugars gave considerable stimulation.

In 58 per cent of the exudates from leaves sprayed with fungicides there was an increase in total-ninhydrin-staining compounds and in 92 per cent of the exudates there was an increase in total carbohydrates (Table 3). With maneb the largest increases recorded for total amino-acids and carbohydrates were 689 and 328 per cent, respectively. The increases were not always associated with the highest fungicide concentration or longest exposure time. The 1,600 p.p.m. treatment was considered equivalent to one field spray of 2 lb. per 100 gal./acre and the higher concentrations to repeated applications. Slight increases in amino-acids in tomato fruit sprayed with maneb and zineb at 8,000 p.p.m. were recorded but there was little or no change in the carbohydrate content in the exudates of sprayed fruit. Part or all of the increase in amino-acids in exudates could be attributed to metal ions in maneb or zineb since the amino-acid content of exudates from leaves sprayed with manganese and zinc salts were found to be increased 1,023 and 690 per cent, respectively. The salts did not increase total carbohydrates.

These results indicate that maneb and zineb may increase the susceptibility of tomatoes to *B. cinerea* by stimulating the growth of the fungus and by the release of nutrients from the host favourable to fungal growth. 'Dyrene' and thiram also release nutrients from the plant but probably do not increase susceptibility to *B. cinerea* due to their greater fungitoxicity to this fungus.

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In vitro Culture of Seedlings of *Striga senegalensis* Benth

It is difficult to germinate the seeds of most obligate, phanerogamic root parasites. Saunders[1] demonstrated that certain factors exuded from the roots of maize could stimulate the germination of *Striga asiatica* (witchweed) seeds. Further development of the seedling occurs only when a host root is penetrated.

In the work recorded here the seeds of *Striga senegalensis* (*S. hermonthica*) were germinated in sterile nutrient media to discover whether the seedlings could grow in the absence of a host.

The seeds were sterilized in 1 per cent sodium hypochlorite solution for 15–20 min and incubated on moist filter paper in Petri dishes for 10–12 days in darkness (25° ± 2° C) for moisture pretreatment[2]. The cultures were raised on nutrient media employing Knop's or modified Murashige and Skoog[3] minerals. Varying amounts of sucrose or other sugars were used as the source of carbon. An aqueous exudate of the host (*Sorghum vulgare*) roots was incorporated into the media to initiate and stimulate the germination of *Striga* seeds. The host exudate was filter-sterilized before adding to the rest of the medium in order to avoid heat-destruction (during autoclaving) of its germination factor[1]. The *p*H of the medium was adjusted to 5·5–5·8 and the medium was gelled with 1·2 per cent Difco 'Bacto' agar. About 100–200 seeds were inoculated on the surface of the agar slant in each culture tube. Cultures were kept in darkness at 25° ± 2° C or in natural daylight in the laboratory or greenhouse.

After growth for 30 days in darkness on Knop's medium containing 1 per cent sucrose, the seedlings attained the maximum size of 2 mm having six pairs of leaves. The length of shoot and number of leaves decreased with increase in sucrose concentration. Adventitious roots with root hairs were produced by seedlings in media having no sugar or lower concentrations of sucrose.

However, the medium containing higher concentration of mineral salts (Murashige–Skoog minerals[3]) and 2 per cent sucrose supported the best growth. Within 30 days



Fig. 1. Four months and nine days old cultures of *Striga senegalensis* seedlings on Murashige and Skoog minerals + *A*: 1 per cent mannose, *B*: 1 per cent glucose, showing flowering in light (× *c.* 0·13)

the seedlings were 18 mm long, had nine pairs of leaves and produced numerous adventitious roots with root hairs.

In other trials with Murashige–Skoog minerals, it was demonstrated that fructose, glucose, mannose, maltose, galactose, lactose, xylose and raffinose can also serve as carbon source for *Striga* seedlings. Ribose, sorbose, rhamnose and mannitol were not effective in growth.

By transfer to large culture tubes, seedlings could be grown for several months on Murashige–Skoog minerals–sugar medium. In this new medium there was no root exudate—only water, agar, minerals and sugar. In light, the seedlings became intensely green and flowered after 4–5 months in culture (Fig. 1). Due apparently to lack of fertilization, they did not produce viable seeds.

To my knowledge, this is the first report of successful culture *in vitro* of seedlings of *Striga*. Worsham *et al.*[4] and Williams[5] reported that kinetin and gibberellic acid stimulated the shoot apices of germinated *Striga* seedlings to elongate beyond the cotyledons, but with no further growth. Williams[5], therefore, suggested that *Striga* seedlings probably depend on the host for a supply of growth factors. Success in the present investigation in obtaining development of normal *Striga* seedlings on media containing only mineral salts and sugar indicates that Williams's[5] suggestion may not be tenable, at least for *S. senegalensis*. It appears that *Striga* seedlings can synthesize their growth factors once they are supplied with mineral salts and a suitable sugar. The enhancement of growth of seedlings on Murashige–Skoog minerals–sugar medium as compared with Knop's minerals–sugar medium could be due to the former's high mineral salts content, or ammonium nitrogen or both. On the basis of my experimental results, it appears that *Striga senegalensis* depends on its host only for minerals and sugar and not for more elaborate substances.

I thank Prof. Ernest Ball of the University of North Carolina, Raleigh, and Dr. P. C. Joshi for their advice.

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ENTOMOLOGY

Independent Development of the Male Gonads and Vasa Deferentia, and the Genitalia of the Australian Earwig, *Labidura riparia truncata* (Dermaptera : Labiduridae)

L. r. truncata Kirby is the Australian-wide form[1] of the cosmopolitan earwig *Labidura riparia* (Pallas). Snodgrass[2] lists literature dealing with the development of the genitalia in several insect orders. His description of the process in the Dermaptera is largely based on the account given by Qadri[3] for *Forficula auricularia* L. (Forficulidae). Snodgrass points out that it is generally held that the genitalia develop from the primary phallic lobes and that the terminal ampullæ of the vasa deferentia are commonly associated with the lobes.

The position in *L. r. truncata* is, however, quite different, for the internal reproductive organs and the genitalia develop independently of each other (at least, during the first three of the five nymphal instars). Their development has been investigated by means of serial sections of the entire abdomen, cut at 5μ and stained with Mallory.

In a specimen at three days (first instar) the testes were found to be in much the same position as in adults: dorso-lateral, near the midgut, and surrounded by fat-body. Each was cigar-shaped and consisted of the usual two follicles. The contents corresponded to the germarium and an early stage of the zone of spermatocytes of a mature testis. Posteriorly the two follicles coalesced and the testis ended blindly, with no sign of a vas deferens.

At this stage, the genitalia consisted of a pair of conical phallic lobes, pointing posteriorly, fairly close together, fused basally and lying in the genital chamber above the ninth sternum. The phallic structure consisted of a solid mass of uniform undifferentiated cells invested with a cuticular intima. There were no ducts associated with the phallic organs. The lining of the genital chamber joined the base of the phallic structure just in front of the phallic lobes, so that they lay outside the body-wall.

In a third instar nymph (exact age uncertain) the testes have grown considerably. Most of the follicular contents have reached the stage of the zone of spermatocytes of a mature testis. The paired follicles are fused basally and a vas deferens leaves each testis and runs posteriorly through the fat body. Each of the vasa deferentia becomes slightly narrower and eventually ends abruptly and independently. The vasa have the same structure as those of *Anisolabis littorea* (White) (Labiduridae)[4]. The genitalia also show growth and differentiation from the condition in the first instar. The organ is still flattened: it is single basally and double apically, each apical portion consisting of a mesomere and a paramere, which projects back beyond the mesomere. The basal portion, and the full length of each mesomere, is traversed by a single ejaculatory duct. Sections of the genitalia and ducts closely resemble those figured by Qadri[3]. No ducts or other structures join the base of the genitalia.

According to Ramamurthi[5] the vasa deferentia of *Euborellia annulipes* (Lucas) (Labiduridae) are present in the first instar, before the genitalia appear. He describes the ultimate fusion of the vasa with the ejaculatory ducts during the fifth and final instar. The present work confirms, to some extent, the process described by Ramamurthi.

Lhoste[6] traces the development of parts of the reproductive system of *Forficula auricularia*, in particular the unusual 'nodiform gland'[6–8] or 'ejaculatory canal'. It is apparent from Qadri's description[3] that, in *F. auricularia*, development of the gonads and genitalia is more rapid (and the two sets of organs are less independent) than in *L. r. truncata*, even after allowing for the additional nymphal instar of *L. r. truncata*. The independence of the ectodermal phallic rudiments from the mesodermal vasa deferentia in *L. r. truncata* does not accord with the

widely held view on the derivation of the tissues of the genitalia. The condition in *L. r. truncata* accords with the views of Abul-Nasr[9] concerning the origin of the tissues of the insect gonads, ducts and genitalia.

As the phallic lobes are first seen after the embryonic limb buds have disappeared, there is no evidence as to which segmental structures, if any, enter into the formation of the phallic rudiments. It is only certain that the phallic lobes are associated with the inner surface of the ninth sternum, the general arrangement in insects. Nothing, therefore, can be done at present to resolve the differences between the divergent opinions of Else[10], Roonwall[11], Snodgrass[2], Gustafson[12] and Bouthier[13].

This work is continuing and will be reported in full elsewhere. I thank Mrs. W. Walker for her help.

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Chiasma Frequency and Gregarization in Locusts

Phase transformation of locusts[1,2] is the reversible group of morphological, biochemical, physiological and behavioural changes in locust hoppers or instars and adults, based to a large extent on population density. The transformation to the swarming or gregaria phase occurs in the field when individuals of the grasshopper or solitaria phase congregate in large numbers due to rapid growth of population. The brown locust, *Locustana pardalina* Walk., is at present in a cycle of intensive gregarization after a period of about seven years of mainly solitaria life. Gregarization started during the summer of 1962–63 in many areas of the approximately 100,000 square miles of the outbreak region in the Karoo plateau, and continued in the formation of swarms over most of the region during early summer of 1963–64. Many of these swarms have consequently passed through at least three generations of gregarization in the field. Through the courtesy of the Locust Control Division our Locust Research Unit has received field collections throughout the past four years so that representative samples of solitaria, congregating populations and swarms with a history of 1, 2 or 3 generations of gregarization have been available.

Cytological investigations of the brown locust have shown that the 11 autosomes of the chromosome complement of the male are very similar in relative mitotic metaphase lengths to those of other locust species[3], and assort into the general classes of three long (3*L*), five medium (5*M*) and three short (3*S*). During meiosis in the male the 3*S* chromosomes regularly pair with 1 chiasma each, but the frequency varies in the 3*L* and 5*M* chromosomes as shown in Table 1 in which mean frequencies for the two groups are given with their error variances. These populations comprise a selection of the many that have been scored for chiasmata, and the amount of gregarization in the field is the minimum estimates based on field counts of population density and the number of generations per season. The data clearly show a rise in chiasma frequency in the 8 largest chromosomes during the course of the swarming cycle. This increase is considerable and the differences between field solitaria and gregaria is of an order much greater than differences which have been found in geographical populations of grasshoppers[4], in wild populations of plants[5], in strains of *Drosophila*[6,7] or in inbred lines of rye[8].

Table 1. Mean Chiasma Frequencies in the Three Long and Five Medium Chromosomes with their Error Variances

Area in outbreak region	Population symbol	Generations of field gregarization	Chiasma frequencies in 3*L*	Chiasma frequencies in 5*M*
North	*Ma* 1	0	5·68	5·21
			0·00478	0·00149
	Ma 2	1 (medium)	6·63	5·56
			0·00494	0·00303
	Ma 3	3	7·44	6·97
			0·00275	0·00429
North central	*H* 1	0	5·79	5·11
			0·00840	0·00129
	H 6	1 (medium)	5·98	5·32
			0·00434	0·00117
	H 7	2 (1 medium)	6·23	5·40
			0·00613	0·00196
	H 8	2 (full)	6·73	6·14
			0·00574	0·00787
	H 9	3	7·17	6·62
			0·00358	0·00405
Central	*A* 1	0	5·40	5·07
			0·01231	0·00091
	A 7	2 (1 medium)	6·99	6·37
			0·00312	0·00447
	A 8	2 (full)	7·11	6·43
			0·00574	0·00563
	R 1	2+	7·48	7·26
			0·00670	0·00898
	Hn 1	3	7·56	7·06
			0·00255	0·00324

Polygenic control has been suggested for differences in chiasma frequency in a species[5–8]. The increased chiasma frequency in gregarized brown locust populations is probably also the result of such a kind of control. Miss M. D. Willemse of the Locust Control Division has provided hoppers from a selection experiment on the colour of the instars in cages with low densities of population. The P_1 of this investigation was derived from egg pods deposited in an egg nest or field by a swarm in about the second generation of gregarization. She selected two lines, the first for typical black gregaria hoppers and the second for green (solitaria-type) hoppers. The hoppers scored for chiasma frequency were the F_2 of selection and yielded the data given in Table 2.

Table 2. Mean Chiasma Frequencies in Lines selected for Instar Colour

Selected line	Chiasma frequencies in 3*L*	Chiasma frequencies in 5*M*
Gregaria hopper colour	6·45	5·71
	0·00402	0·00207
Solitaria hopper colour	5·92	5·44
	0·00329	0·00148

Solitaria females lay eggs which produce small grey hatchlings while gregaria females produce large black hatchlings. In several instances it has been found that in populations which were building-up, that is, starting to congregate, pods were deposited which hatched into either black or grey hatchlings, presumably depending on the previous history of the mother. In one such a mixed population the chiasma frequencies of Table 3 were scored.

Table 3. Mean Chiasma Frequencies in Sub-strains from a Congregating Population

Sub-strain	Chiasma frequencies in 3*L*	Chiasma frequencies in 5*M*
From black hatchlings	6·94	6·34
	0·00540	0·00509
From grey hatchlings	5·87	5·38
	0·00407	0·00224

The rise in chiasma frequency in gregarizing populations of the brown locust indicates a large increase in genetic recombination, but whether this increase is initiated before or is a correlated characteristic of swarming is not quite clear from the present data. Increased recombination through the breaking up of linked congeries of genes may be of great importance in populations due to

changes in selective values of particular sets of genes[9], especially in the case of the locust migrating into an invasion territory with ecological conditions quite different from those of its natural habitat. The first indications in the laboratory breeding of F_1 from field collections are that increases in chiasma frequency are transmitted to offspring, irrespective of the density of populations in the cages.

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Genetically Controlled Variation in Pteridine Content of *Pieris brassicae* L.

IN various insects, simple recessive mutants which have a distinctly different pteridine content from the wild type have long been recognized[1]. This phenomenon has been particularly well investigated for the eye-pteridines of the imago of *Drosophila melanogaster*. In all these cases, the presence or concentration of one or more of the 6-substituted pteridines and/or isoxanthopterin is affected. For Pieridae, a family in which pteridines occur in great variety and large quantities, no such mutants have been described. Sexual differences in pteridine content or composition can be very marked in some species of Pieridae, for example, in *Euchloe cardamines*. Also some geographical races of *Pieris brassicae*, for example, race *cheiranthi* (Hübner) of the Canary Islands, are, because of differences in pigmentation, suspected of having a different pteridine composition.

Recently, Gardiner described two genetically controlled colour-variations of *Pieris brassicae* which appeared as mutations in a laboratory colony: *ab. coerulea* and *ab. jauni*[2]. Both these mutants differ from the wild type in the amount of yellow pigmentation: *ab. coerulea* lacks all yellow, and shows a slight blue iridescence; *ab. jauni* shows a marked increase in yellow pigmentation, especially on the underside of the hind wings. Since the yellow pigmentation of the wing scales of the wild type depends on small amounts of xanthopterin and erythropterin mixed with the much larger quantities of colourless substances, that is, leucopterin, isoxanthopterin and uric acid, it was suspected that the mutants were affected in their pteridine composition.

Wing extracts were chromatographed on paper, and the resulting chromatograms showed a marked difference in pteridine composition when viewed in ultra-violet light. Xanthopterin and erythropterin are completely lacking in *ab. coerulea*, while both these substances, especially erythropterin, are present in increased amounts in *ab. jauni*. Another colour variation in *Pieris brassicae*, *ab. albinensis*[3], does not differ from the wild type in pteridine. composition. The change of colour in this mutant depends entirely on an incomplete melanin formation.

The main point of interest here is that in *Drosophila* the genetic variation in pteridine composition in all described mutants involves the 6-substituted pteridines and isoxanthopterin; while in both *ab. coerulea* and *ab. jauni* of *Pieris brassicae*, a 7-substituted pteridine (erythropterin) and xanthopterin are involved. This linkage of isoxanthopterin to 6-substituted pteridines and of xanthopterin to a 7-substituted pteridine, whenever genetically controlled variations occur, may well be an indication of a rather strong metabolic connexion. It seems that naturally occurring isoxanthopterin is an oxidative breakdown product of the 6-substituted pteridines and that naturally occurring xanthopterin is an end-product of the oxidative breakdown of 7-substituted pteridines. The bioconversion from 7-substituted pteridines to 6-substituted pteridines, as well as the bioconversion from xanthopterin to isoxanthopterin, appear to be metabolic impossibilities.

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MICROBIOLOGY

Occurrence of Nematode-trapping Fungi in the Rhizosphere

ALTHOUGH there are many species of predaceous fungi that capture and kill nematodes in soil, there are little if any data in the literature to indicate if they are preferentially stimulated by roots. Since nematodes tend to accumulate in the root zone or rhizosphere[1] we determined the incidence of nematode-trapping fungi in this region as compared with root-free soil.

Wheat and soybean were grown in pots in the greenhouse for 4–6 weeks. On removal the roots were shaken to dislodge loosely adhering soil and clumps, and sections with rhizosphere soil adhering were placed on the surface of 'Difco' corn meal agar containing 30 mg chlortetracyclene/l. to suppress bacteria. Root-free soil was distributed uniformly on plates of this agar medium at the rate of 10–20 mg per plate. Root material was also washed thoroughly in tap water and then in 5 changes of sterile water and segments of approximately 0·5 cm were placed on the agar medium. Fifty plates were prepared for each sample. To each plate was added a suspension of living nematodes of the genus *Panagrellus* (kindly supplied by Dr. E. Hansen, Kaiser Foundation Research Institute, Richmond, California, U.S.A.) to stimulate trap-formation[2] as an index of the presence of the predaceous fungi. The plates were incubated at 20° C and examined thoroughly after 3 and 6 weeks.

Table 1. PERCENTAGE OF PLATES SHOWING *Arthrobotrys* IN THE ROOT ZONE OF WHEAT AND SOYBEAN. 50 PLATES IN EACH COUNT

Plant	Root segments	Rhizosphere soil	Root-free soil
Soybean	0	86	62
Wheat	0	32	

The data given in Table 1 show an interesting distribution of fungi in the zones indicated. *Arthrobotrys oligospora* Fres. was the only nematode-trapping fungus that was consistently isolated, although in a few plates there was evidence of other types producing constricting rings, and occasionally of the endozoic predator *Harposorium*. Perhaps the most striking feature was the complete absence of *Arthrobotrys* on the root segments compared to its occurrence in the soil samples. The data strongly suggest that this fungus is a soil-inhabiting organism, in the terminology of Garrett[3]. Also noteworthy is the greater abundance of the fungus in the soybean rhizosphere as compared with that of wheat. Repetition of these experiments revealed an essentially similar relationship, implying that the type of plant used exerts a strong influence on the incidence of this fungus. Recent work from our laboratory[4] showed that under certain conditions soybean rhizosphere soil contained four times as many nematodes as wheat rhizosphere, a fact that may account

for the prevalence of these fungi in the root zone of soybean.

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GENETICS

Genetics of Plasmids in *Salmonella typhimurium*

In bacteria certain genetic determinants which may be gained or lost can multiply without incorporation in the bacterial chromosome. Some such plasmids[1], the class of episomes[2], may become reversibly integrated into the chromosome; for others, including the transmissible drug-resistance (*R*) factors[3], integration has not been unequivocally demonstrated. We report observations on the transmission of an *R* factor and of colicine factors by conjugation and transduction in *Salmonella typhimurium*, and show that *R* factor genetic material may become attached to the bacterial chromosome—but only by becoming associated with an episome, prophage *P22*.

The strain to be used as donor was an *LT2 Hfr* derivative[4] (received from Dr. N. D. Zinder of the Rockefeller Institute) which injects its chromosome 'clockwise' from *ile**. It was first infected with three colicine factors, *colI*, *colE*1 and *colE*2, by the high-frequency colicinogeny transfer method[5] and then by conjugation with an *R* factor (isolated in England by Datta[6]) which confers resistance to sulphonamides, streptomycin and tetracycline. The strain so obtained was mated with an *LT*2 *adeC proA ile*, etc., recipient. The relative frequency of different recombinant classes and interrupted-mating results indicated the gene order:

$$-ile-rha-mal-inl-(ara,\ azi)-pro-gal-$$

Some chromosomal recombinants—the proportion varied from 15 to 100 per cent in different classes—were found to have acquired one or more of the colicine factors of the donor; and 0·1–1 per cent had acquired the *R* factor. However, none of the plasmids showed linkage to any of the chromosomal loci which were segregating. It appears that in this mating the four plasmids are transferred unattached to any standard locus on the *Hfr* chromosome; similar results have been reported for transfer of colicine factors[7,8] and of *P*1–*dl* (an aberrant prophage[9]) during *Hfr* mating in *Escherichia coli*.

Some plasmids can be transduced. We grew phage *P22* by the lytic cycle on the *Hfr* strain carrying the three colicine factors and the *R* factor, and used it to infect the *ade pro ile* recipient at a multiplicity of *c.* 5. Sulphonamide-resistant (*sul-r*) and tetracycline-resistant (*tet-r*) transductants were obtained, at frequencies per plaque-forming unit of 10^{-8} and 10^{-9} respectively; frequencies lower than those for transduction of *ile*$^+$ (10^{-6}) and of *pro*$^+$ (10^{-7}). All *tet-r* transductants tested were sensitive to sulphonamides and streptomycin; all of *c.* 500 *sul-r* transductants tested were also *str-r*, but only two were *tet-r*. Separate transduction of the *tet-r* trait and of the other *R*-factor resistance traits has been observed before in *Salmonella*[10,11]. Some *tet-r* and some *sul-r str-r* transductants produced colicine *E*1 or colicine *E*2. This we attribute to co-transduction of the *colE*1 or *colE*2 factor with the drug-resistance determinants. In control experiments we never observed co-transduction of resistance traits or of colicinogeny with the chromosomal genes *ade*$^+$ and *pro*$^+$. The co-transduction suggests that in some donor cells a colicine determinant is closely associated with part of the *R* factor.

Nearly all the resistance-trait transductants were normally lysogenic for *P22*—as is generally the case for *P22* transductants. Most of 15 exceptional transductants not releasing phage *P22* (from a sample of about 200 transductants tested) were, however, resistant to *P22*, and we, therefore, suppose them to be defectively lysogenic; that is, to carry a *P22* prophage which is deficient, at least in function. As *Salmonella* resistance-trait transductants in general do not transmit their drug-resistance by conjugation[10,11], it has been surmised[3] that the transduced fragment of the *R* factor may be integrated into the chromosome of the recipient bacterium, perhaps at a region of genetic homology. We attempted to map the transduced resistance traits of *T*1, a defective lysogenic *tet-r* transductant, and of *T*3, a defective lysogenic *sul-r str-r* transductant. A genetically marked *LT*2 strain as donor was crossed to *T*1 and to *T*3 as recipients by the *col*-factor hybridization method[12]. In each cross more than 95 per cent of recombinants selected for possession of the *pro*$^+$ locus of the donor parent acquired also its *P22*-sensitivity and drug-sensitivity; by contrast, recombinants selected for possession of the donor *ade*$^+$ locus had the phage-resistance and drug-resistance of the recipient parent. That is, both the *P22*-resistance (defective lysogeny) and the transduced drug-resistance trait (*tet-r* in *T*1 and *sul-r str-r* in *T*3) showed very close linkage to *proA*. In normally lysogenic *LT*2 strains *P22* prophage maps close to *proA* (Sylvia Smith and Stocker, unpublished results; Zinder, personal communication). These results and others suggest that in each of these two defective lysogenic transductants the *R*-factor fragment determining drug-resistance is associated with an abnormal *P22* prophage at the usual *P22* prophage site on the chromosome. Thus, as surmised, transduced *R*-factor genes have become located on the bacterial chromosome—but only through association with an episome having an attachment site there, not by crossing-over into the chromosome at a region of homology.

The vegetative multiplication of a prophage which carries genes of non-phage origin may result in production of many abnormal phage particles, able to transduce the character concerned—for example, galactose-fermentation in the case of the abnormal prophage, λ-*dg*. We encountered evidence for such a phenomenon when we examined a *tet-r* transductant, *T*10, which we term a 'semi-defective' lysogenic. It is *P22*-resistant and, though its overnight broth cultures contain < 50 plaque-forming units/ml., normal *P22* can always be recovered by enrichment with a phage-sensitive strain. Unlike ordinary *Salmonella* resistance-trait transductants, line *T*10 grown in mixed culture transmitted *tet-r* at about the same rate (10^{-3}/donor cell) as a strain carrying the whole *R*-factor and able to conjugate. We infer that *T*10 transmits *tet-r* by *P22* transduction, not by conjugation, because: (1) even cell-free filtrates of *T*10 cultures on long incubation evoked *tet-r* clones from smooth (*P22*-sensitive) recipient strains; (2) incubation with *T*10 cultures or filtrates failed to evoke *tet-r* clones from rough recipients, which do not absorb *P22* though they readily accept *R*-factors by conjugation. *T*10 culture filtrates which transmitted *tet-r* to an *ade pro* acceptor contained < 50 plaque-forming units/ml. and did not transmit the *ade*$^+$ or *pro*$^+$ characters of *T*10; the filtrates thus have the high-frequency transduction property in respect of *tet-r* but not of other genes. The addition of *P22* phage grown on a drug-sensitive strain to a mixture of *T*10 filtrate and acceptor bacteria greatly increased the number of *tet-r* clones obtained. Thus, as in other special transduction systems, normal phage helps the abnormal particles to effect transduction. When *T*10 filtrates are incubated with *P22*-sensitive recipient bacteria a high titre of normal phage results from the

* Genotype symbols: *ile, ade, pro*, requirement for, respectively, isoleucine, adenine, or proline; *rha, mal, inl, ara, gal, lac*, fermentation of, respectively, rhamnose, maltose, inositol, arabinose, galactose or lactose; *azi*, azide resistance; *colI, colE*1, *colE*2, factors determining production of, respectively, colicines *I*, *E*1 or *E*2.

multiplication in the recipient cells of the rare normal *P22* particles in the *T10* filtrate; the need for 'helper' phage presumably explains the necessity of long incubation of the mixture before selection for *tet-r*. We infer that in transductant *T10* (as in *T1* and *T3*) the transduced *R* fragment is associated with a *P22* prophage, and is, in consequence, replicated during vegetative prophage multiplication; and that many phage particles carrying the *R* fragment, and so able to transduce *tet-r*, are thereby produced.

Transductants *T10*, *T1* and *T3* may themselves have resulted from recipient bacteria becoming lysogenic for abnormal *P22* genomes with associated *R*-factor genes, introduced by abnormal particles present in the original *P22* lysate of the *Hfr* strain carrying the *R* factor. Possibly all transduction of *R*-factor drug-resistance traits in *Salmonella* is effected by such abnormal phage particles, the normal lysogeny of most transductants resulting from superinfection by normal phage. As has been surmised[3,11] transduction of *R*-factor resistance-traits in *Salmonella* would then be an instance of the transduction by lysogenization observed[13] when the *lac*$^+$ genes of *E. coli* were transduced by phage *P1* to the inherently lactose-negative species *Shigella dysenteriae*, or to *E. coli* mutants in which the *lac* region had been deleted. With such recipients unstable *lac*$^+$ clones were obtained, in which the *lac*$^+$ genes were associated with *P1* genes in an abnormal prophage. Luria *et al.*[13] suggested that in ordinary general transduction the bacterial genes introduced by an abnormal phage particle are readily incorporated into the recipient chromosome by crossing-over at the homologous region; but that when no such region exists this is impossible and the introduced bacterial genes will not be replicated unless they come to form part of a prophage, a plasmid able to ensure its own replication. The *R* agent when transferred *in toto* by conjugation (or by *P1* transduction in *E. coli*) presumably carries genes able to ensure its own replication as a non-chromosomal particle. In *P22* transductants the drug-resistance determinants of the *R*-factor become separated from such genes; lacking homology with the bacterial chromosome, they can then, perhaps, be stabilized only by becoming associated with a *P22* genome, able to attach to the chromosome as prophage.

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Recovery of Monokaryons from Veil Cells of Fruit Bodies of *Coprinus lagopus* sensu Buller

A USEFUL feature of the dikaryon of *Coprinus lagopus* sensu Buller is the ability to recover monokaryons with nuclei of one or other of the component types. It is thus possible to confirm whether the genotype of these nuclei has remained unchanged in the dikaryon during vegetative growth since the two parental monokaryons fused. It also makes possible the investigation of one nuclear type in cytoplasm derived in part from another monokaryon.



Fig. 1. Chains of cells from the veil of *Coprinus lagopus*. ×200

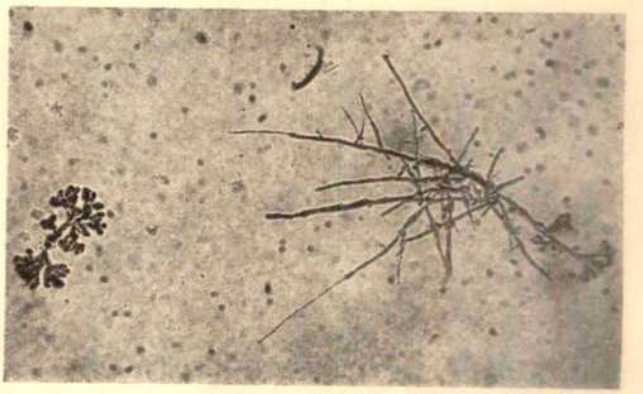


Fig. 2. Germinating veil cells of *Coprinus lagopus*. The one on the left has produced only dendroid mycelium. That on the right has produced both dendroid and normal mycelia. ×*c.* 50

The dikaryon produces chlamydospores which fall into two main classes: (*a*) round spores which germinate by one germ tube to give dikaryon and (*b*) long spores which may germinate from both ends to give monokaryon in the first instance. The hyphæ arising from long chlamydospores soon become dikaryotized, but early isolation of hyphal tips gives a reasonable proportion of monokaryons[1].

A strain of *Coprinus lagopus*, disomic for one chromosome, is under investigation and has been used as one of the parental monokaryons in the production of dikaryons and fruit bodies. Chlamydospore analysis has demonstrated the persistence of disomy in the dikaryon, but the extra chromosome was sometimes lost before the production of basidiospores. It was therefore desirable in subsequent crosses to know whether the disomy had persisted in the fruit body before undertaking an analysis of basidiospores.

Young fruit bodies are covered with a loose veil composed of chains of large cylindrical cells[2] (Fig. 1). I have found that some of these cells are viable and will germinate on agar to produce monokaryotic growth. Veil cells from young fruit bodies are in general more viable than those from mature ones.

A little of the veil from the pileus is placed in sterile water and macerated to break the chains of cells. This is done in a test-tube, used as a mortar, with a glass pestle ground to fit exactly inside the test-tube. The suspension of cells is then sown on complete medium[1] and incubated for about 18 h at 37° C. The cells may germinate from one or both ends to produce monokaryotic or dikaryotic hyphæ. By isolating monokaryotic hyphæ at this stage it is possible to recover monokaryons with the parental nuclear types.

Fig. 2 shows two germinating veil cells from the fruit body of a dikaryon between a haploid strain carrying a recessive morphological mutation 'dendroid' and a disomic strain with the wild-type allele. The dendroid gene was not on the duplicated chromosome. One veil cell has produced only dendroid growth, which must be a monokaryon with nuclei derived from the dendroid parent, and the second has produced some dendroid and some normal growth. Such normal growth has been isolated and those isolates which gave rise to monokaryon of the normal morphological type carry nuclei derived only from the disomic parent. These monokaryons have been tested and found to be disomic, demonstrating the persistence of the disomy in the fruit body. In other cases they have been found to be haploid, demonstrating the loss of the extra chromosome at some stage between the formation of the dikaryon and fruit-body production.

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SOIL SCIENCE

Stability of Granular Dicalcium Phosphate Dihydrate in Soil

Bouldin and Sample[1] considered that the dissolution of dicalcium phosphate in soil was a diffusion-controlled process, and consequently the rate of dissolution would depend on the surface area of the particular sample. Since diffusion is a slow process, granules of dicalcium phosphate may remain in soil for a considerable period after application. Thus these workers observed on numerous occasions that granules of dicalcium phosphate did not dissolve completely after 3–6 months in cropped soil.

Granular dicalcium phosphate dihydrate (2–3 mm diameter) was included in an experiment at this establishment in which the influence of various periods of soil/fertilizer contact on phosphate availability was examined on five different soils. The dicalcium phosphate was mixed with each soil (2 g of granules per 4 kg of soil) 37, 26, 15 and 4 months before examination. The mixtures were stored in the open in 8-in. clay pots, buried up to their rims in sand. At each time of addition of phosphate, the soil within every pot of all treatments was thoroughly mixed. At the end of the storage period approximately 400 g of each soil/phosphate mixture was examined and any remaining white granules removed and their total weight recorded. The results expressed as percentage recovery of dicalcium phosphate are given in Table 1.

Table 1. Percentage Recovery of Granular Dicalcium Phosphate Dihydrate after Various Periods of Soil Contact*

Soil texture†	*p*H	Period of contact (months) 4	15	26	37
(1) Sand	4·7	70	40	0	0
(2) Sandy clay loam	6·5	55	10	10‡	0
(3) Calcareous loamy sand	7·1	55	20	15	0
(4) Humose clay loam	5·7	35	10	0	0
(5) Sandy clay loam	5·0	95	30	20	0

* Mean of two replicates.
† U.S. Department of Agriculture grades.
‡ One replicate only.

To check whether these granules had changed in composition during the period of contact with the soil, X-ray powder diffraction patterns were obtained (using a Nonius 'Guinier' focusing powder camera) for each sample of fertilizer residue. These showed that every sample contained pure dicalcium phosphate dihydrate; no other constituent was detected. Hence in three out of the five soils examined, undissolved dicalcium phosphate granules may remain chemically unchanged, after as much as 26 months contact with the soil.

The percentage of the added phosphate taken up by a crop of barley (phosphate uptake from the phosphate treated soil minus that from the untreated soil) grown on the soils to which the granules had just been added was 4·5, 6·2, 5·8, 3·3 and 0·3 respectively for soils Nos. 1–5. This compares well with the figures for percentage recovery after 4 months soil contact. An exception is soil 4, in which the texture and a dark colour combined to make recovery of intact granules difficult because of staining.

We thank Dr. R. V. Coates and G. Woodard for the X-ray data.

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VIROLOGY

A Rich Source of Mouse Interferon

Considerably the richest source of mouse interferon, out of seven tested, has been found to be the brains of mice infected with the arbovirus West Nile (WN)[1]. The amounts of interferon present in one such brain are large enough to protect two or three other mice against a systemic infection with a large dose of an encephalitis virus[2]. The preparation of such mouse interferon and its properties are here described.

WN virus was obtained from Dr. J. S. Porterfield, Mill Hill, London. The strains of mice used[1] and the method for assaying interferon by quantitative hæmadsorption[3] have been described elsewhere. Interferon titres were standardized in terms of a reference preparation titrated in parallel[1].

Mice were infected intra-cerebrally with WN virus, and their brains were collected 3 or 4 days later. The brains were blended in Hanks's saline, using 2 ml. per brain, and the resultant extracts were twice centrifuged at 44,000*g* for 120 min to sediment virus particles. The top two-thirds of the supernatant from the second centrifugation constituted the interferon preparation.

In a typical experiment, the first centrifugation reduced the content of infective virus in the supernatant to 0·11 per cent of the initial level. The interfering activity of the re-suspended pellet was only 0·35 per cent of that of the final interferon preparation, and thus probably due to contamination with the interferon-containing supernatant. No infective virus could be detected in the final preparation, either directly or after blind passage, by inoculating

it into mice intracerebrally or into tissue cultures of mouse or hamster cells. Since the interfering activities of the final preparations were thus not due to the presence of virus, the preparations could be directly assayed for interferon. The same titres were obtained if preparations were treated at *p*H 2 for 24 h before assay, or if they were mixed with a potent rabbit antiserum to WN virus and held at room temperature for 2 h before assay.

In a growth curve experiment, *BSVS* mice were infected with 600 LD_{50} of virus. Many mice died from the fifth day onwards, and there were no survivors on the tenth day. Titres of virus and interferon in brain extracts are shown in Fig. 1. The interferon reached a peak level 78–90 h after infection, and thereafter declined slowly until the last time of sampling on the seventh day.

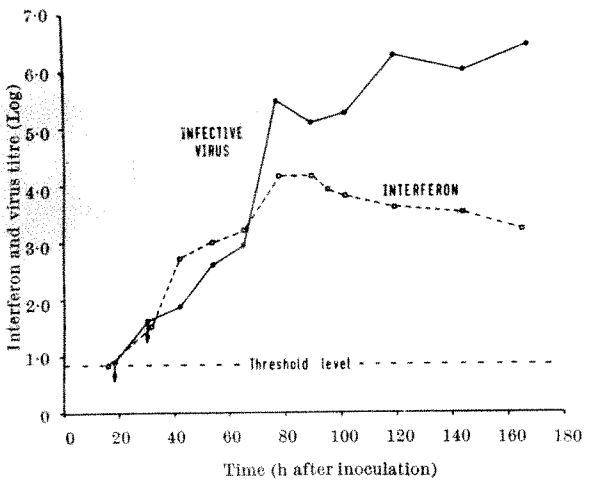


Fig. 1. Formation of infective virus and interferon in the brains of mice infected with 600 LD_{50} of West Nile virus. ●, Infective virus (log plaque-forming units/ml.); □, log interferon titre/ml.

In other experiments it was found that, over a wide range, the exact dose of virus and time of collecting had little effect on the yield of interferon in brain extracts. Mice inoculated with only 3 LD_{50} had small amounts of interferon in their brains 3 days later, but thereafter large amounts until the sixth day, whereas mice inoculated with 300 LD_{50} had large amounts on the third day, but significantly lower amounts on the sixth day after infection. No consistent differences were noted between the results with male and female mice, with mice of weights ranging from 10 to 30 gm, and with mice of the Alderley Park 1 and *BSVS* strains.

The geometric mean titre of 23 batches of such interferon was 9,500 units/ml., equivalent to 17,000 units per mouse brain, and 1,600 units/mg protein.

The interferon in the brain extracts has the typical properties described for many other interferons. Thus it was not appreciably sedimented in 2 h by a centrifugal force of 105,000*g*, and it was stable at *p*H 2. It was rapidly destroyed by incubation with 0·2 per cent trypsin, but not with 0·001 per cent crystalline ribonuclease. It had little activity unless incubated with cells for some hours before addition of challenge virus, though 35 per cent of the maximum activity was obtained after only 3 h (ref. 3). It inhibited the growth of a variety of viruses in mouse cells, for example, Semliki Forest, encephalomyocarditis, Newcastle disease, Sendai and influenza viruses, but was relatively very much less active when tested in tissue cultures of cells derived from other species, for example, chick embryo, primary calf kidney, primary hamster kidney and *BHK*21 hamster cells. Unlike some other interferons, however, it was relatively unstable on heating at 56°, 90 per cent of the activity being lost after 30 min.

The presence of interferon in the brains of mice infected with WN virus was first described by Vainio *et al.*[4], in an investigation in which yields from the brains of genetically virus-susceptible and virus-resistant mice were compared. These authors found a greater content of interferon in the brains of the susceptible mice, but made no comment on the potency of their preparations. Interferon has also been found in the brains of mice infected with other arboviruses, O'nyong-nyong virus[5], and tick-borne encephalitis virus[6]. We have found approximately 100 times less interferon in the brains of mice after infection with Semliki Forest virus than after WN virus[1].

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PSYCHOLOGY

Changes in Psychological Performance associated with Changes in Total Body Water

Changes in body water have been shown to occur in mental illness. Gjessing[1] in his investigations of periodic catatonia showed biochemical changes associated with nitrogen and water balance. Crammer[2] examined a patient with a regular 6–7-day cycle of change in mood. He noted a change in body water associated with a change in body weight. Gibbons[3] has reviewed the field in relation to changes in body water. Brown *et al.*[4] have reported cases of changes of body water (extra cellular fluid) associated with changes in mood, in conditions other than periodic psychosis. Coppen and Shaw[5] published a series of cases associated with change in body water and electrolytes. The work recorded here is associated with a larger series, Kerry[6], investigating the relationship of changes of body water and mood. In all previous series the clinical changes of mood do not always correlate with the changes in total body water in either degree or direction. The aim of the work described here is to see if a more precise association could be obtained using psychological measures.

All admissions to a large mental hospital were screened to select recurrent admissions showing an obvious alteration in mood or psychomotor activity[6]. Of this series 21 patients were given the psychological measures to be described later. The measurements of total body water and the psychological assessments were always performed at the same time. Generally the first tests were performed within a few days of admission when the patient was ill. It should be pointed out that inevitably a patient is never admitted at the beginning of an illness but only after the illness has been in progress for some time. The second, or follow-up, tests were done about a week after clinical recovery. It was not possible strictly to control the patient's diet.

(1) *Total body water measurement.* This was measured by an isotope technique using tritium. A Packard Tricarb counter was used. A prepared dose of 100 μc. of tritium in 30 ml. of water was administered by mouth on a fasting stomach. Samples of urine were collected prior to administration and samples afterwards collected at 2·5, 3, 4, 5 and 6 h.

(2) *Psychological measurements.* A number of personality tests were also given to each patient on the two occasions when total body water was estimated. The tests used were as follows: (*a*) a personality inventory with four short scales measuring anxiety, dissociation, obsessionalism, emotional extraversion; (*b*) a multiple choice Rorschach measuring psychoticism; (*c*) two time estimation measures—the productive estimation of 30 sec and the verbal estimation of 30 min; (*d*) human figure drawings.

This variety of tests was included to cover a range of relatively independent characteristics affected in varying ways by different psychiatric illnesses. It was expected that a number of the measures would not show any association with total body water for two reasons. First, clinical observations associate changes in total body water with changes in mood. Secondly, some of the measures are probably not sufficiently sensitive to changes in the individual.

Table 1. Product Moment Correlation Coefficients ($N = 21$) between Changes in Figure Drawings and Changes in Total Body Water

(1) Area (in.^{2})	−0·646	$P < 0·01$
(2) Time taken (sec)	−0·578	$P < 0·01$
(3) Horizontal deviation (in.)	+0·284	N.S.
(4) Vertical deviation (in.)	+0·477	$P < 0·05$

For these reasons expectations were largely directed to the human figure drawings. These drawings were measured using the method described by Hetherington[7] and Orme[8]. A point is made on each drawing at the place which appears to be its 'centre of gravity'. Distances of this point from the horizontal and vertical median lines of the page were measured in each case. Drawings towards the left of the vertical median line were given positive signs, those towards the right, negative signs. Similarly, drawings above the horizontal median line received positive signs, those below, negative signs. To assess the size of each drawing, the area covered by it was calculated. This was done by means of a transparent grid ruled off in half-inch squares superimposed over each drawing. Previous work with this technique showed it to be a sensitive measure of depression and of recovery from depression. Depressives, compared with non-depressives, place their drawings nearer the left-hand side of the page, nearer the top of the page and cover less area. With recovery from depression these features significantly decrease. Here the time taken to draw was also recorded.

In the present series of patients, changes in test performances were correlated with changes in total body water. It was assumed that the use of such measures would be more meaningful than the use of basal rates. With the 'draw a person' technique, the sign of the changes in position and size were reversed for those seven patients considered (independently) by the psychiatrist (R. J. K.) to have been initially in an elated mood. Of the psychological tests, only the 'draw a person' technique produced significant results. As noted, this was the technique of particular interest. Of the four figure drawing measures, all are in the expected direction, three of them at a statistically significant level.

It can be seen from Table 1 that, with a patient who is initially depressed, the size of his human figure drawing will increase as his total body water decreases. Similarly, he will, as his total body water decreases, take more time over his drawing, and it will move towards the centre of the page.

An examination of the literature shows that changes in total body water during the course of a psychiatric illness do not always follow the change in mood as judged clinically. For example, patients may appear less depressed clinically, but their total body water may have either increased or decreased. These paradoxes have never been satisfactorily explained. Also the degree of change has often been disproportionate. The work presented here, using psychological measurements, was directed to examine these problems. Significant correlation coefficients between changes in total body water and changes in human figure drawings have been obtained. As previously stated, the direction of change in total body water is often paradoxical when compared with the clinical assessment of the mood change. In this sample the measurement of mood, by human figure drawing changes, parallels the changes in total body water. It appears from this finding that further investigations into biochemical changes and their association with psychological measures of mood change are worth carrying out.

We thank Dr. H. Miller and the staff of the Sheffield Medical Physics Department for advice and for carrying out the tritium estimations. We also thank our consultant colleagues for allowing us to examine patients under their care, and Dr. Z .C. Randall and Dr. M. Griffiths for their assistance. This work was supported financially by the Sheffield Regional Hospital Board.

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Semantic and Acoustic Similarity in Short-term Memory

Conrad[1–3] has shown that sequences of items which are difficult to discriminate when heard in noise are also hard to remember, even though presented visually. This result can be interpreted either: (*a*) as showing that there is a very strong acoustic factor in immediate memory, or (*b*) as an example of one of many types of similarity which impair recall. Since long-term sequence learning is hindered by various types of similarity[4,5] if this latter interpretation is correct, it would support the view that long- and short-term memory are basically very similar[6].

The following experiment attempted to decide between these alternatives by comparing the influence of acoustic similarity with that of semantic similarity, a variable which has been shown to be important in the long-term learning of word sequences[4].

The material to be remembered consisted of five-word sequences presented by tape recorder at a rate of one word per sec. No word was repeated in any sequence. At the end of each sequence, subjects were allowed 20 sec to write as much as they could recall in the appropriate order.

A different group of housewives served in each of two conditions. In condition *A* 12 sequences were drawn from a vocabulary of eight acoustically similar words (mad, man, map, mat, cad, can, cap, cat), and eight from acoustically different words of approximately equal frequency of occurrence in English (bar, cow, day, few, hot, pen, pit, sup). In condition *B*, 12 sequences were drawn from a set of eight words with similar meanings (broad, great, large, wide, big, high, long, tall), and 12 from a set of eight semantically different words (hot, old, strong, foul, deep, late, thin, safe). Similar and different sequences were presented in the same random order in both conditions. The relevant sets of words were written on cards which were visible throughout the test session. To ensure that subjects were hearing the words correctly a listening test was given both before and after the memory test. This involved presenting the 16 relevant

words in random order. Subjects were instructed to copy down each word and were allowed 5 sec to do so. Two condition *A* subjects did not score perfectly on this test and were discarded, leaving 20 subjects in condition *A* and 21 in condition *B*.

Table 1. Performance as a Function of Acoustic and Semantic Similarity (Percentage Correct Sequences)

Condition	Similar vocabulary	Different vocabulary	Difference
A (acoustic)	9·6	82·1	72·5
B (semantic)	64·7	71·0	6·3

Results were scored in terms of percentage of sequences correctly recalled and are shown in Table 1.

A large effect of acoustic similarity is shown by all 20 subjects and is thus highly significant ($P < 0.001$). There is a small effect of semantic similarity, which a Wilcoxon test shows to be significant ($P < 0.05$, 2 tail). The effects of acoustic and semantic similarity were then compared by computing a difference score for each subject, based on her performance with the similar and dissimilar sequences. All 20 subjects in condition *A* showed a greater effect of similarity than any subject in condition *B*, showing that the effect of acoustic similarity was very significantly greater than that of semantic similarity ($P < 0.001$).

These results show that semantic similarity does affect short-term as well as long-term memory. Its effect, however, is only one-twelfth as great as that of acoustic similarity. The evidence therefore supports the first interpretation of Conrad's results, that there is a strong acoustic (or quasi-acoustic) element in immediate memory, rather than an explanation in terms of the general effects of similarity shown in long-term memory.

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Imprinting to a Static Feature of the Home Environment

In 1951 Ramsay[1] reported observations which suggest that recognition of the home environment by precocial birds might be regarded as a form of imprinting. This view was also expressed by Thorpe[2] and implies that imprinting can occur not only to a fluctuating stimulus but also to any salient feature of the natural environment. The occurrence of attachment to a stationary object has since been demonstrated, though not beyond controversy, by Hess[3], Gray[4], and Abercrombie and James[5]. Both Hess and Gray used as stimuli a black circle and a black triangle of comparable size. Young domestic chicks were exposed to one or the other of these figures for one day, having previously been reared in visual isolation; when later tested in a choice apparatus with the figures at opposite ends they showed a clear preference, in terms of approach behaviour, for the original over the new object. Abercrombie and James tested their chicks in a runway at each end of which was a continuous light, one of them being accompanied by a stationary polythene ball which served as a 'lure'. They found that in this situation chicks eventually formed an attachment to the lure, although they required more trials for its formation than did others which had been trained with the lure accompanied by a flickering light.

In all these experiments the chicks were reared in visual isolation before being exposed to the test object when one or more days old. This technique may well have strengthened the salience of the test figure and so increased the likelihood of attachment. The present experiment attempts to demonstrate attachment to more complex stationary objects which are, from the beginning, a part of the chicks' environment. The investigation therefore represents a closer analogy with the 'natural' situation.

Chicks of a commercial breed were incubated and hatched in the laboratory and, when dry, removed to individual visual isolation pens 12 × 13 × 11 in. high. Half the 32 chicks were reared with a cardboard box suspended against one of the walls 1·5 in. clear of the floor: the remainder were reared with a foam-rubber object similarly suspended. Troughs were placed in the opposite corners, so that a chick had to move away from food and water to approach its object. After 48 h each subject was tested in a rectangular pen 26 × 28 × 10 in. high, with the two objects suspended opposite one another in the middle of the longer walls. The positions of the objects were evenly distributed over the experiment. The chick was placed under a lid in the middle of the test pen for 1 min, and then observed for not more than 5 min. If the animal did not move within that time the trial was discontinued; if it did move it was observed for a further 5 min and records were kept (*a*) of approach movements into the areas within 8 in. of each object, and (*b*) of any physical contact with either object.

The approach results are shown in Fig. 1. Of the 32 chicks tested, 6 (3 box-reared and 3 foam-reared) stayed still and were removed after the 5-min maximum latency period, and 7 moved but did not approach either object. One box-reared subject approached both objects and was scored as a half-choice for each of them in statistical analysis and in Fig. 1. The remaining 18 birds all approached the object with which they had been reared, and did not approach the other. Thus, the approaches made to original and new objects numbered 18·5 and 0·5 respectively. The binomial test shows this discrepancy to be significant at the 0·001 level. Furthermore, 16 chicks actually contacted the original object and none of them touched the new object, a difference which is equally significant. By both these criteria our subjects demonstrated a virtually exclusive preference for the object with which they had been reared.

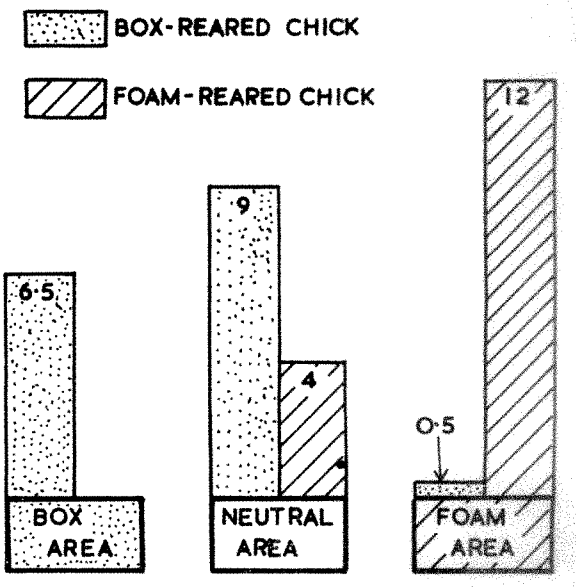


Fig. 1. Numbers of chicks entering approach areas or remaining in neutral area during test

It was observed that chicks which had been reared with the foam object often pecked at and pressed against it to the accompaniment of contentment chirps, while the others generally showed less positive activity when contacting the box. This suggested that the foam provided an intrinsically more satisfying object of attachment than the box. We tested this by comparing the proportions of the two groups which approached and which contacted their original object in the test (for this analysis the chick which approached both objects was excluded as furnishing ambiguous data). Of the 15 box-reared chicks, 6 approached the box and 4 touched it; while of 16 foam-reared chicks, 12 approached and 11 contacted the foam. The proportions test by Brown[6] shows these differences both in approach and in contact to be significant beyond the 0·05 level.

It could be argued that this result was due to chance differences in the general liveliness of the chicks, rather than to differences in the strength of attachments to the two objects, To test this we compared the mean latency times of the two groups, excluding those subjects which did not move at all. For box-reared chicks this was 2·7 min (range 0·8–4·7), for foam-reared it was 2·1 min (range 0·6–4·8). Clearly there is no significant difference between these means nor, in so far as may be inferred from latency times, in the reactivity of the two groups.

Our main result supports the hypothesis that chicks can form attachments of the kind classified as imprinting to static objects forming a part of their environment from the beginning, as well as to objects introduced into, and so disturbing, their familiar world. Although this attachment was demonstrated by our subjects in a novel situation it must have been established previously in their home environment. Nor can this be explained in terms of conventional reinforcement, since food and water were available in the corners of the pens away from the objects and temperature was constant in all areas.

It was noted, however, that the chicks appeared to seek contact with the box or the foam during the two-day period of rearing. They were often discovered sitting pressed against the object or even, in the case of the foam, actually inside it. Consequently the normally stationary objects did move when pushed, and it is possible that both contact and the movement which this produced had reinforcement value which increased the likelihood or the strength of the attachment. But chicks of this age are not particularly mobile, so that while movement of an object might occasionally have been produced by random floundering against it, systematic or frequent movement would have been unlikely unless the bird had specifically sought contact with it. Movement would therefore have been a concomitant rather than a cause of imprinting. The fact that the foam elicited stronger attachments than the box suggests that some variable akin to Harlow's 'contact comfort'[7] may have been of importance in our experiment. Further work on this question is now in progress in our laboratory[8]. We thank Prof. S. G. Lee and Dr. W. Sluckin for advice.

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STATISTICS

Epidemics and Rumours

Goffman and Newill[1] have directed attention to the analogy between the spreading of an infectious disease and the dissemination of information. We have recently examined the spreading of a rumour from the point of view of mathematical epidemiology and wish to report very briefly here on work to be published in detail elsewhere[2]. In particular, we must emphasize that a mathematical model for the spreading of rumours can be constructed in a number of different ways, depending on the mechanism postulated to describe the growth and decay of the actual spreading process. In all these models the mathematical techniques familiar in mathematical epidemiology can be applied, but even the qualitative results so obtained need not necessarily be as expected on the basis of the formal analogy with epidemics.

To illustrate this point (but not to advance the model *per se*), let us divide the (finite, closed, homogeneously mixing) population into three mutually exclusive and exhaustive classes:

Class	No.	Rumour interpretation	Epidemic interpretation
X	x	Has not heard rumour	Susceptible to disease
Y	y	Actively spreading rumour	Infectious case
Z	z	No longer spreading rumour	Dead, isolated, or immune

If we assume that the only transitions are: (1) 'infections' $(x, y, z) \rightarrow (x-1, y+1, z)$, at a rate proportional to xy; (2) 'removals' $(x, y, z) \rightarrow (x, y-1, z+1)$, at a rate proportional to y; then we obtain the deterministic Kermack–McKendrick epidemic[3] in which an initial trace of infection will generate an appreciable epidemic if, and only if, the initial number $x(0)$ of susceptibles exceeds a certain 'threshold' value ρ. In this formulation the decay of spreading is due to a process of 'forgetting'. Another plausible hypothesis is that an active 'spreader' stops telling the rumour because he learns that it has lost its 'news value'; if this happens as soon as he meets another individual knowing the rumour (that is, a member of Z or another member of Y), then transitions from Y to Z occur as a result of YZ and YY encounters (with a double transition in the latter case), and hence at a rate proportional to $yz + 2[\frac{1}{2}y(y-1)] = y(y+z-1)$.

If this assumption is made, however, the behaviour of the system is strikingly different from that of a Kermack–McKendrick epidemic, and could not have been predicted from familiarity with the latter. The fraction f of the population which ultimately learns the rumour (corresponding to the fraction of the population which succumbs to the disease) is always about 0·80, almost irrespective of the value of $x(0)$, so that the familiar threshold effect has disappeared. A stochastic analysis yields very similar results; we find that f (now a random variable) has an expectation of about 0·80 and essentially a variance of approximately $0{\cdot}31/N$, where $x(0) = N$.

'Reluctance to tell stale news' can be incorporated into the model in other ways, some of which we have investigated by similar methods, and the results are then correspondingly modified. However, the object of this communication is not to urge the appropriateness of any one model of rumour-telling; but to stress the danger of transferring formulæ designed for epidemiological use without a thorough re-examination of the hypotheses, which may not always be appropriate in the new situation.

D. J. Daley
D. G. Kendall

Statistical Laboratory,
University of Cambridge.

[1] Goffman, W., and Newill, V. A., *Nature*, **204**, 225 (1964).
[2] Daley, D. J., and Kendall, D. G. (unpublished).
[3] Bailey, N. T. J., *The Mathematical Theory of Epidemics* (Griffin, 1960).

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, December 14

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Du Cane Road, London, W.12), at 4 p.m.—Lord Cohen of Birkenhead: "The Lipidoses".*

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Mr. J. G. Attfield and Mr. D. A. Wheeler: "Vapona Insecticide".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.15 p.m.—Prof. E. H. Thompson, O.B.E.: "An Itinerant Scientist in China".

Tuesday, December 15

UNIVERSITY OF LONDON (at the Institute of Obstetrics and Gynæcology, Hammersmith Hospital, Du Cane Road, London, W.12), at 2 p.m.—Mr. C. J. Dewhurst: "The Uncertainty of Sex".

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, London, S.W.1), at 5.30 p.m.—Mr. A. V. Johnston: "Some Economic and Engineering Aspects of the Construction of New Railway Lines in Northern Canada".

INSTITUTION OF ELECTRICAL ENGINEERS, SCIENCE AND GENERAL DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Transistors—The First Encounter", opened by Mr. V. H. Attree.

PARLIAMENTARY AND SCIENTIFIC COMMITTEE, GENERAL COMMITTEE (in Committee Room 11, House of Commons, Westminster, London, S.W.1), at 5.30 p.m.—Discussion on "Teaching of Science in Schools, with particular regard to the Future Application of Science" initiated by Sir Graham Savage, C.B., and Mr. William G. Rhodes.

UNIVERSITY OF LONDON (in the Physiology Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. R. J. Rossiter: "Biosynthesis of Phospholipids".*

INSTITUTION OF MECHANICAL ENGINEERS, RAILWAY ENGINEERING GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Transport to the London Airports, Present and Future".

ROYAL AERONAUTICAL SOCIETY, ASTRONAUTICS AND GUIDED FLIGHT SECTION (in conjunction with the Institute of Navigation, at 4 Hamilton Place, London, W.1), at 6 p.m.—Dr. G. V. T. Matthews: "Bird Navigation".

Wednesday, December 16

INSTITUTION OF CIVIL ENGINEERS (joint meeting with the Corrosion Group of the Society of Chemical Industry, at 14 Belgrave Square, London, S.W.1), at 4.30 p.m.—Informal Discussion on "Measurement of Thickness of Sprayed Metal Coatings on Steel; Paints for Sprayed Metal Coatings; and Measurement of Thickness of Paints Applied to Steel".

ROYAL METEOROLOGICAL SOCIETY (at 49 Cromwell Road, London, S.W.7), at 5 p.m.—Mr. J. R. Probert-Jones: "An Analysis of the Fluctuations in the Tropical Stratospheric Wind"; Mr. K. R. Ramanathan: "Bi-Annual Variation of Atmospheric Ozone over the Tropics"; Mr. R. C. Smith: "A Derivation of the Meridional Velocity Profile".

ROYAL STATISTICAL SOCIETY (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.15 p.m.—Mr. H. B. Berrington: "The General Election of 1964".

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. H. K. Beale: "Some Economic Aspects of Extra-High-Voltage Underground Cables"; Mr. J. A. M. Gibbons, Dr. P. R. Howard and Mr. D. J. Skipper: "Gas Pressurised Lapped Polythene Dielectric for Extra-High-Voltage Power Cable Systems"; Mr. D. J. Skipper and Mr. P. I. McNeall: "Impulse Strength Measurements on Compressed Gas Insulation for Extra-High-Voltage Power Cables".

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, JOINT I.E.R.E./I.E.E. MEDICAL ELECTRONICS GROUP (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Discussion on "D.C. Defibrillation" opened by Mr. J. Spencer.

INSTITUTION OF MECHANICAL ENGINEERS (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "The Future of Household Appliance Design".

SOCIETY OF ENVIRONMENTAL ENGINEERS (in the Mechanical Engineering Department, Imperial College of Science and Technology, London, S.W.7), at 6 p.m.—Mr. B. G. Toms and Mr. G. E. Johnson: "The Laboratory Simulation of Real Vibration Environments".

SOCIETY OF INSTRUMENT TECHNOLOGY (at Manson House, 26 Portland Place, London, W.1), at 6 p.m.—Mr. L. Rowland: "The Control of a Warehouse by Computer".

Thursday, December 17

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 10 a.m.—Colloquium on "Modern Techniques for Microwave Circuit Synthesis".

LONDON MATHEMATICAL SOCIETY (at the Royal Astronomical Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Prof. B. H. Neumann: "Equations Over Groups".

INSTITUTION OF CIVIL ENGINEERS, ENGINEERING MANAGEMENT GROUP (at Great George Street, London, S.W.1), at 5.30 p.m.—Informal Discussion on "Management Teaching Methods—Programmed Learning" introduced by Mr. C. G. Monk.

CHEMICAL SOCIETY (in the Chemistry Lecture Theatre, King's College, Strand, London, W.C.2), at 6 p.m.—Prof. W. Baker, F.R.S.: "The Widening Outlook in Aromatic Chemistry" (Pedler Lecture).

SOCIETY OF CHEMICAL INDUSTRY, ROAD AND BUILDING MATERIALS GROUP (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Mr. W. R. Oram: "Pre-Fabricated Housing".

INSTITUTION OF MECHANICAL ENGINEERS, GRADUATES' AND STUDENTS' SECTION (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6.30 p.m.—Mr. P. Spear: "Development of the B.R.M.".

INSTITUTION OF MECHANICAL ENGINEERS (in the Council Chamber, F.B.I., 21 Tothill Street, London, S.W.1), at 6.30 p.m.—Discussion on "A Scientific Theory of the Golf Swing".

Friday, December 18

INSTITUTE OF NAVIGATION (at the Royal Institution of Naval Architects, 10 Upper Belgrave Street, London, S.W.1), at 5.30 p.m.—Commandant L. Oudet: "Collisions and the Law".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

READER IN ENGINEERING PRODUCTION—The Secretary, Welsh College of Advanced Technology, Cathay's Park, Cardiff, quoting Ref. No. BZ 464 (December 17).

LECTURER or ASSISTANT LECTURER IN MATHEMATICS; a LECTURER or ASSISTANT LECTURER IN APPLIED MATHEMATICS; and a LECTURER or ASSISTANT LECTURER IN NUMERICAL ANALYSIS in St. Salvator's College—The Secretary, The University, College Gate, St. Andrews, Fife, Scotland (December 19).

SENIOR LECTURER (with a good honours degree in botany, and preferably some teaching and research experience) IN BOTANY to teach students to the B.Sc. General Degree (London)—The Principal, South-West Essex Technical College and School of Art, Forest Road, Walthamstow, London, E.17 (December 19).

LECTURER (Grade II) IN THE DEPARTMENT OF MECHANICAL ENGINEERING—The Registrar and Secretary, The University, Bristol (December 21).

SENIOR CHAIR OF MATHEMATICS—The Secretary, The University, Aberdeen (December 26).

ASSISTANT LECTURER or LECTURER IN PSYCHOLOGY—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1 (December 31).

ASSISTANT LECTURER or LECTURER (preferably with a special research interest in physical organic chemistry) IN CHEMISTRY—The Registrar, The University, Sheffield (December 31).

ASSISTANT LECTURER or LECTURER (with a veterinary or agricultural degree) IN ANIMAL HUSBANDRY IN THE SUB-DEPARTMENT OF ANIMAL HUSBANDRY, Faculty of Veterinary Science—The Registrar, The University, Liverpool, quoting Ref. CV/364/N (December 31).

FIELD ARCHAEOLOGIST to organize and control excavations in Roman sites in the City of Leicester—The Director, Museum and Art Gallery, New Walk, Leicester (December 31).

LECTURERS or ASSISTANT LECTURERS (with interests in organic chemistry, inorganic chemistry, theoretical chemistry or high resolution spectroscopy) IN CHEMISTRY—The Registrar, University of East Anglia, Earlham Hall, Norwich, NOR 05G (December 31).

MASTER TO TEACH GEOGRAPHY—The Headmaster, Leeds Grammar School, Leeds (December 31).

PROFESSOR OF CONTROL ENGINEERING—The Registrar, Ref. 59Y/E, Bradford Institute of Technology, Bradford, 7 (December 31).

PROFESSOR OF SOLID STATE PHYSICS—The Registrar, Ref. 58Y/E, Bradford Institute of Technology, Bradford, 7 (December 31).

SECOND CHAIR OF ELECTRONIC SCIENCE IN THE DEPARTMENT OF ELECTRICAL ENGINEERING—The Registrar, University of Strathclyde, George Street, Glasgow, C.1 (December 31).

SENIOR LECTURER (with qualifications required to teach mathematics for honours degree courses) IN MATHEMATICS—The Registrar, Ref. 64Y/E, Bradford Institute of Technology, Bradford, 7 (December 31).

BIOCHEMIST (with a Ph.D. degree in biochemistry or chemistry and experience in modern methods of protein separation) for work on the purification of proteins which influence the behaviour of animal cells *in vitro*—Dr. C. A. Vernon, Department of Chemistry, University College, Gower Street, London, W.C.1 (January 1).

LECTURER or ASSISTANT LECTURER IN THE DEPARTMENT OF PURE MATHEMATICS—The Registrar, University College of North Wales, Bangor, North Wales (January 1).

LECTURER IN THE DEPARTMENT OF SOCIOLOGY—The Secretary, The University, Aberdeen (January 4).

SENIOR LECTURER (with a special interest in vertebrate zoology) IN THE DEPARTMENT OF ZOOLOGY—The Registrar, The University, Sheffield (January 6).

LECTURER or ASSISTANT LECTURER IN EDUCATION at the University of Basutoland, Bechuanaland Protectorate and Swaziland—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (January 8).

LECTURER or ASSISTANT LECTURER IN MATHEMATICAL STATISTICS—The Registrar, The University, Manchester, 13 (January 9).

LECTURER (preferably with experience in physical electronics or materials science) IN THE DEPARTMENT OF ELECTRICAL ENGINEERING, University of Western Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 9).

TECHNICIAN (with experience in electron microscopy) for full-time work in the electron microscopy section—The Secretary, Institute for Research on Animal Diseases, Compton, near Newbury, Berkshire (January 14).

LECTURER IN BACTERIOLOGY AT THE UNIVERSITY OF SYDNEY, AUSTRALIA—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 15).

LECTURER (with a higher degree in zoology and evidence of competence and interest in research, and experience in university teaching) IN ZOOLOGY AT THE UNIVERSITY OF QUEENSLAND, AUSTRALIA—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, January 15).

POSTDOCTORATE FELLOW IN PHYSICS IN ANY OF THE FOLLOWING RESEARCH FIELDS—NUCLEAR PHYSICS, SOLID STATE PHYSICS, MICROWAVE SPECTROSCOPY, AND THEORETICAL PHYSICS—Head of the Department of Physics, Queen's University, Kingston, Ontario, Canada (January 15).

READER IN EXPERIMENTAL PHYSICS AT QUEEN MARY COLLEGE—The Academic Registrar, University of London, Senate House, London, W.C.1 (January 15).

LECTURER (with a good honours degree in geography, and preferably previous university teaching experience and a higher degree) IN GEOGRAPHY AT NEWCASTLE UNIVERSITY COLLEGE, THE UNIVERSITY OF N.S.W.,

AUSTRALIA—The Warden, Newcastle University College, Tighe's Hill, New South Wales, Australia; and The Agent General for New South Wales, 56–57 Strand, London, W.C.2 (January 16).

LECTURER (with research experience in the field of the physiology of marine animals) IN ZOOLOGY—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (January 18).

READER, and LECTURERS (3) IN THE DEPARTMENT OF MECHANICAL ENGINEERING, Ahmadu Bello University, Northern Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (January 18).

SENIOR LECTURER (with a good honours degree in geography and also a higher degree or actively engaged in research leading to a higher degree, and preferably some lecturing experience) IN GEOGRAPHY at the University College of Townsville, University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, January 29).

LECTURER (Grade II) IN THE DEPARTMENT OF INORGANIC CHEMISTRY—The Registrar, University of Bristol, Senate House, Bristol, 2 (February 14).

ANIMAL HUSBANDRY OFFICER (national of the United Kingdom or the Republic of Ireland, with a degree in agriculture and extensive experience in practical animal husbandry) IN SARAWAK, to develop the livestock industry dealing mainly with pig feeding trials and management—The Appointments Officer, Room 301, Ministry of Overseas Development, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 213/155/06.

ASSISTANT PROFESSOR OR ASSOCIATE PROFESSOR IN THE DEPARTMENT OF PHYSIOLOGY—Prof. M. Beznak, University of Ottawa, Ottawa, Canada.

BIOLOGIST, SCIENTIFIC OFFICER OR SENIOR SCIENTIFIC OFFICER GRADE (with a first- or good second-class honours degree in genetics, botany or zoology) IN THE ISOTOPE RESEARCH DIVISION, Wantage Research Laboratory, A.E.R.E., to undertake a programme of research on the effects of radiation at the cellular level—The Personnel Department (A 5089/34), Atomic Energy Research Establishment, Harwell, Didcot, Berkshire.

CHIEF TECHNICIAN TO TAKE CHARGE OF THE MEDICAL ELECTRONIC WORKSHOP—The House Governor, The Hospital for Sick Children, Great Ormond Street, London, W.C.1.

D.S.I.R. RESEARCH ASSISTANT (honours graduate in chemical engineering, physics or general science) IN THE DEPARTMENT OF CHEMICAL ENGINEERING, for work on the evaluation and comparison of methods of particle size analysis—Mr. J. C. Williams, Department of Chemical Engineering, Ref. 65Y/E, Bradford Institute of Technology, Bradford, 7.

LECTURER IN MATHEMATICS—The Principal, Bede College, Durham.

LECTURER (with a good honours degree or Diploma in Technology and some research experience) IN THE DEPARTMENT OF APPLIED PHYSICS—The Academic Registrar, Northampton College of Advanced Technology, St. John Street, London, E.C.1, quoting Ref. AP/N.

LECTURER (with special interests in nuclear structure problems) IN THEORETICAL NUCLEAR PHYSICS—The Secretary, Battersea College of Technology, London, S.W.11.

MASTER TO TEACH CHEMISTRY TO "A" LEVEL AND SOME ELEMENTARY BIOLOGY—The Head Master, St. John's School, Leatherhead, Surrey.

MASTER TO TEACH MATHEMATICS TO SCHOLARSHIP LEVEL—The Headmaster, Malvern College, Worcestershire.

PRINCIPAL (with high academic qualifications, a wide experience of further education, and preferably some industrial experience) OF THE NORTH STAFFORDSHIRE COLLEGE OF TECHNOLOGY—The Clerk to the Governors, North Staffordshire College of Technology, Education Department, P.O. Box No. 23, Town Hall, Hanley, Stoke-on-Trent.

RESEARCH ASSISTANT (with an honours degree in physics) IN THE PHYSICS DEPARTMENT to examine the process of ruling the lines of a diffraction grating—The Assistant Bursar (Personnel), The University, Reading.

RESEARCH STUDENT IN INORGANIC CHEMISTRY FOR RESEARCH ON GERMANIUM CHEMISTRY UNDER THE DIRECTION OF DR. K. M. MACKAY—Prof. C. C. Addison, Department of Chemistry, The University, Nottingham.

SENIOR TECHNICIAN (preferably with experience of experimental biology or physiology) IN THE DEPARTMENT OF PHARMACOLOGY, mainly for the supervision of practical classes and maintenance of apparatus—The Professor of Pharmacology, Institute of Preventive Medicine, Welsh National School of Medicine, The Parade, Cardiff.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

H. Tinsley and Co., Ltd. Tinsley's History 1904–1964. Pp. 32. (South Norwood, London: H. Tinsley and Co., Ltd., 1964.) [610

The Institution of Chemical Engineers. The Choice of Chemical Engineering at University and Technical College: a Survey of Students Admitted in 1963. Pp. vii + 53. (London: The Institution of Chemical Engineers, 1964.) 15*s.* [610

Sulphur Dioxide as an Atmospheric Pollutant. By the Technical Committee of the National Society for Clean Air. Pp. 32. (London: National Society for Clean Air, 1964.) 2*s.* [610

Symposium on Aspects of the Gene. Edited by Michael D. B. Burt and Peter W. Barlow. Pp. 75. (*The Biological Journal*, Vol. 4, Supplement, August 1964.) (St. Andrews: Biological Society of the University of St. Andrews, 1964.) [610

The Institution of Agricultural Engineers. Year Book 1964. Pp. 168. (Brighton: Fraser Pearce, Ltd., 1964.) [610

Royal Observatory Bulletins. No. 87: Differential Curve-of-Growth Analyses of some Cool Stars. By B. E. J. Pagel. Pp. E227–E280. 8*s.* net. No. 88: Double-Star Observations made with the 28-inch Refractor 1960–63. By Sir Richard Woolley, L. S. T. Symms, D. H. P. Jones and M. P. Candy. Pp. E281–E310. 4*s.* 6*d.* net. (London: H.M. Stationery Office, 1964.) [610

Other Countries

Bulletin of the Museum of Comparative Zoology, Harvard University. Vol. 131, No. 7: Early Evolution of the Dissorophid Amphibians. By Robert L. Carroll. Pp. 161–250 + 2 plates. Vol. 131, No. 8: The Genus *Micrathena* (Araneae, Araneidae) in the West Indies. By Arthur M. Chickering. Pp. 251–281. Vol. 131, No. 9: Revised Generic Diagnosis of the Fossil Fishes Megalichthys and Ectosteorhachis (Family Osteolepidae). By Keith Stewart Thomson. Pp. 283–311 + 1 plate. Vol. 131, No. 10: The Comparative Anatomy of the Snout in Rhipidistian Fishes. By Keith Stewart Thomson. Pp. 313–357. Breviora. No. 205 (July 15, 1964): Redescription of *Amphisbaena dubia* Müller (Amphisbaenia: Reptilia). By Carl Gans. Pp. 11. No. 206 (July 15, 1964): The Aistopod Amphibians Surveys. By Donald Baird. Pp. 17. No. 207 (July 28, 1964): Notes on the Horseshoe Bats *Hipposideros caffer, ruber*, and *beatus*. By Barbara Lawrence. Pp. 5 (Cambridge, Mass.: Museum of Comparative Zoology, Harvard University, 1964.) [610

Institut Royal Météorologique de Belgique. Contributions, No. 91: Le Problème des Hauteurs d'Echelle et de Leurs Gradients dans l'Hétérosphere. Par Dr. G. Kockarts. Pp. 24. Publications, Série A, No. 49: Rayonnement Solaire à Uccle, Année 1963. Pp. v + 60. Publications, Série B, No. 43: Investigation of the Reliability of Radiosonde Measurements in Uccle with the Method of Twin Sondes. By A. G. Maenhout. Pp. 10. (Uccle-Bruxelles: Institut Royal Météorologique de Belgique, 1964.) [610

Union Radio Scientifique Internationale. XIV^e Assemblée Générale, Tokyo. Sept. 9–20, 1963. Vol. XIII—8: Administration et Activités Diverses. Pp. 172. (Bruxelles: Union Radio Scientifique Internationale, 1964.) [610

United States Department of the Interior: Geological Survey. Bulletin 1080: Review and Annotated Bibliography of Ancient Lake Deposits (Precambrian to Pleistocene) in the Western States. By John H. Feth. Pp. iii + 119 + plates 1–4. Bulletin 1108–E: Geology of the Sumdum Copper-Zinc Prospect, Southeastern Alaska. By E. M. MacKevett, Jr., and M. C. Blake, Jr. Pp. iv + 31 + plate 1. Bulletin 1158–D: Bedrock Geology of the Tiverton Quadrangle, Rhode Island—Massachusetts. By Samuel J. Pollock. Pp. iv + 16 + plate 1. Bulletin 1158–E: Bedrock Geology of the Kingston Quadrangle, Rhode Island. By George E. Moore, Jr. Pp. iii + 21 + plate 1. (Washington, D.C.: Government Printing Office, 1964.) [710

United States Department of the Interior: Geological Survey. Bulletin 1160: Studies of Pre-Selma Cretaceous Core Samples from the Outcrop Area in Western Alabama. By W. H. Monroe and 6 other authors. Bulletin 1161–I: Petrochemistry and Bedrock Geology of the Fitchville Quadrangle, Connecticut. By George L. Snyder. Pp. iv + 63 + plate 1. Bulletin 1180–A: Cretaceous Stratigraphy of the McCarthy A–4 Quadrangle, Southern Alaska. By David L. Jones and Henry C. Berg. Pp. iii + 18 + plate 1. Bulletin 1181–E: Fossils in Ordovician Tuffs, Northeastern Maine. By Robert B. Neuman. With a section on Trilobita by Harry B. Whittington. Pp. iii + 38 + plates 1–7. Bulletin 1181–M, N: Geological Sketch of Northwestern Oregon. By P. D. Snavely, Jr., and H. C. Wagner. Pp. iii + 17 + plate 1. Geological Interpretation of Reconnaissance Gravity and Aeromagnetic Surveys in Northwestern Oregon. By R. W. Bromery and P. D. Snavely, Jr. Pp. iii + 13 + plates 1–3. Bulletin 1196–A: Geochemical Exploration Near the Getchell Mine, Humboldt County, Nevada. By R. L. Erickson, A. P. Marranzino, U. Oda and W. W. Janes. Pp. iv + 26 + plates 1 and 2. Water-Supply Paper 1809–E: Galleries and Their Use for Development of Shallow Ground-Water Supplies, with special reference to Alaska. By Alvin J. Feulner. Pp. v + 16. (Washington, D.C.: Government Printing Office, 1964.) [710

United States Department of the Interior: Geological Survey. Professional Paper 313–C: Stratigraphy and Petrology of the Permian Rocks of Southwestern Montana. By Earle R. Cressman and Roger W. Swanson. Pp. vi + 275–569 + plates 14 and 15. Professional Paper 331–C: Gastropods from the Coffee Sand (Upper Cretaceous) of Mississippi. By Norman F. Sohl. Pp. iii + 394 + plates 53–57. Professional Paper 377: Ore Deposits of the St. Lawrence County, Magnetite District, Northwest Adirondacks, New York. By D. F. Leonard and A. F. Buddington. Pp. ix + 259 + plates 1–21. Professional Paper 445: Ore Deposits of the Coeur d'Alene District, Shoshone County, Idaho. By Verne C. Fryklund, Jr. Pp. v + 103 + plates 1–5. Professional Paper 446: Geology of the Guadalupe Mountains, New Mexico. By Philip T. Hayes. Pp. iv + 69 + plates 1–3. Professional Paper 454–D: Glaciation of Little Cottonwood and Bells Canyons, Wasatch Mountains, Utah. By Gerald M. Richmond. Pp. iv + 41 + plates 1 and 2. Professional Paper 454–J: Geology of Bullfrog Quadrangle and Ore Deposits related to Bullfrog Hills Caldera, Nye County, Nevada and Inyo County, California. By Henry R. Cornwall and Frank J. Kelinhampl. Pp. iv + 25 + plates 1–9. (Washington, D.C.: Government Printing Office, 1964.) [710

United States Department of the Interior: Geological Survey. Professional Paper 454–N: Miocene Floras from Fingerrock Wash, Southwestern Nevada. By Jack A. Wolfe. Pp. iii + 36 + plates 1–12. Professional Paper 454–O: Relationship of Precambrian Quartzite–Scist Sequence Along Coal Creek to Idaho Springs Formation, Front Range, Colorado. By John D. Wells, Douglas M. Sheridan and Arden L. Albee. Pp. iii + 25 + plate 1. Professional Paper 483–E: Multinodose Scaphitid Cephalopods from the Lower Part of the Pierre Shale and Equivalent Rocks in the Conterminous United States. By William A. Cobban and Glenn R. Scott. Pp. iii + 13 + plates 1–4. (Washington, D.C.: Government Printing Office, 1964.) [710

International Union Against Cancer. Activities Report, 1962–63. Pp. 47. Manual of the International Union Against Cancer. Pp. 94. (Geneva: International Union Against Cancer, 1964.) [211

Brochure on an Exhibition of Books and Manuscripts by and Relating to Galileo Galilei upon the Quadricentennial of his Birth, 1564—1964. Pp. 32. (Bethlehem, Pennsylvania: Lehigh University Library, 1964.) [211

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Population Reference Bureau. Annual Report, 1963. Pp. 20. (Washington, D.C.: Population Reference Bureau, 1964.) [211

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BRITISH AID TO DEVELOPING COUNTRIES

THE surveys of the Department of Technical Co-operation by the Estimates Committee* and by the Overseas Development Institute† are of major interest from the scientific point of view because of the account given of the support of overseas research. However, that was only one aspect covered in the surveys, and, so far as the report of the Estimates Committee is concerned, it was a comparatively minor function. The Committee was much more concerned with the organization of the Department generally, with the financial control exercised over technical assistance, and with the Overseas Service Aid Scheme. It was also disturbed by education co-operation and particularly the possibility of an overlap with the work of the British Council—Britain's other main source of educational assistance (excluding capital) to the developing countries. A principal recommendation is that the Department and the Council should agree to the drawing up of a comprehensive statement to establish a demarcation of their respective responsibilities.

The Estimates Committee did not discover any duplication in services provided for students or trainees. It was considered that if the present policy were maintained by which there were two distinct bodies working in similar fields, the report of the joint working party, set up in March 1964, should provide a basis for a workable definition of areas of operation in the supply of school teachers. There is nothing in the report to refute the idea that an unofficial body enjoys certain advantages over an official body. Indeed, the Estimates Committee is convinced that Britain would have gone further in supplying educational assistance to the developing countries and would have had a better programme if the British Council had, in the past, been induced to enter into the field of supplying teachers to colonies on the verge of independence, and if it had been granted the necessary financial resources.

The Estimates Committee stresses the importance of solving the problems associated with fitting the activities of the Department of Technical Co-operation into the general pattern of overseas aid. It recognizes that it is not always easy to define the boundaries between the responsibilities of the Department and other forms of aid. Generally it approves of the Overseas Service Aid Scheme as an effective instrument. On organization the Committee recommends the appointment of a Deputy Director-General, accompanied by a review of senior posts, with the view of returning to the three-division system of administration; however, it is recognized that geographical as well as functional departments are needed. The Finance Department should be reconstituted without responsibility for any geographical area, but with responsibility for forward planning and, together with the Treasury, the Department of Technical Co-operation should re-examine the present organization for recruitment with the view of establishing one department with this responsibility. The Establishment Department should examine regularly all the other departments of the organization and, together with the Finance Department, should be made directly responsible to the Director-General or to a Deputy Director-General. The Committee also recommends a survey by Embassies and High Commissions in all countries where the Department's function is to ascertain whether technical assistance at present justifies a rearrangement of duties to allow one member of the staff to be employed full-time on technical assistance work. Any such full-time post should normally be seconded from the Department of Technical Co-operation. The Department should re-examine the work of its Middle East Development Division to see whether the needs of developing countries for technical assistance could not be served better and more economically by advisers from the Department based on London.

The criticism from the Overseas Development Institute is directed not so much at the Department of Technical Co-operation as at the pattern of British technical assistance generally: this was established at the end of the Second World War and is not wholly appropriate for present needs. Some of the institutions and expertise built up in colonial days are not fully geared to meet the new situation, partly because of a failure, particularly during the later 1950's, to build up Britain's reserves of expertise on development problems. The old colonial career services were allowed to run down in numbers, and organizations like the Directorate of Overseas (Geodetic and Topographic) Surveys and the Tropical Products Institute were subjected to years of uncertainty and frustration instead of expansion. As a result, we now face considerable manpower problems. The advisers of the Department of Technical Co-operation are mostly more than fifty, while the Department's administrators are mostly more than forty-five. Many of Britain's technical assistance personnel with any length of experience overseas are nearing retirement.

The suggestions made by the Overseas Development Institute for identifying the needs in technical assistance, for organizing Britain's resources and for improving recruitment, including the organization of a career service, were noted in an earlier article (*Nature*, 204, 715; 1964) discussing overseas research, with the exception of one point. The recent growth in the volunteer movement is regarded as the brightest spot in Britain's whole effort to help developing countries with personnel. However, under the present constitution, the value of this movement's contribution lies as much in the experience gained by the volunteers and the leavening effect on attitudes in Britain towards developing countries as in any significant increase in the skills being made available. So long as volunteers are virtually untrained and stay for such short times, their value will not realize its true potential.

The survey suggests that the voluntary societies and the Government should seriously consider whether the

* Tenth Report from the Estimates Committee together with part of the Minutes of the Evidence taken before Sub-Committee F and appendixes. Session 1963–64. Department of Technical Co-operation. Pp. xxix+243. (London: H.M. Stationery Office, 1964.) 16*s.* 6*d.*

† The Overseas Development Institute. British Aid—4: Technical Assistance. Pp. 196. (London: The Overseas Development Institute, 1964.) 7*s.* 6*d.*

next step after the expansion already announced for 1965 should be to lengthen the period of service to two years or more. This might make it worth while to give the volunteers a longer training and orientation period before leaving Britain. The further suggestion is made that enthusiasm of volunteers and other young people might be directed into more permanent institutional forms, for example, by encouraging the returned volunteer to join a properly constituted service of British technical assistance personnel, or recruiting young people under thirty-five as 'associate experts' for the United Nations in the same way as the Dutch and Germans have been doing. Longer contracts or a career service structure would also make it worth while for the Department of Technical Co-operation to arrange special training, particularly for younger recruits, and to extend the range of studentships it offers for such training. Greater provision for training relevant to overseas conditions, coupled with attachment to senior experienced workers in the field of a counselling service for sixth forms and first-year students on opportunities overseas, should facilitate rapid expansion of opportunities.

Although it was announced at the time of forming Mr. Harold Wilson's Government that a new Ministry of Overseas Development would assume the functions of the Department of Technical Co-operation, it was not until November 10 that the Minister, Mrs. Barbara Castle, was able to make a full statement on the scope of the new Ministry. The Ministry's responsibility for carrying out the Government's policies for development overseas would include the Aid Programme as a whole: its distribution between bilateral and multilateral aid and between capital aid and technical assistance; the terms and conditions of capital aid and the principles on which technical assistance is granted; the size and nature of the aid programme for each country; the management of capital aid and technical assistance; relations with international aid organizations; Britain's interest in the United Nations' programmes of technical assistance; and liaison with voluntary bodies operating in the same fields. In reply to questions, Mrs. Castle said that the voluntary bodies would include not only Oxfam but also such bodies as Voluntary Service Overseas, and that the scientific work overseas formerly undertaken by the Overseas Research Council would also be a part of the technical assistance for which the Ministry would be responsible.

In dealing with all these matters the Ministry would work in harmony with the policies of the Overseas Departments and other Departments concerned, and the purpose of the new Ministry would be to promote the progress of the developing countries, bearing in mind Britain's special ties with Commonwealth countries. The Ministry would be responsible for the Commonwealth Development Corporation and would have general responsibility for co-ordinating British policy in respect of the aid and development work of the United Nations and its Specialized Agencies and for aid questions arising from the United Nations Conference on Trade and Development. It would also assume the prime responsibility for relations with the United Nations Educational, Scientific and Cultural Organization and the Food and Agriculture Organization from the Department of Education and Science and the Ministry of Agriculture, Fisheries and Food, respectively. The Treasury would continue to deal with the International Bank for Reconstruction and Development, in close consultation with the new Ministry.

Mrs. Castle added that the whole aim in establishing the Ministry had been to create a body to bring to an end the proliferation of responsibility for matters of aid. The whole tenor of her statement and her replies to questions arising out of it shows that the changes should meet much of the criticism which the Overseas Development Institute has advanced in a series of constructive reports. She also indicated that contact on aid matters would be maintained with the Organization for Economic Co-operation and Development. Apart from a specific question about the Colonial Office and Commonwealth Relations Department arising out of the Plowden Report on overseas services (*Nature*, **203**, 439; 1964), the statement largely answered the enquiries voiced by Mr. N. Fisher in a speech on November 6 in the debate on the Address to the Throne.

Mrs. Castle should be assured of the firm support of all who appreciate the critical importance of this question of aid to the developing countries. Wisely she declined to commit the Government, at this stage, to any specific increase in Britain's contribution; however, her recognition of the great challenge which the situation presents warrants the hope that the Minister will bring a fresh and constructive mind to bear not only on the question of finance but also on the numerous other aspects on which authoritative and informed criticism has been advanced in recent months.

SYNTHETIC ORGANIC CHEMISTRY

Methoden der Organischen Chemie

Von Houben-Weyl. Vierte, völlig neu gestaltete auflage. Herausgegeben von Eugen Müller. Unter besonderer mitwirkung von O. Bayer, H. Meerwein und K. Ziegler. Band XII/1: Organische Phosphorverbindungen, Teil 1. Bearbeitet von K. Sasse. Pp. lxxii+683. (Stuttgart: Georg Thieme Verlag, 1963.) Moleskin, 166 D.M.

Methoden der Organischen Chemie

Von Houben-Weyl. Vierte, völlig neu gestaltete auflage. Herausgegeben von Eugen Müller. Unter besonderer mitwirkung von O. Bayer, H. Meerwein und K. Ziegler. Band VI/2: Sauerstoffverbindungen 1, Teil 2. Bearbeitet von O. Bayer, A. Berthmann, A. Hausweiler, W. Herrmann, R. Köster, H. Kröper, R. Ratz, K. Schimmelschmidt, F. Schmidt, K. Schwarzer, W. Simmler und F. Sinn. Pp. xlviii+952. (Stuttgart: Georg Thieme Verlag, 1963.) 220 D.M.

Methoden der Organischen Chemie

Von Houben-Weyl. Vierte, völlig neu gestaltete auflage. Herausgegeben von Eugen Müller. Unter besonderer von O. Bayer, H. Meerwein und K. Ziegler. Band XIV/2: Makromolekulare Stoffe, Teil 2. Bearbeitet von H. Herlinger, M. Hoffmann, E. Husemann, E. Istel, W. Kern, H. Krimm, E. Müller, H. Rinke, R. Schmitz-Josten, P. Schneider, R. C. Schulz, G. Spielberger, R. Wegler und R. Werner. Pp. lxxii+1251. (Stuttgart: Georg Thieme Verlag, 1963.) 272 D.M.

BAND XII/1. In the period 1848–64 Prof. W. von Hofmann, working in the Royal College of Chemistry, carried out some extraordinarily able researches on the organic derivatives of phosphorus. The subject was afterwards comparatively neglected for very many years, being largely replaced by a much wider interest in similar arsenic derivatives. Interest in organo-phosphorus compounds began to quicken again some 25–30 years ago, and has since developed at a rapidly increasing rate; a very considerable volume of knowledge is now scattered in the chemical literature. Workers in this subject were greatly helped in 1950 by Kosalopoff's book, in which

synthetic methods were classified and described. The importance of the subject—both theoretical and technical—clearly warranted a much fuller and more modern treatment, and this is most excellently provided by Band XII/1 of the new edition of "Houben-Weyl". This volume is devoted (as the main title of the series implies) to synthetic methods, and the chemical properties of the final products are only briefly indicated. The systematic classification of the various methods for the preparation of each class of phosphorus compound, including specific experimental detail to illustrate typical preparations, occasional tables of melting-points and yields, and very full references, has been carried out with remarkable thoroughness: indeed, it would be very difficult to find any relevant matter in chemical literature which has escaped the authors. Moreover, the presentation of all this information is so attractive that the reader can with equal pleasure either 'browse' through the volume or concentrate his attention on any specific subject. The book will delight all organo-phosphorus chemists.

Band VI/2. This volume will ultimately be the second of three Parts devoted to organic derivatives of oxygen. Its contents are unusual, for the greater part (503 pp.) deals with compounds on the border-line of inorganic chemistry. Thus there are separate chapters on metal-alcoholates, -phenolates, -enolates and -chelates, on organic derivatives of silicic acid, of boric acid, and of the inorganic acids of phosphorus, arsenic, antimony and sulphur, and on the esters of nitrous and nitric acid, of the hypohalogenous acids and of perchloric acid. I am myself unaware of any other single volume in which all the foregoing subjects are discussed in such detail, and they make absorbing reading, partly because they are so far removed from the common well-worn tracks of most organic chemical texts.

The remainder (342 pp.) is devoted to two chapters, on β-lactones and other lactones respectively. The syntheses of lactones having rings of various sizes are carefully classified and described in great detail. The total information on lactones is so considerable that these two chapters, if published together, would alone make a valuable volume. This Part 2, therefore, will appeal strongly to the orthodox organic chemist and to the organic chemist having rather wider interests.

Band XIV/2. The earlier Band XIV/1 dealt in considerable detail with the nomenclature and terminology of macromolecular substances, with their preparation by the polymerization of vinyl and divinyl compounds in solution and in heterogenous systems, and with special polymerization processes for the more important monomers. Band XIV/2, a volume as massive as Part 1, is divided into three main sections. Section A, the largest of the three, deals with the preparation of macromolecular products by 'poly-condensation' and 'poly-addition', including the use of comparatively simple heterocyclic units such as substituted epoxides and ethyleneimines. This section has, therefore, a wide range: it discusses the condensation of, for example, carboxylic acid esters, organic carbonates, urethanes and amides, and that of carbonyl and thiocarbonyl compounds with phenols, amines and various other types of nitrogen compounds such as ureas, melamine and urethanes. It consequently contains detailed information on many processes of the greatest technical and theoretical value. Section B deals with the chemical properties of various types of macromolecular products and Section C with the determination of their structure. Pages 960–1069 contain a list of references (in addition to those given as footnotes in the text) classified under thirty-six subjects.

This volume, the work of many contributors, must, with Part 1, be the outstanding work on this vast subject.

The publishers and printers of the foregoing three volumes deserve warm praise for the impressive and attractive appearance, printing and binding of these works.

F. G. MANN

PHONON INTERACTIONS

Phonons and Phonon Interactions

Edited by Thor A. Bak. (Aarhüs Summer School Lectures. 1963.) Pp. xiv+640. (New York and Amsterdam: W. A. Benjamin, Inc., 1964.) 10.45 dollars.

THIS volume contains almost all the lectures given at a summer school at Aarhüs University during August 1963. The school must have been most stimulating in providing a review of all the extremely varied phenomena of the physics of condensed systems in which phonon interactions play a fundamental part.

As is inevitable in a collection of this kind, there are repetitions, and the notation is not always uniform, but these are the only adverse comments that one can make, apart from regretting that the highlights of the discussion, said to have been 'lofty', have not been included. The collection is outstanding and will be consulted with great profit by both experts and beginners. It should be particularly useful to experimentalists who wish to acquire a sound knowledge of the underlying theory. Since ample references are given, it will be used also as a work of reference.

The word 'phonon' is repeated twice in the title, as it were to stress that the relevant quantum theory is mostly expressed in terms of creation and annihilation operators. The fundamental ideas are set out very clearly in three introductory lectures, the first by H. H. Jensen on the free phonon field, the second by W. Ludwig on some aspects of phonon-phonon interaction, that is on the effects of anharmonicity, and the third by A. Sjölander on "phonons and external radiation", that is on how to obtain information on the lattice vibrations of the solid. Van Hove's space-time correlation function, which has proved so general, and which provides physical clarity, is introduced here.

In the first of the more special contributions, W. Cochran deals with the interaction of phonons with X-rays, reaching the conclusion that X-ray scattering is generally obsolete as a method of investigating the thermal spectrum. Such investigation can, however, be carried out with excellent results using neutron scattering. This is treated in detail by B. N. Brockhouse, who has himself done much to develop the method. He discusses the theoretical and experimental factors involved in the determination of the dispersion relations, frequency distributions and decay properties of the lattice vibrations. One has the impression that a complete theoretical interpretation of all the results obtained has still to be given.

J. J. Quinn discusses theoretically the phonon-electron interactions, while H. G. Reik shows how, in semiconductors, transport properties can be used to obtain information on such interactions. Additional information can be reached from a study of the absorption of long-wave phonons, and this is discussed by A. R. Mackintosh. The interaction of phonons with photons is considered by E. Burstein, who analyses in detail the infra-red, Raman and Brillouin spectra of solids arising from lattice vibrations. The theoretical treatment is particularly clear in this contribution.

The lectures by J. R. Schrieffer deal authoritatively with those aspects of the theory of superconductivity which are directly related to the phonon field and its interaction with the electrons. J. de Boer gives the theory of phonons in liquids: this problem becomes easier for liquids at the absolute zero, thus ^{3}He and ^{4}He are considered; it is shown that the excitation model proposed by Landau is very promising.

A. A. Maradudin, in a lengthy and comprehensive contribution, deals with the interaction of phonons with lattice imperfections: these lectures will be particularly useful to those wishing to become familiar with the use of Green's function methods. E. H. Jacobsen examines the generation, detection and propagation of elastic waves

in solids at frequencies higher than 10^{10} sec^{-1}. The discussion of spin-phonon interactions is especially interesting here. There is also a short contribution by K. S. Singwi on the influence of thermal motion on the Mössbauer effect, and a monograph by R. G. Kepler on the conductivity, photoconductivity and luminescence of organic molecular crystals.

In a brief summary it is inevitable that only a few points can be mentioned, but it is hoped that it has indicated how many fascinating topics are covered in these lectures and how high is the standard of treatment. Almost all the contributions are outstanding for their clarity and for bringing out the essential points, besides including the latest results. Warmest congratulations should be extended to the organizers of the school for their happy choice of topics and lecturers, to the lecturers themselves and to the careful editor, Dr. T. A. Bak, who has produced this volume in a very reasonable time with a minimum of blemishes. L. Pincherle

ALGORITHMIC DEEP BLUE SEA

The Mathematical Theory of Optimal Processes

By L. S. Pontryagin, V. G. Boltyanskii, R. V. Gamkrelidze and E. F. Mishchenko. Translated from the Russian. Pp. viii+360. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, Inc., 1962.) 90*s*.

THE calculus of variations and the science of control have developed side by side over the centuries but have only recently begun to affect one another. Norbert Wiener's classical war-time report on the filtering of noise was one of the first to introduce to control engineers some of the mathematical ideas involved in the optimization of dynamic systems. Wiener wrote in a terse and eccentric style, often using concepts belonging to nineteenth-century mathematical physics. Indeed, so incomprehensible did the bewildered engineers find the yellow-bound report that they called it "the yellow peril". Nevertheless, they discovered that it contained an algorithm which might help them to minimize the effects of random disturbances in control systems.

Now another eminent mathematician, the Russian L. S. Pontryagin, has inflicted an algorithm on the engineers. The algorithm is an extension of the calculus of variations and is called Pontryagin's maximum principle. Pontryagin and his colleagues developed their elegant and useful method in a series of masterly papers begun in 1956, and collected the results in book form in 1961. The present book is a translation from the Russian. Although prompted by engineering problems, the book is written as pure mathematics. Engineers are going to have almost as much trouble in understanding the abstract style as they had with Wiener's yellow peril. Will they call this blue-bound translation 'the deep blue sea'?

The motivation for the work stems from difficulties encountered in the application of linear theory to servo-mechanisms during the Second World War. The controlling variable (throttle opening, amplifier output, etc.) had only a limited range of variation and it was found that this saturation effect caused the performance to be worse than expected. When the saturation was taken into account the theory became non-linear. The problem was the formidable one of designing an optimum non-linear controller for the system. At first sight it seemed that the classical calculus of variations was not applicable to the problem, owing to the sharp limitations on the allowable variations in control. Nevertheless, by 1956 engineers had obtained a number of useful preliminary results, using various *ad hoc* techniques. What Pontryagin has done is to show that all these results, and many more, are encompassed by a method which virtually involves recasting the calculus of variations.

Pontryagin's maximum principle is, roughly speaking, as follows. The differential equations of the system are written in 'phase space' form, that is, as a set of first-order equations. From this a related set of differential equations, termed the adjoint system, is obtained. The maximum principle now states that, at each instant of time, the control variables must be chosen so that a certain function of the phase co-ordinates and of the adjoint variables becomes a maximum. When this is done the problem reduces to the solution of a set of ordinary differential equations of twice the order of the original system. (These differential equations have as analogues in the classical calculus of variations, the Euler-Lagrange equations when written in Hamiltonian form.)

Actually the calculus of variations had already been extended to deal with problems with inequality constraints, before the Second World War. This was done by Valentine of the Chicago school in 1937. However, Valentine's algorithm is decidedly less elegant than Pontryagin's. Also Valentine's proof depended on adding an algebraic trick to the classical calculus of variations which was already composed largely of algebraic tricks (two examples: the integration by parts in deriving the Euler–Lagrange equations, and the introduction of Lagrange multipliers). Pontryagin's method, on the other hand, potentially makes it possible to use geometric intuition and to obtain the results more 'naturally'. All the well-known necessary conditions of the classical calculus of variations follow readily from Pontryagin's results. Indeed, in the future we may well regard the classical calculus of variations as a branch of Pontryagin's theory, rather than vice-versa.

The authors, not surprisingly, have chosen to address their work to mathematicians rather than engineers, and have endeavoured to establish their theorems with utmost rigour. Unfortunately this has resulted in an extremely abstract treatment. Often a proof depends on elaborate initial constructions, the point of which does not emerge until the end of the proof, some 20–30 pages later. Little attempt is made to help the reader by preliminary heuristic outlines of arguments. Usually results are proved with maximum generality, and then simpler special cases are considered, rather than the other way round. The reader will continually find difficulty in distinguishing the wood from the trees.

For example, in Chapter 2, devoted to the proof of the maximum principle, the authors assume that the control signal is a measurable function of time. The entire proof becomes obscured with the paraphernalia of measure theory. It would have been preferable to give, at least as a preliminary, the proof for the simpler case when the control signal is assumed piece-wise continuous.

The authors also take for granted familiarity with certain mathematical concepts, especially adjoints and convexity. It would have been helpful if an exposition and perhaps a history of these concepts, in relation to the calculus of variations, had been given. If it had been pointed out that the adjoints are the differential equations satisfied by Green's functions, and that the Green's functions are the impulse responses of the linear variational system, the essence of the argument would have been simpler to grasp. Admittedly, in Chapter 1, Pontryagin and his co-authors make concessions to readability. This is an admirable survey of results, written with great care and lucidity.

The book contributes a large number of results to the theory of optimal control. Control engineers will have to make the effort to discover what these results are, even if they are unable to follow the proofs. Typical examples are as follows. The solution of Bushaw's problem when there are two control variables. The problem of attaining a given circle in the phase space in minimum time. (In connexion with problems like this one, the maximum principle yields a simple geometric interpretation of the transversality conditions of the calculus of

variations.) Optimal control of systems with time-varying parameters. Optimal control of systems with initially adjustable parameters.

One chapter deals with systems with restricted phase co-ordinates. Unfortunately the proofs are extremely elaborate, and this chapter is very unreadable. It would have been helpful if some simple examples had been given (the so-called examples given are actually extensions of the theory to related problems.) Some of the results, while of considerable academic interest, seem unlikely to be of practical use owing to the complexity of the algorithms arrived at, for example, the problem of optimal processes with a delay, and the problem of optimal pursuit of a controllable target.

The final chapter deals with a statistical optimal control problem. It is a pursuit problem, in which the pursued object is assumed to move randomly in the phase space, and only its initial position is known. The pursuer is to be controlled so that the probability of interception is maximized. The chapter is unsatisfying, because the problem itself seems unrealistic, the mathematics is extremely involved, and the final result is too complex to be used, yet is only approximate. However, as an exercise in mathematical manipulation it compels admiration.

The standard of translation is excellent. We must be grateful to the translators for their scholarly attention to detail, and to the publishers for the rapidity with which the book was brought out.

To sum up, Pontryagin's maximum principle has effected a revolution in the calculus of variations, and in the theory of control. The present book is the most complete account of the theory yet available, and is likely to become a classic. Simpler expositions will be written, but we shall often find ourselves returning to this book to discover what has or has not been proved.

A. T. FULLER

ALASKAN ARCHÆOLOGY

The Archæology of Cape Denbigh

By Prof. J. L. Giddings. Pp. xv+331+73 plates. (Providence, R.I.: Brown University Press, 1964.) 12.50 dollars.

CAPE DENBIGH is on the northern Behring Sea coast of Alaska, and at several places interesting Stone Age sites have been discovered and studied. There are two main areas where stone tools occur, namely, at Nukleet and Iyatayet, on the north-west side of the Cape. The former has yielded an Eskimo industry, and the place seemingly was occupied from the twelfth to the eighteenth century. Iyatayet is much more interesting. On top there is a level similar to that at Nukleet, but below there are two older levels, the upper of which has been called Norton and the one below it the Denbigh Flint Complex. The Norton level is not too sharply defined, as it seems to have been partially cut into by the overlying Nukleet folk, and in turn the Norton culture itself has disturbed the top of the Denbigh Flint Complex below.

The Norton people seem to be early Eskimo, and their industry includes bone and flint tools, as well as stone lamps and pottery. The latter differs somewhat from that found at Nukleet. But the level underneath is something different. The stone industry contains a number of undoubted burins of various types and there is no pottery. Indeed, in Europe one could consider it to be a late palæolithic or early mesolithic culture. At the same time, there is little or no evidence to suggest that there was any vast gap in time between the Norton level and that below. Carbon-14 dating points to a considerable antiquity, however, suggesting an age of nearly 6,000 years ago.

Are we to suggest that the Denbigh Flint Complex represents a very early pre-Eskimo stage of culture without pottery itself ultimately derived from an upper palæolithic stage of Western Europe? As the climate in Western Europe ameliorated at the close of Magdalenian times, these reindeer-loving peoples seem to have moved north following the animals. Did they penetrate into what is now northern Russia and Siberia? In Siberia early Eskimo industries have been recognized; will further investigation unearth still earlier pre-Eskimo levels? So far, there is no direct evidence for this, but little investigation has yet been made. The author of this very interesting volume has demonstrated at Cape Denbigh a succession of cultures with more or less normal Eskimo industries on top and reaching down in the lowest level to an industry which shows distinct late palæolithic affinities.

The theory that the latest palæolithic cultures of Western Europe migrated north and east following the reindeer when the climate ameliorated is not new; it was suggested many decades ago, but at that time there was no scrap of evidence to uphold it, except the fact that the culture of the Eskimo seemed not too unlike that of our Magdalenians. There are still many difficult questions to answer and the matter is by no means clear; it is to be hoped that continued investigation will throw more light on the problem and provide some satisfactory explanation of the interesting facts disclosed.

The volume is well illustrated with more than 75 full-page half-tones. A few good drawings of the implements would perhaps have helped. Prof. Giddings is to be congratulated on his work.

M. C. BURKITT

RIVER POLLUTION

Fish and River Pollution

By Dr. J. R. Erichsen-Jones. Pp. viii+203. (London: Butterworth and Co. (Publishers), Ltd., 1964.) 52*s.* 6*d.*

THIS most useful book should be read by all concerned with the well-being of our fishes and all industrialists who are, or may be, passing trade effluents into our natural waters.

Dr. Jones has reviewed very competently the research work on the toxicity to fish of a large variety of substances. There are chapters on fish and oxygen and the reactions of fish to water of low oxygen concentration. Here are described several pieces of apparatus to test these responses. It is of interest that some fish do not have an innate ability to avoid low oxygen concentrations and in fact seem to be unaware of the lack of oxygen. On the other hand, Chinook salmon (*Oncorhynchus tschawytscha*), Coho salmon (*O. kisutch*) and the large mouth bass (*Micropterus salmoides*) under experimental conditions avoided low oxygen concentrations, the degree of avoidance increasing with the reduction in oxygen concentration.

A chapter on the measurement of toxicity in the laboratory is mainly of academic interest, as these results cannot be applied without qualification in the field because most of our rivers are already polluted. If a substance is found to be toxic it should not be put into rivers until it has been exonerated by long-term field experiments.

There is a most useful chapter on the effects of lead, zinc and copper; here Dr. Jones includes many of his own excellent observations on a zinc-polluted stream in Wales. When discussing the salts of metals the author stresses "that concentration on tolerance limits must be applied with caution, for the extreme limits of toxicity, even at one specified temperature, have been determined for few substances and few fish", and that experimentally it has been shown that different fish react very differently to metallic salts at toxic concentrations.

As many salmon poachers nowadays use cyanide it is of interest to note that the threshold toxicity is as low as about 0·1 p.p.m. and that the gills of the fish dying in cyanide solutions become bright red. I agree that this is an important symptom of the cause of death, but unfortunately in a few hours the gill colour returns to normal.

Other chapters deal with organic gases, acids, alkalis, *p*H tolerance limits, synthetic detergents and soaps, complex effluents, and thermal pollution.

A discussion on the growth rates of fish in acid and alkaline waters ends with a quotation from Brown that evidence of better growth in alkaline water is circumstantial and that "no one has demonstrated unequivocally that growth rates are directly affected by the ionic composition of the water". To this I would add that most acid waters I have investigated have been overcrowded and the population thereby underfed. Given enough food, salmonids will grow well in acid waters.

The 'newer' pollutants in the form of synthetic detergents, herbicides and insecticides are dealt with adequately. The high toxicity and long life of some of the components of these substances make sad reading for anyone interested in the preservation of our fishes. It is equally distressing to learn that fish are not capable of avoiding phenol in solution at any concentration.

To the full discussion on the effect of pollution on fish eggs I would like to add that the absence or very reduced flow of water at the spot where the salmon or trout eggs are about to be laid is temporary. Once the female fish has covered her eggs with gravel the water will once again flow through the gravel. Also, the bed-making activities of the female salmon and trout are such that a great deal of the silt and detritus in the gravel is carried downstream. Consequently the gravel around the eggs is reasonably clean and will itself form an adequate filter to prevent the eggs being silted.

Dr. Jones is to be complimented on his achievement, but I wonder if the book could be made more acceptable to the less scientifically minded by the addition of simple abstracts at the beginning of each chapter. I would also like to see the generic and specific names of the fish given in the text, not as an appendix, especially as the American common names can be confusing.

J. W. Jones

TIDAL COMPUTATIONS

Tidal Computations in Rivers and Coastal Waters

By J. J. Dronkers. Pp. xii+518. (Amsterdam: North-Holland Publishing Company, 1964.) 110*s*.

"... the effect of either luminary doth likewise depend upon its declination or distance from the equator: for if the luminary were placed at the pole, it would constantly attract all parts of the waters without any intensification or remission of its action, and could cause no reciprocation of motion. And, therefore, as the luminaries decline from the equator towards either pole, they will, by degrees, lose their force; and on this account will excite lesser tides in the solsticial than in the equinoctial syzygies ..."*.

Dr. J. J. Dronkers, of the Netherlands Rijkwaterstaat, begins *Tidal Computations in Rivers and Coastal Waters* with a historical outline which may well tempt the reader to turn to the *Principia* and savour such arguments as this before following with Dr. Dronkers the development of tidal theory through the work of D. Bernoulli, Laplace, Airy and Kelvin to the present time. In his introductory outline, Dr. Dronkers states that "this book is devoted to those tidal computations in rivers and coastal waters which are of great use in the planning and construction of engineering works in tidal waters". Thus, the book is primarily addressed to engineers, and so is largely confined to shallow water phenomena: for constructional work in the oceans is not yet envisaged.

Under the term 'tidal computations' Dr. Dronkers includes the harmonic analysis of tides, and the broad range of calculations concerned with tidal propagation. Such a statement, however, gives little indication of the actual scope of the work; and a brief résumé will therefore be attempted.

Chapter 1, in addition to the historical sketch, contains reference to problems arising when civil engineering works are contemplated in tidal régimes: for example, new navigation channels, land reclamation works and tidal barrages for power or water-supply purposes. The vital 'closure' problems arising in tidal barrage works are treated more fully later in the book. In solving such problems, the engineer will nowadays make use of the hydraulic scale-model, the electrical analogue, or the mathematical model with computer. Chapter 1 contains a general comparison of these devices.

The remaining chapters of the book are distributed between four main divisions. Part 1, "The Generation and Harmonic Analysis of the Tides", occupies about one-fifth of the text. It contains sections on the necessary basic astronomy, develops the tidal potential by vector methods, and considers the inertia-less 'equilibrium tide', the constituents of which are always in advance of the real ones. There is a list of 29 tidal constituents, and a chapter devoted to practical harmonic analysis, which leads to phase and amplitude determinations for the real constituents. The work of Doodson occupies a conspicuous position.

Part 2, "Basic Hydrodynamics and Hydraulics", leads from continuity, through the Euler equations, and thence by tensor methods to the Navier–Stokes equations. There follow an introduction to concepts of turbulent motion, and the velocity-distribution arising from the mixing-length theory of Prandtl. Chapter 6 discusses both long and short waves, with the approximations appropriate to each class, and also gives some consideration to the propagation of a semi-diurnal tide on a flowing river. Chapter 7 deals with the equations for long waves in shallow water; and, in particular, with the propagation across important discontinuities in the profile, as, for example, through a gap.

In Part 3, which deals with tidal computations in rivers and coastal waters, complete chapters are devoted to each basic method of solution; that is, to the harmonic, characteristic and finite-difference methods. It occupies nearly two-fifths of the book, and presupposes in the reader a considerable mathematical background. However, in the excellent bibliography, reference is made to some basic text-books. Chapter 9, on the method of characteristics, contains some sixteen pages devoted to the fascinating question of the propagation of a tidal bore. Here, as elsewhere, the combination of physical and mathematical reasoning is impressive.

Part 4, "Practical Applications", contains condensed descriptions of the solution by the harmonic method of the problem of tides in the River Lek; and of an application of the method of characteristics to a sluice problem. The first part of the final chapter deals in outline with 'closure' problems. In the second paragraph of p. 460 the words 'ebb' and 'flood' should apparently be interchanged. The work closes with a discussion of very high tides, followed by a further short contribution on hydraulic and analogue models. Reference is made to the impressive *Delta* model at Delft, with its battery of Magnus-Coriolis cylinders.

One would expect to find this book in the libraries of all civil engineering schools and hydraulic research institutes because of its wide scope and coverage of modern analytical and computational techniques. Many readers would doubtless welcome a second (perhaps shorter) volume devoted to the detailed solution of specific problems.

R. B. Whittington

* Isaac Newton, *Principia*, Book 3, prop. XXIV (1686): Translated by A. Motte, edit. by W. Davis (1803).

The Role of Science in Civilization

By Robert Bruce Lindsay. Pp. vii+318. (New York and London: Harper and Row, 1963.) 6.50 dollars; 49*s*.

PROF. LINDSAY, who is dean of the Graduate School at Brown University, is well known for his joint contributions (with Prof. Henry Margenau) to the philosophy of physical science, notably in the book *Foundations of Physics*, now widely available in the Dover paper-back series. The present work is a sequel to these earlier books.

Although the title leads one to expect a discussion centred on the sociological and historical aspects of science, what Dean Lindsay gives us turns out to be, for the most part, a fairly straightforward summary of the philosophical positions with which his name is already associated: that is to say, a variety of the 'logical empiricism' which was transplanted in the 'thirties to the United States from Vienna, where it was inspired by the teachings of the late Ernst Mach. To this summary, Prof. Lindsay adds, first, a concise discussion of 'information-theory', in its relations to language and thermodynamics; next, a brief survey of the problems arising out of the contemporary involvement of science with public affairs; and finally, an account of the author's own 'thermodynamic' theory of ethics, which revives the attempt originally made at the turn of the century by the distinguished chemist, Wilhelm Ostwald, to define an objective moral imperative in terms of (or on the analogy of) the physical ideas of 'order' and 'negative entropy'.

Having chosen to cover so much ground, Prof. Lindsay is compelled to proceed throughout at a gallop. We are taken from subject to subject at the double, and as a result scarcely one of the hundred-odd questions put up from under our feet is pursued for more than a few pages at a time. This compression will greatly reduce the book's value for serious students of the history, philosophy and sociology of science: too often, in place of cogent and careful arguments, we have to make do with summaries or with exclamation-marks (four in two paragraphs on pp. 68–69). To elementary students, the work can be recommended chiefly for the compact account it provides of the fundamental doctrines of the logical empiricist school—particularly on the axiomatic structure of physical theories. Like others of the same school, Dean Lindsay is led by his preference for this kind of logical articulation to adopt a rather patronizing attitude towards "the scientific immaturity of biology", while his chapter on the politics of science takes us little way beyond the pre-war views of Michael Polanyi and John R. Baker about the essential autonomy of science.

STEPHEN TOULMIN

Advances in Organometallic Chemistry

Vol. 1. Edited by F. G. A. Stone and Robert West. Pp. xi+334. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1964.) 88*s*.

IT is a pity that the publishers of this book have seen fit to bring out yet another new "Advances" series. There is less justification in this case than in some others, since the type of chemistry involved could well have been fitted into the publishers' own *Advances in Inorganic and Radiochemistry*—indeed, such organometallic articles have appeared there in past issues. There are also numerous other existing review journals which could easily cope with the amount of material available.

This volume contains articles entitled "Diene-Iron Carbonyl Complexes and Related Species" (43 pages, Pettit and Emerson), "Reactions of Organotin Hydrides with Organic Compounds" (39 pages, Kuivila), "Organic Substituted *Cyclo*silanes" (48 pages, Gilman and Schwebke), "Fluorocarbon Derivatives of Metals" (74 pages, Treichel and Stone), "Conjugate Addition of Grignard Reagents to Aromatic Systems" (17 pages, Fuson), "Infra-red and Raman Spectral Studies of π Complexes formed between Metals and C_nH_n Rings" (72 pages, Fritz). The general level among these rather diverse topics appears to be quite high and the type of treatment is comparable to that in other review articles elsewhere; the last-mentioned article is perhaps the weakest in that it attempts to cover too much ground and is accordingly too discursive.

The book is well produced with adequate subject and author indexes. It will be of most use to research workers in the organometallic area, although, on account of the breadth of the field, ranging over widely different types of compounds, it may obviously not be of interest for personal libraries.

G. WILKINSON

Organic Syntheses

Collective Volume 4. Editor-in-Chief: Prof. Norman Rabjohn. A revised edition of Annual Volumes 30–39. Pp. xiv+1036. (New York and London: John Wiley and Sons, Inc., 1963.) 125*s*.

DURING the past forty-two years, organic chemists have been greatly indebted to the contributors and editors of the annual volumes of *Organic Syntheses*, in which the practical details of each synthesis were independently checked by at least two chemists; there must be exceedingly few organic research workers who have not used these volumes. The production of *Collective Volumes* every nine or ten years has been particularly valuable, because it has afforded an opportunity to incorporate any modifications in the experimental detail which later experience has shown desirable.

Collective Volume 4 is dedicated to Prof. Roger Adams, "one of the founders of Organic Syntheses, Inc., and its leader during the last forty-odd years". The book contains the usual wide spread of syntheses, which, even at this stage of development, still include the preparation of simple (but toxic) compounds such as cyanogen iodide and diazomethane. A special note is made of all compounds the preparations of which are described and which have since become commercially available. There is an increased number of warning notices of hazardous procedures or chemicals, based on information supplied by the users of the annual volumes, and indeed a printed slip has been inserted to direct attention to one dangerous operation. *Collective Volume* 4, like its predecessors, is excellent and should be in every chemical library.

F. G. MANN

Switching Theory in Space Technology

Edited by Prof. Howard Aiken and William F. Main. Pp. x+357. (Stanford, Calif.: Stanford University Press; London: Oxford University Press, 1963.) 92*s*. net.

A SYMPOSIUM on "Application of Switching Theory in Space Technology" was held in California during February 27–March 1, 1962, sponsored by the American Air Force Office of Scientific Research and the Lockheed Missiles and Space Co. Twenty-five papers were presented during the course of an introductory and four working sessions; these are reprinted in full here but without recording whether there was any concomitant discussion.

About half the papers are concerned with theoretical aspects of circuit logics and switching systems, treating both analysis and synthesis of logical functions under the constraints introduced by the eventual need to turn the theory into practice. The topics include sequential digital networks, threshold logic, modular arithmetic and matrix algebra. The subject matter of papers dealing more with the practice than the theory covers engineering techniques (for example, an abrasive technique for machining ferrites), system design (for example, digital data bandwidth compressor), and switching and stor-

age devices (multi-aperture ferrites, cryogenic stores, etc.).

The quality of the papers taken as a whole is high (which perhaps is a reflexion of the fact that many of the writers are the established authorities), and all have useful bibliographies. The topics cover a wide range, from the logically mathematical to the severely practical, and it is unlikely that any one reader will be interested in them all, but this is not unusual in the *Proceedings* of symposia. In many cases the material presented is of much wider significance than the title of the book would suggest, and while the principles and techniques are undoubtedly of use in spacecraft, they could also be of considerable value in other applications where digital control and information processing are used.

G. J. Herring

Random Essays on Mathematics, Education and Computers

By John G. Kemeny. Pp. ix+163. (Englewood Cliffs, New Jersey, U.S.A., and London: Prentice-Hall, Inc., 1964.) 40*s*.

Dr. Kemeny, head of the Department of Mathematics at Dartmouth College (U.S.A.), has made many successful experiments in presenting modern mathematics to his advanced pupils. The books on *Finite Mathematics* of which he is part-author show how far he has gone in removing dead wood and in introducing and using quite difficult concepts at an early stage. In *Random Essays on Mathematics, Education and Computers* he has collected a number of his essays, all more or less grouped around his own experiences, under three headings.

In the first section Dr. Kemeny writes convincingly about mathematical values, of his efforts as a mathematical missionary, of how to encourage rigour in demonstration without stifling intuition, of why it is not undemocratic to pick out and cherish the pupil who shows himself to be mathematically gifted. These essays, like the rest of the book, are intensely human and readable, and it is difficult to resist the temptation to quote from so much wise and vivid material. Envy compels me to make one extract: in this decade, Dartmouth College Library should reach one million volumes, it takes more than one hundred mathematical periodicals, and spends more than 100 dollars a month on mathematical books; yet Dartmouth is not one of the larger colleges in the United States.

The second section deals with matters of organization, in an idiom of units and grades that will not be very familiar to the British reader, but, for example, in setting the well-rounded man against the specialist, Kemeny makes his discussion of universal interest to teachers. In the essay on "The Vanishing College Teacher", his ten points to remedy the fact that "as far as the average undergraduate is concerned, the well-qualified science professor is nearly extinct" may well be of interest to Britain's new universities. The final section considers, among other matters, the place of the computer in colleges of liberal arts and science, and the way in which computers may be expected to be of service to libraries by A.D. 2000.

This is a lively, entertaining and instructive book, which will be of value to anyone who is concerned in any way with mathematical education.

T. A. A. Broadbent

Solid Lubricants and Surfaces

By E. R. Braithwaite. Pp. viii+286. (London and New York: Pergamon Press, 1964.) 84*s*.

Since the Second World War there has been a rapid growth in the use of solid lubricants by industry, and the appearance of a text-book specifically dealing with this subject is not only welcome but also long overdue.

Research in this field embraces a number of scientific disciplines, and there is little doubt that this book will interest a wide range of readers. The author has clearly recognized this fact in compiling his manuscript, and he has devoted a number of chapters to a general introduction of friction and wear phenomena together with a survey of the many other techniques available to-day for studying surfaces. Some of this material could have been arranged with more care. One chapter, in particular, attempts to cover such diverse topics as friction and wear-testing machines, surface profilometers, X-ray and electron diffraction investigations and particle size–surface area measurements. Such close grouping requires careful attention to sub-headings, and it is confusing, for example, to be led from a consideration of surface profilometry to the question of stacking faults and other crystal defects without adequate warning.

The second half of the book provides a useful survey of the properties and uses of the many solid lubricants available to-day. The modern repertoire of the lubrication engineer is so extensive that the author has not found it possible to direct equal attention to every aspect of the field, and he has concentrated on the two most widely used materials, namely, graphite and molybdenum disulphide. The author has been particularly closely associated with the production and use of these two important lubricants, and the subject has been tackled in an authoritative manner.

The style of presentation is extremely variable, and it is to be hoped that the many clumsy passages and tiresome errors present in the text will be eradicated by the time the second edition appears. K. H. R. Wright

Location and Land Use

Toward a General Theory of Land Rent. By William Alonso. Pp. xi+204. (Cambridge, Massachusetts: Harvard University Press; London: Oxford University Press, 1964.) 44*s*.

This volume is concerned with the basic economic theory relating to the price and use of land in urban areas. It is not a book for the layman, but for all economists working in the field it is essential reading. The argument is presented mainly in mathematical terms, but Alonso goes to a good deal of trouble to make the broad sweep of his thinking clear to those who do not have a strong mathematical background, and such persons should not be put off by a quick glance at the rather formidable array of symbols and graphs which the book contains.

Alonso begins by considering the factors important in determining the demand for urban land both by enterprises and by consumers. He then proceeds to show how the forces of demand and supply will, as part of the process of clearing the market, produce a certain pattern of land use. Some of the implications of the free market solution and also some of the implications of interference with it are then considered both in a static and dynamic context.

Probably the most interesting feature of the book is the treatment of the factors determining the demand for residential land. Alonso shows in a most careful and elegant manner how the price, location and quantity of land demanded are linked together. The price of land tends to decrease as one moves away from an urban centre, but the cost of transport to such a centre goes up. Alonso shows how these two opposing influences affect the eventual demand for both quantity and location and how a consumer's expenditure on land plus transport varies in relation to his income and other expenditures. Although this is the section which is most original, the book also makes a valuable contribution to our understanding of the factors which are important in influencing the demand for urban land by traders and manufacturers, and as a whole it is a significant contribution to a difficult and somewhat neglected field. D. Walker

THE ROYAL SOCIETY

ANNIVERSARY ADDRESS*

By Sir HOWARD FLOREY, P.R.S.

AWARD OF MEDALS, 1964

Copley Medal : Prof. S. Chapman, F.R.S.

PROF. S. CHAPMAN is distinguished for his contributions to many aspects of physics, astronomy and geophysics. His work on the kinetic theory of gases now forms the basis for much detailed study of gaseous phenomena, and more than forty years ago his interest in these problems led him to consider the processes of diffusion and viscosity in stars. In addition to these important studies Chapman's name has, for more than half a century, been inseparably linked with problems in terrestrial magnetism and with a wide range of studies concerned with the high atmosphere, ionosphere and interplanetary space. From a statistical analysis of a great mass of data Chapman succeeded in demonstrating the existence of a small lunar component in the Earth's quiet magnetic field variations, and he showed that the solar and lunar diurnal variations could be separated into a predominant component originating above the Earth's surface and a subsidiary component within the Earth itself. From this he developed the general theory of the relation of the magnetic variations to the solar and lunar atmospheric tidal effects, a theory which has been fundamental to all subsequent work. After the elucidation of these quiet magnetic daily variations, Chapman began his great work on the magnetic storm effects. In a classical sequence of papers he showed how the magnetic storm variations observed on Earth could be interpreted in terms of a corpuscular stream emitted from localized regions of the Sun. This work of Chapman has stimulated a great deal of work by himself and others on the general problem of solar terrestrial relationships and particularly on the auroral phenomena. His studies have recently attained a special significance in the interpretation of the nature and consequences of the trapped radiation zones around the Earth.

Wherever one turns in geophysics or in the physics of the upper atmosphere one finds that Chapman has made fundamental contributions. For example, his theory of ionospheric layer formation is basic to the subject. He demonstrated that atomic oxygen should be dominant in the high atmosphere, and he has contributed to the theories of the ozone layer and to the problem of the night air glow. The depth and extent of Chapman's researches, which have continued undiminished for more than half a century, have brought both him and British science international renown. Indeed, his world-wide associations became of critical importance in the promulgation of the 1957–58 International Geophysical Year and he was the president of the Special Committee for the International Geophysical Year (CSAGI) from 1953 until 1959.

Rumford Medal : Prof. H. C. van de Hulst

Prof. van de Hulst is distinguished for his contributions to theoretical astrophysics. Nowadays it is unusual for a single name to be linked decisively and unambiguously with a prediction of major scientific significance, but the name of van de Hulst has this distinction. In 1944, under conditions of occupation by an enemy in which many would find scientific work impossible, van de Hulst predicted that it should be possible to observe the spectral line emitted by the interstellar clouds of neutral hydrogen in the radio wave part of the spectrum. The radiation in question arises in a transition between two closely spaced energy-levels in the hyperfine structure of the ground state of the hydrogen atom. Although, in 1944, the techniques of radio astronomy were in a rudimentary state and little was known about radio emissions from space, van de Hulst predicted that it should be possible to observe this 21-cm spectral line from the interstellar clouds of neutral hydrogen. In 1951 this line radiation was detected almost simultaneously in Holland, the United States and Australia. The episode marked the vindication of van de Hulst's predictions and immediately opened observational possibilities of great importance in astronomy. The existence of clouds of neutral hydrogen gas in the galaxy had been presumed, but no means of observing them had been available. The ability to measure the frequency displacement of the line caused by the motions of the clouds with respect to the solar system quickly led to a great clarification in our knowledge of galactic structure.

The consequences of van de Hulst's work have extended beyond the galactic system, and a great increase in our knowledge of the extragalactic nebulæ has resulted from the recent studies of the hydrogen line emission from the Magellanic Clouds, the *M*31 nebula in Andromeda and several other extragalactic systems. Further, the Zeeman splitting of the line has been measured in the gas clouds of the Milky Way and the galactic magnetic field thereby determined. Van de Hulst's prediction and subsequent work on this spectral line from neutral hydrogen have somewhat overshadowed his other contributions. He is, however, apart from this, distinguished for his theoretical work on the scattering and interaction of light and particles in the solar corona and interstellar space. Van de Hulst is a person whose great insight into physical processes has been matched by an overall integrity which has led him to a position of high esteem in the international community. In particular he was elected the first president of the Committee on Space Research (COSPAR) and he served in this capacity with distinction through many years of difficulty.

A Royal Medal : Prof. M. J. Lighthill, F.R.S.

Prof. Lighthill's earliest papers were in the field of supersonic flow, where he provided, for the first time, a rigorous theory for thin wings of finite span and for slender bodies of revolution. In later work he adopted a non-linearized theory, applying it to the effects of blast waves and the position of a detached shock wave in front of an aerofoil. Later still he initiated the aerodynamic theory of gases ionized by passage through a shock wave. In these and other problems Lighthill has shown great power in discussing the solutions of a non-linear differential equation with a singular perturbation. It was in this way that he was able to place on a firm basis the domains of analyticity of the various partial solutions which arise, and solved a number of important problems in boundary-layer flow and shock-wave theory.

Among Lighthill's most important papers are those which first gave the theory of the aerodynamic generation of sound. This has had a profound influence on the design of 'silencers' for jet exhausts. In the field of classical, that is to say, low-speed, aerodynamics Lighthill has studied the theory of jets and cavities, and developed a new method for the design of aerofoils. He has also provided a theory of the swimming of nearly spherical organisms by means of a 'squirming' motion. Prof.

* Delivered at the Royal Society on November 30.

Lighthill's interests have recently penetrated into regions of pure mathematics, where he has made significant contributions to the generalized theory of Fourier analysis. It is rare to find someone so thoroughly at home both in the applied world where engineers need guidance in the design of important apparatus, and in the pure mathematical world. His ability to bring these two worlds together shows him to be one of the outstanding mathematicians of our day.

A Royal Medal : Prof. F. W. Rogers Brambell, F.R.S.

Prof. Rogers Brambell and his research school are widely acknowledged to be the world's foremost authorities on the problems of how protein molecules enter the mammalian fœtus from the mother. The work that earned Brambell this high reputation began in the Second World War when he was trying to work out the causes of the unexpectedly high mortality of embryonic rabbits. He soon found that maternal proteins—fibrinogen and globulins—were among the normal contents of the embryonic yolk sac. How did they get there? Did they reach the embryo itself? Brambell's first major contribution was to show that the pathway of entry of maternal protein was not the placenta, as everyone had until then assumed, but the wall of the yolk sac; maternal antibodies, for example, were secreted into the uterine lumen and passed through the wall of the yolk sac into the fœtal circulation. The same turned out to be true of guinea-pigs and—before birth—mice and rats. In man and primates generally, the size and anatomical relations of the yolk sac showed it could not fulfil that function; in them the placenta might indeed be the route of entry.

The second of Brambell's major contributions to the analysis of the relations between mother and fœtus was his study of the 'selective' entry of proteins into the fœtus or infant rodent. Discrimination between one protein and another was certainly not based on mere molecular size, for γ-globulin entered more rapidly than the smaller molecules of albumin, and the fragments of γ-globulin produced by digestion with papain sometimes entered more slowly than the parent protein. 'Foreignness' had something to do with it, since in the main homologous proteins were admitted more readily than foreign proteins. Discrimination was not based on the exclusion of some proteins and the admittance of others, for proteins of all types actually entered the cells of the endodermal membrane; what happened was that some proteins passed through to the fœtal blood with their biological activity unimpaired, while others were broken down. The investigation is still in progress.

Brambell's own work and the work he has inspired in a group of distinguished colleagues and pupils have made use of the methods and ideas of experimental surgery, embryology, immunology, biochemistry and biophysics. It throws light not merely on the physiological rapport between mother and fœtus, but on every situation in which there is a selective transmission of large molecules across biological membranes.

Davy Medal : Prof. M. Calvin, For.Mem.R.S.

As a Nobel Laureate and a Foreign Member of the Royal Societies of London and Edinburgh, it is scarcely necessary to give a detailed description of the reasons for the award of the Davy Medal to Prof. Melvin Calvin, whose achievements in the fields of physical, organic and biological chemistry have won for him international recognition.

He was co-author of the first comprehensive book on the theory of structural effects and of reaction mechanism. His contribution to our knowledge of homogeneous and heterogeneous catalysis is outstanding in the extent to which he explored the border regions between organic, inorganic and physical chemistry and biochemistry, leading to the solution of problems of profound importance in biology. He initiated many enquiries, all characterized by breadth of concept and penetration of method, into the biosynthesis of peptides, nucleotides and lipids, but his name is probably best known in connexion with his work on the mechanism of photosynthesis in the higher plants, algae and the photosynthetic bacteria. These investigations have involved the development of delicate isotopic tracer techniques and are characterized by an ingenious co-ordinated use of methods derived from analytical, physical and organic chemistry demanding a profound appreciation of the underlying fundamental principles. Calvin and his colleagues have succeeded in delineating most of the processes involved in the linked assemblage of reactions by which carbon dioxide is assimilated by plants and transformed into carbohydrates and oxygen; and, in addition, by his investigations into the role of thio-octoic acid, he has linked up the photosynthetic cycle with the Krebs cycle.

Calvin's work has already had, and is continuing to have, a profound influence on the approach to both chemical and biological problems.

Darwin Medal : Prof. K. Mather, C.B.E., F.R.S.

Prof. Mather is distinguished for many original contributions to genetics, but more particularly for his study of quantitative characters and their significance in evolution and development. Prof. Mather's early work included the discovery and interpretation of interlocking chromosomes which provided important evidence for the understanding of the mechanism of crossing-over. He was one of the first to use X-rays in the study of chromosome replication and, in a different field, he made important contributions to the genetics of natural breeding systems, a subject of great interest to Darwin.

In the study of quantitative inheritance, in which the characters do not show discontinuous variation and therefore are not amenable to Mendelian analysis, Prof. Mather has developed an analytical tool whereby the components of variation, both heritable and non-heritable, may be estimated. With this method it has also been possible to detect gene interactions of the types found in Mendelian analysis. The work has led to the theory that heritable differences in quantitative characters, such as height, depend on numerous genes each with effects similar and supplementary to one another, but individually small in relation to the non-heritable variation. Using different methods of selection, important contributions to other aspects of genetics and evolution have been made. A system of disruptive selection tends to split a population into locally adapted groups and this can lead to polymorphisms such as are frequently found in many species, including man. In a different way, disruptive selection can lead to instability in development of the individual. These selection experiments have shown that both segregation of a population into discrete groups and the orderly development of an individual are under genetic control.

Prof. Mather's wide-ranging research is of special interest and value to plant and animal breeding, to genetical theory, to the understanding of the mechanism of evolution and to the control of development.

Sylvester Medal : Dr. Mary L. Cartwright, F.R.S.

Dr. Mary Cartwright is distinguished for her contributions to the theory of functions (particularly integral functions) and differential equations and topology.

After a few years of school-teaching, Dr. Cartwright returned to Oxford as a research pupil of Hardy; she also acquired and maintained an interest in the work of Titchmarsh. The intensive cultivation of the theory of integral functions stems from Riemann's famous hypothesis (1859) that a particular integral function (immediately related to the zeta function) has real zeros only. The theory, having among its founders Weierstrass, Picard and Hadamard, has attracted the efforts of the

leading analysts up to the present day, and Dr. Cartwright has earned an honoured place in the list. To break new ground in this deep and elaborate theory has needed great analytical power. A few of the headings of her work, much of which is based on results of Phragmén and Lindelöf, are: (1) zeros of integral functions; (2) maximum and minimum moduli; (3) functions of finite order in an angle; (4) directions of Borel.

Several of Dr. Cartwright's outstanding contributions, particularly those dealing with maximum and minimum moduli, have given rise to great advances by herself and others. The shadow of war deflected Dr. Cartwright's work into a different field. Early in 1938 the Department of Scientific and Industrial Research circulated a memorandum to pure mathematicians appealing for theoretical guidance in investigating solutions of non-linear differential equations encountered by physicists and engineers dealing with defence problems. Well-known equations of Appleton and van der Pol were among those which awaited a more substantial basis of pure mathematics. The appeal received a handsome response. A stream of weighty papers by Littlewood and Cartwright, jointly and individually, has continued until to-day. They show that a proper understanding of non-linear equations needs topological insight as well as powerful analysis. A powerful fixed point theorem due to Littlewood and Cartwright plays a vital part in the authors' proofs of the existence of periodic and almost periodic solutions of differential equations. At the same time, with Sir Edward Collingwood, Dr. Cartwright was making fundamental contributions to the modern theory of cluster sets.

Dr. Cartwright has proved that the administrative and social duties of the Mistress of Girton are compatible with the advancement of knowledge by profound discoveries in mathematical analysis and topology. By the depth of her discoveries as well as their extremely wide range she has established herself as one of the world's leading mathematicians and, among her sex, the leading one.

Hughes Medal : Prof. A. Salam, F.R.S.

There are very few physicists in the world who have maintained such a constant and fertile flow of brilliant ideas as Abdus Salam has achieved during the past thirteen years. His Ph.D. thesis, published in 1951, contained fundamental work in quantum electrodynamics (renormalization theory), which had already gained for him an international reputation of a very high order. On two subsequent occasions he has made contributions to the theory of elementary particles which are in the absolutely highest class.

In 1957, Salam proposed a deep and intimate connexion between the zero rest mass of the neutrino and the parity non-conserving effects in weak interactions. This theory makes a large number of very specific predictions about neutrino properties, and of the way in which parity is violated in neutrino interactions, all of which have since been confirmed by experiment. In 1961, Salam, in collaboration with J. C. Ward, proposed that the strong interactions of elementary particles should satisfy unitary symmetry. This implies that the particles and resonances should appear in super-multiplets of the same spin and parity, and well-defined isotopic properties. In particular, Salam and Ward predicted an octet of spin one mesons, playing an analogous part in strong interactions to the photon in electrodynamics. All these mesons have since been found, and the general ideas of the theory recently confirmed by the spectacular discovery of Ω^-.

These two pieces of work are both contributions to the basic laws of physics. They are not isolated achievements, but the high points in more than ten years of prolific and sustained performance. During this period Salam has published about a hundred papers and maintained his position at the fore of this rapidly advancing frontier of the fundamentals of the subject.

PRESIDENTIAL ADDRESS

Administration

A case can be made out that Council does not keep the Fellows adequately informed of what is being undertaken on their behalf within a reasonable time of its taking place, and in this connexion an enquiry is at present being made to see if practical means can be devised to overcome what at least some Fellows consider to be a shortcoming in the administration of the Society. But the lack of a regular explanatory news bulletin gives me a chance on November 30 to consider in more detail some of the activities of the Society which are somewhat baldly chronicled in the annual report of Council.

This year there are three matters I should like to direct particularly to your attention—the progress in providing new accommodation for the Society, the scope of the Fellowship, and the relationship of the Society to the organization of science.

Accommodation

Last year I gave a description of the difficulties of accommodation that have faced the Society on and off ever since its foundation, and I was able to say that most of the problems of moving from Burlington House to Carlton House Terrace were on the way to solution*.

You may remember that at this time last year a number of generous donors had undertaken to give us £361,500 towards the £500,000 that was estimated to be necessary for the suitable conversion of the interior for our purposes. I am very happy to say that the Goldsmiths' Company has now offered a very generous gift of up to £50,000 for the reconstruction of that part of the building which will contain the Council Room and the President's and other offices. We are extremely grateful to the Company for this magnificent benefaction which will bind still further the Royal Society of London to one of the great City Companies.

The social amenities have been further assured by a gift of £5,000 for what might be vulgarly called a bar, but which in fact will be a very pleasant meeting-place for the Fellows. We are indebted to Sir Ellerton Becker for this benefaction, and this gives me particular pleasure, for Sir Ellerton has already placed science in his debt by his splendid contributions to the building of the Australian Academy of Science in Canberra.

Those who are mathematically minded will calculate that we still need some £83,500 to reach our target of £500,000, and I hope that this gap can soon be closed.

During the year a building committee of Fellows has been working with Sir William Holford and his associates on plans for the alteration of the interior. These are now complete in outline, and a final examination of the condition of the interior, now that the houses are vacant, is being made. I hope that it will be possible some time next year to arrange for the plans to be exhibited to the Fellows. If all goes well building operations should start in about nine months' time, and we should be able to occupy our new apartments in eighteen months to two years after that. I hope you noted the first phrase, "if all goes well", because I have been impressed during the past year with the very many hurdles, often of a trivial character, that have to be surmounted in such an undertaking. There can be no flying leaps in this business, so that I hope that Fellows will not become impatient with the apparently slow progress that has so far been made.

Scope of the Fellowship

During the long history of the Society the composition of the fellowship has by no means been uniform or stereotyped. Indeed, we take pride in the fact that among the early Fellows were men such as Evelyn and Pepys as well as those more directly concerned with the emerging natural philosophy.

* *Nature*, **200**, 1256 (1963).

When I look out of my study window at Queen's College, Oxford, I can see a row of statues in the façade of the library. Among the kings, queens and clerics stands Joseph Williamson, our second president, who, although he was a Fellow of Queen's, could not by any stretch of the imagination have been called a scientist nor indeed even a distinguished scholar. He was in one sense a man of business and a not unsuccessful one at that. It is unnecessary now to go into the question why he, a non-scientist, was made president of a scientific society or why, by the eighteenth and early nineteenth centuries, more than half the Fellows of the Society had no scientific qualifications whatever. It is sufficient to note that in the middle of the nineteenth century the Society, adapting itself to the growing importance of scientific studies in the life of the nation and the increasing number of admirable scientists then being educated, reformed itself, so that before the end of the century it consisted of active scientists and Council contained no one who was not closely connected with the practice of science.

At the end of the nineteenth century the big decision was made to set up the British Academy, which was to be composed of Fellows engaged in activities now loosely described as arts subjects which were not then considered to be of importance or even of interest to the Fellows of the Royal Society. It was to look after disciplines not then recognized among the strictly natural sciences. This division, which is not paralleled in the structure of most foreign academies, leaves some areas in which the interests of the British Academy and the Royal Society overlap.

Fellows of our Society have for many years been elected by machinery which, though no doubt imperfect, gives scope for the Fellows at large to determine who shall join them. That this has been moderately successful is perhaps attested by the fact that Fellows who still retain a vestige of modesty are surprised and gratified when they are elected, but that with the passage of time they begin to detect signs that the scientific attainments of newly elected Fellows are not quite what they were in previous generations. This attitude of mind is by no means to be deprecated, for it is a powerful support to the determination of the fellowship not to let the standard of attainment of the Fellows decline. It is one reason why proposals for increasing the number of Fellows elected annually are closely scrutinized by the Fellows.

You might like to be reminded of the number of Fellows elected annually during the past hundred years or so. Up to and including 1847 the numbers were unlimited, but in 1848 the number of annual elections was limited to 15, in 1931 it was raised to 17, in 1938 to 20 and in 1946 to 25. This year, after many discussions, it was decided by the due processes of the Society's constitution to increase the number of annual elections from 25 to 32.

It may be as well to have on record some of the arguments that led to this decision, not only for our own purposes but for the information of the public interested in scientific matters and, in particular, in the part that the Society can play, if it wishes, in the expanding complexity of modern social organization.

It is commonly believed that present-day civilization is becoming more and more science-based and that the speed of change is greater than the human mind can comfortably cope with. What we really mean is that technology, which is based on science, is changing our lives. The study of the elucidation of the structure of the atom can perhaps be considered as a philosophical abstraction, but when technology translates the resulting discoveries into electronic gadgets our lives are profoundly, and so far on the whole agreeably, altered. Thus it is the technologist who is transforming at least the outward trappings of modern civilization, and no hard-and-fast line can or should be drawn between those who apply science, and in the process make discoveries, and those who pursue what is sometimes called basic science. The relative increase in the number of applied scientists, and the increasing importance of their discoveries, has directed the attention of many Fellows to the question whether the Society was taking sufficient note of present-day developments. In the eighteenth century the Society reflected the general lassitude of scientific performance; in the nineteenth century it adapted its components to the great increase in scientific activities characteristic of that century; and it can now be maintained that we must reflect in an adequate way the driving forces that are so rapidly changing our physical environment. These forces are to a large extent technological.

We are fortunate and privileged to have among our Fellows some of the most distinguished engineers and metallurgists in the country; all are Fellows who have made outstanding contributions to their subjects. Nevertheless, it is certain that there are many applied scientists, particularly in government and industrial environments, who if they became Fellows would greatly strengthen the Society in its relationship to the contemporary milieu. It is for the Society to devise machinery by which their originality and contributions to contemporary civilization can be assessed as against the more familiar claims of those who have hitherto formed the great majority of our potential Fellows.

We still have to work out in detail the best procedure to adopt in assessing the merits of those whose principal field of work is in technology, but I am convinced that it is by no means impossible to solve the problem to everyone's satisfaction.

We should not conceive of applied scientists as being those associated particularly with the physical sciences. Perhaps it is the biologists and those dealing with human characteristics who will have to obtain and apply new knowledge if we are to have lives which will be really satisfactory and not an unendurable succession of frustrations. Calculations have been made that if the present rate of increase of population should continue, our descendants in 600 years' time will each have one square yard of land on which to conduct their affairs. This is manifestly absurd and something is bound to happen before that time, but what? It is all too easy for both politicians and scientists to leave a problem like this alone until the position is disastrous. More than frequent loud cries and metaphorical wringing of hands is now required. Ought we as a Society to be considering how science and scientists can contribute to a solution of the great problem of bringing the human population into satisfactory, even if dynamic, equilibrium with its surroundings? Or should we wait for these matters to be tackled by people who may have little connexion with us? I have no doubt myself that we should try to lead scientific advances by positive action. Of course, we are quite accustomed to doing so in many time-honoured and valuable directions such as the mounting of expeditions to distant lands, or participating in international 'years' or 'programmes', but is it wise for the Society at this time not to have among its Fellows those skilled in demographic work or social anthropology, or even a substantial representation of psychology and psychiatry? The investigation and understanding of the psychology and even the psychiatry of rapidly expanding populations may well be of paramount importance in the near future.

The Society has not in the past been irrevocably determined not to have anthropologists among the Fellows—one can mention *Golden Bough* Frazer—nor has it been implacably against psychiatry, for Freud was a Foreign Member. However, if the standard applied in these instances were used for judging Fellows in the more usual subjects we could safely diminish the number of annual elections to not more than five.

It would perhaps be well for us not to adopt the frame of mind of our illustrious Founder who, you will remember, made fun of those who weighed air.

It was considerations of the kind I have just outlined that led Council to put before our corporate body the desirability of increasing the number of Fellows to be elected annually. It is not the policy of the Society to allot a special number of places to any one subject or group of subjects, but it was the firm intention of this year's Council that a substantial number of the new places should be given to applied scientists on both the physical and biological sides, and that consideration should be given to electing some Fellows in some of the disciplines at present not represented or, if represented, inadequately so.

The Society by its present action is deliberately proclaiming its special interest in the marvellous developments of modern technology and in those who are responsible for them. We are confident that new technologically oriented Fellows will strengthen the Society, and we also hope that our increased interest in these matters will go some way to convince our technological colleagues that we are interested in them and all they mean to the nation.

It has been argued and indeed publicly proposed that Britain needs a new Academy, similar to the Royal Society, but devoted to the application of science, particularly in engineering. The feasibility of such a proposal clearly depends in the first instance on whether applied scientists feel the need for a body with the structure of an Academy which would look after their particular interests and which could serve the country during the coming critical years. My personal view, for what it is worth, is that the Royal Society, having made its present dispositions, should watch the position carefully in the next year or two and should not shut its mind to considering how it can best help in furthering technology if there should be a genuine and widespread demand for such action. What forms this further help might take I am not prepared to say, but as the problem of dealing with the growing importance of applied science is not confined to Britain it would be advisable to pay some attention to what is happening elsewhere.

The last thing I believe we want to see in Britain is two possibly antagonistic Academies representing what might come to be thought of as incompatible interests.

In the meantime, to emphasize that the Royal Society, while not diminishing in any way its interest in the more traditional natural sciences, is in earnest about widening its scope, there are to be four special occasions during next year at which two lectures on applied science will be given particularly for the information of Fellows, and there will be two lectures on economics and social anthropology, financed by a generous donation from the Nuffield Foundation. It is planned to arrange a similar series of lectures for 1966.

In conjunction with the Engineering Institutions Joint Council, the Society is arranging a conference for headmasters and others interested in education, with the object of letting it be known as widely as possible that the applied sciences offer just as splendid careers as the so-called 'basic sciences'.

Government and Science

The Society, ever since its foundation, has given advice to the Government when called on to do so. Indeed, we owe our rent-free accommodation to the fact that H.M. King George III was very much gratified at the assistance that the Society gave him.

In the penultimate paragraph of his last anniversary address, Sir Cyril Hinshelwood said: "There are plenty of problems ahead. For example, our long, and, though informal, immensely important relations with the Government are in process of adaptation to the vast scale and complexity of scientific activity to-day. The optimum solution of this problem is of the greatest importance both to the Society and to the country. Of equal importance is the establishment among the Fellows of the right balance between the pure and the applied sciences to enable the Royal Society to maintain its full influence in the modern world". You will have noted that we have done something about his last suggestion, and I should now like to spend a few minutes on his first.

We are at a stage in the organization of science in which the involvement of Government in the affairs with which scientists traditionally deal, and the dependence of science on the public purse, introduce important problems because the days have largely passed when scientific enquiry is conducted by individuals in isolation—indeed, in many respects the ability to conduct research at all now depends on complicated Government organization.

In the present age there has been an efflorescence of Government-sponsored councils, committees, boards and authorities, and on these many Fellows of the Society sit and thus help science and the country by their advice and activities in one way or another. But the fact must be faced that because of the establishment of these numerous bodies to which the Government turns for advice in the first instance, the direct relation of the Royal Society to Government has been diminishing during the development of the scientific activity of the twentieth century.

It thus becomes of particular interest to examine what part the Royal Society can play specifically in the contemporary scene, or at least in that part of it which I am going to mention to-day.

Scientists are perhaps becoming more self-conscious about their activities and their aspirations, and about how these can optimally be fitted into the social structure. The Society as a collection of individuals from a wide spectrum of scientific activities has the advantage of being closely in contact with those who actually make discoveries, and I hope that with our new arrangements for increased representation of applied scientists in the Society we shall be in even closer touch with technology than we are at present. It may well be that we can increase our usefulness to science if we review at this time the methods by which we can furnish information and advice to the Government on a variety of topics if it so wishes.

During the past few years the Council of the Society has made its opinions known to Government about the way in which it would like to see governmental science organized, and I am happy to say that in broad outline the proposals of the present Government have taken note of the opinions that we have expressed. The pattern is emerging that there is to be a Secretary of State for Education and Science with a Minister of State who deals with the universities and Research Councils. These Ministers are to be advised by a Council for Scientific Policy which will be concerned with the consideration in broad terms of how the nation's resources are to be deployed for civilian science. In addition to the Medical and Agricultural Research Councils there is to be a Science Research Council, which will take over some of the functions of the Department of Scientific and Industrial Research, and a Natural Environment Research Council. In addition there is a Minister of Technology who is to have an advisory body composed of scientists and others. The object of this arrangement is to see whether further impetus can be given to our use of technology, and that not necessarily of the so-called 'advanced' variety.

These bodies with their numerous sub-committees now require the time and energy of a substantial number of scientists, and this imposes a severe dilemma on many of our colleagues who are still very active in their research and do not wish to be swallowed up in an administrative machine. Some scientists have for this reason a most difficult problem to face in determining the allotment of priorities for the time and energy they have available.

I am sure that Fellows would like to see the establishment of a body or bodies which would have their confidence and on which they could rely to see that organizational matters were kept constantly under review, in order that big decisions on the deployment of national resources for scientific and technological purposes should only be taken

after the best possible advice had been given to politicians, and in order that reasons for decisions should be made clear. But the supply of suitable people for these advisory jobs is somewhat limited, and it will become still more limited if many more good scientists become vice-chancellors, for I am sure it has not escaped your attention that the last three to be appointed are distinguished Fellows of the Society.

Nevertheless, over the years it has been well established that scientists are prepared to devote some of their time to helping the organization of scientific effort at the Government level. We have probably all sat on various committees and councils and tried to do our best in the interests of science and of the country. Many of us have kept up with the products of that menace of modern civilization, the duplicator, by hastily thumbing over the papers for a meeting in a train, or, if we live in London, at the meeting itself.

We should perhaps be asking ourselves whether the complications of modern organization and the size of Government commitments to science and technology any longer permit this somewhat amateur approach. But our Fellows should be aware of what they may be attempting by becoming more involved in Government, for, as R. C. Wood has said: "Men steeped in the tradition of empirical investigation undertake to compete with men versed in the dialectic whose scholarship is heavy with syntax and intrinsically barren of the scientific perspectives of even the social sciences. Men oriented towards the concept of change face men who by tradition are history oriented". Nevertheless, I consider it the duty of the Fellows of the Society to do all they possibly can to assist Government in the present somewhat critical stage of Britain's development.

Perhaps one of the services that the Society could now render to science would be to initiate a study of just how scientific opinion is brought to bear on government, so that we can have some clearer guide than exists at present through the complexities by which opinions are formed and decisions reached on scientific matters.

The Society as such has assumed no stance in the matters that I have been discussing. I merely wish to convey to you that active thought is being given to them, for they may well be of great importance to the well-being of science in the future. One reason for the Society to take an increasing interest in these matters is that the direct link with the Treasury which has existed for more than a hundred years has been broken, for we now deal with the Department of Education and Science. I cannot let the occasion pass without recording the Society's gratitude to the many able and sympathetic men it has dealt with in the Treasury. I am happy to say that we enjoy good relations with the Government, and I am sure that, while not becoming an arm of Government, we can perform a useful national service by maintaining a close collaboration with those who control our destinies.

In spite of some new commitments the traditional activities of the Society are not diminishing, indeed it can be said that we have had more than the usual number of stimulating discussions and lectures during the past year or two. As you know, the work of the officers has been spread by the appointment of what are termed active 'vice-presidents'. Prof. W. T. J. Morgan has been one of these during the past three years, and the Society owes him a great debt for the service he has given, particularly as chairman of the Hooke Committee which helps so much with the arrangements of our meetings. Although he is no longer to be on Council he has consented to continue as chairman of the Hooke Committee. We are all indebted to him and I wish to thank him personally for the help he has given me, always in such a cheerful fashion.

The other active vice-president, Prof. H. W. Thompson, is also ceasing to be a member of Council. He has been of the greatest assistance to the Society, especially when he acted as 'A' Secretary during the absence of Sir William Hodge. In a wider sphere, as president of the International Council of Scientific Unions, he is rendering a great service to international science, and I hear his praises sung in many quarters for the vigour and the inspiration which he is putting into this task. We wish him well in the continuance of this work and thank him for what he has already done.

Although the officers may at times consider that they are working hard, I hope that we never lose sight of the fact that any increase in the Society's activities places great additional burdens on Dr. D. C. Martin and his staff who, as always, have laboured mightily to sustain the work of the Society. We can count ourselves very fortunate to have such an able and experienced executive staff. We are all profoundly grateful to them for the way in which they help us in so many ways, and I am sure you would wish to convey your thanks to them on this our Anniversary Day.

THE COUNCIL FOR NATIONAL ACADEMIC AWARDS

THE Council for National Academic Awards was established by Royal Charter in September 1964. Its president is Sir Harold Roxbee Cox, and there are 21 members. The Secretary of State for Education and Science has appointed Mr. C. R. English and Mr. J. A. R. Pimlott as assessors, and Dr. J. G. Strachan has been appointed an assessor by the Secretary of State for Scotland.

The Council has powers to award first and higher degrees to students in educational establishments other than universities. It intends to use the powers given to it, and its work will thus extend beyond that of its predecessor, the National Council for Technological Awards, whose responsibilities it will take over. The latter has, with outstanding success, developed awards in technology at honours degree level for students at educational establishments other than universities: the Robbins Committee stated that the Council has been one of the influences which have brought the work of the colleges of advanced technology to its present high standards. The new Council intends to continue the work of the National Council for Technological Awards and to extend the provision of awards at honours degree level to other fields of higher education outside the universities. At the same time the Council intends to give attention to the desirability of degrees of a different type and at a different level as mentioned in the Robbins Committee's Report.

It will thus be apparent that, from the outset, the Council will need to consider a number of matters of the highest importance relating to the development of higher education in Great Britain. Thus it would be unrealistic to expect the Council to announce in the immediate future its final policy on all the aspects of higher education with which it will have to deal. At the same time, the Council appreciates it is important that the degrees it will offer should make a significant contribution to solving the problems which will arise during what the Robbins Committee described as the "short-term emergency" when children born in 1947 and the years immediately following become of university age. Therefore, it is the Council's intention to publish statements from time to time indicating those matters on which it has reached decisions and those which are being considered, with the view of enabling colleges to submit proposed courses to the Council as soon

as possible and to plan other courses which they expect will lead to the Council's degrees.

The Council wishes colleges which will offer courses leading to its degrees, and other interested organizations, to have an opportunity of contributing to its discussions, and, as will be seen, it invites comment on a number of the matters mentioned later in this article. The Council believes that the most useful form of comment from colleges would be brief statements of the appropriate courses which they have in mind as leading to the Council's degrees: it would be convenient to the Council if such statements dealt very briefly with the aims of a proposed course, the way in which the course is intended to achieve those aims, the entry qualification to the course, and any other items which the college thinks will enable the Council to make a preliminary appreciation of the proposed course.

The Council has decided, in the first instance, to set up two Boards of Studies to be known as: (1) the Committee for Science and Technology; (2) the Committee for Arts and Social Studies. This is not a final decision for all time; the Council will create further Boards of Studies if its work makes this essential.

The Council will not expect these committees to be able to deal with other than broad questions of policy relating to the nature of courses, examination arrangements, etc.; therefore, subject Boards working to the committees will also be set up. For example, working to the Committee for Science and Technology there will be an Electrical Engineering Board, a Chemistry Board and so on. These Boards will exert a powerful influence in setting and maintaining the standards of the Council's degrees in the several fields of study.

The Council appreciates that schools, colleges and present and prospective students will be anxious to know as soon as possible what degrees the Council will make available. The Council is giving high priority to a decision about the titles of its first degrees and hopes to give more information at an early date. The Council is empowered by its Charter to grant degrees to holders of the Diploma in Technology and to students who complete successfully such diploma courses which they are now attending; the Council will exercise these powers when establishing any new scheme for degrees.

The Council will take over the Diploma in Technology scheme. Until a further announcement is made about the degrees which the Council will grant to students who successfully complete Diploma in Technology courses, recommendations by colleges for the award of Diplomas in Technology to successful students should continue to be submitted in the normal way to the secretary of the National Council for Technological Awards.

Apart from any changes in the title of the award granted, the Council will continue the Diploma in Technology scheme in its present form, leaving any possible changes to emerge as the Council gains experience of the working of the scheme.

The Council is anxious that there should be no pause in the consideration of proposed new Diploma in Technology courses and is able to announce that the Boards of Studies and Subject Panels of the National Council for Technological Awards have agreed to consider proposed new courses until the Council's Committee for Science and Technology and subject Boards have been established. The Council hopes that this arrangement may need to continue for only a short period.

What is stated here about the Council's degrees applies to Scotland as well as England and Wales. Up to the present certain Scottish colleges have offered full-time courses in technology leading to their own associateships and not to the Diploma in Technology. The Council's Charter gives it power to grant degrees to students who have successfully completed or who are at present attending those courses at Scottish Central Institutions which lead to an award comparable, in the opinion of the Council, to the Diploma in Technology. The Council is considering this matter and a further announcement will be made as soon as possible.

The Council will make degrees available for students completing courses at honours level in science and technology whether organized on a full-time or a sandwich basis. The Council hopes that colleges will continue to develop the sandwich courses so successfully established under the auspices of the National Council for Technological Awards and that they will not be prevented from doing so by a shortage of places for industrial training. Colleges are invited to submit proposed courses for the Council's consideration; principals wishing to do so should consult the Council's registrar and secretary about the mechanism for the submission of the courses; but, in general, submissions should, in the first instance, be on the lines used for the Diploma in Technology courses.

As soon as a Business Studies Board has been established the Council will consider proposed honours courses in business studies planned in accordance with the report of the National Advisory Council on Education for Industry and Commerce entitled *A Higher Award in Business Studies* (the Crick Report). Colleges are therefore invited to submit proposed courses for the Council's consideration. Principals wishing to submit proposed courses should consult the Council's registrar and secretary about the mechanism for the submission of courses; but, in general, submissions should, in the first instance, be on the lines used for Diploma in Technology courses.

While the Council will not preclude full-time courses in Business Studies from leading to its degrees, it will, as a first priority, consider sandwich courses of the type recommended in the Crick Report.

If a large volume of courses in business studies is submitted for consideration the Council envisages that it may have to give priority to the consideration of courses submitted by those colleges which have already had some experience of conducting advanced courses in business studies.

The Council believes that colleges will be looking to it to provide degrees in a variety of arts subjects and the social sciences, and hopes that its Committee for Arts and Social Studies will be able in the near future to advise it on the types of courses in those fields of study for which it should grant degrees. The Council is sure that any comments which colleges, teachers' organizations, professional bodies and other interested organizations may care to submit would be of great value to the Committee in its deliberations.

Experience seems to indicate that colleges may wish to offer courses which extend over more than one field of study, for example, courses embracing studies in technology and social sciences. The Council would like to give thought to such courses and would be interested to hear the comments of colleges and other interested organizations who may wish to submit observations.

The Council proposes to examine the case for the development of courses in science and technology at ordinary degree level and would be interested to see outline proposals from colleges which have in mind to offer courses at this level.

Undoubtedly the Council will for some time be preoccupied with the consideration of the honours degree courses recommended in the Crick Report, and there may be a delay before the Council can examine the case for ordinary degree courses in business studies. However, if a college submitting a proposed honours course on the lines of the Crick Report intends to offer in the future also a complementary course at ordinary level, the Council would be interested to see outline proposals of the latter at the same time as it examines the honours course.

The Council believes that part-time students should have the opportunity of gaining its degrees. Further consideration will be given to the nature and length of the part-time study which will be required.

In addition to granting degrees the Council is empowered by its Charter to grant diplomas and certificates comparable with those awarded by universities. The Council will consider the ways in which this power shall be exercised.

The Council believes that it will need to offer higher degrees both at master and doctor level. However, some time may elapse before the Council can make a firm statement on this point, since it must obviously give first priority to the establishment of its first degrees.

The Council will ensure continuity of the award of membership of the College of Technologists by taking over this work from the National Council for Technological Awards as soon as this can be arranged, and details will be announced in the very near future. As part of the long-term consideration of the higher degrees it will offer, the Council will consider whether in due course one of its own higher degrees shall be granted for programmes of work of the type which now lead to the award of membership of the College of Technologists. If any changes are made these will apply also to the holders of the award and to students working for it.

Further information, including fees, etc., can be obtained from the registrar and secretary, Mr. F. R. Hornby, Council for National Academic Awards, 24 Park Crescent, London, W.1.

THE *SPARTINA* STORY

TO-DAY, even the layman is often aware that a vigorous new maritime grass has become increasingly widespread around the coasts of Britain, and now forms conspicuous swards on many tidal flats. It is less commonly realized, however, that this large and aggressive newcomer, of the genus *Spartina*, was completely unknown a century ago. Under the title of "The *Spartina* Story", current work concerning its origin, present ecological status and economic value was brought together at a meeting of Section K (Botany) of the British Association for the Advancement of Science on August 26 at Southampton; since the grass originated on the shores of Southampton Water, the location of the symposium was particularly apt.

The symposium was introduced and chaired by Dr. C. E. Hubbard (deputy director of the Royal Botanic Gardens and keeper of the Herbarium at the Royal Botanical Gardens, Kew), who discussed the general taxonomy and distribution of the grass; Dr. C. J. Marchant (also of Kew and formerly of the University of Southampton) considered its origin in the light of recent cytological investigations; Dr. J. M. Lambert and Prof. W. T. Williams (both of the University of Southampton) dealt respectively with ecological work on the grass and with possible physiological causes of the incidence of 'die-back' in some swards; and Dr. D. S. Ranwell (Nature Conservancy) summarized the broader aspects of the establishment of *Spartina*, with particular reference to its use for economic purposes and its general significance to man.

The story formally begins with the recognition and description of the grass as a new species, under the name of *Spartina townsendi* (later *townsendii*), by the brothers H. and J. Groves, from material collected near Hythe in 1878. Investigations have since revealed that previous collections were made in 1870 and possibly even before, and that the early material (including the type specimen) consisted of sterile plants. Later collections, however, were principally of a more robust, fertile grass, and—pending taxonomic revision—the name *Spartina townsendii* is commonly still used indiscriminately to cover both sterile and fertile material.

From the time of its first discovery as a hitherto unknown British plant, there has been much speculation concerning the origin of the grass. Suggestions included accidental introduction from the United States, mutation of an existing species, and hybridization. The last possibility was made particularly plausible by the fact that the new *Spartina* was morphologically intermediate between two other species which existed together in the locality at the time, namely *S. maritima*, native to Britain, and *S. alterniflora*, a North American species believed to have been introduced accidentally by shipping into the Southampton area in the early nineteenth century.

In 1931, C. L. Huskins published an investigation of hereditary relationships in 'British' representatives of *Spartina* from cytological findings. Unaware of the existence of the sterile plant, he gave the chromosome complement of fertile *S. townsendii* as $2n = 126$, and derived it by chromosome doubling from a hypothetical 63-chromosome hybrid between *S. maritima* ($2n = 56$) and *S. alterniflora* ($2n = 70$). Unfortunately, Huskins's results were thrown open to doubt by the fact that, believing *S. alterniflora* to have become extinct in its former British locality, he made his counts for this polymorphic species on American material; moreover, the cytological techniques available at that time were inadequate for examining the small chromosomes of *Spartina* with any degree of precision.

In his recent re-investigation of the situation, C. J. Marchant was not only able to use modern techniques, but also to find and examine British material of *S. alterniflora* still surviving in the Southampton Water area. Both sterile and fertile plants of *S. townsendii* were included in his investigations, and chromosome counts from a wide range of material gave the following numbers: *S. maritima* 60 (never 56); *S. alterniflora* 62 (never 70); sterile *S. townsendii* 62; fertile *S. townsendii* variable with 120, 122 (commonest) and 124. Although the new counts provided a numerically less-exact scheme of relationships than that suggested by Huskins, the postulated origin of fertile *S. townsendii* by hybridization and subsequent chromosome doubling was nevertheless supported. The hybrid nature of the sterile plant was attested by irregularities in chromosome pairing at meiosis, while careful comparison of chromosome morphology confirmed its derivation from *S. maritima* and *S. alterniflora*; in contrast, the 120- to 124-chromosome form of *S. townsendii* showed almost regular pairing, while the discovery of other *Spartina* plants, intermediate in external form and chromosome number (90 and 76), suggested back-cross links between *S. townsendii* and *S. alterniflora*.

Because of the interest aroused by the first appearance of *S. townsendii*, the subsequent spread of the grass has been recorded by botanists in detail. Much of its early spread from its original station near Hythe was by natural means, and by 1915 it was established in every south-coast estuary from Chichester to Poole. From the early 1920's onwards its range was greatly extended by artificial plantings, and it now covers at least 30,000 acres of tidal flats around the British Isles alone.

The story of *Spartina* in Britain, however, is not solely one of uninterrupted success in the occupation of new habitats. Within the past 40 years there have been a number of reports of patchy degeneration—'die-back'—of previously healthy *Spartina* swards. This problem was recently investigated by P. J. Goodman and Mrs. E. M. Braybrooks at the University of Southampton. Their detailed surveys showed that the pattern of die-back seemed remarkably constant in affected areas, with 'channel die-back' along the edges of the major creeks, 'pan die-back' in low-lying areas in the middle of marsh segments, and healthy *Spartina* on slightly raised banks

or *levées* nearer the creeks. There was no evidence to associate the incidence of die-back with pollution or pathogen attack; indeed, both field observations and transplant experiments suggested strongly that die-back resulted from the establishment of locally unfavourable habitat conditions.

The reason for die-back pan formation in previously healthy swards was elucidated by means of soil borings. In a number of different sites, levelled lines of bores showed that a decrease had occurred in the general slope of the marsh from back to front following colonization by *Spartina*; the grass had caused greater mud accretion at the front, and drainage of the marsh surface had been progressively impaired. Similarly, bores across marsh segments lying between adjacent creeks gave evidence of a change from a convex to a concave profile for the intervening marsh, resulting in more or less permanently waterlogged conditions in the centres of the segments. The appearance of die-back pans thus resulted from the normal physiographical development of the sward: *levées* had been produced by mud filtered from the tidal water by the *Spartina* plants themselves, and surface run-off from the marsh had thereby been locally impeded. The cause of channel die-back was less certain; but it may have been due to the slipping of soft mud back into the channels, bringing the neighbouring marsh surface down to a level too constantly flooded for healthy *Spartina* growth.

Although the field evidence strongly suggested an ecological connexion between *Spartina* die-back and excessive wetness of the soil, the physiological explanation of such die-back was by no means clear. Symptoms of die-back included death of underground buds and soft-rotting of the rhizome apices, but such symptoms could be due to a number of causes. The deleterious effect of water-logging on plants is commonly attributed to lack of oxygen in the soil; but this again could operate in several different ways—by causing toxic substances like alcohol or acetaldehyde to accumulate in the tissues as a result of anaerobic respiration, by interfering with some important vital process, by producing poisonous substances like ferrous salts, sulphides or nitrites in the soil, or by predisposing the plant to bacterial or fungal attack by any of these mechanisms.

Laboratory experiments, carried out at Southampton by P. J. Goodman, Miss S. Barker and A. Ivemy, and discussed by Prof. Williams, have now reduced these possibilities to that of interference with some vital but unidentified function in the plant. Alcohol- and acetaldehyde-levels in die-back plants were too low to be critical for growth; the presence of bacteria in *Spartina* rhizomes was not consistently associated with die-back; and, although high concentrations of sulphide were present in die-back soils, no quantitative relationship could be established with rhizome apices cultures at different sulphide-levels, while, on the other hand, die-back symptoms could be produced in the absence of sulphide when oxygen was excluded from the cultures. It is certainly possible that soil sulphides are indirectly responsible for die-back by forming an oxygen sink, but the basic physiological effect of lack of oxygen still remains essentially unresolved.

Whatever may be the ultimate cause of die-back in *Spartina*, once it is initiated in a sward the weakened areas may be enlarged by tidal scour so that the sward becomes progressively disintegrated. At present, the phenomenon of die-back is very restricted in extent, and limited to marshes on fine-textured soils with exceptionally poor subsoil drainage. In other *Spartina* areas with coarser soils it is probable that more stable conditions will result; most swards are still too young for their ultimate fate to be predicted with any certainty.

Although *Spartina* seems susceptible to continuous water-logging, it nevertheless appears more tolerant of wet conditions than many other salt-marsh plants. It thus tended in the first place to colonize bare mud flats in front of existing salt-marshes, and this type of situation is sometimes regarded as its exclusive habitat. However, there is increasing evidence that it is now also migrating backwards into older saltings and ousting the original salt-marsh vegetation.

One such investigation of the successful invasion of pre-existing saltings was carried out by Miss Barker at Southampton, with particular reference to the mechanism by which *Spartina* replaces *Juncus maritimus*, a tall-growing species which normally occupies the higher and drier salt-marsh zones. A number of soil borings made in critical sites established that the entry of *Spartina* had greatly accelerated the rate of mud deposition where it had invaded, so that, whereas the high-lying *Juncus* zone had previously been freely drained, it was now thickly flanked by recently accumulated mud. Morphological and anatomical investigations of plants from the disappearing *Juncus* zone suggested that *Juncus* was showing the same die-back symptoms as were exhibited by *Spartina* under more extreme conditions; its replacement by *Spartina*, therefore, appeared to hinge on its greater susceptibility to impeded drainage rather than on a more direct competitive effect.

The power of rapid mud accretion by *Spartina* has long been recognized as one of its outstanding characteristics as a salt-marsh plant. In fact, its potential value for stabilizing and reclaiming inter-tidal flats was realized as early as the beginning of this century, and many thousands of cuttings and seeds have since been distributed to various parts of Britain and to more than 40 countries in different parts of the world. Dr. D. S. Ranwell and his colleagues in the Nature Conservancy have recently collected much information on the distribution and use of *Spartina* for economic purposes, and their survey indicates that *S. townsendii* now covers a world total of some 70 square miles or more.

Outside the British Isles, the establishment of *S. townsendii* has been most successful in the Netherlands, France and Germany. Its present known geographical limits are between 48° and 58° N. on European coasts. It has also been introduced successfully at 48° N. on the western seaboard of the United States, but plantings have failed at 45° N. on the colder eastern side. In Australia and New Zealand it survives between 35° and 46° S. Where otherwise suitable habitats exist, it seems to be restricted by frost damage on the polar sides of these limits. On the equatorial side, it apparently succeeded for a few years on the coast of British Guiana only 7° N. of the equator, but was finally smothered in competition with the native species *S. brasiliensis*; so far as is known, it has not survived in any other tropical sites.

In Britain itself, the primary use of *Spartina* has been for coast protection. A very successful example of this is seen at Bridgwater Bay on the Somerset coast, where a 2·5-mile length of the coastline has been effectively protected and the foreshore-level raised by 5 ft. since *Spartina* was planted in 1929. A similar use has been made of the grass in the Mitchell estuary and around the Gippsland lakes in Australia, and in the South Island of New Zealand.

In the Netherlands, Denmark and Germany, and also on a smaller scale in Britain round the Wash, *Spartina* has been used mainly for reclamation purposes. Perhaps the biggest and most successful reclamation experiment was that carried out in the Sloe Dam area in Holland, where large-scale planting started in 1925; by 1949 the first *Spartina* polder was ripe for reclamation, and the whole area is now a settled agricultural district. In northern Holland, planting of *Spartina* was at first less successful, and all but about 1 per cent of the initial transplants from south Holland were killed by winter frosts; with later selection and propagation of winter-hardy forms, however, there are now extensive *Spartina* meadows along the Groningen coast.

It was Prof. F. W. Oliver, one of the pioneer investigators of *Spartina* in Britain, who realized that the new

British grass had potentialities not only for reclamation but also as a crop. Its food value to stock is roughly equivalent to that of medium-quality hay, and it seems to be relished by a wide variety of herbivores. Although it has proved too coarse for the highly bred livestock of countries like Holland and Denmark, the older breeds of domestic animals graze freely on *Spartina* when it is available. Because of the difficult terrain on which it grows, there have been few attempts actually to harvest the grass. However, an experiment at Bridgwater Bay has shown that in suitable areas it is possible to cut and transport *Spartina* using light machinery, and the cut material is being tested for possible use as silage.

In spite of its economic value, the advent of *Spartina* is not an unmixed blessing. The very rapidity with which it can spread and transform a habitat can itself be a cause of trouble in certain circumstances. Fears that it would block major navigation channels in harbours have proved unfounded; but in estuarine marshes it sometimes chokes minor channels used by yachtsmen. Again, where it invades mud-flats near holiday beaches, the amenities of the area may be spoiled, and the beaches themselves eventually obliterated. Further, from a conservation angle, there is a very real danger that certain forms of wild-life may be lost in coastal areas overrun by the grass; other salt-marsh plants may be eliminated, the feeding grounds for wintering wild-fowl covered, and variety in the marshes reduced at the expense of the spread of a single species. Control measures for *Spartina* are under investigation, however, and some success has been achieved with carefully timed applications of some of the more innocuous herbicides.

In all, the *Spartina townsendii* story is clearly one of importance from both an academic and a practical point of view. Its main scientific interest lies in its exemplification of the evolution of a new species under natural conditions by hybridization between two geographically isolated species brought together by chance, restoration of fertility by chromosome doubling, and successful establishment in a vacant ecological niche. Man has not been slow to take advantage of the particular ecological and physiological properties of the new species which have contributed to its success; and it is perhaps ironic to note that it is just these marsh-building properties which may have hastened reclamation and industrial development along the Southampton Water shores, so that the classic birthplace of *Spartina townsendii* is now threatened with extinction.

J. M. Lambert

NEWS and VIEWS

Genetics in the University of Birmingham: Prof. J. L. Jinks

Dr. J. L. Jinks, at present honorary reader in genetics in the University of Birmingham, has been appointed to the chair of genetics in succession to Prof. K. Mather, who has been appointed Vice-Chancellor of the University of Southampton (*Nature*, 203, 24; 1964). Dr. Jinks gained both his B.Sc. and his Ph.D. at the University of Birmingham. He graduated in botany in 1950 and was awarded a research studentship in genetics by the Agricultural Research Council, which he held in the Department of Genetics in the University, obtaining his Ph.D. in 1952. Following his research studentship, he was appointed to the staff of the Agricultural Research Council's Unit of Biometrical Genetics, attached to the Department of Genetics, and has continued in this appointment up to the present time. He spent a year as a Harkness Fellow at the California Institute of Technology, and has worked for shorter periods in Copenhagen and Milan. Dr. Jinks's research has been mainly concerned with inheritance in moulds, bacteria and viruses, how they adjust themselves to their environments and how those which cause diseases of domestic plants adapt themselves to attack their hosts. He has also investigated the inheritance of characters which, like yield in domestic plants and animals, vary by imperceptible gradations over a wide range of phenotypic expressions. The methods of analysis that he has devised for such characters are now in wide use by plant breeders and have also led to important advances in the understanding of the hereditary determination of behaviour in animals. Dr. Jinks's appointment will take effect from October 1, 1965.

Agricultural Chemistry in the University of Leeds: Prof. J. A. F. Rook

Dr. J. A. F. Rook, principal scientific officer in the Chemistry Department of the National Institute for Research in Dairying, Shinfield, has been appointed to the chair of agricultural chemistry in the University of Leeds. Dr. Rook was educated at Scarborough High School for Boys and the University College of Wales, where he graduated with first-class honours in chemistry with agricultural chemistry in 1947. He held an Agricultural Research Council research training grant from 1947 until 1950 and spent two years in the Chemistry and Physics Department of the National Institute for Research in Dairying, followed by one year in the Department of Animal Physiology and Biochemistry of the Danish State Agricultural Research Laboratories, Copenhagen. He gained a Ph.D. degree at the University of Glasgow in 1957 for a thesis on "Some Studies on Energy Metabolism". In 1950 he was appointed scientific officer at the Hannah Dairy Research Institute, Ayr, and three years later was promoted to senior scientific officer. He returned to the National Institute for Research in Dairying in 1954, as senior scientific officer, and has held his present appointment as principal scientific officer in the Chemistry Department of that establishment since 1961. Dr. Rook's research interests include magnesium metabolism in calves and dairy cows, milk composition and metabolism and milk secretion.

Applied Mathematics in the University of Liverpool: Prof. J. G. Oldroyd

Prof. J. G. Oldroyd, professor of applied mathematics in the University College of Swansea, has been appointed to the newly established second chair of applied mathematics in the University of Liverpool. Prof. Oldroyd is forty-three years of age and was educated at Bradford Grammar School and Trinity College, Cambridge, where he won the Rouse Ball Mathematical Prize in 1941, the Mathison Prize in 1942, and successfully completed the Cambridge Mathematical Tripos Part III with distinction in 1942; he was awarded a B.A. degree in 1942, M.A. in 1946, Ph.D. in 1949 and Sc.D. in 1958. From 1942 until 1945 he served as an experimental officer with the Ministry of Supply, undertaking theoretical research mainly on the internal ballistics of rockets. From 1945 until 1953 he held the post of mathematical physicist at Courtaulds' Fundamental Research Laboratory at Maidenhead. He was a Fellow of Trinity College, Cambridge, from 1947 until 1951. In 1953 he was appointed professor of applied mathematics at University College of Swansea and became head of the Department of Applied Mathematics when it was created in 1957. Prof. Oldroyd has served as Dean of the Faculty of Science at Swansea, as a member of the College Council, a member of the Academic Board of the University of Wales and a member of the University of Wales Commission. From 1955 until 1957 he was president of the British Society of Rheology. Prof. Oldroyd will take up his new appointment on January 1.

Physiology in the University College of Rhodesia and Nyasaland : Dr. J. D. Nelms

Dr. J. D. Nelms, at present research medical officer in the Climatic Research Laboratory of the Institute of Aviation Medicine attached to the University of Birmingham, has been appointed head of the Department of Physiology in the University College of Rhodesia and Nyasaland from a date to be arranged. Dr. Nelms gained an honours B.Sc. degree in physiology at University College, London, in 1953, and qualified with the degrees of M.B., B.S., in 1955. He joined the Royal Air Force in 1957 and has since that time worked in the Institute of Aviation Medicine, during the past four years as a research medical officer. His research work has been mainly concerned with the effects of climatic environment on man. He was largely responsible for the development of new and modern methods of heating the body by electrically warmed underclothing. He has collaborated in two international expeditions to investigate the physiological basis of acclimatization to cold—one among Arctic Indians in the Yukon Territory.

Pharmacy in the Hebrew University, Jerusalem: Prof. M. Donbrow

Dr. Max Donbrow, principal lecturer in the School of Pharmacy of Chelsea College of Science and Technology, has been appointed professor, and head of the Department of Pharmacy in the Hebrew University of Jerusalem as from January 1965. His published research is concerned with the application of physical methods to the analysis of drugs, the properties of surface-active agents and the uptake of drugs by non-ionic surfactants. He is at present working on complex-formation between drugs. Dr. Donbrow spent several months in Israel in 1963 advising on the development of the Pharmacy School in the Hebrew University, and modifying the curriculum. The foundation stone for the new Pharmacy School was laid in August this year at the Medical Centre in Ein Kerem, and the existing Pharmacy School is expanding at once into premises vacated by the Medical School in the Russian Compound in Jerusalem.

Nuclear Physics in the Weizmann Institute of Science: Dr. Peter Hillman

Dr. Peter Hillman has been appointed head of the Department of Nuclear Physics at the Weizmann Institute of Science at Rehovoth, in succession to Prof. Amos de-Shalit, who has held the post since 1954. Prof. de-Shalit remains in the service of the Institute but has assumed a wider range of duties. Dr. Hillman has been on the staff of the Institute since 1960 and has acted as head of the Department on several occasions. Born in Durban, he was educated in South Africa and at Harvard University, where he received the M.A. and Ph.D. degrees. He later worked in Britain and in Sweden, and was for a time lecturer in the University of Witwatersrand.

Marsa el Brega : an International Oil Port

Some remarkable records of discovery, development, exploitation and marketing of crude petroleum from a new oilfield are disclosed in an article in the summer issue of *Esso Magazine* (13, No. 3, 1964; Esso Petroleum Co., Ltd., London), judged even by modern oil-company standards. It was in 1954 that geological search for oil in the then wilderness of northern Libya started in earnest, resulting in the discovery in 1959 of what is now known as the Zelten field, some 100 miles or so inland from the Mediterranean coastline. The problem of getting the oil away to the tankers came next. The intervening territory was barren and desolate, save for some half a million land mines strewn around, left over from World War II. Aerial surveys of the Gulf of Sirte coastline pointed to a place called Marsa el Brega, some 114 miles S.W. of Benghazi, as a potential harbour, with reasonable shelter and off-shore depth of water to accommodate the largest tankers afloat. In 1960, 110 miles of road between Marsa el Brega and Zelten were completed and soon afterwards some 115 miles of 30-in. pipeline were emplaced alongside the road. In September 1961, the first shipment of oil left the port of Marsa el Brega for the Esso refinery at Fawley. In the meantime, another commercial-size oilfield was discovered at Raguba, 45 miles N.W. of Zelten, and a spur pipeline from this area was completed early in 1963. "As a measure of what had been achieved in a very short span of time, over 6 million tons of oil were exported from Marsa el Brega in 1962, the first full year of operation. . . . As a result of . . . continued activity, over 14 million tons of oil were exported during the 12 months of 1963." Marsa el Brega, which so short a time ago was nothing more than rock, sand and water, is to-day an international oil port in every sense of the word; it is used by tankers of many different countries, including Britain, West Germany, Italy, France, Holland, Belgium, Spain and Egypt; it has helped to establish Libya as a major oil-producing country, a timely addition to the diverse and ever-growing oil resources available to the world.

The Museums Journal

The September issue of *The Museums Journal* (64, No. 2; September 1964) includes an article on the extensive modernization which has been carried out at the Whitworth Gallery, Manchester. The collections include early and contemporary water-colours and textiles with some sculpture and oil paintings. The main objects of the work were to raise existing facilities in the Gallery to advanced modern standards in respect of conservation, display, circulation and supervising, and storage facilities. Dr. M. Eager describes an excellent and modern display concerning the Moon which has been set up in the University Museum at Manchester, and Mr. W. A. Seaby details the proposed extensions to the Ulster Museum in Belfast. A report of the annual conference in Nottingham in July completes the issue.

Caspian Sea and Siberian Rivers

Until 1929 the level of the Caspian Sea was more or less constant, but since then its level has fallen by 2·5 m, corresponding to a loss of 960 cubic kilometres of water—four times the volume of the Volga's annual discharge. According to B. A. Apollov and S. N. Bobrov (*Priroda*, 2, 68; 1963) such an enormous fall in level is seriously threatening navigation, fisheries and other industries. Several projects aimed at raising the level of the Sea have been proposed. One of them was to dam the Rivers Yenisey and Ob and discharge them into the Aral Sea and then let the waters flow into the Caspian along the old drainage channel of Uzboy. Another project was to increase the Volga's volume of water by diverting into it the Rivers Pechora and Vychegda. Apollov and Bobrov are of the opinion that both these projects are impracticable, and instead suggest the construction of an earth dam across the northern part of the Caspian Sea so as to form a separate North Caspian reservoir. Another project is that of I. I. Stas' (*Priroda*, 2, 75; 1963), who proposed to link up by canals the Azov, Caspian and Black Seas. Projects of damming the Siberian Rivers Ob, Yenisey and Lena are discussed by V. S. Antonov (*Priroda*, 6, 25; 1963), Yu. I. Gordeev (*Priroda*, 6, 51; 1963), M. A. Shargaev (*Priroda*, 6, 54; 1963) and V. I. Orlov (*Priroda*, 6, 59; 1963).

Glued Formwork for Concrete

In a recent note on modern gluing techniques as applied to laminated wood in timber structures for buildings (*Nature*, 204, 129; 1964), attention was directed to the versatility of this form of construction under certain conditions where its entry had proved architecturally admissible. It was but a step to adapt the principle

involved to another constructional problem, this time a vital question of formwork for concrete as used in the 540-MW hydro-electric scheme in New Zealand; this is what is known as the Benmore project on the Waitak River, South Island, designed to ". . . contain six 90,000-kilowatt generators producing 540 MW of electricity" and ". . . will feed into the national grid, which, by means of a cable under Cook Strait, will supply the North Island as well . . .". According to an article entitled "Aerolite-glued Formwork for Concrete", some 450,000 cubic yards of concrete are involved in this project, most of which was cast using timber formwork (Ciba (A.R.L.), Ltd. *Technical Notes*, No. 259, July 1964. Pp. 8. Duxford: Ciba (A.R.L.), Ltd., 1964). In concrete construction, especially for river dams such as this, there is a recent tendency towards larger and higher pours; formwork therefore has to withstand greater pressures than before. When the motive is also economy, there is a rational trend towards the use of units which can be used repeatedly; essential criteria are extra strength, quick release and easy handling. "It was found that all these characteristics could be facilitated by the use of glue laminated timber structures incorporating Aerolite adhesive." In a report by D. R. Douglas, structural design draughtsman on this project, quoted in the article, he remarks on the big advantages obtained by the use of glue laminated work. "The choice of laminating or of using solid timber for forms was determined mainly by the importance of the component in relation to the whole form or by the need for stability or alignment. With a rainfall averaging 15 in. or less per year, and with the very low humidity of the district, all timbers dried out and tended to distort more than they would have in many other places. For this reason alone, the lamination of critical members was preferred." The several excellent illustrations accompanying this article clearly show the manner in which this glued formwork is prepared, assembled and used; it is a technique which will become increasingly popular in similar circumstances elsewhere.

Palæontology in the U.S.S.R.

The recent volumes of the *Transactions of the Palæontological Institute (Moscow)* (*Nature*, 197, 854; 1963; 199, 1139; 1963) are: (98) Bryozoa of the Borshchovsky and Chortkovsky horizons of Podolia, by G. G. Astrova (1964). These two horizons were originally placed in the Upper Silurian, but the present author proves them to be of Lower Devonian age; 150 specimens belonging to 24 species are described. (99) Biometric study of Pliocene vivipari of the southern part of the U.S.S.R. by V. Ya. Taboyarkova (1964). (100) The historical development of Diptera, by B. B. Rodendorf (1964). (101) Tabulates of the Lower and Middle Devonian age in the Kuznetsk Basin, by I. I. Chudinova (1964). (102) Conditions of life of the Silurian and Devonian marine fauna in the Kuznetsk, Minusinsk and Tuva Basins, by E. A. Ivanova, T. N. Bel'skaya and I. I. Chudinova (1964).

Bleeding in the Surgical Patient

Surgeons throughout the world can scarcely fail to be interested in the recent issue of the *Annals of the New York Academy of Sciences* entitled *Bleeding in the Surgical Patient*, in which 68 experts, who met at a conference held by the Academy in May 1963, discuss various aspects of the general problem of bleeding during, or after, surgical operations (115, Article 1. By Seymour Gollub, Alex W. Ulin and 77 other authors. Pp. 1–542. New York: New York Academy of Sciences, 1964. 7 dollars). This has been and, as the first paper in this series explains, still is, a fundamental problem for the surgeon. Great advances in the control of hæmorrhage have, of course, been made since Lister showed that blood vessels could be ligatured. Nowadays the detailed biochemical investigation of the various factors concerned in blood coagulation would seem to be in the foreground, but hypothermia, blood transfusion, advances in anæsthetics and a number of other factors discussed here have also played important parts in the reduction of the consequences of operations and the mortality formerly due to them.

Congenital Absence of Pain

Dr. H. J. Kingsley and Dr. J. E. A. David of Bulawayo have described (*Central African Journal of Medicine*, 10 (8), 1964) the case of a girl aged 22 months. She was subject to temper tantrums, and during these tantrums would bang herself on the head or bite herself. She appeared to be completely insensitive to pain. She is a well-built, sturdy little girl and, apart from the bandiness of her legs and a photosensitive skin rash, no physical abnormalities were found. Mentally, it was thought she might be a little backward. She was admitted to hospital for investigation and was noticed to have periods of blankness which were thought to be some type of petit mal. Many investigations were made and all results were normal. While she was in hospital her sensitivity to pain was tested and it was found that the child was insensitive to pain almost all over the trunk, limbs and face, and a sterile hypodermic needle could be stuck through the skin to the subcutaneous tissues without any flinching. She was apparently insensitive to heat and cold and to light touch, but these were difficult to evaluate. Winkelmann and his colleagues have previously reported a case of congenital universal indifference to pain in a 9-year-old white boy, with detailed anatomical, clinical and physiological studies. Confusion exists in the literature about congenital absence of pain, and a complete neurological examination should be carried out in these cases; specific attention should be directed towards the discovery of isolated islands of normal sensation, particularly round the trunk, genitals and face. This type of insensitivity is usually congenital and the patient is usually of normal intelligence, development and psychic adjustment. In a case described by Winkelmann, Lamber and Hayles no nerve end organs (Meissner or Vater-Pacini corpuscles) were found in any of the skin specimens studied. Dr. Walter B. Shelley of Philadelphia thinks that these cases are not as rare as is supposed and that there are people who experience coronary thrombosis or a perforating appendicitis, or have babies, without pain. Apparently, where pain is absent, itching is also absent.

University News: Bradford

The Governing Body and the Academic Advisory Committee of the Bradford Institute of Technology have announced the establishment of 30 professorships. The following heads of department become the first 12 professors in the fields concerned: Dr. E. Lees (biological sciences); Dr. R. L. Elliott (chemical technology); C. W. Page (chemical engineering); Dr. C. B. Wilby (civil engineering); Dr. G. N. Patchett (electrical engineering); J. G. Jagger (mechanical engineering); T. Kempner (management); Dr. J. G. Freeman (mathematics); Dr. J. M. Rowson (pharmacy); Dr. D. L. Smare (physics); P. H. Coles (social sciences); Dr. F. Happey (textiles).

London

Dr. J. W. Boag, physicist in the Research Unit in Radiobiology at Mount Vernon Hospital, has been appointed to the chair of physics as applied to medicine tenable at the Institute of Cancer Research, Royal Cancer Hospital. Dr. E. W. Emery, senior lecturer in medical physics in the University of Manchester, has been appointed to the readership in medical physics tenable at the Postgraduate Medical School of London from April 1. The following titles have been conferred: *Professor*, on Dr. D. R. Laurence (pharmacology and therapeutics), in respect of his post at University College and University College Hospital Medical School. *Reader*, on Dr. P. N. Campbell (biochemistry), in respect of his post at Middlesex Hospital Medical School; on Miss N. F. T. Crowley (bacteriology), in respect of her post at the Royal Free

Hospital School of Medicine; on Dr. H. Urich (neuropathology), in respect of his post at the London Hospital Medical College; on Dr. P. L. Williams (experimental neuroanatomy), in respect of his post at Guy's Hospital Medical School.

Oxford

In an oration published in the annual report as a supplement to the *Oxford University Gazette* of October 2, 1964, the Vice-Chancellor announced that it had been possible, thanks to the initiative of Merton, All Souls, Christchurch and St. John's Colleges, to make a start with the creation of two new graduate societies for non-Fellows (Supplement No. 3209. Pp. 51–77. Oxford: The University, 1964. 2*s*.). A capital grant of £29,000 had been promised to the University with recurrent grants for ten years to ensure an annual income of £18,000. The Vice-Chancellor expressed the view that doors should be left open, as in the past, for those who contribute to the life of the University in other ways than as tutors or research workers. The annual report records an increase of 174 in the number of matriculated students in residence compared with 1962–63; of the 8,963 students in 1963–64, 7,558 were men. Of the total, 412 men and 60 women came from Commonwealth countries and 550 men and 80 women from other countries. Besides the establishment of two posts of University lecturer status in each of the Faculties of Physical and Biological Sciences, a new professorship was established in the Department of Engineering Science through funds made available by Messrs. Stewart and Lloyds, to cover a period of seven years in the first instance, and this professorship of structural engineering will be known as the Stewart and Lloyds professorship.

Canadian Awards in Chemical Engineering

G. C. M. MacKay and S. G. Mason (McGill University and the Pulp and Paper Institute, Montreal) have received the award of the Chemical Engineering Division of the Chemical Institute of Canada for the best paper published in *The Canadian Journal of Chemical Engineering* during the year, in respect of their paper entitled "The Gravity Approach and Coalescence of Fluid Droplets at Liquid Interfaces". In the Division's 1964 Student Paper Competition, J. Morgan (University of British Columbia) was awarded first place and I. Patterson (McGill University), second.

IBM Scholarships

Five new scholarships, together amounting to about £10,000, are to be awarded each year by IBM United Kingdom, Ltd. The scholarships will provide grants for students wishing to attend any British university. The grants will be worth about £600 per annum each, and will be available for covering a maintenance allowance and certain university fees and expenses. Such grants will be given to candidates who have obtained places at residential universities; reduced grants will be available for successful applicants who will live at home during the term. Although there will be no obligation on either side, IBM scholars will be given preferential consideration if they wish to join the Company after graduation. Initial interviews will be held in February and March, and final interviews will be held during the Easter vacation. Application forms, which should be submitted through headmasters and headmistresses, and further details are available from the Director of Personnel, IBM United Kingdom, Ltd., 101 Wigmore Street, London, W.1.

Smithsonian Observatory Post-doctoral Research Programme

A post-doctoral fellowship programme for research associates has been established at the Smithsonian Astrophysical Observatory, Cambridge, Massachusetts. Two appointments for visiting scientists from U.S. or overseas institutions will be available in January 1965 with two additional appointments available in July. The Astrophysical Observatory is one of several Smithsonian Institution bureaux which will offer such a programme in co-operation with the U.S. National Academy of Sciences–National Research Council. Research associateships will be offered to scientists pursuing post-doctoral investigations in the general fields of applied and theoretical space sciences, astronomy, astrophysics, and celestial mechanics. Two types of appointment will be available: regular associateships for scientists at the immediate post-doctoral level, and senior associateships for scientists who are five years past their doctorates and who are already engaged in research activities resulting in publication or recognition in their fields. Associateships are available to scientists from the United States and overseas. The stipend for the regular post-doctoral research associateships is 10,000 dollars per annum. Appointments will normally be for one year. The U.S. National Academy of Sciences–National Research Council will administer the programme. Further information and application forms can be obtained from the U.S. National Academy of Sciences–National Research Council, 2101 Constitution Avenue N.W., Washington, D.C. 20560.

Announcements

A symposium on "Human Palaeopathology", organized by the U.S. National Academy of Sciences—National Research Council, will be held in Washington on January 14. Further information can be obtained from the U.S. National Academy of Sciences—National Research Council, 2101 Constitution Avenue, Washington D.C. 20418.

The fifth summer research institute of the Australian Mathematical Society will be held at the Australian National University, Canberra, during January 11 - February 19. Further information can be obtained from the secretary of the Institute, J. S. Sandiford, School of Mathematics, University of New South Wales, Kensington, New South Wales.

The fifth seminar of "Electrochemistry" will be held at the Central Electrochemical Research Institute, Karaikudi, during January 27–30. The programme will include technical sessions on: electrode kinetics, electrochemical equilibria and electroanalysis; electro-organic and -inorganic products; electrothermics and electrometallurgy; batteries; electrodeposition and metal finishing; corrosion; miscellaneous. Further information can be obtained from Dr. M. A. V. Devanathan, Central Electrochemical Research Institute, Karaikudi 3.

Corrigendum. In the communication by Prof. J. L. R. Candela *et al.* entitled "Effect of Glucose on Insulin Secretion *in vitro* by Rabbit Pancreas pre-incubated with β-Hydroxybutyrate" (*Nature*, 203, 758; 1964) lines 16 and 17 should read "... Krebs–Henseleit buffer containing β-hydroxybutyrate 1×10^{-2} M . . .".

Corrigendum. In the article entitled "Distribution of Particle Size in Dust" (*Nature*, 203, 1057; 1964), equation (3) should read:

$$p(D) = (\gamma - 1)\, D^{-\gamma} / D_S^{1-\gamma} - D_L^{1-\gamma}$$

Equation (4) should read:

$$P(D) = (D_S^{1-\gamma} - D^{1-\gamma}) / (D_S^{1-\gamma} - D_L^{1-\gamma})$$

and equation (5) should read:

$$\frac{\mathrm{d}P(D)}{\mathrm{d}D} = (\gamma - 1) D^{-\gamma} / (D_S^{1-\gamma} - D_L^{1-\gamma})$$

Erratum. In the review of the book *Classification and Human Evolution*, edited by Sherwood L. Washburn (*Nature*, December 5, p. 951), the heading of the chapter by G. A. Harrison and J. S. Weiner was unfortunately printed as "Some Considerations in the Formulation of Theories of Human Physiology"; for 'Physiology' read 'Phylogeny'.

U.S. INSTITUTE FOR BIOMEDICAL RESEARCH

AN Institute for Biomedical Research is to be established by the American Medical Association Education and Research Foundation and will be officially opened on July 1, 1965. Laboratory and animal research facilities for the Institute will be located in a new addition to the American Medical Association headquarters building in Chicago, now under construction.

A committee has been set up under the chairmanship of Prof. Maurice B. Visscher, chairman of the Department of Physiology of the University of Minnesota Medical School, to advise the Board of the American Association Education and Research Foundation on the qualifications of prospective members of the Institute's scientific staff and to recommend specific applications of policies.

Other members of the committee are Prof. William H. Feldman, chief of laboratory research in pulmonary diseases for the Veterans Administration, Washington, D.C.; Prof. Chauncey D. Leake, Department of Pharmacology, University of California School of Medicine, San Francisco; Prof. Donald D. Van Slyke, member emeritus of the Rockefeller Institute, of Brookhaven National Laboratory, Upton, New York; Prof. Henry Eyring, dean of the University of Utah Graduate School, Salt Lake City, and president-elect of the American Association for the Advancement of Science.

The purposes and policies of the Institute are delineated as follows:

(1) "To provide appropriate personnel, facilities, and conditions, conducive to obtaining significant, basic biomedical information which eventually may be applied to promoting the good health of people.

(2) "The personnel, nominated by the Committee of Scientific Advisors, will include biomedical scientists of the best possible stature or promise with such secretarial and technical assistance as may be appropriate to their respective activities, and with such Post-doctoral Fellows as may be attracted by the individual investigators in relation to their facilities and scientific interests.

(3) "Initial laboratory facilities will include 30,000 square feet of space to be set up and equipped on the recommendations of the scientific personnel and the Committee of Scientific Advisors, together with appropriate quarters and care for experimental animals. Extensive library facilities will be available through the headquarters organization of the American Medical Association.

(4) "Every possible effort will be made to establish such conditions in the Institute as will be stimulating for basic biomedical investigation in accordance with the highest scientific standards and ideals. The organizational policies of the American Medical Association provide liberal financial security and perquisites. Responsible freedom in research and publication is assured. The Institute is expected to maintain the best possible intellectual environment for supporting investigation into fundamental biomedical scientific problems."

C.S.I.R.O. PHYSICS IN AUSTRALIA

THE Divisions of Applied Physics and of Physics of the Commonwealth Scientific and Industrial Research Organization, Australia, are situated at Sydney and form the National Standards Laboratory. The Applied Physics Division is responsible for the establishment and maintenance of mechanical standards of measurement of length, mass and time, and associated measurements, and of electrical standards of measurement. The Division of Physics has similar responsibility for standards in heat, light, viscometry and hygrometry. The annual reports of the Divisions for 1962–63, together with those of the Divisions of Tribophysics and of Meteorological Physics situated at Melbourne have recently been issued*.

The work done in the Division of Applied Physics on the reproducibility of the orange-red radiation of krypton-86 as the primary standard of length, and on other secondary standards, was reported at the 1962 meeting in Paris of the advisory committee on the definition of the metre. A new version of the Fabry–Perot scanning interferometer in use in the division is being designed. A precise quartz-scale and photoelectric microscope for rapid measurement of changes of plate separation to within one order of interference and up to 500 mm are to be incorporated. Alterations are being made to the 1-m line standard comparator. The 0·1-in.-wide thin soft-metal strip to be used in the determination of the gyromagnetic ratio of the proton has been measured and an accuracy of 8×10^{-6} in. achieved in the measurement of the thickness. Steady progress has been made on the construction of the equipment for the absolute determination of gravity by the rise-and-fall method. An accurate bolometer bridge has been developed for the measurement of power at radio and microwave frequencies, and work on magnetic viscosity has been extended by the provision of equipment for the measurement of both loss and permeability as a function of either time or frequency at temperatures up to the Curie temperature. In connexion with the 210-ft. radio telescope at Parkes, New South Wales, a detailed study has been made of the vibration modes of telescope structures.

In the Division of Physics results have been obtained which indicate that the freezing-points of pure tin (231·912° C) and pure cadmium (321·032° C) are worthy of consideration as reference points for platinum resistance thermometry on the International Practical Temperature Scale. There is a continuous demand for consultations on techniques of temperature measurement, and inspections of laboratories on behalf of the National Association of Testing Authorities for the purpose of registration in the field of heat and temperature measurement have continued. Additional qualified laboratories are still needed. The measurement of the thermal conductivity of unirradiated and irradiated BeO at low temperatures, the specific heat of silver below 4° K, and the linear expansion of alkali-halide crystals, germanium and silicon and anisotropic metals at temperatures below 20° K, form part of the solid-state physics research activities of the division. There are facilities for a wide range of precision optical measurements, particularly for interferometry and the

* Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Applied Physics, 1962–63. Pp. vi+45. Annual Report of the Division of Physics, 1962–63. Pp. v+28. Annual Report of the Division of Meteorological Physics, 1962–63. Pp. ii+20. Annual Report of the Division of Tribophysics, 1962–63. Pp. i+18. (Commonwealth Scientific and Industrial Research Organization: Sydney and Melbourne, 1963.).

assessment of optical quality. Two-beam interferometers are used for the measurement of the transfer functions of optical systems, and a modified Michelson interferometer, in which the mirror movement is controlled by the application of a uniformly increasing voltage to barium titanate piezoelectric supports, is used for interference spectroscopy. A new technique is being developed for the measurement of solar magnetic fields in which a series of four Fabry–Perot interferometers are used as a tunable monochromater with a 1/50-Å pass band, and the data processed rapidly by an adaptation of television techniques. The Division of Tribophysics is concerned with fundamental investigations in the physics and chemistry of surfaces of solids and in metal physics. The behaviour of solid materials depends to a large extent on defects in the atomic arrangement in the crystals, and strength and ductility in particular depend critically on the imperfections. Point defects in plastically deformed metals and in quenched metals, and the dislocation rearrangements and recovery which occur after deformation, have been determined by the correlation of the results of measurement of many different physical properties. The effect of plastic deformation on the Hall coefficient of copper has been measured. Polycrystalline films of silver have been produced by evaporation on a mica substrate at pressures of the order of 10^{-8} torr and the investigation of damage produced in gold crystals by bombardment with ions of the inert gases has been completed. A thin film of silicon monoxide on an aluminium reflector is a selective surface which emits and absorbs radiation between 8 and 13μ only. This corresponds to one of the several infra-red windows of the atmosphere, and thus the film, which radiates more heat than it absorbs, could be used to form the surface of a refrigerator which requires no supply of power. Patents for such a refrigerator have been granted by Australia and the United States.

The report of the Division of Meteorological Physics deals with investigations under the headings of dynamic meteorology, general micrometeorology, and agricultural meteorology and evaporation. The study of radiative flux divergence in the lowest 15 m of the Earth's atmosphere during clear nights has been continued, and previous results confirmed. A new fast and accurate method for the measurement of radiative heat exchange of human subjects has been developed. The equipment used consists of two balanced net radiometers, which face each other at the same level on opposite sides of the subject and which move at a constant speed in a helical path. Each complete scan, taking about 1 min, sweeps out a vertical cylindrical surface enclosing the subject. Detailed investigations of radioactive fall-out using improved apparatus have been carried out, and the resumption of atomic testing in the Pacific during 1962 provided an opportunity to re-examine features of the fall-out observed following the 1958 tests. On the average, debris from tests at the equator is detected in Melbourne about 11 weeks after release. Information on the structure and lateral extent of rain systems is being obtained by a 10-cm surveillance radar, and a 3-cm wind-finding radar is to be installed.

KINETICS OF PYROLYTIC REACTIONS

UNDER the auspices of the Chemical Institute of Canada a symposium on the kinetics of decompositions and isomerizations was held at the University of Ottawa during September 9–11. There had been no symposium on this topic for a considerable time, and there was lively discussion of a number of questions, including isomerizations of ring compounds, the stability of radicals, over-all mechanisms for inhibited and uninhibited decompositions, and surface effects. It was revealed that a number of major problems are still far from solution, and many specific proposals for future work were put forward.

Several papers dealt with the mechanism of the gas-phase isomerizations of small ring compounds, which in most cases appear to be largely homogeneous and elementary. H. M. Frey (University of Southampton) described six types of thermal gas-phase reactions involving substituted cyclopropanes; these include geometrical and structural isomerizations and ring expansions, and all appear to be unimolecular. Similar conclusions were drawn by W. D. Walters, A. F. Pataracchia and P. C. Ropoli (University of Rochester) in the case of substituted *cyclo*butanes; they studied the reactions in their fall-off regions and compared the Arrhenius parameters.

J. P. Chesick (Haverford College) and M. L. Halberstedt (National Bureau of Standards) described results on the pyrolysis of ethyl*cyclo*propane that could be satisfactorily interpreted in terms of the Marcus quantum modification of the Rice–Ramsperger–Kassel theory of unimolecular reactions.

S. W. Benson (Stanford Research Institute) and P. S. Nangia (University of Southern California) reviewed the kinetic evidence on the decompositions of a number of small-ring compounds. Two extreme modes of isomerization have been proposed for the *cyclo*propane ring compounds: (*a*) cleavage of one C—C bond to form a biradical; (*b*) H migration followed by C—C bond rupture. Similarly, two modes of reaction have been considered in *cyclo*butane studies: (*a*) the biradical mechanism; (*b*) simultaneous cleavage of two C—C bonds to yield the two product molecules. It was argued by Benson and Nangia, and generally agreed, that the evidence favours the biradical mechanism. The reaction path for a group of compounds with activation energy much lower than the usual values for ring isomerization was shown to be consistent with the biradical mechanism if the radical formed is stabilized appreciably by allylic resonance. The value of the resonance energy of the allyl radical was suggested to be about 12 kcal/mole, somewhat lower than previously estimated. Benson and Nangia also proposed that in certain reactions of unsaturated molecules there is the formation of intimate ion-pairs before the biradical configuration is reached. S. G. Cohen and C. Steel (Brandeis University) showed that their results on certain bicyclic hydrocarbons are largely consistent with the biradical mechanism, but suggested a concerted mechanism in which H transfer does contribute slightly to the C—C bond splitting.

The decomposition of ethylene oxide, on the other hand, proceeds by a complicated free-radical mechanism, and O. O. Bernardini and E. A. Cherniak (Carleton University) described results which lead to conclusions about the details of the mechanism.

A completely new approach to the study of unimolecular reactions was described in a paper by I. P. Fisher, J. B. Homer, B. Roberts and F. P. Lossing (National Research Council, Ottawa). A new type of flow reactor, for use with a mass spectrometer, has been constructed, and preliminary results on a number of decompositions were presented. The rate constants measured were in the range 50–5,000 sec^{-1} and were for the most part consistent with those obtained from more conventional investigations on reactions the rate constants of which are of the order of 10^{-3} sec^{-1}. Evidence was obtained for a falling off of the first-order rate coefficients at high temperatures; some discrepancies between the results and the Kassel theory were noted.

The stability and reactivity of free radicals were discussed by P. Gray (University of Leeds) on the basis of the iso-electronic principle and of thermochemistry; reliable data on radical reactions have now accumulated to the point where correlation of structure and reactivity can provide insight into the detailed pattern of radical reactivity. M. Szwarc (State University of New York, Syracuse) considered theoretical and experimental aspects of the decompositions of substances such as metal alkyls, where there may be simultaneous rupture of two bonds if the radical produced by the breaking of one bond is thermodynamically unstable.

The free-radical chain mechanisms for the decomposition of ethane and acetaldehyde were discussed with much vigour, but many unsolved features of these problems remain. Considerable discussion was devoted to the kinetic order of the combination of methyl radicals under various conditions of pressure and temperature, and of the decomposition of the ethyl radical into C_2H_4 and a hydrogen atom. M. H. Back, M. Eusuf and K. J. Laidler (University of Ottawa) reviewed the evidence from various sources and showed that some of it appears contradictory. A. S. Gordon (U.S. Naval Ordnance Test Station, China Lake) described new results on the ethane pyrolysis that favour a mechanism due to Quinn, according to which the ethane dissociation is first-order and the ethyl radical decomposition rate varies with the square root of the pressure of foreign gas; surface effects are, however, important and the rate of diffusion may play a significant part in the over-all mechanism.

M. C. Lin and M. H. Back (University of Ottawa) presented results on the rates of the methane and butane productions in the ethane pyrolysis that were also consistent with Quinn's mechanism. In discussion it was, however, pointed out that there are some difficulties and that further work is necessary on this important reaction. C. P. Quinn (University of Cambridge) described work on the propylene-inhibited decomposition of ethane that indicates that the propylene and the ethane are decomposing simultaneously, by two mutually interacting chain processes, the allyl radicals playing an important role in the inhibition. A paper by M. Niclause, R. Martin, A. Combes and M. Dzierzynski (Université de Nancy) on the pyrolysis of propane and of isopentane emphasized the important accelerating effect of very small traces of oxygen on the reaction rate, and the fact that in some cases the effect of surface is very much less when the reaction proceeds in the absence of oxygen.

Two papers were concerned with the pyrolysis of acetaldehyde. A. B. Trenwith (University of Newcastle upon Tyne) described a study of the formation of minor products of the reaction. He found that acetone was produced more rapidly than ethane and concluded that the main chain-termination step is $CH_3 + CH_3CO \rightarrow CH_3COCH_3$; other interpretations were also suggested, and there is still no agreement on the over-all mechanism of this reaction. D. G. Milne and O. K. Rice (University of North Carolina) described experiments on the decompositions of mixtures of acetaldehyde and azomethane, and proposed mechanisms involving mutually interacting chain processes.

A somewhat different aspect of pyrolysis reactions was described in a paper by F. S. Rowland, E. K. C. Lee, F. Schmidt-Bleek and Y. N. Tang (University of Kansas), who caused high-energy tritium atoms to replace hydrogen atoms in a wide variety of molecules, and examined by radioactive techniques the decompositions of the highly energetic molecules produced in this way. They concluded that the decomposition mechanisms are surprisingly like those deduced on the basis of more conventional investigations.

Azomethane and diazomethane both decompose by free-radical mechanisms. W. Forst (Université de Laval) described results on the azomethane decomposition that presented difficulties of interpretation; the methane production, for example, he found to be 3/2 order and this seems to be incompatible with the high activation energy. A paper by W. J. Dunning and C. McCain (University of Bristol) described work on the thermal decomposition of diazomethane in the presence of hydrogen; the results are interpreted in terms of a mechanism in which methylene forms vibrationally excited methane which afterwards decomposes into a methyl radical. B. G. Gowenlock and R. M. Haynes (University of Birmingham) proposed a mechanism for their results on the pyrolysis of acetaldazine, $CH_3CH=N-N=CHCH_3$.

Reactions involving alkoxyl radicals and nitric oxide were the subject of a paper by E. A. Arden, L. Phillips and R. Shaw (Ministry of Aviation, Waltham Abbey), who suggested mechanisms for the formation of N_2, NO_2 and N_2O in such systems.

A paper by S. J. W. Price and M. C. Jacko (University of Windsor) dealt with the decompositions of certain metal alkyls, use being made of a flow system. Breaks in the Arrhenius plots were taken to give evidence with regard to whether the two bonds were broken simultaneously.

Three papers at the symposium were concerned with the pyrolysis of alkyl halides. K. A. Holbrook (University of Hull) described an examination of the role of surface in chloride pyrolyses, and showed that different methods of producing a carbon deposit on the surface led to different kinetic behaviour; electron-spin-resonance investigations on some surfaces lead to conclusions about the presence of free radicals. J. L. Holmes and G. Choudhary (University of Ottawa) described recent work on the pyrolysis of alkyl iodides, and proposed reaction mechanisms to explain the kinetic results. M. R. Bridge and A. Maccoll (University College, London) showed that their results on the effect of *p*-halogen substituents on the pyrolysis of α-phenyl ethyl chlorides were consistent with the view that an ionic type of activated complex is involved. Discussion of the halide pyrolyses was centred about two main problems: the elementary nature of the process and the uncertain role of surface. For some halides the evidence for decomposition by molecular elimination of hydrogen halide appears convincing, but for others the interpretation is doubtful. Many of the customary tests for chain reactions, such as the addition of inhibitors, may have little effect on the over-all rates, and decisive tests have generally not yet been made. Similarly, satisfactory tests for surface effects have usually not been made, and it was emphasized that if initiation and termination are both surface reactions the changing of the surface/volume ratio may have no effect on over-all rates. More convincing evidence would be provided by investigations involving changes in the chemical nature of the surface, as well as its area, and investigations of the elementary reactions that are believed to occur.

Inhibition in pyrolysis mechanisms was discussed at an informal session on the final afternoon. It seems to be generally agreed that the effect of inhibitors is frequently to generate new chain processes. This is a feature of mechanisms proposed by Laidler, Wojciechowski *et al.*, but their suggested chain-ending steps, postulated to explain the over-all kinetic behaviour, were the object of criticism. The alternative proposal of Pratt and Norrish, who emphasize the role of the decomposition of oximes formed as intermediates when nitric oxide is present, was also debated vigorously. It became clear that the matter can only be elucidated by experimental studies of the kinetics of the formation of the minor products of the decompositions, including those products formed from the inhibitor itself.

Constantly throughout the symposium it became apparent that there are many points where there are serious discrepancies as to the facts, as well as many differences of opinion and interpretation. By bringing these matters into sharp focus the symposium will undoubtedly have a significant effect on future work in this field.

M. H. Back
K. J. Laidler

UNIVERSITY APPOINTMENTS BOARDS IN BRITAIN

IN 1961, at the suggestion of the University Grants Committee and with the approval of the Committee of Vice-Chancellors and Principals, Nuffield College agreed to undertake and finance an enquiry into the scope, purpose, constitution and organization of university appointments boards, and Lord Heyworth agreed to supervise the enquiry with the assistance of a steering committee.

To a large extent Lord Heyworth, in coming to his conclusions and in framing his recommendations, has relied on the results of surveys carried out of the control, composition, finance and activities of appointments boards through a questionnaire to vice-chancellors, a review of the present and projected work of appointments boards, based on papers written by the secretaries of the boards, and on surveys of fact and opinion relating to the use and utility of the boards among students, recent graduates and employers. This is pointed out by Lord Heyworth in the report of his enquiry which has now been published *.

The report sets out, in successive chapters, the need for appointments boards and considerations of organization, finance and staff, before discussing the employers of graduates, the students searching for a job, appointments services at work, and the future of appointments services. In a later chapter the cost of future work is reviewed, and finally it is emphasized that appointments boards represent a joint responsibility of academic staff, students and employers.

Appointments services have been overtaken by the influx of students and also by the increase in the number of employers of graduates. Invariably they suffer from a shortage of appointments officers and of clerical staff, as well as of accommodation and of excessive concentration of work in the spring term. As a result, the services vary from fairly adequate to frankly rudimentary. The report maintains that, if standards are to be put right and kept right, efforts should be made to see that the ratio of appointments officers to students available for employment should not rise far above 1 : 100; as the number of students increases so the resulting demand for clerical staff and accommodation should be met. It recommends that certain functions now performed separately by various services should be performed centrally for the benefit of all, thus lightening the burden of labour and reducing the cost. This refers particularly to certain information services, and especially those connected with a register of employers. To bring to a proper state of efficiency the services now offered by appointments boards would require an increase in the total expenditure of all boards of upwards of 40 per cent on the average for 1961–62 of £14·2 a head. This would bring the sum *per capita* to £25, which should be set against the £1,500 - £3,000 a year which to-day the education of a university student costs the State (depending on his subject). The lowest figure of fixed expenditure for running an appointments service is likely to be about £5,000 a year, but with growth of the service economies of scale might reduce somewhat the expenditure *per capita*.

The overriding purpose of the report in making these recommendations regarding appointments boards is better service both to students and to employers so that students may develop their abilities to the full and make their fullest contribution to the national life. To this end the closest co-operation is essential between the boards and their staffs and the other parties mainly concerned, namely, the academic staff of the universities, the students and the employers.

* University Grants Committee. *University Appointments Boards:* a Report by the Rt. Hon. the Lord Heyworth. Pp. xii+127. (London: H.M. Stationery Office, 1964.) 12*s*. 6*d*. net.

PRIMARY EDUCATION IN BRITAIN

THE National Union of Teachers has published, under the title *First Things First**, a memorandum of evidence submitted to the Central Advisory Council for Education (England), under the chairmanship of Lady Plowden, which presents the results of extensive consultation with teachers serving in the primary schools. It is based on replies to a questionnaire which was circulated among Union members. The questionnaire, which had been drawn up by the Plowden Committee, covered the stages of primary education, the transition from primary to secondary education, the work of the primary schools, their size and organization, teachers and their training, handicapped children and the relation of home, school and community.

Points of special interest in the memorandum include a request to the Council to investigate the possible development of the present services available to mothers through the child welfare clinics, so that advice is available not only on the nutritional and medical aspects of child nurture, but also on those quasi-educational functions which only the mother can render to the child at this stage of growth. No claim is made that the evidence presented on the transition from primary to secondary education represents the opinion of all primary-school teachers, but the lack of general agreement disclosed by the questionnaire reveals that many quite definite patterns of thought exist.

The pamphlet observes that, if the value of the comprehensive school is to be properly tested, certain necessary conditions must be established: first, the intake must be genuinely comprehensive with no subtraction of children regarded as having higher ability, and, secondly, its buildings must be designed or satisfactorily adapted for their purpose. In very few areas have both these conditions been fulfilled. The memorandum stresses the need to create adaptable minds well furnished with the basic concepts which underlie modern social organization and its technology. So far as the primary school is concerned, the problem is one of using its free and informal methodology in a curricula context that is directed towards the new aims of the secondary school.

The memorandum welcomes the many experiments now being carried out into ways in which mathematical and scientific ideas can be developed inside the framework of primary method. The use of primary teachers without professional training or teaching in the primary school is not simply a question of the best internal organization. Highly trained teachers are the key to success in solving the problems affecting the balance between internal freedom and the responsibility of the school to society. The memorandum argues that, if the social role of the primary school is to be continued to be regarded as the great

* National Union of Teachers. *First Things First:* A Memorandum of Evidence Submitted to the Central Advisory Committee for Education (England) under the chairmanship of Lady Plowden. (An inquiry into primary education in all its aspects and the transition to secondary education.) Pp. 44. (London: National Union of Teachers, 1964.) 2*s*. 6*d*.

provider of equal opportunity for all children, it is imperative that the great majority of children should have opportunities of enjoying the benefits that highly skilled and, therefore, highly trained teachers can confer.

The National Union of Teachers fears most that, although the evidence for the maximum provision of educational opportunity at the earliest stages may be accepted, the form of school organization indicated may be rejected because of the anxiety that now exists about the supply of teachers in the immediate future. The Union urges that no purely economic considerations should be allowed to lead to legislation that will permanently warp the present primary-school system—a system which for many reasons is educationally desirable.

INTRODUCED SPECIES OF AMPHIBIA AND REPTILIA IN MAINLAND BRITAIN

THE native fauna of mainland Britain is small compared with that of continental Europe. In the last glaciation of the Ice Age, the great ice sheets stretched almost as far south as the Thames, and only with their recession could the fauna of warmer climates spread back from the southern part of Europe. At that time the area which is now the English Channel was occupied by dry land, so that movement across it was relatively easy. With the invasion of the sea and the opening of the Channel Gap some 5,000 years ago, those species which had already entered the country were separated from those which had not yet reached so far north. Since then, any changes in the British fauna can only be due to extinction of some, and further passage across the sea by others, whether aided or unaided by man. In the case of amphibia and reptiles, such passage (apart from the occasional turtles) has invariably been aided.

The surviving species of amphibia and reptiles which reached Britain unaided comprise only two toads, one frog, three newts, three snakes and three lizards. Species used in attempts at naturalization far outnumber these, yet success has been infrequent. The motives for the enlargements of alien species appear to have been varied, ranging between curiosity to see if they could establish themselves, or the liberation of captive specimens, the discarding of unwanted pets or even the escape of captive specimens. Casual escapes are unlikely to have taken place in such numbers as to form breeding populations except in the case of common pets such as tortoises. In the *British Journal of Herpetology* (3; June 1964), J. F. D. Frazer, of the Nature Conservancy, has classified the introduced species as follows: (*a*) Species which cannot survive Britain's normal conditions of weather, terrain and soil. These must be presumed to include the Madeiran wall lizard, the eyed lizard, the European wall lizard *L. campestos* (at least under conditions where it has been liberated hitherto), and the platanna, *Xenopus laevis*. (*b*) Species which can survive under normal conditions, but which are killed in exceptionally severe winters, for example, Ewing's tree-frog. (*c*) Species of which the adults can survive even under severe conditions, but where breeding never takes place, or only in exceptional summers. These include the snapper and other American terrapins, the Greek tortoise, the geckos, the green lizard and the bullfrog. The fire-bellied toad is also included here. (*d*) Species of which the eggs can normally hatch or the young be born, but cannot reach adulthood. These may possibly include the green tree-frog in some parts of Britain. (*e*) Species which have not been introduced originally in sufficient numbers to produce success. The painted frog and fire salamander come into this group, which also probably includes a number of introductions of green tree-frogs, where not enough have been liberated in one place to allow sufficient females to survive until the next breeding season. There is the further complication that this species is imported in large numbers when taken at the breeding ponds, so that there will be far fewer females than males, and a whole consignment of 500 may contain only one or two females. At best, an unsorted batch of 100 liberated would only be expected to contain about ten females. (*f*) Species which apparently succeed in establishing themselves, but then go into decline. The major example of this is the edible frog, assuming that it was never native to the fen district. Its final decline can be associated with the destruction of its habitat, and it may be significant that its disappearance in East Anglia coincided with changes in land management and with increasing use and pollution of the waterways, while the decline in the traditional cutting and use of reed was associated with a great silting-up process. It may be that the fading fortunes of such a species are due to its changing environment becoming unsuitable for it. (*g*) Finally, the species which seem to have colonized the land successfully have either been deposited in a suitable habitat, as in the case of the marsh frog, wall lizard (*L. muralis* and *L. nigriventris*) and some successful colonies of green tree-frog; or, as with the alpine newt, midwife and yellow-bellied toads, have been fairly inconspicuous species which are not particularly specialized in their requirements. In this connexion it is interesting to contrast the breeding failures of the fire-bellied toad, which lives in and around shallow water in lowland terrain, with its congener, the yellow-bellied toad. This is more a creature of mountain streams, and it must have found the Devon hills to its liking.

SPECULATION ON THE ORIGIN AND NON-SYMMETRY OF TWO THIN LAYERS *D″* AND *F* EMBRACING THE LIQUID CORE INSIDE THE EARTH

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IN general the Earth is considered to be formed of a thin crustal layer covering a fairly thick mantle which embraces its core. Bullen[1] utilized seismic data to form a model of Earth subdivided into eight regions. The crustal layers form region *A*, which has an average of 33 km thickness. Mantle, forming the regions *B*, *C* and *D′*, extends to a depth of 2,700 km and is followed by a region *D″*, which extends from 2,700 to 2,900 km. The core is subdivided into three regions, *E*, *F* and *G*.

The two layers *D″* and *F* embrace the outer core, layer *E*, which is generally considered to be liquid because it does not transmit the shear waves[2]. The nature and

composition of D'' and F, however, are subject to considerable conjecture[3–11].

Pfann *et al.*, in 1957 (ref. 12), and Bardeen *et al.*, in 1958 (ref. 13), published the results of some experiments on the Peltier effect, the principle of which will be utilized to speculate on the origin of D'' and F layers. Both Pfann and Bardeen show that Peltier cooling between a metal and its melt gives rise to a further growth of the solid phase, while the reverse process, Peltier heating, will increase the volume of the melt. It is necessary to estimate conditions which would give rise to Peltier heating and cooling at the core–mantle boundary. These results could easily be extended to cover the boundary conditions at the solid and the liquid cores.

Theoretical estimation of the Peltier coefficients. The basic ideas of Peltier effect as applied to solid and its melt were developed by Reiss[12]. These ideas are utilized to estimate the Peltier coefficients, which will indicate whether melting or freezing occurs at the core–mantle boundary due to electric currents.

If the D'' layer and the F layer are due to Peltier precipitation and melting, the velocity of the interface must be larger than zero:

$$v = \frac{\frac{PI}{J} + K_c\tau_c - K_m\tau_m}{H} > 0$$

where K is the thermal conductivity, τ the thermal gradient, I the current density, and H the heat of fusion.

From this we obtain for:

$$I = 10^{-3}\ \text{amp/cm}^2 - P > 3{\cdot}9 \times 10^{-3}\ \text{V, hot zone}$$

$$I = 10^{-3}\ \text{amp/cm}^2 - P > 12{\cdot}3 \times 10^{-3}\ \text{V, cold zone}$$

$$I = 10^{-6}\ \text{amp/cm}^2 - P > 8{\cdot}0\ \text{V, hot zone}$$

$$I = 10^{-6}\ \text{amp/cm}^2 - P > 8{\cdot}0\ \text{V, cold zone}$$

The Peltier coefficients of solid and its melt measured by Pfann and Bardeen are Bi = −0·013 V, Cd and Zn = 0·003 V, and Ge = 0·200 V. In general the Peltier coefficients of semiconductors are considerably larger than the coefficients of conductors. It is therefore feasible that the Peltier coefficient of the mantle (a semiconductor) against the molten iron is quite large. The Peltier range $-8 > P > 12{\cdot}3 \times 10^{-3}$ V, as calculated for boundary conditions, is within the range of the Peltier coefficients observed under laboratory conditions.

Pressure and temperature conditions prevailing at the core–mantle boundary are considerably different from those under which Pfann's and Bardeen's data were obtained. Bridgman[14] has shown that, in general, the value of the Peltier coefficient increases with the increase of pressure. The behaviour of the Peltier coefficient of iron as a function of temperature is fairly complicated, and it is not possible at present to predict its behaviour at very high temperatures. Therefore, if a current density $10^{-3} > I > 10^{-6}$ amp/cm^2 flows through the boundaries, it is reasonable to presume that Peltier heating or precipitation would occur.

Speculations on the origin of the D'' and F layers. Let us speculate that some of the electric current loops circulating in the molten core are in the vicinity of the core–mantle boundary, and that these currents partially penetrate into the mantle. At the boundary, depending on the direction of the electric currents, either a Peltier cooling or a Peltier heating will occur. If the conditions are suitable, on one side of the electric current loop the Peltier heating will raise the temperature of this zone above the melting-point of the mantle material, producing a melt of mantle. This melt, being of lighter silicate material, will float as a sludge over the material of the core at the core–mantle boundary. If the silicon metal is quite abundant, 10–20 atomic per cent in the molten core as Ringwood[9] has suggested, then it will indeed react with the iron oxide of the mantle melt giving rise to silica. The floating melt of the mantle, which is now enriched in silica at the expense of losing some iron to the core, will become even lighter than the original mixture. This aggregate of the molten sludge may eventually solidify in the relatively cold zone at the surface of the mantle, or it may remain as an aggregate of molten material in the vicinity of the hot zone.

The other side of the loop, the region at the core–mantle boundary, will be cooled by the Peltier effect. If the cooling continues faster than the rate at which heat is supplied by the floating mantle melt or other agents, then eventually the temperature of this locality may drop to the freezing point of the iron, giving rise to a partial solidification. The iron crystals with their impurities will grow on the solid mantle. If the cohesive forces between these crystals and the solid mantle are strong, so long as the temperature conditions prevail they may form a solid iron layer as a permanent feature of this locality. Runcorn[3] also has postulated the existence of a solid iron layer with varying thickness of up to 10 km. If the freezing-point temperature of the molten core is appreciably below the standard average temperature of the core–mantle boundary, then even a partial solidification of iron would be an unstable condition.

Similarly, the electric current loops, if they exist in the vicinity of the solid–liquid core (the F region), will give rise to partial melting of the solid core at one point, while freezing a portion of the molten core at another point. Such a process will form a series of undulations (hills and valleys) and thus produce a non-spherical boundary.

The coriolis forces due to the rotation of the Earth probably will increase interaction between the core and the mantle in the plane of the equator. Consequently, if the conditions are suitable, the Peltier activity should be maximal in the equatorial belt.

Based on these speculations, D'' layer is composed partially of mantle material (slightly enriched in silica and extruding non-symmetrically into the molten core), and partially of molten core material intruding into the base of the mantle. Bolt recently has suggested that F layer has an intermediate seismic velocity between the two cores[5–7]. An intermediate velocity would be obtained from the path of a ray passing partially through liquid and partially through solid core. According to this model, the two layers D'' and F are not continuous layers but probably have a maximum anomalous thickness in the vicinity of the equatorial belt.

Other geophysical evidence supporting the model. According to Miss Lehmann[15], the seismic data from the record at the epicentral distance of 105°–115° indicate the variation of the depth of the core–mantle boundary and possibly a variation in the constitution of the bordering region. On the basis of *PcP*, *ScS*, *PcS* and *ScP* waves, Vogel[16] has shown that the surface of the outer core is undulated with amplitudes up to 200 km.

Jeffreys[17] has a map of free air gravity anomalies which indicate some broad features of about 20 milligals spread around the Earth in the equatorial belt. I have maintained for a long while that the source of these anomalies can be in the neighbourhood of the core–mantle boundary. Recently published calculations by Egyed[16], which are based on the work of other investigators[18–20], also support this view. The striking similarity between the isopores of vertical magnetic intensity for the years 1942.5. pointed out by Egyed[16] was also observed by me and it was discussed with several colleagues of Stanford University. These magnetic anomalies may be utilized to locate the geometrical position of the major current loop within the molten core interacting with the mantle.

The three independent geophysical observations (seismic, gravity, magnetic) seem to support the model presented in this article.

Concluding remarks. For a current density $10^{-6} < I < 10^{-3}$ amp/cm^2 this mechanism is physically feasible when

the corresponding Peltier coefficient between the mantle and the core is:

$$-8 > P > -12{\cdot}3 \times 10^{-3}\,\mathrm{V}$$

To produce the layer D'' several million years of sustained electric current flow is necessary.

It is likely that the process of melting slightly outbalances the process of freezing; thus we can speculate that a molten sludge of mantle material has accumulated gradually. It follows that the layer D'' is made of non-continuous aggregate of molten sludges which travel with the current cells, which in turn give rise to magnetic secular variations. Such a model predicts a secular westward drift of gravity anomalies. However, if the conditions are such that the process of melting balances the process of freezing, then the layer D'' would represent predominantly a solid layer formed of mantle material enriched in silica. Consequently, the undulations would be a permanent feature of core–mantle boundary. If such undulations are produced by Peltier effect, we must seek the origin of some electric currents which are of more permanent nature and seek another explanation for the similarity between the gravity and the magnetic anomalies.

I thank Profs. G. A. Parks, G. A. Thompson, B. A. Bolt, J. Vergoogen and S. Ward for their advice. I also thank Dr. Francis Everitt and Margaret S. Guthrie for their assistance.

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LONG-PERIOD PULSATIONS OF AURORAL-ZONE X-RAYS

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During a series of balloon flights from Andenes in northern Norway, several observations were made of nearly periodic intensity variations of bremsstrahlung X-rays, with periods of about 8–10 min. This is approximately equal to the estimated period for the fundamental mode of oscillation of the geomagnetic field, and is also in good agreement with previous observations of long-period geomagnetic pulsations.

Observations of this kind may be of considerable theoretical interest, because of the information they may contain about the processes in the Earth's magnetosphere which accelerate electrons and precipitate them into the atmosphere. In this article we shall not, however, try to suggest or discuss possible acceleration mechanisms, but merely report the observations which have been made, and see how they fit in with other observations of long-period geomagnetic pulsations, and with our present ideas about the magnetic régime in the Earth's immediate neighbourhood.

Precipitation into the atmosphere of electrons with energies of the order of 25–100 keV was recorded through observation of the associated bremsstrahlung. Whereas electrons with such energies are stopped at altitudes of about 100 km, the X-rays produced in the process penetrate down to balloon altitudes of 25–35 km. The balloons were of the now common non-pressurized polyethylene type, which can float for about a day at a nearly constant pressure-level. The size of the balloons was about 4,200 m^3 and they usually floated at atmospheric depths of about 8–10 g/cm^2.

Most balloons carried a standard instrumentation consisting of two Victoreen type 1*B*85 and one type 6306 Geiger–Müller counters, all mounted horizontally and immediately above each other, with the 6306 counter in the middle position. The counting rates of the upper type 1*B*85 counter, of the type 6306 counter, and of the triple coincidence counter telescope formed by all three counters, were recorded. Some balloons carried more complicated systems of counters, with up to 7 counting channels. However, as all the results to be presented in this preliminary note are based on the records from one single unshielded counter channel, there is here no need for any more detailed description of the other detector systems.

Andenes is located at 69·3° N., 16·1° E., and lies very close to the line of maximum auroral activity. Altogether 29 balloons were launched during July 17–September 27, 1963. In spite of several balloon failures X-rays were observed during 17 of these flights. The average levels of solar activity and associated geophysical disturbances were unusually high during this period, considering how close it was to a solar cycle minimum.

Three obvious cases of nearly periodic X-ray intensity variations were observed. These are shown in Figs. 1*a*, 2*a* and 3*a*. It should be noted that the intensity scale is logarithmic in all diagrams, which reduces the heights of the peaks as compared with a linear plot. No associated variations in the counting rate of the counter telescope were observed, proving that the intensity increases above the cosmic-ray background were not caused by particle radiations.

In the first of the three cases here reported (August 5, Fig. 1*a*), the periodic intensity variation seems to be superimposed on a more slowly varying X-ray event, of the type which is often associated with magnetic bays[1]. Examination of magnetograms from the observatory of Tromsø, about 100 km geomagnetically east of Andenes, reveals that a magnetic bay with $\Delta H = -500\gamma$ did indeed occur at the time of this event, even though there was no detailed correspondence between magnetic variations at Tromsø and the X-ray intensity peaks recorded by the balloon-borne detectors.

The event of September 23 (Fig. 2*a*) also occurred in connexion with a strong negative magnetic bay ($\Delta H = -600\gamma$ at Tromsø). In this case there is no indication of a more slowly varying X-ray event on which the pulsations are superimposed, except that one peak, which

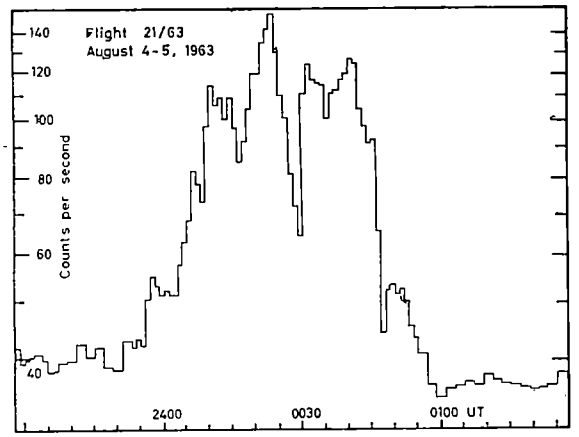


Fig. 1a*

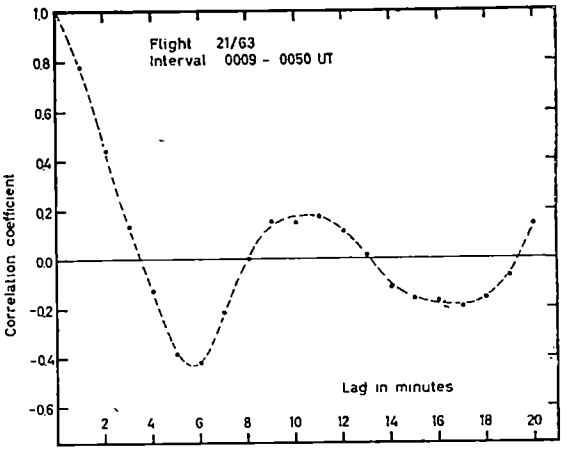


Fig. 1b

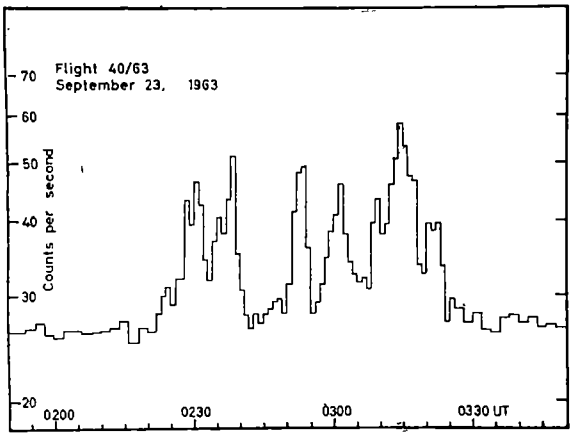


Fig. 2a

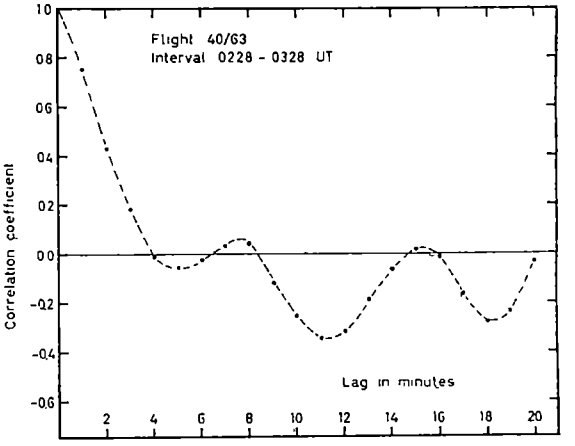


Fig. 2b

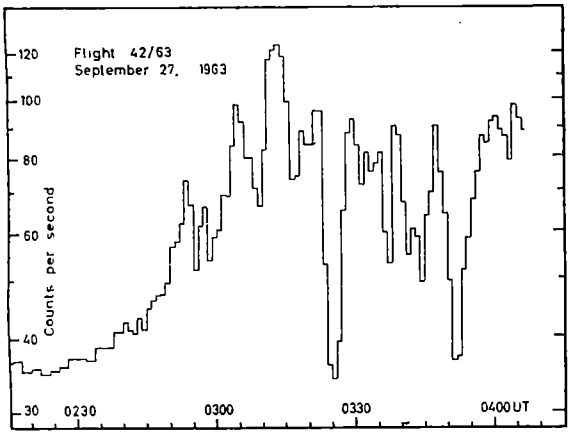


Fig. 3a

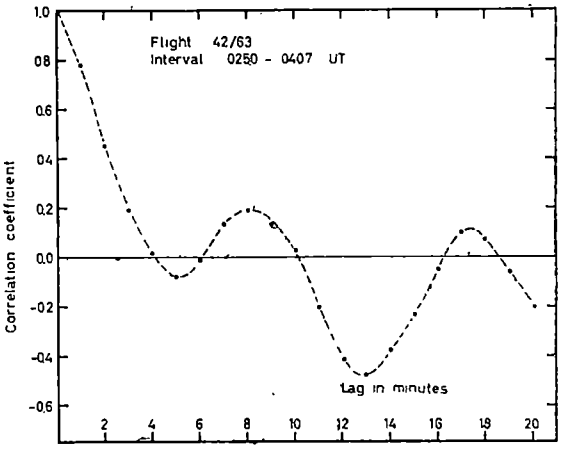


Fig. 3b

Fig. 1a. The X-ray event of August 5, 1963. Fig. 1b. Autocorrelogram for the event of August 5, for the interval of 0009–0050 U.T. Fig. 2a. The X-ray event of September 23, 1963. Fig. 2b. Autocorrelogram for the event of September 23, for the interval of 0228–0328 U.T. Fig. 3a. The X-ray event of September 27, 1963. Fig. 3b. Autocorrelogram for the event of September 27, for the interval of 0250–0407 U.T.
* The times given in this figure should be increased by 5 min due to an error in plotting

would have been the third of the series, appears to be missing. Again no detailed correspondence was found between magnetic and X-ray variations.

The third case (September 27, Fig. 3a) resembles the first one in that there again appears to be a superposition of a slowly varying X-ray event and a pulsating one, but at this time only a weak magnetic disturbance was recorded at Tromsø. There had, however, been considerable magnetic activity earlier during the night. This event was the most long-lasting of those here reported, and altogether 8 major peaks occurred between 0250 and 0403 U.T. Unfortunately the high-voltage power supply for the Geiger counters failed at 0407 U.T., and it is not known how long the pulsations continued.

The periods of the three pulsating X-ray events can be determined quite well from the average spacing of consecutive major intensity peaks, but it can be done more accurately by examining the corresponding autocorrelation functions. These have therefore been computed, and are presented in Figs. 1b–3b, corresponding to the events of Figs. 1a–3a. The periods are seen to be approximately 650, 450 and 520 sec, respectively.

Even though no detailed correspondence was found between any of the observed X-ray events and the mag-

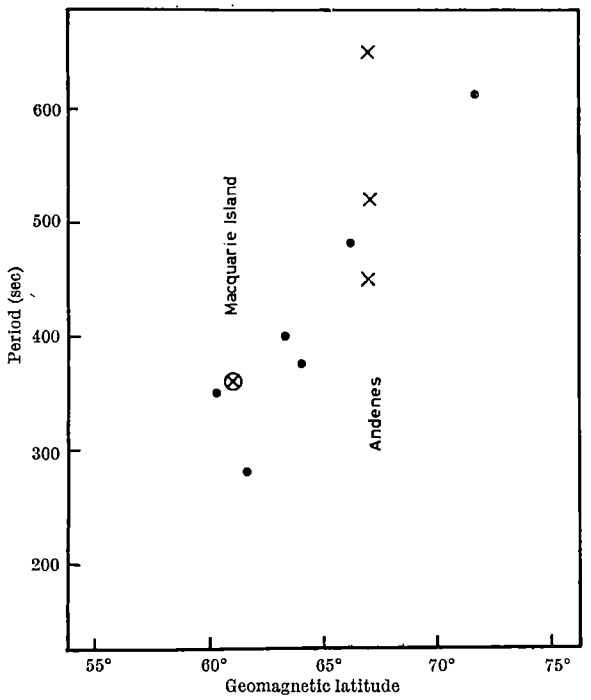


Fig. 4. Periods of geomagnetic pulsations and X-ray events as a function of geomagnetic latitude. Black dots represent periods of geomagnetic pulsations as summarized by Ohl (ref. 2), open crosses represent the X-ray pulsation periods reported in this paper, and the circled cross represents the X-ray event reported by Anger *et al.* (ref. 5)

netic variations recorded at Tromsø, we still think that the observed electron precipitation may have been associated with oscillations of corresponding periods in the Earth's magnetosphere, because of the near equality between the periods of these X-ray intensity variations and those of long-period giant magnetic pulsations.

In Fig. 4 we have plotted the observed periods of such magnetic pulsations against geomagnetic latitude, using data taken from Ohl[2], and the periods of the X-ray intensity variations observed by us, which are seen to be in fair agreement with the periods of geomagnetic pulsations.

Dungey[3] has suggested that geomagnetic micropulsations may be due to hydromagnetic oscillations in the magnetosphere. He showed that the fundamental mode would be an oscillation of the whole of a geomagnetic line of force in outer space, and estimated the corresponding period to be of the order of several minutes for lines of force connecting with the auroral zone. Furthermore, the period would increase rapidly with increasing geomagnetic latitude, because of the increasing length of the geomagnetic lines of force. Jacobs[4] showed that the period of oscillation would also depend on the size of the magnetosphere, and computed theoretical estimates for several different assumed values of the equatorial radius of the magnetosphere. The period would obviously increase with increasing radius, because this would extend the lines of force. For lines of force connecting with the auroral zones, Jacobs found periods between 100 and 1,000 sec, depending on the assumed size of the magnetosphere.

It is therefore interesting to note that of the three X-ray events reported here, that with the shortest period was associated with the highest value of the planetary K_p-index. This was the event of September 23, when the K_p-index for the 3-h interval encompassing the X-ray event was 9^-. The event of September 27, with a period of 520 sec, occurred at a time when K_p was only 3^+, and so also did the event of August 5, which had a period of 650 sec. However, the average K_p-indices for the 24-h intervals preceding these two events were 4 and 3^-, respectively. For the event of September 23, the corresponding 24-h average was 6^+. If the X-ray events discussed here are actually associated with oscillations of magnetic lines of force, this inverse relationship between K_p-index and X-ray pulsation period would be consistent with the expected dependence of the hydromagnetic oscillation period, on the size of the magnetosphere, because the higher the K_p-index the more should the magnetosphere be compressed by the solar wind.

The difference in periods between the events of September 27 and August 5 could, however, also be due to a diurnal variation of the period of oscillation of a magnetic line of force, associated with a diurnal change in the length of such a line. One would expect the length to be a maximum at geomagnetic midnight, when the line of force lies in the tail of the magnetosphere, and a minimum at geomagnetic noon when it lies in the compressed part of the magnetosphere. Now the event of September 27 occurred at about 0600 h local geomagnetic time, whereas the event of August 5 occurred at about 0300 h local geomagnetic time, and this might conceivably explain the difference in periods. The event of September 23, however, occurred at the same local time as the event of September 27, and no diurnal variation can account for the difference in periods between these two events. But these were the cases which were associated with such very different K_p-indices, as mentioned above.

Only two direct observations of long-period pulsating X-ray events have previously been reported. One event was observed by Anger *et al.*[5] during a balloon flight from Macquarie Island, Australia, between 61° and 62° S. geomagnetic latitude. The observed period was about 360 sec, which has also been plotted in Fig. 4 at the appropriate latitude, and is seen to fit in very well with the other observations. This event is the only one so far which has been definitely associated with corresponding geomagnetic pulsations. It occurred at approximately 8 h local geomagnetic time, and the K_p-index at the time was about 5^-. The other previously reported long-period pulsating X-ray event was observed by Evans[6], and was the most nearly periodic of all those so far reported. It had, however, a period of only 100 sec, but was recorded during a balloon flight from College, Alaska, at about 65° N geomagnetic latitude, and therefore definitely falls outside the group of events included in Fig. 4. The event reported by Evans may perhaps be associated with a different mode of oscillation of the geomagnetic field.

The three cases of periodic X-ray events here reported are the most conspicuous among several other cases of long-period (several minutes) pulsating events recorded during our 1963 series of balloon flights from Andenes. A systematic search for such periodicities is now being conducted.

Further investigations will be needed to decide whether the observed events are caused by periodic acceleration of electrons by hydromagnetic waves, or whether magnetic oscillations merely perturb the orbits of electrons already trapped in the radiation belts and thus cause them to enter the atmosphere with nearly periodically varying intensities.

It is also possible that the observed pulsations may be due to a periodic movement of the region of electron precipitation, which should be investigated by means of X-ray recordings performed simultaneously from two different balloons, separated by a suitable distance.

This work was financed mainly by a research grant from the NATO Scientific Committee in Paris, with additional contributions from the Norwegian Council for Technological and Scientific Research, and from the University of Bergen.

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COLOUR OF PRECIOUS OPAL

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SOME Australian opals are renowned for the glorious displays of colours which they exhibit. They are consequently known as precious opals, and are valued as gem stones.

It is known that the colours from precious opals are spectral and polarized, and it is commonly conceded that they are caused by 'diffraction' rather than 'interference'[1-4]. In 1932 Baier[3] made an extensive optical investigation of these colours, and concluded that they were produced by a lamellar structure. Later, Raman[4] suggested that opals displaying colours were made up of regularly spaced layers of two types of silica with different refractive indices. However, the work recorded here using electron microscopy has shown that, in fact, the colours are caused by diffraction from a regular three-dimensional structure arising from the regular packing of spherical particles of silica, 1500–4000 Å in diameter.

Structure of opals. Precious opals are brittle and easily fractured. When a shadowed replica of a freshly fractured surface is examined in an electron microscope, a regularly spaced array of regularly shaped holes can be seen (Figs. 1, 2 and 3). Replicas of surfaces etched in dilute hydrofluoric acid revealed the amorphous silica spheres of which these opals are composed[5] (Fig. 4). The holes in the fracture surface are presumed to be sections of the voids between the spheres, and although this is not generally obvious from their shapes (such as in Fig. 3) it is reasonably clear in the particular section shown in Fig. 2.

In any opal displaying colours, the spheres are remarkably uniform in size, and they may, therefore, pack regularly. That they do so can be seen in Fig. 4; but it is demonstrated more convincingly by the extensive regularity of the patterns of holes, such as in Fig. 1. Because this regularity appeared on all freshly fractured surfaces of precious opal which have been examined, it may be concluded generally that the voids and spheres lie on a regular three-dimensional lattice. Micrographs suggest that the packing is face-centred cubic, but that it may be faulted and twinned.

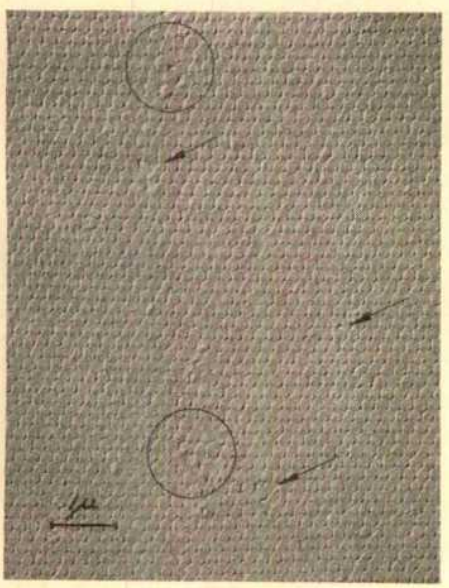


Fig. 1. Shadowed replica (Pt-C 20°) of a freshly fractured surface of an opal from Lightning Ridge (× *c.* 8,700)

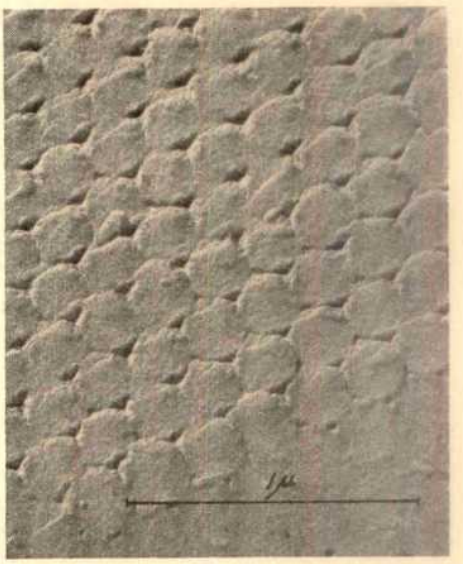


Fig. 2. Section of an area similar to Fig. 1 showing details of the holes (× *c.* 39,000)

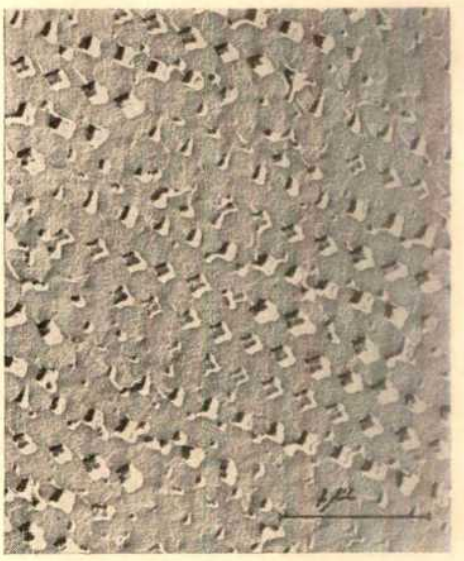


Fig. 3. Alternative section through the lattice of voids showing varying shapes of holes (C replica shadowed with uranium oxide, × *c.* 20,000)

Ideally, the voids between spheres in a close-packed array are either 'tetrahedral' or 'octahedral' and lie on the same lattice as the spheres. However, micrographs (Figs. 2 and 3) show that the holes have non-ideal shapes, and that the smaller tetrahedral voids are commonly missing.

It is considered that the matrix is generally optically transparent but, because the refractive index of silica is relatively high (~1·45), light should be scattered at the surface of the voids. Since the voids are regularly arranged, with a suitable lattice parameter, the whole array should act as a three-dimensional optical diffraction grating. The monochromatic diffracted beams produced by this grating are the colours which can be seen in opals.

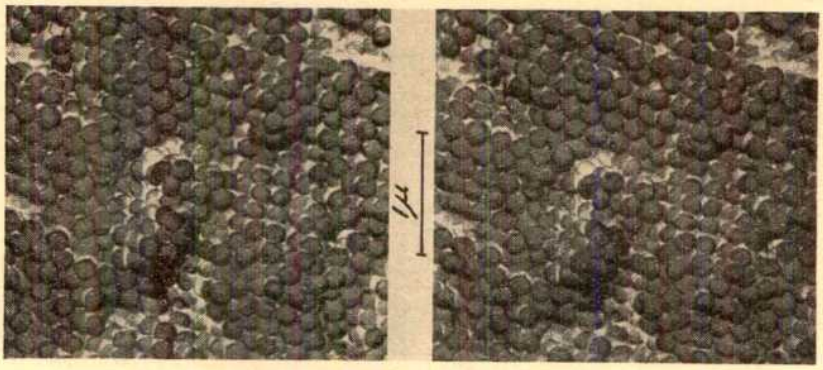


Fig. 4. Heavily etched (30 sec, 10 per cent HF) fracture surface of a coloured opal (Pt-C shadow + C, replica ; stereo pair, × 16,000)

Diffraction by the lattice of voids. The geometry of the diffraction conditions here is very similar to that which occurs in X-ray crystallography, because the wave-length of visible light (0·4–0·8μ) is about the same as the parameter (a_0 = 0·2–0·5μ) of the lattice produced by the spheres. A similar situation has been investigated for 'crystals' of latex spheres[6], and TIV virus[7,8], where it was established that the packing was face centred cubic rather than hexagonally close packed.

The range of colours which can be seen in a given specimen of opal will be determined by two factors. First, the size of the spheres will determine the lattice parameter, and hence the maximum wave-length which can appear. Secondly, the range of colours will be limited by refraction at the surface. In contrast to X-ray diffraction the refractive index is appreciably greater than one, and light rays are bent at the interface so that only a restricted range of Bragg angles is possible within the specimen.

The longest wave-length which can be diffracted by a face-centred-cubic arrangement (λ_{max}) is a beam which can be diffracted back by (111) planes, that is, the Bragg angle, θ, is π/2 and sin θ = 1. This maximum wave-length is related to the sphere diameter (d) and the refractive index (μ = 1·45 for amorphous silica) by:

$$\lambda_{max} = \mu \cdot 2a_0/\sqrt{3} = 2{\cdot}37d$$

for face-centred-cubic packing, when $a_0 = d\sqrt{2}$.

For a plane surface, refraction at the interface restricts the range of colours so that the minimum wave-length (λ_{min}) which can escape after being diffracted is:

$$\lambda_{min} = \lambda_{max}\sqrt{1 - 1/\mu^2} = 0{\cdot}72\ \lambda_{max}$$

Thus, for an opal which can just give red (λ_{max} = 7000 Å), the shortest wave-length which can be seen from it is green (λ_{min} = 5000 Å) if it has a plane surface.

For a given sample, with a fixed sphere diameter (and hence fixed a_0), shorter wave-lengths will appear at smaller Bragg angles. This effect is readily observed by taking an opal, looking at it with the incident light behind one's head, and rotating the opal until the colour of maximum wave-length is observed from a particular area. Movement of the observer about the opal, which is simultaneously rotated but at half the angular velocity, then gives the spectral colours in order of decreasing wave-lengths.

Faulting and twinning will modify slightly the conclusions drawn from a model in which silica spheres pack perfectly in a face-centred-cubic manner.

Verification of the explanation. This explanation of the cause of the colours was confirmed by examining three samples from Coober Pedy. They were: (1) a nearly transparent opal, which exhibited only a violet colour for all angles of incidence and observation; (2) a milky opal which gave only green or blue colours for all angles of incidence and observation; (3) a milky opal which by back diffraction was a homogeneous red for a given orientation of the sample. This colour changed to spectrally shorter wave-lengths as the angle between the observer and incident light was increased.

Replicas of these samples were examined and the spacing between the holes measured on areas selected for the simplicity of orientation. Other similar samples were etched (0·5 min in 10 per cent hydrofluoric acid) and sphere diameters measured from micrographs of shadowed replicas. In the table, these measurements are given together with estimated values for the maximum wave-length observed in the diffracted beams. The difference in the diameters of the spheres is clear from a comparison of Figs. 5*a*, *b* and *c*.

Table 1

Sample	Spacing between holes (Å)	Sphere diam. (Å)	λ_{max} calc. (Å)	λ_{max} obs. (Å)
I	1750	1750	4150	violet, 4000
II	2100	1900–2100*	4500–5000	green, 5000
III	2900	2500–3500*	5900–8300	red, 7000

* This is the scatter from several different samples.

Relationship between appearance and structure. It has now become clear that the play of colours which is so characteristic of precious opals occurs because the diffraction conditions are satisfied at different angles for different wave-lengths, so that, if an opal is viewed in an extended source of white light, the colour of the diffracted beams changes continuously as it is tilted. Areas over which the colour is the same have the same orientation of the lattice of voids. Such patches are like grains in polycrystals and

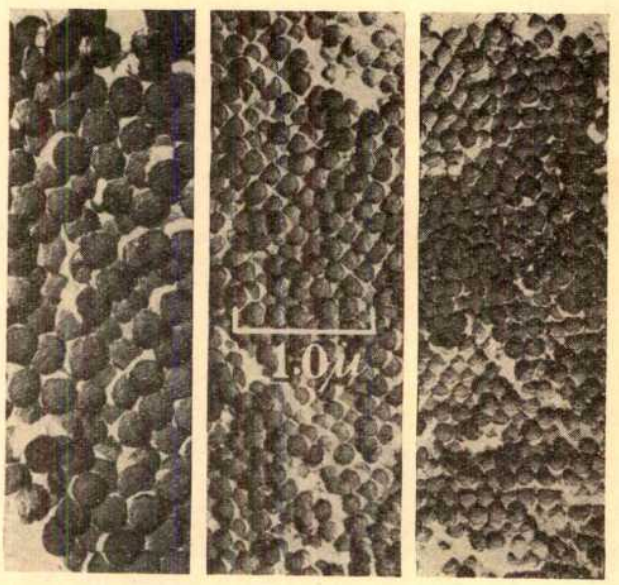


Fig. 5. Heavily etched fracture surfaces of coloured opals *a*, Type I (violet); *b*, type II (green); *c*, type III (red) (Pt-C replica, × 24,000

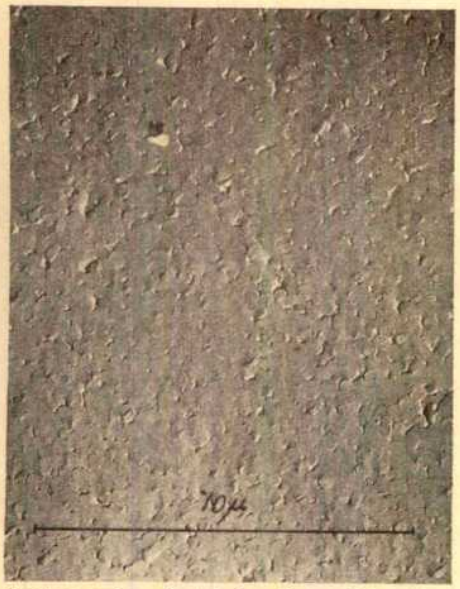


Fig. 6. Fracture surface of potch from Coober Pedy, not exhibiting colours, because there is no regularity of packing of the spheres (Pt-C shadowed replica, × 5,000)

their differences in size and distribution produce variety to the appearance of different samples of opals.

I have already explained how the range of colours depends on the size of the spheres, and their refractive index. This range may be extended to shorter wavelengths if refraction is eliminated by immersing an opal in a fluid of the same or greater refractive index. A dome of transparent plastic on a flat opal would enhance the colour-range in the same way.

When the packing of the spheres is not regular, voids still occur and scatter light; if the irregularities are too great, interference to produce diffracted beams of colour is not possible and the opals look homogeneously milky. Fig. 6 shows that such material (potch) contains voids, but they are irregular; etching confirms that potch are still made from spheres, but that their size and packing is not uniform. Where coloured patches or seams appear in potch, the packing of the spheres must have become regular. Rarely the packing is nearly perfect over a complete specimen and it is, therefore, transparent but diffracting. Thus the most precious opals are made from large spheres regularly packed with little disorder.

It is interesting to note that there are defects in packing of the silica spheres in opals, just as there are in the atomic or molecular packing in crystals. For example, Fig. 1 has been selected because it contains examples of dislocations (in circles) and point defects (arrows).

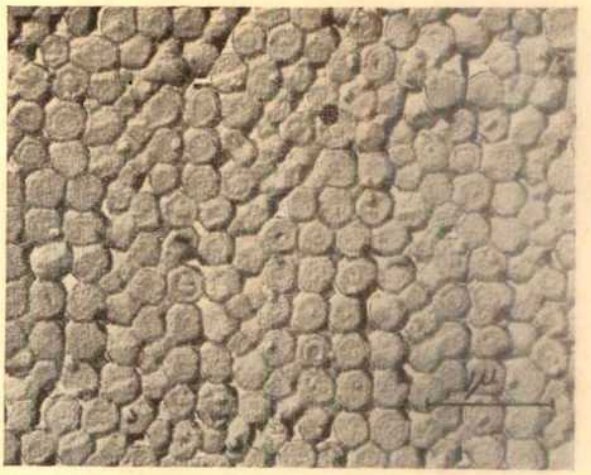


Fig. 7. Lightly etched (10 sec, 10 per cent HF) fracture surface of an opal from Coober Pedy (Pt-C shadowed replica, × 17,000)

Formation of the structure. The size of the holes is generally less than that expected from the close packing of hard spheres. However, etching revealed spheres of apparently almost perfect shape, and consequently it seems unlikely that the size of the voids has been reduced by a homogeneous compression. Furthermore, light etching revealed a detailed structure of sections through the spheres. The central dot and concentric rings (Fig. 7) are consistent with the spheres having grown in suspension by deposition of silica on to a precipitated nucleus. The spheres must then have grown to some size, constant for a given environment, and later packed together, possibly during a filtration process. The way in which the etchant reveals spheres of almost perfect shape suggests that it dissolves silica which has been deposited following the packing of the spheres, and which cements them in position. The extent to which the voids are filled should determine their 'scattering factor', and hence the intensity of the diffracted light. If too much 'cement' were precipitated the voids would become completely filled and the opal would be transparent and not coloured.

I thank Dr. E. R. Segnit, Mr. A. J. Gaskin and Mr. P. J. Darragh for their advice.

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PHOTOSENSITIZING PROPERTIES OF DYES AND WHITE PIGMENTS

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IN his investigations of the photosensitized oxidation of leuco malachite green by acriflavine, Kautsky[1] demonstrated the existence of an active oxidizing entity, but its nature has not been definitely established[1–4]. Evidence that this entity is probably O_2^- is provided by an examination of the similarities of the photosensitizing properties of eosin, acriflavine, the white pigments, zinc oxide, zinc sulphide and titanium dioxide, and various vat dyes. In the presence of oxygen and water the irradiation of these sensitizers by near ultra-violet and visible radiation results in the formation of hydrogen peroxide in solution[5–10]. Neither a dye peroxide[6] nor a metal peroxide[11] is formed. When the water is in the vapour phase the formation of a volatile oxidizing agent some distance from the sensitizer can be demonstrated by its effect on a textile fibre[9,10], or on filter paper containing starch and potassium iodide[10,12]. The sensitizer may be present on a textile fibre[9], or as a solid film which has been pasted with water on a glass plate and dried.

The results in Table 1 were obtained by exposing undyed cotton yarn to sunlight over solid films made with various sensitizers. The marked effect of the peroxide vapour

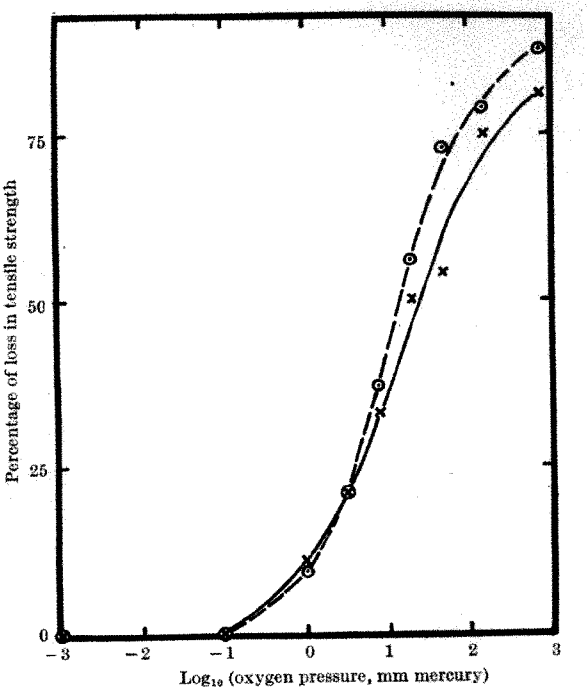


Fig. 1. Nylon yarn dyed with eosin (×) and 'Durindone Orange *R*' (⊙) exposed to sunlight for 24 days at different pressures of dry air and oxygen

produced by these sensitizers in moist air is clear. The degradation of undyed cotton yarn irradiated in the proximity of, but not in contact with, cotton yarn containing any of these sensitizers is increased in the presence of oxygen and water vapour, but it has not been possible to detect the volatile peroxide in the absence of water vapour[9,10]. The presence of oxygen, however, is necessary for the degradation of the dyed and undyed yarns whether or not water vapour is present[13]. These results are in agreement with the observations[7,8,11,14] that the formation of hydrogen peroxide requires the presence of both oxygen and water.

Kautsky[1] rigorously excluded water from his system, so that his entity is different from and actually has a very much shorter life than the volatile peroxide produced in the presence of oxygen and water vapour. Many of his experiments were carried out with the sensitizer and acceptor on separate granules of silica gel, but the sensitizing effect occurred over a much wider range of oxygen pressure when both were adsorbed on the same granule. A similar system would seem to be that in which the sensitizer is on a textile substrate and water vapour is excluded. Many dyes sensitize the degradation of textile fibres in dry oxygen-containing atmospheres by near ultra-violet and visible radiation, and it has been suggested[10] that the degradation is due to attack of the fibre by the oxidizing entity of Kautsky. If this view is correct, as recent results[16] would suggest, then measurement of the degradation of the textile fibre represents a much less sensitive but more quantitative method of assessing the effect of this entity than the oxidation of leuco malachite green. Nylon is particularly sensitive to photosensitized degradation in dry oxygen-containing atmospheres. Some results in which nylon yarns (Du Pont de Nemours, four-fold 45 den. 13 fil., free from delustrant) dyed with eosin and the sensitizing vat dye, 'Durindone Orange *R*', were exposed to sunlight behind glass at various pressures of dry air and in dry oxygen are shown in Fig. 1. The degradation of the dyed nylon is dependent on the oxygen content of the atmosphere and is negligible at low pressures of air. The similarity of the relations between oxygen pressure and degradation suggests that the same mechanism must be involved with both dyes. Similar results to those shown in Fig. 1 have also been obtained with other dyes on nylon, and with dyeings on cotton, silk and viscose rayon yarns. Comparison of the influence of oxygen on the photochemical degradation of undyed nylon containing titanium dioxide (incorporated in manufacture) and nylon free from delustrant but dyed with sensitizing dyes also suggests that the mechanism of degradation in dry oxygen-containing atmospheres is the same for titanium dioxide and dyes.

It is possible to impregnate textile fibres with aqueous suspensions of white pigments, but these treatments have no effect on the photochemical degradation of the textile fibre in dry air, although they may have a very marked effect in moist air[9]. However, if zinc sulphide is precipitated on the textile fibre by double decomposition, then the sensitizer appears to be deposited in sufficiently intimate contact with the textile material to cause sensitized degradation in dry air. This is illustrated in Table 2 for viscose rayon yarn. Similar results have also been obtained with cotton and silk yarns treated in the same manner. In moist air the degradation in the presence of zinc sulphide is the result of attack of the cellulose by the volatile peroxide, which is effective over appreciable distances, and consequently the degradation is not dependent on the state of the sensitizer in the fibre. On the other hand, in dry air photosensitized degradation only occurs when the sensitizer is in intimate contact with the fibre. This is presumably the result of the very limited range of the active oxidizing entity. The results in Table 3 show that the photosensitized oxidation of viscose rayon and cotton yarns by zinc sulphide in intimate contact with these fibres depends on the oxygen content of the atmosphere in exactly the same way as it does with a dyeing of 'Caledon Yellow 5*G*'.

The work described strongly indicates that in the formation of hydrogen peroxide in the presence of oxygen and water, and in the photosensitized degradation of textile fibres in dry and moist air, a common mechanism must

Table 1. Degradation of Undyed Cotton Yarn* exposed to Sunlight over Films of Sensitizer in Air at 100 per cent Relative Humidity

	% Loss in tensile strength	Rise in fluidity†
No film‡	10	6·6
Titanium dioxide	30	23·9
Zinc oxide	66	36·4
Zinc sulphide	67	34·6
No film‡	13	8·0
'Caledon Yellow 5*G*'	47	30·1
Eosin	76	43·7
Acriflavine	100	46·0

* 90s/3 Egyptian (Sakel) cotton, scoured and bleached; yarn about 4 mm from solid film.
† Increase in fluidity (poise^{-1}) over that of unexposed cotton; measurements made with 0·5 per cent solution of cotton in cuprammonium, according to standard method[15].
‡ Different exposures (about 6 weeks each).

Table 2. Degradation of Viscose Rayon Yarn* treated with Zinc Sulphide and exposed to Sunlight for 1 Month in Air at 0 per cent and 100 per cent Relative Humidity (R.H.)

Yarn	% Loss in tensile strength 0% R.H.	100% R.H.
Untreated	2	3
Pigmented with zinc sulphide	2	57
Zinc sulphide precipitated on fibre†	41	69

* Courtaulds, 3-fold, 150 den., scoured with warm dilute aqueous solution of soap and ammonia.
† Yarn treated with cold aqueous solution of sodium sulphide for 45 min, squeezed, treated with cold aqueous solution of zinc acetate for 45 min, washed and dried.

Table 3. Degradation of Cotton and Viscose Rayon Yarns after Exposure for 70 h to 250-W High-pressure Mercury-vapour Lamp

Dry gas	Rise in fluidity* Cotton: Zinc sulphide‡	Cotton: 'Caledon Yellow 5*G*'§	Viscose rayon†: Zinc sulphide‡	Viscose rayon†: 'Caledon Yellow 5*G*'§
Carbon dioxide	1·6	1·5	0·5	0·3
Air	16·2	16·5	7·9	5·2
Oxygen	26·4	30·1	15·1	13·7

* See Table 1.
† Measurements made with 2 per cent solution of rayon in cuprammonium.
‡ Yarn treated with successive solutions of sodium sulphide and zinc acetate, as Table 2.
§ Yarn dyed with vat dye, 'Caledon Yellow 5*G*', in normal way.

sensitizing dyes and white pigments. s should therefore all be capable of replac- in the Kautsky experiments involving ite green. Moreover, investigations[10] of the cal degradation of nylon dyed with vat dyes hat the active oxidizing entity involved in sky phenomenon must be an intermediate in the on (in the presence of water vapour) of the volatile de. The most conclusive investigations of the anism of the photochemical formation of hydrogen roxide have been made with zinc oxide[11,14,17,18]. The mechanism would seem to involve the capture of a photo-excited electron from the zinc oxide by an adsorbed oxygen molecule[17,18]:

$$\text{Sens} + O_2 \rightarrow \text{Sens}^+ + O_2^- \quad (1)$$

followed by peroxide forming reactions:

$$H^+ + O_2^- \rightarrow HO_2 \quad (2)$$

$$2HO_2 \rightarrow H_2O_2 + O_2 \quad (3)$$

in conformity with the observations that one molecule of oxygen disappears for each molecule of hydrogen peroxide formed[11], and that the oxygen in the peroxide originates entirely from the oxygen gas[17]. These conclusions should apply equally well to zinc sulphide and titanium dioxide. It is to be expected that the method of preparation of these white pigments will influence the removal of electrons from the photoexcited pigment, and so lead to the production of different amounts of hydrogen peroxide[8,14]. An analogous mechanism should apply to the photochemical formation of hydrogen peroxide by dyes, probably:

$$D \text{ (triplet)} + O_2 \rightarrow D^+ + O_2^- \quad (4)$$

a reaction suggested by Uri[19] as the initial step in the photochemical formation of hydrogen peroxide by eosin. The results of Rosenberg and Shombert[2] suggest that with solid adsorbates of acriflavine and fluorescein the excited dye molecule reacts in the triplet state with oxygen; but it is not certain that the triplet state of the dye is always the reactive state[20]. Terenin[21] has obtained some results with solid dye films which indicate that oxygen reduces the photoconductivity of a film of crystal violet by catching the conductivity electrons and is converted in the process into O_2^-.

If these views on the mechanism of the formation of hydrogen peroxide are correct, then the active oxidizing entity involved in the Kautsky phenomenon and the activated oxygen postulated as the initial step in the photochemical degradation of dyed textile fibres[10] must be O_2^-. Little is known about its oxidizing ability; but it is presumably due to hydrogen abstraction from organic molecules to form the ion HO_2^-. It would seem that in the photosensitized degradation of textile fibres by near ultra-violet and visible radiation the fibre is acting in most cases merely as a support medium for the sensitizer during the conversion of oxygen of the air into hydrogen peroxide. During this process the textile fibre is subject to attack by the intermediate (O_2^-) produced during the formation of hydrogen peroxide and by the entities (OH and HO_2) formed during its subsequent thermal or photochemical decomposition. The extent of degradation with any particular sensitizer depends on such factors as temperature[22], humidity of the air, which affects the amount of peroxide produced, and the chemical stability of the textile fibre. In some cases where the formation of the volatile peroxide by the dye seems to be enhanced by a particular substrate it may be that the abstraction by an oxygen molecule of an electron from the excited dye molecule is assisted by the state of the dye in the polymer material in somewhat the same way as the method of preparation influences the photoconducting properties of the white pigments.

These results indicate the importance of the work of Kautsky in the elucidation of reactions involving photo-sensitized oxidation in gas-solid systems.

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FINE STRUCTURE OF NATIVE CELLULOSE MICROFIBRILS

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THE molecular organization of native cellulose microfibrils has generally been described in terms of the fringed micellar theory, the microfibrils being thus regarded as an assembly of crystalline and amorphous regions. Although this concept has been widely used to explain the behaviour of cellulose fibres, there has recently been increasing evidence[1-3] that it is an over-simplification.

During the past few years investigations have been carried out in this laboratory to elucidate further the molecular morphology of native cellulose fibrils. The purpose of this article is to give a preliminary account of some of the results obtained.

Preparations of microfibrils of ramie, cotton, wood, *Valonia* and bacterial cellulose have been examined in the electron microscope using the negative staining technique[4-6]. Aqueous suspensions of microfibrils were obtained by ultrasonic disintegration. After suitable dilution a drop of suspension was placed on a carbon-coated grid and allowed to dry down. Afterwards a drop of staining agent (1 per cent sodium phosphotungstate or uranyl acetate) was applied to the grid and then withdrawn after a few seconds.

The specimens were examined in a *JEM 6A* electron microscope using the double condenser system and an accelerating voltage of 80 kV. Photographs were taken at an instrumental magnification of 80,000.

Fig. 1 shows a bundle of ramie microfibrils. The individual filaments are 35 Å in diameter and appear to

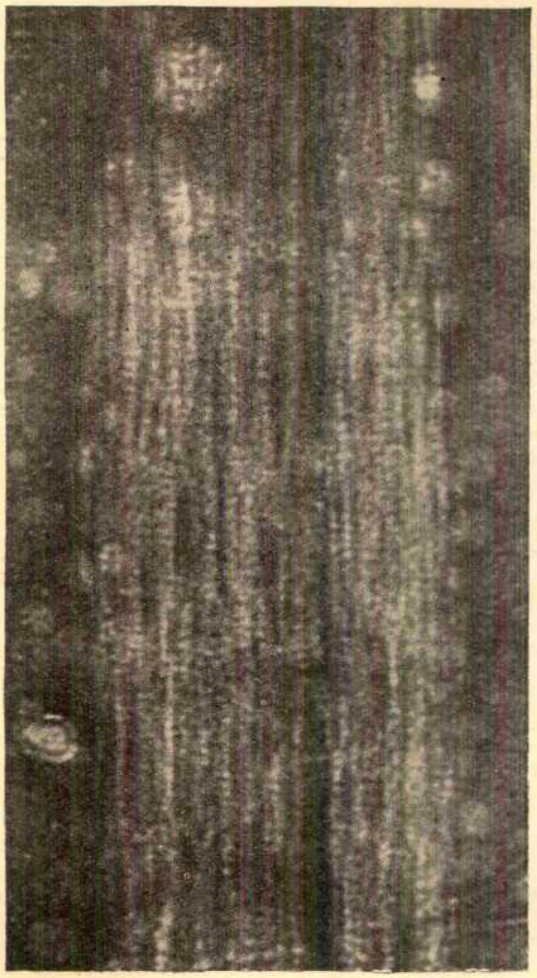


Fig. 1. Electron micrograph showing a bundle of parallel strings of ramie cellulose microfibrils. Negatively stained with sodium phosphotungstate (× 320,000)

have a periodic variation in structure along their length. The effect is rather reminiscent of parallel strings of pearls. Similar observations have been made on cotton, wood, and bacterial cellulose.

The structure of *Valonia* cellulose microfibrils is somewhat more complex. Previous studies in the electron microscope using metal shadowing indicated that these microfibrils are 150–300 Å in diameter[7]. Finer structural elements were not visible. New structural features are revealed by negative staining. Fig. 2 shows a *Valonia* microfibril which contains oval-shaped bodies separated by a short distance along the axis. The diameter of the fibril is 175 Å and the oval-shaped bodies vary in length from 200 to 400 Å. In some cases the microfibrils were wholly or partially devoid of the oval-shaped bodies mentioned above. In these circumstances it became evident that these large *Valonia* microfibrils are in fact composed of smaller filaments 35 Å in diameter. When seen isolated in favourable preparations, they appeared to have the same 'string of pearls' axial organization as seen in the microfibrils of ramie, cotton and wood cellulose.

Attention should be directed to the bent shape of the microfibril in Fig. 2. Normally these microfibrils are more or less straight; but in some cases, presumably as a result of stress on drying down on the substrate, or of ultrasonically induced compression or tension stresses on removal from the cell wall, they become deformed and kink without buckling. It has been repeatedly observed that the angle between the deformed portions is about 120°. At the moment we shall not go deeper into the significance of this observation or the equally interesting question of the nature of the oval s
microfibrils. This will be discussed fu

For the present our main concern is
filaments. Our observations on these stru
confirm and extend the findings of Mühlet
now seems reasonable to propose that these a
structural elements in all native celluloses. A
indicated here, these filaments appear to have a
variation in structure along their axis. At the
stage of our research the possibility that this b
appearance is an electron optical artefact cannot
completely excluded. However, it may still be useful
speculate on its significance, if real. A possible inte
pretation is that the 'beads' are not basic structura
singularities but result from the fact that the microfibril
is composed of a flat ribbon wound in the form of a tight
helix (Fig. 3). If this proposal is accepted, it is pertinent
to enquire how the cellulose chain molecules are disposed
within the coiled microfibril.

From X-ray diffraction investigations it is known that the molecular chain axis is arranged parallel to the length of the microfibrils. Accordingly, if the microfibril is indeed in the form of a tightly wound helix, it follows that there is but one way in which the cellulose molecules can be arranged so as to be parallel to the fibril axis. As seen in Fig. 3*b*, the molecules form flat ribbons by

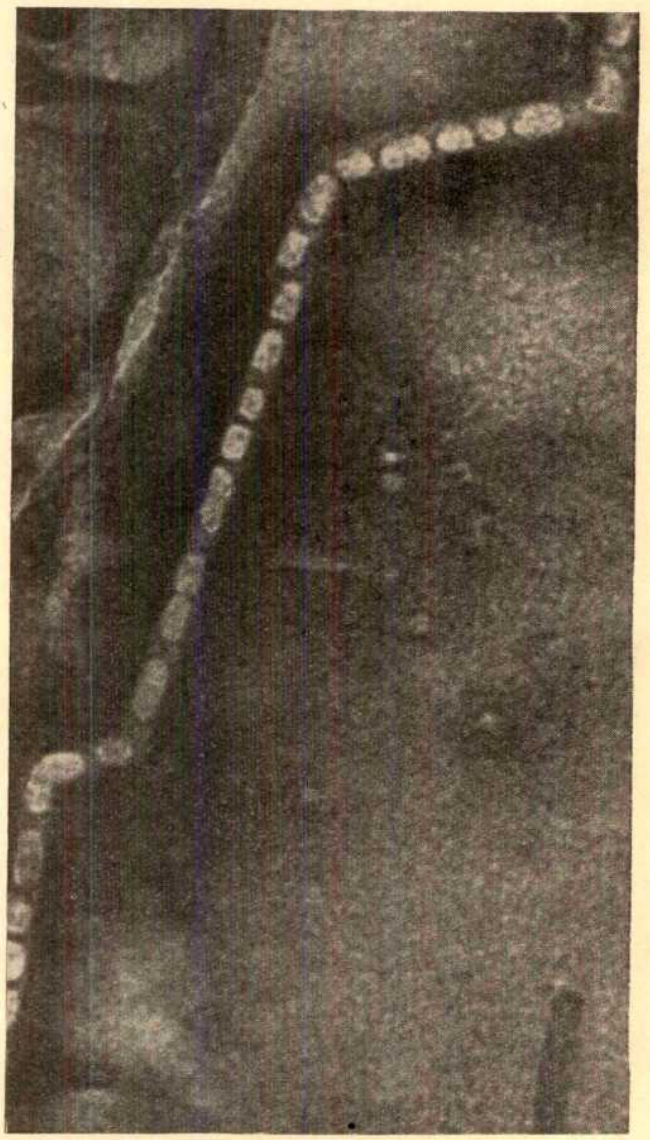


Fig. 2. Electron micrograph of a *Valonia* cellulose microfibril. Negatively stained with sodium phosphotungstate (× 248,000)

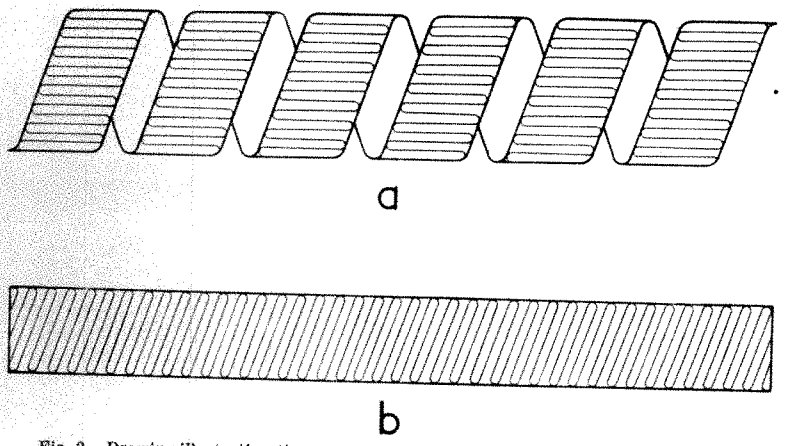


Fig. 3. Drawing illustrating the proposed morphology of a native cellulose microfibril

folding back and forth concertina fashion, and the molecular chain axis is tilted at some preferred angle to the ribbon axis. With the ribbon wound as a helix the molecular chain axis becomes parallel to the fibril axis (Fig. 3*a*).

The question arises whether the cellulose molecule is able to fold in the manner indicated. An examination of Stuart–Briegleb atomic models suggests that it is not only possible, but that only two glucose units would be involved in each fold.

The concept of twisted fibrillar crystals described here is not new. A similar type of helically wound crystalline ribbon has been proposed by Keller[9] for the component fibrils in polymer spherulites. However, in that model the molecules were oriented parallel to the ribbon and therefore nearly normal to the helix axis.

In order to obtain unequivocal evidence for the proposed folded chain configuration I have looked for X-ray low-angle meridional reflexions corresponding to a spacing of about 35 Å. All attempts to observe such a spacing have been unsuccessful. However, the absence of such reflexions is not a proof that the folded structure does not exist. Other methods of proving the structure are at present under investigation.

It is not possible to obtain an accurate measure of the cross-sectional dimensions of the ribbon from the electron micrographs. However, a reasonable estimate would be 10 Å × 35 Å. From the generally accepted Meyer-Misch unit cell dimensions[10] (a = 8·35, b = 10·3, c = 7·9, β = 84°) it is clear that the number of unit cells which could be accommodated within the ribbon cross-section is very small indeed. It seems paradoxical that so small a structure can give rise to discrete crystallographic interferences. Perhaps it would be profitable to consider the X-ray diffraction patterns of cellulose to arise from two different ordered systems, namely, the cellulose chains ordered within the microfibrils and an ordered arrangement of the microfibrils themselves. In this context, it is noteworthy that we have observed a 35 Å equatorial reflexion in low-angle X-ray diagrams of *Valonia* cell walls with the beam oriented parallel to the plane of the wall. This evidently arises from the lateral packing of the microfibrils.

An important characteristic of the model is that the microfibrils do not contain amorphous regions, at least as a separate phase. Each microfibril is really a quasi single crystal, and the diffuseness of the reflexions in the X-ray patterns of cellulose fibres would arise from various types of lattice imperfections; for example, the chain folds and disorder in the mutual arrangement of the microfibrils.

The proposed model has many interesting implications for the interpretation of the properties of cellulose fibres; but as the work is still in progress it would be premature to discuss these matters at the present time.

I thank Dr. S. Inoue for his assistance in obtaining the electron micrographs.

The work is supported under the Pioneering Research Program administered by the Institute of Paper Chemistry, Appleton, Wisconsin.

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BRAIN MATURATION MEASURED BY ELECTROSHOCK SEIZURES IN RATS AT HIGH ALTITUDE (12,470 ft.; 3,800 m)

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INCREASED brain convulsibility, as measured by electrically or chemically induced convulsions, has been reported in adult rats during acclimatization to moderate high altitude[1]. The work recorded here explores the effects of altitude acclimatization on brain maturation in developing rats taken to, or born at, 12,470 ft. (3,800 m). Brain maturation was measured by electroshock techniques in animals: (1) born and maintained at sea-level; (2) born at sea-level and taken to altitude during infancy; (3) born at altitude. Because previous investigations have demonstrated several physiological differences between sea-level and altitude rats[2], other indices of development were also measured in some animals, for example, body growth and weights of organs.

This work was conducted at the University of California, Berkeley, elevation 250 ft.; 76 m (755 mm mercury), and at the Barcroft Laboratory, White Mountain Research Station of the University of California, elevation 12,470 ft.; 3,800 m (480 mm mercury).

An average of 5 litters of Long–Evans rats were placed into each of the following groups: (1) sea-level controls; (2) rats (P) born at sea-level and taken to the Barcroft Laboratory at 4 days of age and maintained there for the entire experimental period; (3) and (4) rats of the first (F_1) and second (F_2) generation born at the Barcroft Laboratory. Both F_1 and F_2 rats as well as sea-level controls were killed at 28 days of age and the weights of total body and of the hypophysis, adrenal glands, thymus, heart and spleen were recorded.

Because in previous experiments brain maturation did not differ in male and female animals younger than 21

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Table 1. Brain Maturation as measured by Electroshock Responses in Sea-level Infant Rats and Several Generations of Rats at High Altitude (12,470 ft.)

Procedure	Time (days) of appearance of first hind-limb extension in 50% of rats	Duration (sec) of first hind-limb extension	Time (days) of appearance of adult seizure pattern in 50% of rats	Duration (sec) of tonic extension of fully mature adult seizure
Sea-level controls	17·8 (17·5–18·1)*	8·62 ± 0·42†	22·1 (21·7–22·5)	10·98 ± 0·35
Rats (P) born at sea-level and taken to 12,470 ft. at 4 days of age	19·7 (19·1–20·3)	11·37 ± 0·93	21·8 (21·1–22·5)	13·64 ± 0·37
P‡	< 0·05	< 0·001	> 0·05	< 0·001
Rats (F_1) born at 12,470 ft. first generation	19·0 (18·4–19·6)	11·07 ± 0·98	23·0 (22·3–23·7)	13·18 ± 0·63
P	< 0·05	< 0·02	< 0·05	< 0·001
Rats (F_2) born at 12,470 ft. second generation	20·8 (19·4–22·3)	15·25 ± 1·27	23·9 (22·1–25·8)	14·18 ± 1·01
P	< 0·05	< 0·001	< 0·05	< 0·001

* 95 per cent confidence limits. † Mean ± $S.E.$ ‡ Values based on t-test for significance of difference between sea-level controls and altitude rats.

Table 2. Organ- and Body-weights of Young Rats (F_1 and F_2) at 28 Days of Age born at Sea-level and at High Altitude (12,470 ft.)

Procedure	Whole body-weight (g)	Hypophysis		Adrenals		Thymus		Heart		Spleen	
		mg	mg/100 g body-weight	mg	mg/100 g body-weight	mg	mg/100 g body-weight	mg	mg/100 g body-weight	mg	mg/100 g body-weight
Sea-level controls	82·1 ± 0·9†	5·4 ± 0·7	6·6	26·2 ± 1·4	32·0	301·0 ± 13·0	367·0	394·0 ± 11·0	480·0	331·0 ± 12·0	403·0
Rats (F_1) born at 12,470 ft. first generation	46·4 ± 2·1	1·6 ± 0·2	3·5	15·9 ± 1·1	34·0	159·0 ± 15·0	343·0	757·0 ± 38·0	1,631·0	559·0 ± 38·0	1,205·0
P*	< 0·001	< 0·001		< 0·001		< 0·001		< 0·001		< 0·001	
Rats (F_2) born at 12,470 ft. second generation	34·9 ± 1·3	1·89 ± 0·1	5·2	11·1 ± 0·6	32·0	99·3 ± 4·5	286·0	1,375·0 ± 8·8	3,990·0	378·0 ± 17·8	1,014·0
P	< 0·001	< 0·001		< 0·001		< 0·001		< 0·001		< 0·05	

* Values based on t-test for significance of difference between sea-level controls and altitude rats. † Mean ± $S.E.$

days of age[3], animals of either sex were used. Young rats remained with their mothers until the termination of the experiment. Food (the standard rat pellet) and water were given *ad libitum* to the mothers. Conditions of nutrition, caging, temperature and illumination in the animal rooms at sea-level and at altitude were identical.

Electroshock seizures were produced through corneal electrodes as described by Woodbury and Davenport[4]. The stimulus, 50 m.amp alternating current delivered for 0·2 sec duration, activates a series of electronic timers which measure in msec the duration of each of the seizure components. Shocking was begun at 15 days of age. Since mortality is great in young rats shocked daily[3], each group of animals was further subdivided into 2 sub-groups, each sub-group being shocked on alternate days.

The brain of the rat does not reach functional maturity until about 3 weeks of age. During the sequential stages of development the types of responses to electroshock are in chronological succession: hyperkinesis, clonus, fore-limb tonic flexion, hind-limb tonic flexion, fore-limb tonic extension and hind-limb tonic extension[3]. These responses may be associated with biochemical and structural changes in the developing brain[5] and have been used in the young rat to assess brain development under various environmental conditions[3].

The time (days) of appearance of hind-limb extension and fully mature adult seizure pattern in 50 per cent of control and high-altitude rats, calculated by the method of Litchfield and Wilcoxon[6], is presented in Table 1. In high-altitude rats the appearance of hind-limb extension was delayed 2–3 days as compared with the controls. The fully mature seizure pattern appeared 1–2 days later than in controls in rats born at high altitude (F_1 and F_2) but occurred at the same time as the controls in those animals (P) born at sea-level and taken to altitude at 4 days of age. In general the delay in the appearance of adult seizures was proportional to the duration of high-altitude sojourn: it was longest in F_2 rats and shortest in P rats.

When brain maturation is attained at about 22 days of age, animals respond to electrical stimulation with the adult-type maximal electroshock seizure pattern[3]. The sequence of the main components of this pattern is hind-limb tonic flexion, hind-limb tonic extension and whole-body clonus[4]. The durations of the first hind-limb extension and of the adult tonic extension are given in Table 1. Tonic extension was significantly prolonged in all high-altitude rats as compared with controls. The greatest lengthening of this phase occurred in F_2 animals.

The sequential development of seizure patterns correlates with myelination of neuronal pathways involved[5]. Thus, a full tonic–clonic seizure is exhibited by rats in which the nervous system is functionally matured. The results presented here show that the appearance of the adult tonic–clonic seizure is significantly delayed in altitude-born rats. It is suggested that this delay may b due to retardation of brain maturation. Brain glycoge concentration is reduced in adult rats exposed to a altitude of 12,470 ft. for a period of 3–30 days[7]. Exposur to high altitude at an early age might also impair cerebral enzymatic activity because of a diminution of metabolic substrates and thereby delay maturation.

In electroshock seizures the shorter the duration of flexion and/or the longer the duration of extension the more severe is the convulsion[1]. Seizure intensity was markedly increased in adult rats exposed to an altitude of 12,470 ft.[1]. In the experiment described here the prolongation of extension indicates that although brain maturation was delayed, altitude exposure increased the severity of seizures once maturation was achieved.

Retardation in brain development can be related to other signs of altered growth at altitude. Sea-level species which breed at altitude produce litters which are less strong and have less chance for survival than corresponding controls[2]. Inhibition of body growth at altitude for P rats has already been reported[2], and was demonstrated here to be even greater in F_1 and F_2 rats (Table 2). Delayed maturation was further evidenced by significantly decreased hypophysis, adrenal and thymus weights in the animals born at high altitude (Table 2). Other effects of high altitude indicative of altered development were represented by marked cardiac and splenic hypertrophy (Table 2) as part of the cardiovascular compensatory mechanism to hypoxia.

Inasmuch as laboratory conditions for the rat were similar at high altitude and sea-level in terms of nutrition, temperature and illumination, the role of hypoxia and/or hypocapnia in causing neurological changes and other physiological manifestations is now being investigated.

We thank Dr. A. Vernadakis for her advice, and Mr. W. Roche for the care of the rat colony at Barcroft Laboratory.

This work was supported by U.S. Public Health Service grant *GM*–09267.

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EFFECT OF CIGARETTE-SMOKE CONDENSATES ON HOMOGRAFTS OF NEONATAL LUNG TISSUE IN MICE

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THE susceptibility of lungs of laboratory animals to the induction of bronchogenic carcinoma by the administration of polycyclic aromatic hydrocarbons, certain metals, radioactive substances and oncogenic viruses has been recently reviewed[1]. It was concluded that the histopathological characteristics of the tumours produced, including the squamous variety, are frequently similar to those observed in man. As yet tumours have not been produced in the lungs of experimental animals by direct inhalation of cigarette-smoke.

The direct effect of cigarette-smoke on lung tissue is still being investigated. Using human fœtal lung in organ culture technique, Lasnitzki[2,3] found that the condensate of cigarette-smoke produced hyperplasia and abnormal mitoses. More recently, Blacklock[4] reported that the direct inoculation with warmed tobacco condensate produced 11 per cent (8/72) malignant tumours of the lung in rats which were allowed to live until death. Hou and Willis[5], using a modification of Horning's[6,7] technique of inducing tumours by the direct application of a carcinogenic compound, found that in rat lung methylcholanthrene pellets produced sarcomas but that tobacco produced only one small papilloma. Since Horning had demonstrated that known carcinogens initiated a high incidence of bronchogenic carcinomas in mouse lung, it was considered important to determine whether lung homografts would respond in a neoplastic manner to cigarette condensate. Unlike most species of laboratory animals, mice develop spontaneous lung tumours. Since genetic susceptibility plays a significant part in the development of pulmonary adenomas in mice, two strains of mice known to have different genetic susceptibilities were used.

New-born strain *A/St* and *DBA/2* mice were used. They were killed with chloroform, and the lungs were removed intact. Each lung was placed in a sterile Petri dish containing a few drops of Ringer's solution. Marginal areas of the lung were removed to increase surface area exposure of the bronchial tissue. The remaining lung was cut into small pieces which would fit into a No. 13 trocar, and partly sliced horizontally with an iridectomy knife. A drop of the material to be tested was applied to each slice with a No. 4 camel-hair brush. The tissue was rolled up with the painted area inside to prevent contact between tar and host tissue. With a No. 13 transplanting trocar the grafts were implanted subcutaneously on either side of the ventral part of the body or in the thigh of the host. Host animals were 6–8 weeks of age at the time of implantation and were of the same sex and inbred strain as the animals from which grafts were taken. Each host animal carried a control graft treated with only a drop of saline.

Graft-bearing animals were not handled for the first two weeks subsequent to the transplant. Afterwards, the graft-bearing area was palpated weekly and recorded as being either firm and well-defined or soft and loose. A few of the host mice were killed as early as 8 weeks after transplant, since obvious growths several times the size of the original graft were noted; however, all remaining mice were killed when the grafts were 6–7 months old. Tumours and other grafts were sectioned, stained with hæmatoxylin and eosin and the diagnosis was confirmed histologically.

The cigarette-smoke condensate was prepared at the Roswell Park Memorial Institute by Dr. F. Bock. One hundred cigarettes of a variety of brands were smoked simultaneously in a manifold type machine having a negative pressure source controlled by an automatic valve[8]. The smoke was collected, condensed, removed with acetone and was condensed at reduced pressure to provide the crude tar, *A*. The *B* tar fraction was obtained by diluting the *A* fraction so that the final concentration of cigarette tar was 15 ml. acetone per carton of cigarettes. The heptane fraction was obtained by dissolving the crude tar in the minimal amount of acetone required and slowly adding eight parts of heptane. The heptane soluble fraction was decanted, condensed, and redissolved in 15 ml. acetone per carton of cigarettes. Solutions were replaced every two weeks.

The numbers of grafts which survived in the treated groups are shown in Table 1. In each series of experiments, 120 lung pieces were obtained from strain *A/St* mice and *DBA/2* mice of either sex and implanted into 30 host mice of the same strain and sex. In all, 180 host mice received 720 grafts of which 90 grafts (12·5 per cent) survived. The proportion of grafts surviving was greatest for tar fraction *A*, which contained the least amount of acetone.

Of the 120 control grafts implanted subcutaneously with saline, 43 (36 per cent) survived, and none developed tumours. Recognizable lung tissue was recovered from both the thigh and the abdominal area.

Successful grafts were firm, and histological examination showed various combinations of complete lung tissue and bronchial elements (Fig. 1). In some instances the alveolar parenchyma was distended while in others it was collapsed. Grafts that failed to grow felt soft when palpated and appeared to be undergoing resorption. The

nuclei were pyknotic, the cytoplasm homogeneous, and areas of necrosis and inflammation were noted.

A positive carcinogenic effect of tar was observed with 23 of the 90 successful grafts (25·6 per cent). Of these neoplasms, 63 per cent were associated with the heptane fraction. With the exception of the pulmonary adenomas, mammary adenocarcinomas and the one possible bronchogenic carcinoma, the tumorous growths are referred to as solid masses, since there is no indication of the tissue of origin. All the tumours were found in strain *A* mice except four adenocarcinomas found in the *DBA* mice, three of which were definitely associated with mammary tissue.

Although primary tumours arose in the grafted area as early as 8 weeks after implantation, the majority took 12 weeks. The one possible bronchogenic carcinoma occurred in lung tissue which had been painted with the heptane fraction. Under low magnification, bronchioles with ciliated epithelium were seen as well as lymphoid tissue and a tumour mass. Under high magnification, this tissue (Fig. 2) appeared to be composed of tumour cells which formed solid sheets of large, polygonal, closely packed cells with abundant cytoplasm, small nuclei and many mitotic figures. Figs. 3 and 4 show examples of remaining tumour masses. A graft from a strain *A* female treated with tar *A* fraction developed a tumour mass composed of coils of stratified squamous epithelium with marked keratinization in some of the coils. This was an adenocarcinoma, probably of mammary gland in origin, with squamous metaplasia. A strain *A* male



Fig. 1. Subcutaneous lung implant treated with heptane tar fraction. Section from strain *A* male shows two bronchioles with ciliated epithelium in middle of loose connective tissue. No tumour noted. (× *c.* 70)

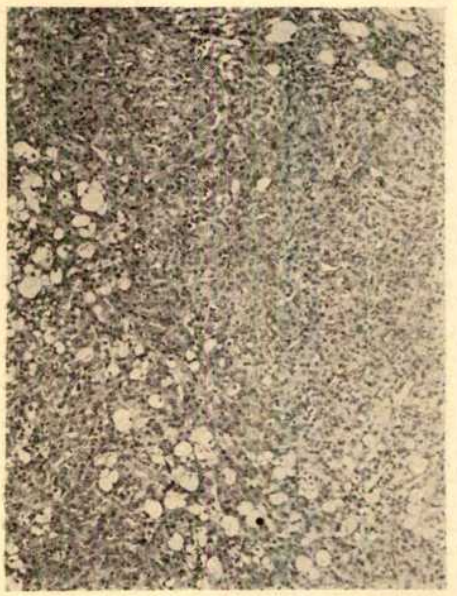


Fig. 2. Subcutaneous lung implant treated with heptane tar fraction. Tumour cells form solid sheets of large, polygonal, closely packed cells with abundant cytoplasm, small nuclei and many mitotic figures. Vacuolated spaces probably represent fat. Compatible with bronchogenic carcinoma. (× *c.* 70)



Fig. 3. Large tumour mass (adenocanthoma) from a strain *A* female which had received lung graft treated with tar *A*. Mass contains glandular elements and pearls of stratified squamous epithelium with marked keratinization in some. Other sections had tubular structures suggesting mammary origin. (× *c.* 70)

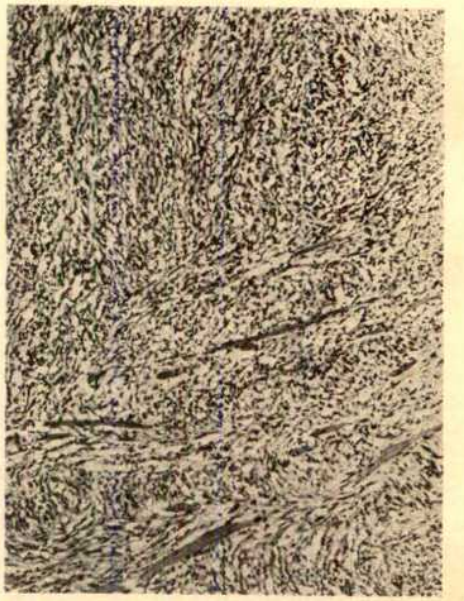


Fig. 4. Subcutaneous tumour mass containing whorls and sheets of small spindle cells. Fibrosarcoma from strain *A* which had received heptane-treated lung. (× *c.* 70)

Table 1. NUMBER OF TAR-TREATED GRAFTS SURVIVING TO SIX MONTHS AND INCIDENCE OF TUMOURS

Treatment	Mouse				Normal		Tumours										
	Strain		Sex				Total tumours	Fibrosarcoma	Bronchogenic carcinoma	Adenoma	Adenocanthoma	Sarcoma	Carcinoma	Adenocarcinoma	Adenocarcinoma, mammary	Undifferentiated tumour	Anaplastic carcinoma mammary
	A/St	*DBA/2*	F	M	Bronchi only	Lung											
Tar *A*	23			23	14	4	5			2			1			1	1
		15	8	7	5	8	2								2		
Tar *B*	11			11	8	1	2						1			1	
		11	2	9	11												
Heptane fraction	18		11	7	4	2	12	1	1		1	1	4	3		1	
		12	8	4	6	4	2							1	1		

bearing a heptane-treated lung graft developed, after 12 weeks, a tumour mass consisting of whorls and sheets of small spindle cells compatible with a fibrosarcoma, possibly a spontaneous tumour (Fig. 4).

Tar application to lung homografts was toxic to the grafts since the permanently implanted rate for such treated grafts was only one-third that of the control group.

As indicated in Table 1, many grafts, particularly those which did not develop tumours, retained their normal lung structures such as mucus-secreting epithelium, ciliated epithelium, and, at times, normally distended alveolar architecture. The majority of the tumour masses, however, could not be identified as having originated from the lung.

Since no tumorous growths were found in any of the control grafts or in the area where these grafts had been implanted in control animals, it must be concluded that the tumour masses were associated with the tobacco tar condensate. Subcutaneous injection of cigarette-smoke condensate or alcohol extracts of the cigarette tobacco have been shown to induce sarcomas at the injection site in the rat[9].

The results obtained in our experiments may be compared to those of Hou and Willis[5] and to those of Horning[6,7]. With the exception of one small intracystic papilloma, Hou and Willis reported negative results with whole tar and with the other tobacco fractions used, while methylcholanthrene elicited occasional neoplastic change in grafted bronchial epithelium. In other cases involving methylcholanthrene, various types of sarcomas developed, probably arising from the connective tissue of both the grafts and the host. Horning found that methylcholanthrene crystals applied to homografts of young adult mouse lung results in a high incidence of adenocarcinomas which were considered bronchogenic. Horning's results with chemical carcinogens and our finding that most of the tumours associated with tobacco tar occurred in strain *A* mice are consistent with the greater susceptibility of certain strains of mice to the development of tumours.

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AUTORADIOGRAPHIC EXAMINATION OF THE EFFECTS OF ENRICHED ENVIRONMENT ON THE RATE OF GLIAL MULTIPLICATION IN THE ADULT RAT BRAIN

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THIS pilot experiment was prompted by the results of a recent autoradiographic investigation[1] in which we injected tritiated thymidine into normal adult rats and cats and obtained evidence of the incorporation of this specific DNA precursor by the nuclei of many glia cells. This indicated that glia cells are multiplying in the normal adult brain. Recent electron microscopic and biochemical investigations have suggested that neuroglia form an insulating matrix and a sustaining medium for the neurones embedded in them, and there has been a resurgent interest in the possible role of glia cells in the organization of neural function and behaviour[2]. Is glial multiplication an aspect in the organization and re-organization of brain and behaviour? Since, through their multiplication, neuroglia could modify the electrical and chemical properties of neighbouring neurones, and through their paramount role in myelin formation they could recruit new neurones for newly organized circuits, we undertook to determine if changes occur in the rate of glial multiplication as a consequence of a sustained and radical behavioural manipulation. The procedure utilized in this work was one originally introduced by Hebb[3] in which rats are reared in 'impoverished' and 'enriched' environments, representing different conditions for the development of behaviour. This procedure was shown to affect the subsequent learning ability of animals[4,5] as well as the concentration of cholinesterase in brain structures[6,7] and the size of the cortex[8,9].

Laboratory-bred, normally raised Long–Evans hooded rats were weaned at the age of 23–26 days. From each litter, half of the males were transferred into isolation cages 10 in. deep, 4 in. wide and 6·5 in. high, covered with opaque material on all sides except the bottom. This represented the 'restricted' environment. The other half of the males, and all the females, were put into a large communal cage, 30 in. deep, 45 in. wide and 70 in. high, with a 'Plexiglass' front. This latter cage, which represented the 'enriched' environment, had three levels. The food trays and water bottles were initially placed at the first level which could be reached by the animals by climbing a metal strip 1 in. wide. The food trays and water bottles were gradually shifted from level to level and their position was changed every second or third day, forcing the animals to climb higher and follow varied routes to changing locations of food and water. Later, some of the metal strips forming the passageways were removed and replaced by less easily negotiable chains, then gaps were left across which the animals had to jump. In addition to this regimen, the animals, from the age of 1·5 months, were made to run 5 days every week in a

motor-driven activity wheel 14 in. in diameter, the speed of which was gradually increased from 3 to 8 r.p.m. while the duration of daily running was increased from 15 min to 1 h.

At the age of about 4·5 months 3 restricted and 3 enriched rats, matched as nearly as possible for weight, were injected intraperitoneally on two successive days with 2 mc. of tritiated thymidine (specific activity 6·0 c./mmole, dissolved in 2 ml. of sterile water). After the injections the animals continued to live in their respective environments for 1 week and were then killed by cardiac perfusion with 10 per cent neutral formalin. In a similar manner 3 'restricted' and 3 'enriched' rats were injected with 2·4 mc. of tritiated leucine (specific activity 5·4 c./mmole; dissolved in 2·4 ml. of sterile isotonic saline) and killed after an exchange period of 60 min by cardiac perfusion with neutral formalin. While an attempt was made to match the restricted and enriched animals for weight, the mean body-weight of the 6 enriched rats was 5·2 per cent below the body-weight of the 6 restricted rats. In contrast, the brain-weight of the enriched rats was 9·7 per cent above the brain-weight of the restricted rats ($P < 0·01$). The fixed brains were embedded in 'Paraplast' and 6-μ sections were cut serially. The deparaffinized sections from the brains of the thymidine-injected rats were stained with gallocyanin chromalum and all sections were coated in the dark by the dipping technique with *NTB*-3 nuclear emulsion. The coated slides were exposed for 13 weeks at 5° C and were then developed in the usual manner. In this article we shall deal essentially with autoradiographic results obtained from the animals injected with tritiated thymidine, with occasional references to findings with the animals injected with tritiated leucine.

The glial nuclei incorporating tritiated thymidine are easily recognizable by the overlying reduced silver grains (Fig. 1). Such 'labelled' glial cells were identified in varied numbers in all the animals, both in white and grey matter, over the entire neuraxis. However, the frequency of labelled glial cells varied over different regions. For quantitative evaluation, the number of labelled glial cells in circular areas 310μ in diameter was counted at a high magnification (500 times) over the entire surface of several brain structures. To obtain sufficient samples, some structures with identifiable components were counted as single entities (for example, 'dorsal thalamus'). Table 1 gives a summary of the results, showing a significant increase in the number of labelled glial cells over the neocortex in the group of enriched rats. A trend of statistically non-significant increase was also obtained in the rhinencephalon, thalamus, inferior colliculus and medulla, with the exception of the hypothalamus in which the number of labelled cells in the enriched and impoverished animals was identical. Table 2 gives a breakdown for the neocortex at different coronal levels, indicating greater increases in some regions than others. But in all instances the increases were higher in the neocortex than in other structures.

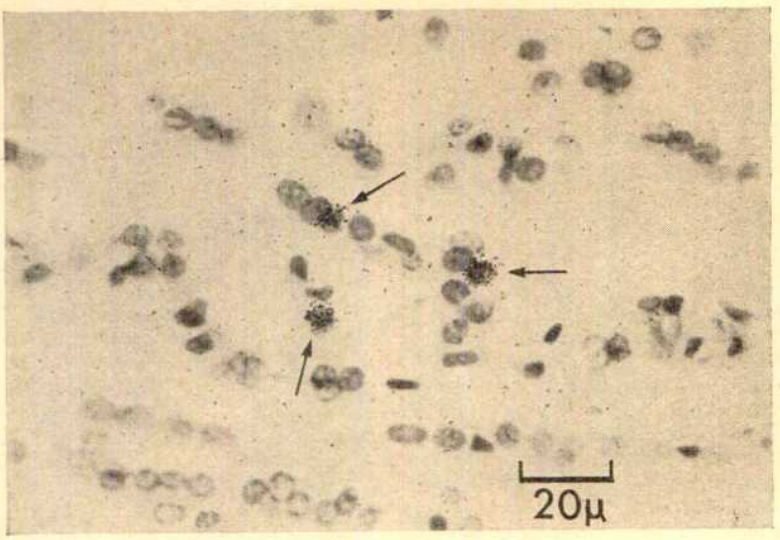


Fig. 1. Photomicrograph of a gallocyanin and chromalum stained autoradiogram. Arrows point to labelled glia cells in cortical radiation

In addition to an increase in labelled cells per unit area in the neocortex of enriched rats, areal measurements at 160 times magnification gave significant increases in the size of the cortex of enriched animals at all coronal levels examined (Table 3). This increase in the area of the cortex should further contribute to the absolute increase in the number of labelled glia cells. A comparison of the mean total number of labelled cells at different cortical levels is given in Table 4, which indicates a pooled increase of 75 per cent in the total number of labelled glia cells in the sampled sections of the cortex of enriched rats. The number of labelled cells given in Table 4 represents cells preparing for multiplication at the time of injection (and possibly some of their daughter cells) in an average section of the cortex with a nominal thickness of 1–2μ, which is the range of penetration of the electrons emitted by tritium into the emulsion.

We did not attempt a breakdown of the type of neuroglia cells labelled in the cortex, but it was clear from our results that the great increase in labelled cells in the cortex of enriched animals was to be attributed largely to cells situated in the cortical radiation and corpus callosum rather than the grey matter of the cortex. This observation can be interpreted in at least two ways. First, the increased labelling of cells in these fibrous structures may be a regional phenomenon, perhaps reflecting the proliferation of interfascicular glia cells. An alternative interpretation is that the labelled cells in these tracts are undifferentiated cells which are in the process of migration to undetermined cortical sites. This latter possibility was suggested by earlier histological observations of the presence of an ependymal and sub-ependymal germinal matrix in adult rats in the dorsal and lateral walls of the lateral ventricle[11–13], a finding which was confirmed more recently in colchicine-treated animals[14] and in animals injected with tritiated thymidine[15–18]. According to recent autoradiographic evidence from our laboratory (unpublished), the cells of the sub-ependymal layer of the dorsal

Table 1. Mean Number of Labelled Glia Cells in Circular Areas 310μ in Diameter in the Brains of Rats Reared in Enriched and Impoverished Environments

In all instances the designated structures were scanned in their entirety bilaterally.

Structure	Enriched group	Impoverished group	% increase in enriched group	*t*-test *P*
Neocortex	0·44	0·28	59	< 0·001
Amygdala and pyriform cortex	0·38	0·34	12	n.s.
'Dorsal thalamus'	0·28	0·24	17	n.s.
Hypothalamus	0·25	0·25	0	n.s.
Inferior colliculus	0·34	0·28	21	n.s.
Medulla	0·20	0·18	11	n.s.

Table 2. Mean Number of Labelled Glia Cells in Circular Areas 310μ in Diameter at Different Coronal Levels in the Neocortex

Coronal sections of neocortex at the level of:	Stereotaxic co-ordinate (ref. 10)	Enriched group	Impoverished group	% increase in enriched group	*t*-test *P*
(*a*) Inferior colliculus	—	0·37	0·17	118	< 0·01
(*b*) Oculomotor nucleus	*A* 1·4	0·45	0·34	32	< 0·05
(*c*) Parafascicular nucleus	*A* 3·0	0·47	0·28	68	< 0·10
(*d*) Posterior mamillary nucleus	*A* 3·4	0·52	0·31	68	< 0·02
(*e*) Mediodorsal nucleus of thalamus	*A* 4·6	0·48	0·32	50	< 0·01
(*f*) Paraventricular nucleus of hypothalamus	*A* 6·2	0·41	0·28	46	< 0·10
(*g*) Caudate nucleus	*A* 9·4	0·41	0·31	32	< 0·20

Table 3. Mean Area of Neocortex at Different Coronal Levels

Coronal sections of neocortex at the level of:	Stereotaxic co-ordinate (ref. 10)	Enriched group $\times 10^3$ square μ	Impoverished group $\times 10^3$ square μ	% increase in enriched group	t-test P
(*a*) Oculomotor nucleus	*A* 1·4	537	477	12·58	< 0·010
(*b*) Parafascicular nucleus	*A* 3·0	596	502	18·73	< 0·005
(*c*) Posterior mamillary nucleus	*A* 3·4	596	544	9·56	< 0·050
(*d*) Mediodorsal nucleus of thalamus	*A* 4·6	660	576	14·58	< 0·005
(*e*) Paraventricular nucleus of hypothalamus	*A* 6·2	675	624	8·17	< 0·005
(*f*) Optic chiasma	*A* 7·8	661	605	9·26	< 0·005

Table 4. Mean Total Number of Labelled Glia Cells at Different Coronal Levels of the Neocortex

Coronal sections of neocortex at level of:	Stereotaxic co-ordinate (ref. 10)	Enriched group	Impoverished group	% increase in enriched group	t-test P
(*a*) Inferior colliculus	—	57·5	23·8	142	< 0·10
(*b*) Oculomotor nucleus	*A* 1·4	68·8	39·7	73	< 0·001
(*c*) Parafascicular nucleus	*A* 3·0	79·7	38·8	105	< 0·05
(*d*) Posterior mamillary nucleus	*A* 3·4	76·5	43·8	75	< 0·005
(*e*) Mediodorsal nucleus of thalamus	*A* 4·6	83·5	47·2	69	< 0·005
(*f*) Paraventricular nucleus of hypothalamus	*A* 6·2	68·5	41·3	65	< 0·10
(*g*) Caudate nucleus	*A* 9·4	68·8	53·7	28	< 0·30
Neocortex as a whole	—	71·9	41·2	75	< 0·001

and lateral walls of the anterior lateral ventricle are in a rapid process of turnover in young as well as adult rats. At this site the majority of labelled cells in the adult rat disappear after survival for 2 weeks, whence the labelled cells migrate to as yet undetermined sites along the cortical radiation over the wall of the caudate nucleus. Since the animals in this investigation were permitted to survive for 1 week only after injection of tritiated thymidine, the cells produced along the wall of the lateral ventricle may have been caught in their migration along the cortical tracts. If this hypothesis were correct, we could expect a more rapid depletion of labelled cells in the wall of the lateral ventricle in the enriched than in the impoverished animals. Accordingly, we counted the number of labelled cells in the dorso-lateral wall of the lateral ventricle and in the adjacent portion of the cortical radiation which is characteristically studded with dark-staining nuclei (the presumed path of migration). Supporting the hypothesis, we found a smaller proportion of labelled cells in this germinal region in the enriched animals (15·6 per cent) than in the impoverished animals (21·1 per cent), though the difference between the two groups was not statistically significant.

It could be argued that the uptake of labelled thymidine by a larger number of glia cells in the cortex of enriched animals is not due to a higher rate of cellular proliferation but to an increased availability of exogenous chemicals as a consequence of changed circulation or a modified 'blood-brain barrier'. This possibility is counter-indicated by our finding of an absence of increased uptake of tritiated leucine in the brain of enriched animals. Indeed, paralleling the findings of Krech *et al.*[6,7], preliminary evaluation of our results indicates a decline in 'protein metabolism' per unit area in the cortex of enriched rats. Our findings also confirm the report of these investigators, based on much larger samples, of an increase in the weight and the volume of the cortex in the enriched animals. The increased addition of new glia cells to the cortex might be one of the variables contributing to this increase in cortical volume, but other variables (such as increased outgrowth of neuronal processes and increased vascularization) may also be involved.

In summary, this pilot investigation brought evidence of the addition of a higher number of newly formed glia cells to the cortex of rats reared in an enriched environment than in rats reared in an impoverished milieu. A trend of less pronounced increase in labelled glia cells was also observed in most of the subcortical structures sampled, the hypothalamus excepted. Since the hypothalamus is the only structure in the series which is practically devoid of corticopetal and corticofugal fibres, the increase in the other subcortical structures may be attributed to cortical fibres passing through them. As a final point we stress that these results cannot be interpreted as representing morphological changes produced by learning. The raising of animals in an enriched environment represents a complex of behavioural and related variables, and increased opportunity for learning and for the storage of experience may or may not have been among the factors that produced the demonstrated change.

This investigation was supported by the U.S. Atomic Energy Commission.

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EFFECTS OF HYPOTHALAMIC STIMULATION ON THE ERYTHROPOIETIC RESPONSE IN THE RHESUS MONKEY

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ALTHOUGH there are some differences of opinion as to the location of the hypothalamic areas regulating pituitary hormone secretion, there is general agreement that the output of adrenocorticotrophic (ACTH), thyrotrophic (TSH) and gonadotrophic hormone is regulated via the hypothalamus. The investigations of Reichlin[1] and of Franz *et al.*[2] have provided evidence that the hypothalamus also regulates growth hormone (GH) secretion. Both electrical stimulation and injury of the hypothalamic complex have been reported to induce increases in the number of erythrocytes and reticulocytes[3–5]. More recent investigations by Halvorsen[6–8] and Siep[9] indicated that the hypothalamus may participate in the regulation of erythropoiesis in rabbits. They noted that electrical

stimulation of the hypothalamus increased the reticulocyte level and red cell mass in some of their rabbits. Plasma from such rabbits increased erythrocyte uptake of iron-59 and reticulocytes when injected into starved mice[6].

Piliero *et al.*[10] investigated the erythropoietic response to long-term hypoxia in rats with hypothalamic lesions and found no difference between the operated and sham-operated animals.

In the light of the findings of the foregoing investigators, we became interested in examining the effect of electrical stimulation of the hypothalamus on erythropoietin production in rhesus monkeys.

Eleven female rhesus monkeys, in normal health and nutrition, provided the pooled plasma specimens used, and plasmas from these animals were assayed for erythropoietin levels in polycythæmic mice. The monkeys were non-uræmic (plasma urea nitrogen values < 25 mg per cent) and non-anæmic (venous hæmatocrits > 38 vol. per cent).

Two nickel–chromium wire electrodes were stereotaxically inserted into the left side of the brain of a rhesus monkey under general anæsthesia. These electrodes were fully insulated, except at the tip, and were 0·09 mm in diameter. The electrodes were designed to operate as bipolar electrodes. Forty-eight hours following electrode insertion, the two electrodes (designated anterior and posterior) were stimulated on a BRS (Behavioural Research Systems) constant current stimulator. The following currents were individually used on each electrode: *Anterior electrode*, a current varying from 0·6 to 0·7 m.amp was administered for a 300 msec duration every 30 sec for 3-h periods on six days; *Posterior electrode*, a current varying from 0·28 to 0·30 m.amp was administered for 300 msec duration every 30 sec for 3-h periods on four days.

Blood was collected before and throughout stimulation; however, care was taken to prevent monkeys from becoming anæmic.

Following completion of the stimulation of the brain, a lesion was burned in each area to assist in later anatomical localization of the electrode position. For the anterior lead a 6-m.amp current was administered. A 4-m.amp current was used for the posterior lead.

The electrodes were designed to be inserted anteriorly into the area preopticus and posteriorly slightly anterior to the mammillary body of the hypothalamus. The electrodes were fixed utilizing the stereotaxic localization co-ordinates of Snyder and Lee[11], and a correction factor for the skull cap based on animal body-weight charts was used. Two weeks after the creation of the electrode lesions, killing was accomplished under pentothal anæsthesia. The brain was fixed and perfused in buffered formalin solutions. Serial sections and multiple staining procedures were then used to locate the lesion.

Five-week-old Swiss *Ha/ICR* mice 30–35 g in weight were used for assay animals. Each assay animal was injected intraperitoneally with a total of 2·5 c.c. of washed red blood cells from the same strain to induce polycythæmia; the hæmatocrits had to be in the 75–85 per cent range before using them as assay animals. The animals were checked for polycythæmia prior to receiving the pooled rhesus monkey plasmas to assure the cessation of erythropoietin production and, therefore, erythropoiesis[12,13]. Each polycythæmic mouse received 0·5 c.c. of rhesus plasma subcutaneously on days 1, 2 and 3, and on the fourth day 0·5 μc of iron-59 in 0·2 ml. of saline was injected intravenously. On the fifth day a 24-h uptake of iron-59 in the blood was determined[14,15].

Table 1. Erythropoietin Assay of Pooled Plasmas from Rhesus Monkeys with Hypothalamic Stimulation versus Controls

Mice	% 24-h ^{59}Fe uptake by the blood in polycythæmic mice			
	Saline control	Pooled plasma prior to stimulation	Pooled plasma during stimulation	
			Run 1	Run 2
1	0·64	0·90	19·27	11·96
2	0·66	1·13	8·99	19·05
3	0·42	0·33	0·98	8·26
4	0·45	0·25	0·74	8·45
5	0·68	0·35	3·89	7·23
6		0·95		
Mean ± *S.D.*	0·59 ± 0·10	0·65 ± 0·40	6·77 ± 7·7	10·99 ± 4·8

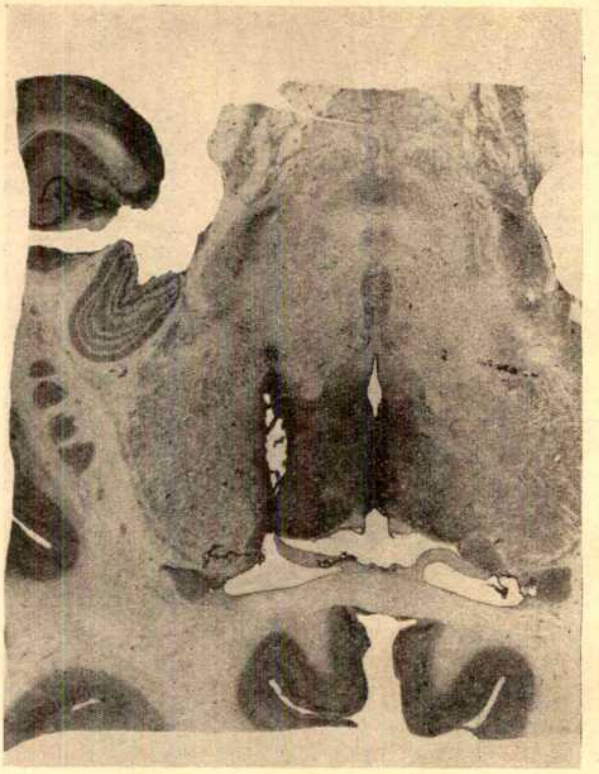


Fig. 1. Histopathological section of the midbrain of monkey *C*–728. Area of posterior electrode lesion is located near the peripeduncular nucleus of the midbrain and the lateral geniculate body

The results summarized in Table 1 show that plasmas from rhesus monkeys responding to hypothalamic stimulation have an increased erythrocyte uptake of iron-59. In runs Nos. 1 and 2 the 24-h iron-59 uptakes by the blood were 10–15 times greater than control uptakes. Hæmatocrit levels of the polycythæmic mice at the conclusion of the experiment ranged from 64 to 71 per cent and the average was 68 per cent.

Plasma collected from rhesus monkeys with hypothalamic lesions and assayed in polycythæmic mice did not show any increases in the 24-h iron-59 uptakes by the blood. The uptakes were comparable with control values.

Serial sections in the electrode stimulation investigation confirmed that two areas were affected; the two were simultaneously stimulated. The anterior electrode was placed in the supraoptic nucleus of the hypothalamus. The posterior electrode (Fig. 1) was located near the peripeduncular nucleus of the midbrain and the lateral geniculate body.

This work demonstrates that high levels of plasma erythropoietin can be obtained from rhesus monkeys subjected to hypothalamic stimulation. Although hypothalamic stimulation increases erythropoietin production, the way in which this is accomplished is unknown. Perhaps oxygen tension is registered in the hypothalamic area and target organs for erythropoietin production are stimulated via the hypothalamus by neurohormones.

The functional significance of the two areas of the brain in terms of the precise localization of a focus for erythropoietin stimulation must remain unsettled from these investigations; however, it is suspected, in view of other findings, that the anterior lead located precisely in

the supraoptic nucleus of the hypothalamus is the more likely site of the two chosen[8].

Piliero *et al.*[10] found no difference in the erythropoietic response of rats with hypothalamic lesions and normal rats; however, he did not stimulate the hypothalamic areas since he used voltage only to produce lesions which can be located histologically. Therefore, our results reaffirm that it is the stimulation, not the placement of the electrodes or the creation of hypothalamic lesions, that determines the absence or presence of an erythropoietic response. For these reasons in this investigation, we felt it was important that animals be allowed to recover from the effects of the electrode insertion and anæsthesia for two days before stimulation began.

It might be worth while to point out that Halvorsen[6–8] obtained an erythropoietic response in rabbits when he stimulated his electrodes bilaterally inserted into the hypothalamus, whereas in this investigation we placed electrodes on only one side and stimulated these individually at a later time.

We thank Edwin Dywinski, Andre Bulba, Helen Fox, Joyce Jividen, Ruth Deckert and A. G. Mirand for their assistance.

This work was supported in part by U.S. Public Health Service grants, the U.S. Atomic Energy Commission, and the John A. Hartford Foundation.

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METHYLHISTAMINE AND HISTAMINE IN THE URINE OF WOMEN DURING THE ELABORATION OF ŒSTROGEN

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MOST of the histamine in man is catabolized[1–4] either to methylhistamine (that is, 1-methyl-4-(β-aminoethyl) imidazole), a reaction catalysed by histamine-*N*-methyl transferase, or to imidazoleacetic acid, a reaction initiated by diamine oxidase (Fig. 1). The major route is methylation[1], and the methylating enzyme[5], in contrast with diamine oxidase[1,2,6], has no known substrate except histamine. Methylhistamine is oxidatively deaminated by monoamine oxidase[1] to methylimidazoleacetic acid which, along with imidazoleacetic acid, is the major metabolite found in urine[1]. Although no methods are available to measure methylimidazoleacetic acid or imidazoleacetic acid, the availability[7] of a method to measure methylhistamine provided an opportunity to gain some insight into the rate of methylation of endogenous histamine.

Measurements[7] on the urine of laboratory personnel showed that the ratio, methylhistamine to histamine (on a weight basis), ranged from 6 to 15, with no difference between sexes. Surprisingly, the urine of two women showed ratios of 3·2 and 1·3, both due to a relative increase in urinary histamine. The woman with a ratio of 3·2 was five months pregnant; the other woman was taking a preparation containing œstradiol and hydroxyprogesterone, and, after withdrawal of these hormones, the ratio rose to 7 (ref. 7). These observations prompted us to examine urine to see if the ratio of methylhistamine to histamine was correlated with the excretion of œstrogens. To this end, these amines and œstrogen were measured in the urine of hypogonadotrophic hypogonadal women during the elaboration of œstrogen by previously inactive ovarian follicles stimulated by exogenous gonadotrophin[8]. Measurements were also made on two normally menstruating women, one of whom has a history of infertility.

Patient 1, who had never been pregnant, developed total amenorrhœa of three years duration following progressive oligomenorrhœa. During the course of amenorrhœa, slight but persistent bilateral lactation was noted. The only significant findings on physical examination were lactation, a profoundly atrophic uterus, and small firm ovaries. Laboratory studies revealed absent endometrial tissues, absent urinary gonadotrophins, and low urinary œstrogens; there was no evidence of intrasellar disease. This patient was treated with human menopausal urinary gonadotrophin (HMGFSH; 'Pergonal'; Cutter Laboratories) and human chorionic gonadotrophin (HCG; 'APL'; Ayerst Co.).

Patient 2, a 34-year-old housewife, complained of amenorrhœa of eight years duration, unassociated with lactation, following an otherwise uncomplicated early miscarriage. The only significant findings on physical examination were small ovaries and uterus. Laboratory examination revealed absent endometrial tissue, absent urinary gonadotrophins, and low urinary œstrogens. This patient was given daily an amount of human pituitary follicle stimulating hormone (HPFSH; kindly provided by Dr. A. Albert of Merck and Co.) equivalent to 20 mg of Armour standard '264-151*X*' FSH; this was combined with HCG ('APL'-Ayerst and Co.).

Patient 3 is a 26-year-old married woman with proved ovulatory cycles by measurements of basal-body temperature and measurements of urinary pregnanediol. However, a two-year history of infertility of unknown cause exists. On examination, the only remarkable findings were urinary levels of 17-ketosteroids which were repeatedly in the upper range of normal (15 mg/24 h) and elevated plasma levels of dehydroepiandrosterone sulphate in excess of 350 μg/100 ml.; the latter compares with normal values of < 200 μg/100 ml. Since these abnormalities are totally suppressible with exogenous corticoids and ovulatory cycles persist, no sustained suppressive therapy has been carried out.

Patient 4 is a 23-year-old single woman with regular ovulatory cycles confirmed by repeated measurements of basal-body temperature and by measurements of an elevated excretion of pregnanediol during luteal phase. No evidence of endocrinopathy was noted.

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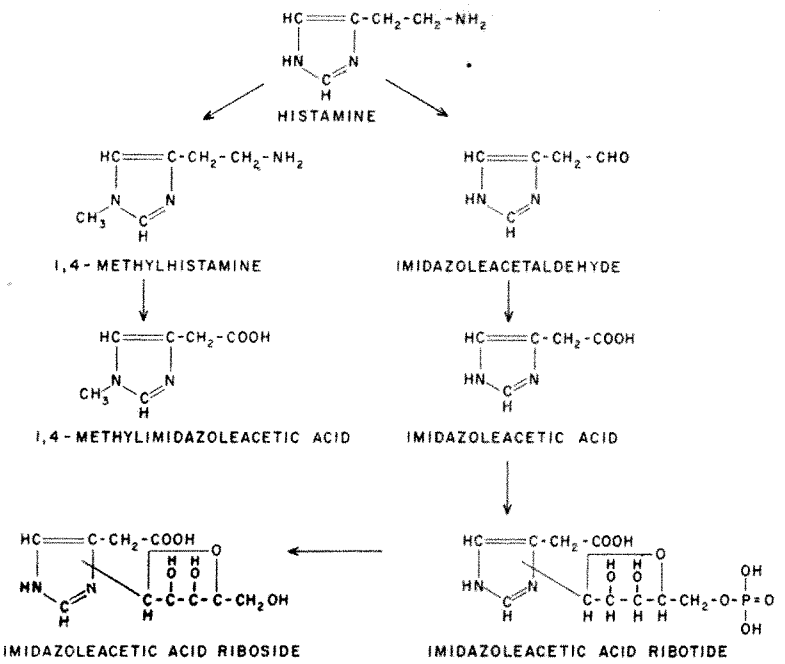


Fig. 1. The major pathways for the metabolism of histamine (refs. 1–4).

Total œstrogens were measured[9] in 24-h urine specimens. Histamine was measured on 24-h urine specimens without knowledge of the source of the urines or of œstrogen-levels. Histamine was measured in duplicate samples of 10 ml. of urine by an adaptation[10] of the fluorometric method[11]. Internal and external standards of 0·25 and 0·50 μg were routinely run, in addition to reagent blanks. Normal women excrete 15–46 μg of histamine/24 h[7].

Methylhistamine, also, was measured on 24-h specimens without any knowledge of the source of the urine or of œstrogen-levels. Methylhistamine was measured in duplicate samples of 10 ml. of urine by a method[7] based on the formation and extraction of dinitrophenylmethylhistamine[12]. Duplicate samples usually agreed within 2–3 per cent and never deviated by more than 10 per cent. The validity of the method has been established by various chromatographic procedures and by experiments in which specific activity of the product was measured[7]. Reagent blanks, together with internal and external standards of 1–4 μg, were routinely run. Normal women excrete 137–444 μg of methylhistamine/24 h[7].

Samples of urine were taken to *p*H 13 or greater with 10 N sodium hydroxide, saturated with sodium chloride, and shaken for 10 min with 1 vol. of a solution containing equal parts, by vol., of 1-butanol and chloroform. After centrifugation at 10,000*g* for 5 min the organic (upper) layer was added to 1·5 vol. heptane and 0·1 vol. of 0·2 N hydrochloric acid. The mixture was shaken for 5 min and then centrifuged at 500*g* for 2 min. The aqueous (lower) layer was dried under reduced pressure in an evaporator. The material was dissolved in 0·5 ml. of water, adjusted to *p*H 9 with 2 M sodium carbonate, and dried under reduced pressure. To the residue was added 0·08 ml. of an aqueous solution containing 0·2 M sodium carbonate–sodium bicarbonate buffer, *p*H 10·6, and 0·004 M sodium diethyldithiocarbamate; to this mixture was then added 0·08 ml. of a solution containing 0·24 ml. of fluoro-2,4-dinitrobenzene in 5 ml. of absolute ethanol. After 20 min at room temperature, the reaction was stopped by the addition of 1·4 ml. of 0·1 N hydrochloric acid. To this solution were added 0·5 ml. of 2 M sodium carbonate and 1 ml. of *sym*-tetrachloroethane; the mixture was shaken for 30 sec. The upper (aqueous) layer was aspirated and discarded, and the lower (organic) layer washed twice with 1 ml. of 0·05 M sodium carbonate. To the organic layer, 1 ml. of a mixture of 0·5 N hydrochloric acid : 1-butanol (97 : 3, v/v) was added, and the mixture was shaken for 30 sec. From the upper (aqueous) layer, 0·85 ml. was removed, transferred to another tube, and evaporated to dryness under reduced pressure. The yellow residue was dissolved in 0·04 ml. methanol and spotted on plates layered with 0·25 mm of silica gel ('Adsorbosil 1', Applied Science Laboratories). The plates were developed for 90 min in a mixture of 1-butanol : acetic acid : water (60 : 15 : 25). At the R_F corresponding to 2, 4 - dinitrophenylmethylhistamine, the silica gel was scraped from the plate and shaken with 0·35 ml. of a mixture of 0·5 N hydrochloric acid : 1-butanol (97 : 3). The solution was centrifuged at 500*g* for 5 min to remove the silica gel, the supernatant solution was placed in a micro-cuvette and the absorption was read in a Beckman *DU* spectrophotometer at a wave-length of 358 mμ.

Excretion of methylhistamine, histamine, and œstrogen after the administration of gonadotrophic hormones. The administration of human menopausal gonadotrophin or human chorionic gonadotrophin to Patient 1 (Fig. 2) caused no significant increase in urinary œstrogens: over the course of therapy they rose from 14·8 only to 19·9 μg/24 h. During this period, the amounts of histamine fell from 61 to 48 μg/24 h, values which are still above the normal range of 15–46 μg/24 h; methylhistamine decreased from 270 to 200 μg/24 h; and the ratio of methylhistamine to histamine, which was abnormally low (4·4) at the start of therapy, never exceeded 5·5, still a low value.

After 4 months, Patient 1 was again treated with human chorionic gonadotrophin, this time at a higher dose (Fig. 3). Again there was only a slight increase in œstrogen excretion, which rose from 19 to 31·5 and then fell to 14·4 μg/24 h. During this period, the amount of urinary methylhistamine also changed slightly, falling from 210

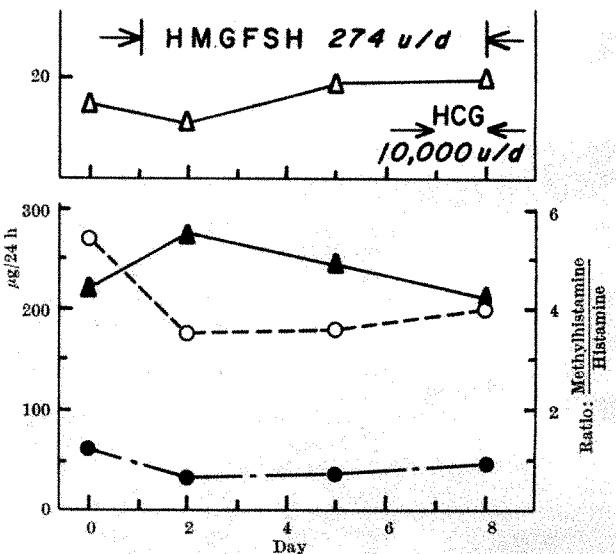


Fig. 2. The urinary excretion of œstrogen and methylhistamine, and the ratio of methylhistamine to histamine, after the administration of human menopausal urinary gonadotrophin (HMGFSH) and human chorionic gonadotrophin (HCG) to Patient 1. There was no consistent change induced by the trophic hormones
△, total œstrogens; ▲, the ratio of methylhistamine to histamine; ○, methylhistamine; ● histamine

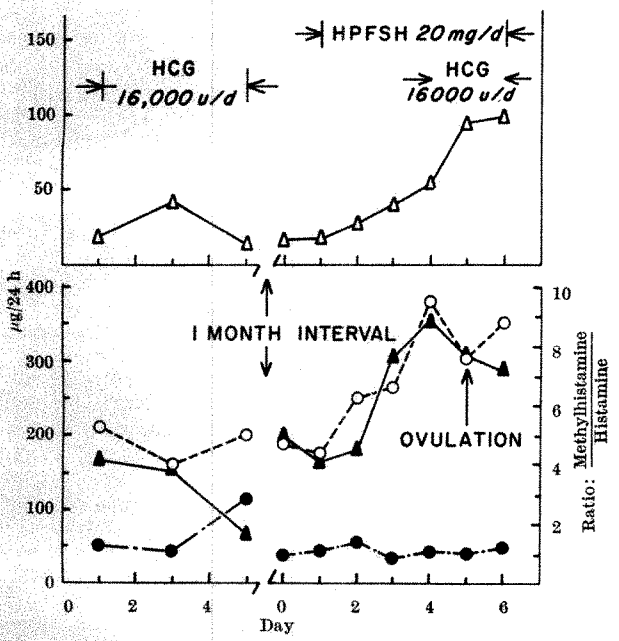


Fig. 3. The increase in the ratio of methylhistamine to histamine in the urine of Patient 1, coincident with the response to human pituitary follicle stimulating hormone (HPFSH) and human chorionic gonadotrophin (HCG), as manifest by the increase in œstrogen excretion. The change in ratio is attributable to a rise in methylhistamine. Symbols are the same as in Fig. 2

to 160 μg/24 h, and then rising to 200 μg/24 h. But there was a significant increase in histamine, which rose from 50 to 115 μg/24 h, causing a significant reduction in the ratio of methylhistamine to histamine, from 4·2 to 1·7, a fall which is not correlated with the excretion of œstrogen and remains inexplicable.

After an interval of 1 month, the same patient was given a course of therapy with human pituitary follicle stimulating hormone and, later, human chorionic gonadotrophin was added (Fig. 3). Œstrogen excretion rose from hypogonadal levels of 15·6 to 102·9 μg/24 h. During this increase, the excretion of histamine was slightly but inconsistently altered, remaining higher than normal. But the excretion of methylhistamine increased from 190 to 380 μg/24 h, a rise that closely paralleled the increase in the ratio of methylhistamine to histamine, which attained a peak value of 8·9. The highest values of both these measurements were obtained the day before ovulation when the œstrogen excretion was 54·7 μg/24 h. At ovulation the ratio of methylhistamine to histamine was 7·7, within the normal range.

Patient 2 responded to treatment with human pituitary follicle stimulating hormone and human chorionic gonadotrophin with a marked increase in the excretion of œstrogen, from the hypogonadal value of 13·2 μg/24 h (Fig. 4). More striking than the elevation in urinary œstrogen was the rise in the ratio of methylhistamine to histamine, which reached a peak of 8·9 at ovulation, when the œstrogen excretion was 167·3 μg/24 h. Three days after ovulation, the œstrogen excretion was 232 μg/24 h; at this time the ratio of methylhistamine to histamine had fallen to 7·0. When this ratio had fallen further, to the abnormally low value of 4·3, the œstrogen excretion had fallen to hypogonadal values of 19·9 μg/24 h.

The change in ratio in this patient (Fig. 4) was almost entirely attributable to a change in urinary histamine; this fell from the initial and abnormally high value of 140 μg/24 h to a low value of 18 μg/24 h which coincided with ovulation. The change in urinary methylhistamine was neither consistent nor marked. This pattern of response in Patient 2 may be compared with that obtained in Patient 1 (Fig. 3), whose changing ratios of methylhistamine to histamine reflected changes in the excretion of methylhistamine. Patient 2 (Fig. 4) also excreted much greater quantities of œstrogen than did Patient 1 (Fig. 3).

Excretion of methylhistamine, histamine, and œstrogen by two normally menstruating women (Table 1). Patient 3 showed normal urinary œstrogen levels, but the ratio of methylhistamine to histamine, in both the pre-ovulatory and post-ovulatory samples, were abnormally low, 3·5 and 2·3 respectively. Both these low values are attributable to abnormally high urinary histamine; the excretion of methylhistamine was within the normal range. Patient 4 had normal values of œstrogen, histamine, and methylhistamine, and normal ratios of methylhistamine to histamine. Patient 4 has no known endocrine problem. Patient 3 has a fertility problem and has abnormally high plasma levels of androgens.

Table 1. Estimates of Urinary Excretion of Methylhistamine, Histamine, and Œstrogen in Two Normally Menstruating Women

	Methylhistamine (μg/24 h)	Histamine (μg/24 h)	Ratio, $\frac{\text{Methylhistamine}}{\text{Histamine}}$	Œstrogen (μg/24 h)
Patient 3				
Pre-ovulatory (day 10)	330	94	3·5	28·3
Post-ovulatory (day 23)	324	140	2·3	45·3
Patient 4				
Pre-ovulatory (day 10)	270	30	9·0	29·9
Post-ovulatory (day 23)	310	40	7·7	54·6

Before therapy with gonadotrophic hormones, the two hypogonadal women had low ratios of methylhistamine to histamine, attributable mostly to high urinary histamine. One of the patients had a ratio of 1·1 (Fig. 4); the other, in three separate measurements, had ratios of 4·4, 4·2 and 4·9 (Figs. 2 and 3). These compare with ratios of 7–9·1 in normal women and of 6–15 in normal men[7]. After receiving gonadotrophic hormones, these ratios increased, coincident with the production of œstrogen by the ovarian follicle, and reached a peak at ovulation (Figs. 3 and 4). It is interesting that the ratio became normal when the amount of œstrogen became normal, and these ratios did not further increase even though the amount of œstrogens increased further (Figs. 3 and 4). With a fall in œstrogen excretion, the ratio of methylhistamine to histamine in urine also fell (Fig. 4).

That factors other than œstrogen are important in contributing to the ratio is indicated in Table 1. Patient 3, who was having ovulatory menses but having an infertility problem, showed normal œstrogen excretion but had a low ratio of methylhistamine to histamine, which was attributable to high urinary histamine; it may be significant that this patient shows abnormally high plasma-levels of androgens, which can be synthesized by the human ovary[13]. Patient 4 had normal œstrogen excretion, normal amine-levels and normal ratios. After ovulation, both patients 3 and 4 showed increased amounts of œstrogen, with no rise in the ratio of methylhistamine to histamine (Table 1). Further evidence that the œstrogens themselves are not wholly responsible for the relative increase in the ratio was obtained in a patient whose ratios fell after she received œstrogen[7]. It is suggested that the high urinary ratio of methylhistamine to histamine is in part due to a direct action of œstrogen and in part due to other factors and events contributing to the œstrogen response, perhaps to other activities of the ovarian follicle such as the production of androgens.

In the absence of an œstrogenic response, the ratio was not altered (Fig. 2), but concomitant with an œstrogenic response the ratio increased (Figs. 3 and 4). In the patient with a relatively weak œstrogenic response, the increase in the ratio was attributable to a rise in methylhistamine (Fig. 3); in the patient with a strong œstrogenic response the increase in ratio was due to a fall in histamine (Fig. 4). This difference suggests that factors contributing to and accompanying the œstrogenic response may influence

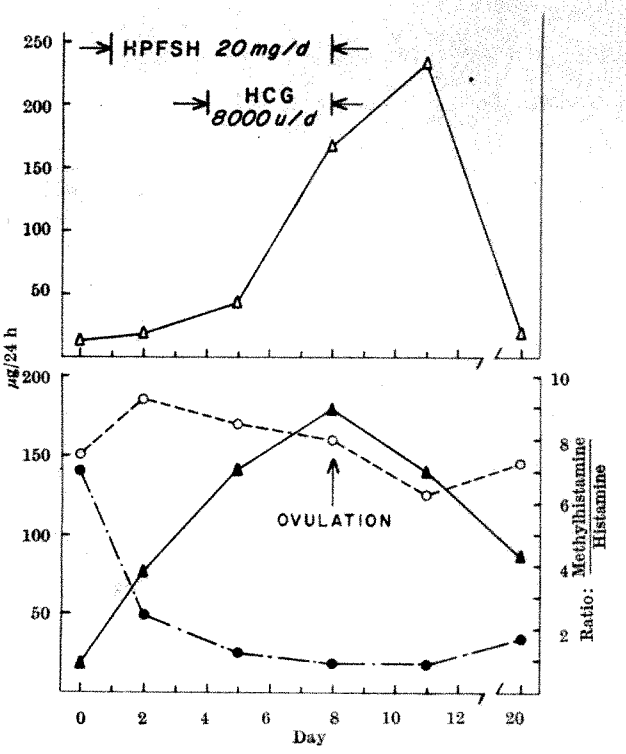


Fig. 4. The increase in the ratio of methylhistamine to histamine in Patient 2, coincident with the response to human pituitary follicle stimulating hormone (HPFSH) and human chorionic gonadotrophin (HCG) as manifest by the increase in œstrogen excretion. The change in ratio is attributable to a fall in histamine. The symbols and abbreviations are the same as in Fig. 2

the disposition of histamine by acting at more than one site. One such site, it may be suggested, could be an inhibition of monoamine oxidase activity, which would result in a rising methylhistamine, but such inhibition is most unlikely, for one would expect that with a further increase in the œstrogenic response (Fig. 4) the methylhistamine would increase further, but it did not.

The sites are not immediately discernible, for although much is known of the effects of hormones on histamine metabolism in the rat[14,15], caution must be exercised in extrapolating these results to man: a pertinent and conspicuous example of the species differences is that female rats excrete much more histamine that do males[14,15], but in man there is no difference between the sexes[7,10]. However, there are investigations of histamine metabolism in man which may help to explain our findings.

Œstrogen disrupts mast cells in both the endometrium and myometrium of the human being[16], guinea-pig[17], and rat[18] uteri. As a consequence of the destruction of the mast cells (and perhaps because of an increased synthesis of histamine by other cells[19,20]) histamine is released[18,21]. An œstrogen-induced release of histamine is further indicated by the increased excretion of histamine seen in pregnancy[7,22] and after the administration of an œstrogen preparation[7]. It has, in fact, been suggested that œstrogen (and luteinizing hormone) owe at least some of their effects on the uterus to a release of histamine[18,21,23], which has been detected in the discharge from the human uterus[24]. As judged from the guinea-pig uterus, the mast cells of which respond to œstrogen as do the mast cells in human uterus[17], the uterine mast cells more readily fragment and release their histamine than do mast cells in other organs[25,26]. It may be presumed that there is a continual formation of mast cells and, with the œstrogenic response, a continual dissolution.

In order to explain the increase in urinary methylhistamine concomitant with a relatively slight change in histamine (Fig. 3), it is suggested that the histamine released by the relatively low œstrogenic response is catabolized in the normal manner to methylhistamine. When the œstrogenic response is increased still further (Fig. 4), a greater portion of the released histamine is catabolized by diamine oxidase, which results in a diminished histamine but unchanged methylhistamine.

There is no definitive evidence that the œstrogenic response either inhibits the methylation of histamine or activates its oxidation. The only relevant investigations on human beings were carried out with exogenous histamine, the disposition of which does not necessarily reflect the disposition of endogenous histamine[27–31]. However, in the experiments with exogenous histamine, it was shown that in human pregnancy, where the œstrogenic response is high, the capacity of the uterus to methylate histamine is reduced, an unusually high proportion of histamine being oxidized[32,33]. Moreover, the plasma of pregnant women is extremely high in diamine oxidase activity[6]. An increase in uterine diamine oxidase activity, induced by a strong œstrogenic response, together with high plasma diamine oxidase activity could explain the falling levels of histamine in urine (Fig. 4); this postulate can be tested when a method becomes available to measure imidazoleacetic acid. Although these observations on pregnant women may help to explain our findings, it should be emphasized that histamine metabolism in pregnancy is influenced by factors other than an œstrogenic response, as manifest by the high levels of histamine found in the urine of pregnant women[7,22], a rise that may be reflective of fœtal growth[20,34].

The tests of the postulates presented here must await the development of methods to measure other endogenously formed catabolites of histamine. Whether or not the postulates are correct does not detract from the salient fact: stimulation of the ovarian follicle in women is correlated with the metabolism of endogenous histamine.

This work was supported by grants 2*K3–GM*–2459–06, *GM*–10313–02 and *AM*–06129 from the U.S. Public Health Service.

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NAD$^+$-GLYCOHYDROLASE OF THYROID HOMOGENATES

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RECENT investigations have shown the importance of the pyridine nucleotides in the energetic[1,2] and iodine[3–8] metabolism of the thyroid gland.

The work recorded here demonstrates the enzymatic inactivation of di- and tri-phosphopyridine nucleotides in thyroid homogenates by NAD$^+$-glycohydrolase. The existence, mode of action, *trans*glycosidase activity, analogue-formation capacity and specific inhibition of this enzyme have been described for different tissues in earlier publications[9–25].

The experiments presented here fall into three groups:

Group A. Thyroid homogenates were prepared from glands of human beings and pigs, as described by Zatman *et al.* for the bovine spleen[18], and the protein content was measured[26]; 0·2-ml. samples were added to 1 ml. of 0·1 M phosphate buffer at *p*H 7·4, containing NAD or NADP at 20 different concentrations (0·1–2 mmoles/l.) and incubations were carried on for 10 min at 37° C in a Dubnoff metabolic incubator. The pyridine nucleotides were determined by their absorption at 325 mμ in a Beckman *DB* spectrophotometer, after addition of M KCN[27]. The results indicated a gradual disappearance of NAD and NADP, increasing with the pyridine nucleotide concentration (Fig. 1).

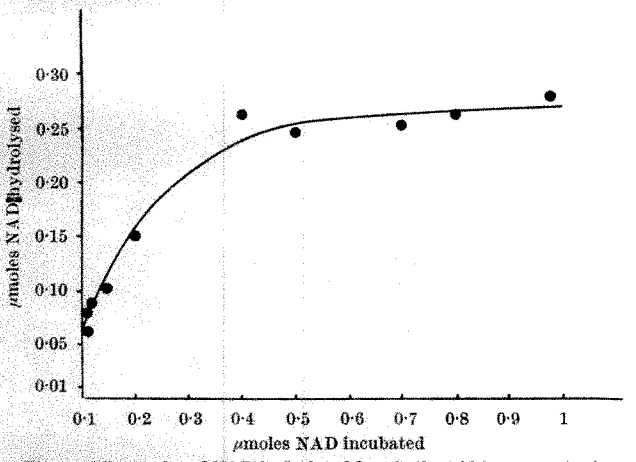


Fig. 1. Micromoles of NAD hydrolysed by pig thyroid homogenates in presence of various concentrations of NAD

When the results were plotted as $1/s$ over $1/v$ following the Lineweaver and Burk method[28], the *Km* from several determinations was between $1·5–3·5 \times 10^{-4}$ M for NAD and $1·9 \times 10^{-4}$ M for NADP. Addition of nicotinamide at a final concentration of $7·5 \times 10^{-2}$ M inhibited 90 per cent of the pyridine nucleotide disappearance. Incubations with 16 different concentrations of nicotinamide (1×10^{-5} M – $7·5 \times 10^{-2}$ M) indicated 50 per cent inhibition with $1·5 \times 10^{-3}$ M for NAD and NADP in agreement with previous findings for the enzyme of other tissues[14,15,29].

Table 1. Percentage of NAD and NADP disappearing during incubation for 10 min with 0·2 ml. pig thyroid homogenate in medium containing $1·5 \times 10^{-2}$ M nicotinamide or nicotinamide-free. The results indicate the non-competitive character of the nicotinamide inhibition

	NAD				NADP		
Incub.	End of incubation 0	End of incubation Nicotinamide	% of disap.	Incub.	End of incubation 0	End of incubation Nicotinamide	% of disap.
1 μM	0·063 μM	0·033 μM	52·0	1 μM	0·061 μM	0·029 μM	47·5
2 μM	0·154 μM	0·07 μM	48·7	2 μM	0·160 μM	0·083 μM	51·8
5 μM	0·240 μM	0·119 μM	49·5	5 μM	0·231 μM	0·123 μM	48·9

Incubation of 20 different concentrations of NAD and NADP in the presence of four different concentrations of nicotinamide indicated the non-competitive nature of this inhibition (Table 1). When data resulting from incubation of NAD and NADP in media free of nicotinamide (*A*) and containing nicotinamide (*B*) were plotted as $1/s$ over $1/v$, the intercept of *A* with $1/v$ was at a lower point than of *B* with $1/v$, confirming the non-competitive nature of the nicotinamide inhibition (Fig. 2).

Confirmatory evidence of NAD$^+$-glycohydrolase presence was brought by incubation at 37° C of 1 ml. of human or pig thyroid homogenate in 3·5 ml. of 0·1 M phosphate buffer at *p*H 7·4 in presence of 6×10^{-4} M NAD or NADP and 2×10^{-2} M isoniazid (INH) leading to the gradual formation of INH analogue, demonstrated by the appearance of yellow colour and of the absorption spectrum with a peak of 385 mμ in alkaline *p*H (refs. 17 and 18). 6-*N*-Propylthiouracil, methyl-mercaptoimidazole, perchlorate, thiocyanate, iodine, monoiodotyrosine, L-diiodotyrosine, triiodothyronine and thyroxine in concentrations between 1×10^{-5} M and 1×10^{-2} M did not inhibit NAD or NADP hydrolysis by thyroid homogenates. No analogue formations with substances other than INH were followed in the work recorded here.

Group B. Results in the literature[30] report, with one exception[12], the inability of the NAD$^+$-glycohydrolase to hydrolyse the reduced forms of NAD and NADP.

Incubating 3 ml. of 0·1 M phosphate buffer *p*H 7·4 containing 1 μmole of NADH or NADPH with 0·2 ml. human or pig thyroid homogenate for 10 min at 37° C, spectrophotometric readings at 340 mμ and fluorometric determinations[31] indicated disappearance of the reduced pyridine nucleotides. As this could have been the result of oxidative processes in the thyroid homogenates[5,27], retrieval of the oxidized forms was attempted either by addition of corresponding reducing systems or by fluorometric determinations[31]. Both methods demonstrated a marked disappearance of NADH and NADPH. It was therefore supposed that the reduced forms of NAD and NADP are first oxidized and afterwards hydrolysed by thyroid homogenates.

The following experiment was designed to substantiate this supposition:

One ml. of human or pig thyroid homogenate was incubated for 24 h at 37° C in 0·1 M phosphate buffer *p*H 7·4,

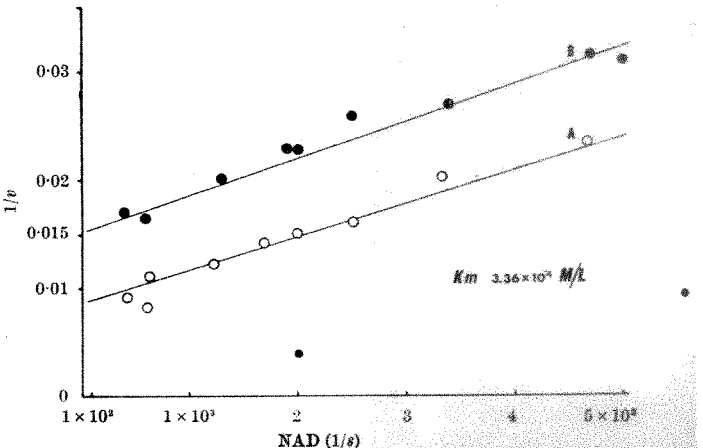


Fig. 2. *Km* of NAD for pig thyroid homogenates. *A*, incubated in 0·1 M phosphate buffer; *B*, 5×10^{-2} M nicotinamide was added to the incubation medium

containing 6×10^{-3} M NADH or NADPH and 2×10^{-2} M INH. After destroying the reduced pyridine nucleotides by addition of 0·1 N HCl, the medium was brought to alkaline *p*H (9·5) by addition of 1 N NaOH. Appearance of a yellow colour and an absorption spectrum with a peak of 385 mμ demonstrated the presence of the INH analogue in the medium.

I think this experiment demonstrates that, under the conditions prevailing in a thyroid homogenate, the concentration or availability of reduced pyridine nucleotides might be influenced by the NAD^+-glycohydrolase activity, subsequent to their oxidation.

Group C. Enzymatic deiodination of the iodotyrosines requires the presence of NADPH[3,4]; its rate is dependent on the quantity or availability of this co-factor[8,32]. Liver homogenates incubated in medium containing nicotinamide exhibit an increased deiodinating capacity[3]. NADase of *Neurospora crassa* added to a thyroid homogenate induces a fall in its deiodinating capacity[3].

These findings justify the presumption that the NAD^+-glycohydrolase of the thyroid tissue may influence the thyroidal enzymatic deiodination of iodotyrosines. The NAD^+-glycohydrolase inhibition by nicotinamide was used to demonstrate this point.

The deiodinating activity of thyroid glands of normal rats was examined, as previously described[8], and found to be markedly enhanced when $7{\cdot}5 \times 10^{-2}$ M nicotinamide was present in the medium (Table 2).

Table 2. Micrograms of L-DIT deiodinated per mg in 2 h by rat thyroids homogenized in 10 vol. of buffer in presence of various concentrations of nicotinamide

Nicotinamide	
0	0·0019 ± 0·0001
$7{\cdot}5 \times 10^{-3}$ M	0·0126 ± 0·0004
$7{\cdot}5 \times 10^{-2}$ M	0·0212 ± 0·0006

The deiodinating capacity of thyroid homogenates increased linearly with the nicotinamide concentration (Fig. 3).

When thyroid homogenates were incubated in NADPH-enriched media, and 5×10^{-2} M nicotinamide added, the resulting *Km* for the co-factor was 5×10^{-5} moles/l. instead of $1–2 \times 10^{-4}$ moles/l. observed in a nicotinamide-free medium[32], that is, a 10 times reduction of the optimal concentration of NADPH for 50 per cent of the maximum velocity of the deiodinating enzyme (Fig. 4).

The enhancing effect on deiodination of nicotinamide addition disappeared when:

(*a*) The rat thyroid glands were homogenized in 100 volumes of the incubation medium, presumably because the NAD^+-glycohydrolase is too weak to hydrolyse the co-factor substantially so that its inhibition is ineffective.

(*b*) The rat thyroid glands were homogenized in 10 volumes of buffer containing a NADPH oxidizing system (oxidized glutathione and glutathione reductase) showing

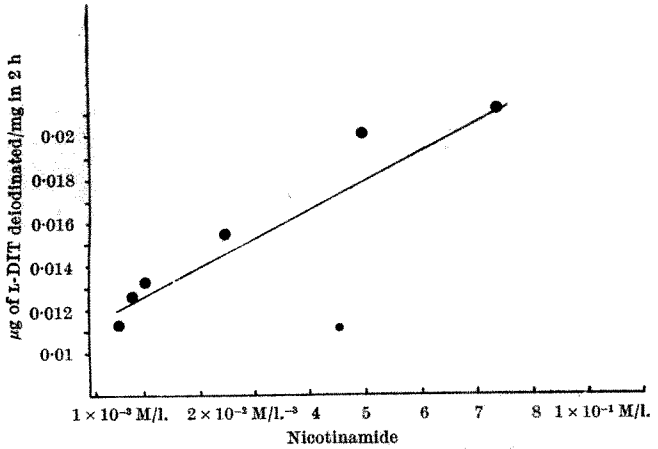


Fig. 3. Micrograms of L-DIT deiodinated per mg of rat thyroid tissue homogenized in ten volumes of buffer containing various concentrations of nicotinamide

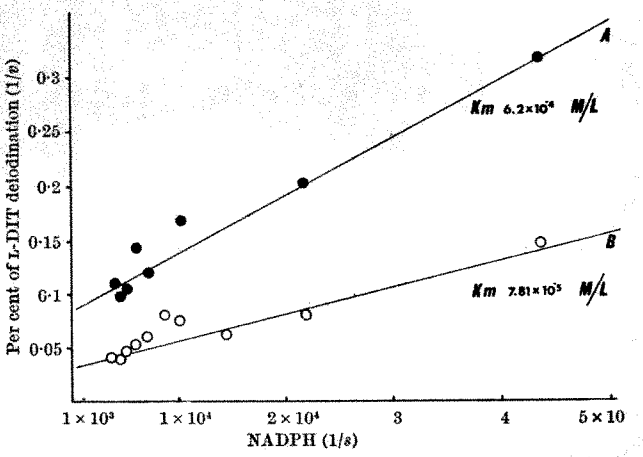


Fig. 4. *Km* of TPNH in L-DIT deiodination by rat thyroids homogenized in ten volumes of medium nicotinamide-free (*A*) and containing 5×10^{-2} M nicotinamide (*B*); 10 min incubation

that nicotinamide does not directly enhance deiodination in the absence of reduced NADP.

It may, therefore, be concluded that:

(*a*) NAD^+-glycohydrolase activity, demonstrated in thyroid homogenates, might influence the tissue concentration of pyridine nucleotides and, thus, those steps of the thyroidal metabolism which required the presence of co-factors[1–9].

(*b*) NAD^+-glycohydrolase transglycosidase capacity to form NAD or NADP analogues might prove useful in future experiments designed to explain the mechanism of action of antithyroid drugs.

I thank Dr. M. Raben for his assistance and advice.

This work was supported by grant *HE*–06924 from the U.S. National Institutes of Health.

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MECHANISMS OF REGULATION OF HEPATIC GLYCOGEN SYNTHESIS

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MANY factors influence glycogen storage in the liver by ultimately affecting either the rate of synthesis or of degradation of the α 1,4 linkages of the glucose polymer. Both the enzymes concerned with this process, UDPG-glycogen glucosyl-transferase (*EC* 2.4.1.11) and phosphorylase (*b*-form, *EC* 2.4.1.1), are now known to have binding sites for certain intra-cellular metabolites which act as allosteric regulators of enzyme activity[1]. Glucose-6-P is the only such metabolite known to regulate the synthesis of glycogen by combining specifically with the enzyme protein. This property of glucosyltransferase appears to be a universal feature of the enzyme from widely divergent sources. Several structural features essential for stimulation by aldohexose phosphates have been defined[2].

Measurements of hepatic glucose-6-P levels in various metabolic states such as repletion, fasting, and diabetes[3] indicate that the steady-state concentration of this metabolite fluctuates widely over a range which coincides closely with the *Km* concentration for glucose-6-P of hepatic glucosyltransferase, and which could, therefore, significantly modify enzyme activity *in vivo*. Several lines of indirect evidence suggest that this mechanism plays an important part in the increased glycogen synthesis which occurs when normal animals are given glucose after a short fast, and also when fasted normal or adrenalectomized animals are given cortisol. In both instances the concentration of hepatic glucose-6-P rises several-fold from significantly depressed levels[3,4]. That the rise in glucose-6-P in normal fasted animals is due primarily to increased glucose phosphorylation is shown by the data of Hers[5], who found that glucose-1-^{14}C is incorporated into liver glycogen without significant redistribution of label when administered to rats fasted 24 h or less. The increase in glucose phosphorylation appears to be due to the presence of a specific glucokinase in liver which has a high *Km* for glucose[6], and which can therefore respond to variations in plasma glucose concentration. The results of an experiment are presented in Table 1, in which fasted normal rats were given glucose injections. At later intervals the livers were removed and the activities of both glucokinase and UDPG-glycogen glucosyltransferase measured. Glucosyltransferase activity increased only slightly after 60 and 100 min, and no change in glucokinase activity occurred. Similar observations with respect to glucosyltransferase activity have been made in animals treated with cortisol[4,7]. However, with cortisol, increased glucose-6-P is derived primarily from the stimulation of gluconeogenesis rather than of glucose phosphorylation although blood glucose concentration is increased by cortisol. In both these instances it is evident that the synthesis of increased amounts of glycogen is initiated by changes in concentration of critical extra-cellular and intra-cellular metabolites rather than by changes in concentration of enzymes.

By contrast, when diabetic or starved animals are given insulin or food respectively, initiation of glycogen synthesis is attendant with the synthesis of glucosyltransferase in increased amounts without an increase in either glucose-6-P concentration or glucose phosphorylation. Fed alloxan diabetic animals have low liver glycogen and glucose-6-P levels despite increased gluconeogenesis and marked alimentary hyperglycæmia[3]. Hepatic glucokinase is markedly reduced and glucose-6-P phosphatase increased in the diabetic organism. When insulin is given to these animals, glycogen repletion begins within about 40 min and enormous amounts of glycogen accumulate

Table 1. Effect of Glucose on Hepatic UDPG-Glycogen Glucosyltransferase Activity, Glucokinase Activity, Glycogen Concentration and Blood Glucose Concentration in Fasted Normal Rats

Group	No. of animals	Glucosyltransferase activity (μmoles/g protein/h) − G-6-P	Glucosyltransferase activity (μmoles/g protein/h) + G-6-P	Glucokinase activity (μmoles/g protein/h)	Glycogen conc. (mg/g)	Blood glucose (mg/100 ml.)
Control (saline)	7	74 ± 3·8	375 ± 17	235 ± 12	3·4 ± 0·4	79 ± 1·3
Glucose, 60 min	4	115 ± 2·7	390 ± 10	—	5·5 ± 0·7	99 ± 3·1
Glucose, 100 min	3	127 ± 14	455 ± 19	246 ± 7·0	9·6 ± 0·7	124 ± 5·2

Female Sprague-Dawley rats, fasted 18–20 h, were given intraperitoneal injections of 5 c.c. of 5% dextrose solution or 0·9% saline solution. The animals were killed at the times indicated and the livers perfused with cold 0·3 M sucrose, 0·005 M EDTA, *p*H 7·4, via the portal vein. The preparation of homogenates, assay and other analytical procedures have been described (refs. 7 and 8).

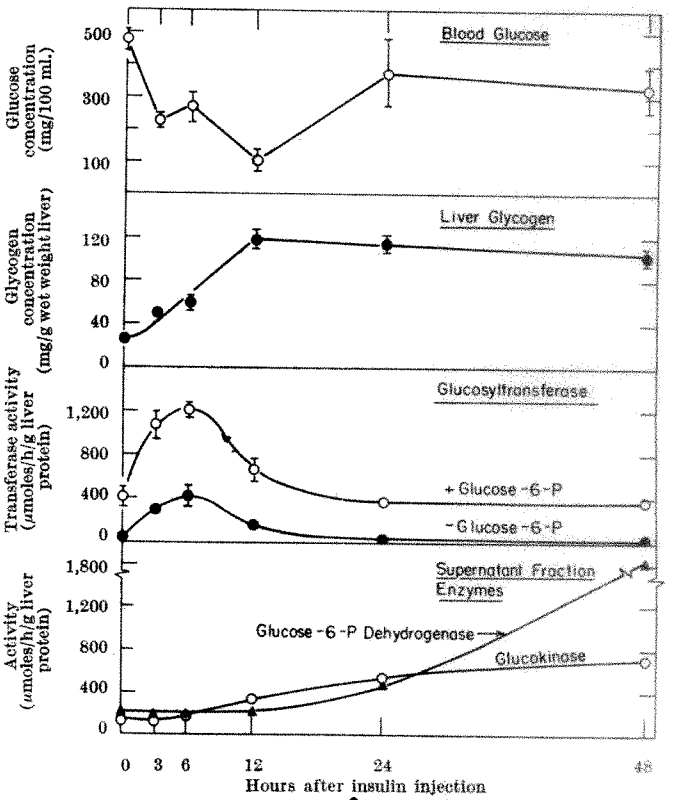


Fig. 1. Effect of insulin administration for 48 h on blood glucose and liver glycogen concentration, UDPG-glycogen glucosyltransferase activity, and the activities of several enzymes of the supernatant fraction in severely diabetic rats. The animals received intraperitoneal injections of 5·0 units of glucagon-free insulin and subcutaneous injections of 6 units of protamine zinc insulin (Lilly) at zero time. The rats to be killed at 48 h were given another injection of 4 units of protamine zinc insulin subcutaneously at 24 h. The animals were killed at the times indicated, and assays were carried out as described (refs. 7 and 8). Each point represents the average of at least three determinations, and brackets indicate *S.E.M.*

* Holder of a Research Career Development Award of the U.S. Public Health Service.

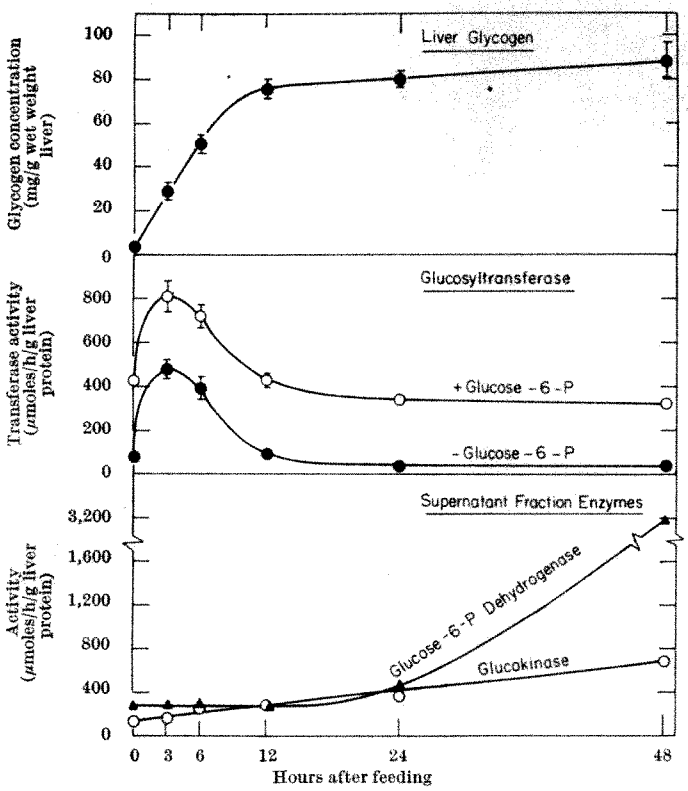


Fig. 2. Effect of re-feeding with a standard laboratory diet (Rockland Rat Diet) for 48 h on blood glucose and liver glycogen concentration, UDPG-glycogen glucosyltransferase activity, and the activities of several enzymes of the supernatant fraction in normal rats fasted 72 h. The animals were killed at the times indicated, and assays carried out as described (refs. 7 and 8). Each point represents the average of four determinations, and brackets indicate *S.E.M.*

during the first 12-h interval of administration of insulin[8]. These deposits persist in the liver for several days while other metabolic transitions ensue, but are eventually dispersed when normal blood glucose-levels are achieved. Glucose-6-P measurements during the initial period of rapid glycogen deposition show only a transient decrease in concentration[3] in contradistinction to the marked rise seen in the two circumstances discussed before.

This is not surprising in view of the recent finding in several laboratories[9] that insulin-dependent synthesis of liver glucokinase does not begin in diabetic animals until about 6 h after insulin is first administered. Restoration of this enzyme to normal levels probably accounts for the increased glucose phosphorylation observed 6–24 h after insulin administration by Renold *et al.*[10] during examination of liver slices from diabetic rats. The early stimulation of glycogen synthesis under these conditions cannot therefore be ascribed to increased glucose phosphorylation, but rather, appears to result from operation of a second control mechanism which acts to increase the rate of synthesis of the enzyme, UDPG-glycogen glucosyltransferase. The results in Fig. 1 illustrate the relationship of changes in liver glycogen concentration to changes in activity of glucosyltransferase, glucokinase and glucose-6-P dehydrogenase following administration of insulin to alloxan diabetic rats. The glucosyltransferase activity rises sharply beginning about 40 min after the first injection of insulin and reaches peak levels by about 6 h at which time glucokinase activity is just beginning to increase. Glucose-6-P dehydrogenase also increases dramatically after insulin but does not begin to rise until 12 or more hours after the first injection of insulin. The glucosyltransferase increase is inhibited completely by both puromycin and actinomycin *D*, and partially by ethionine, *in vivo*[8].

These findings are regarded as strongly suggestive evidence for the existence of a control mechanism for enzyme synthesis which operates at the level of transcription of messenger RNA from the structural gene for the enzyme protein. In contrast to the induction of many other mammalian enzymes, including the others shown in Fig. 1, the induction of glucosyltransferase has a short lag period of only 40 min, and it later decays with a relatively short half-life of about 4 h. Since the inductions of glucosyltransferase, glucokinase, and glucose-6-P dehydrogenase follow sequentially, and with highly individual patterns, it seems quite unlikely that the genes for these three enzymes are grouped within a single operon, as has been suggested for several other groups of closely related hepatic enzymes[11].

The metabolic transitions brought about by insulin in diabetic rats are almost identical to those which follow the restoration of a balanced diet to normal animals fasted for prolonged periods (3–4 days). Fig. 2 presents the results of an experiment in which liver glycogen concentration and the activities of UDPG-glycogen glucosyltransferase, glucokinase, and glucose-6-P dehydrogenase were followed for 48 h after re-feeding rats fasted for three days. It is noteworthy that the glucosyltransferase increase as well as the peak glycogen concentration are both smaller in magnitude than in the insulin-treated severely diabetic rats (Fig. 1) and, also, the peak enzyme-level is reached at an earlier time. Salas *et al.*[9] have observed similarly that liver glucokinase responds more rapidly to re-feeding in normal rats than in insulin-treated diabetic rats, rising as early as 3–4 h after re-feeding. Why this is seen in the induction of both these enzymes is not clear, but may be related to the greater severity of the metabolic derangements in chronic diabetes. Measurements of hepatic glucose-6-P concentration in rats fasted three days and then re-fed a balanced diet or given glucose solution[3,12] indicate that, despite a considerable elevation of the glucose concentration in the blood, no significant rise in concentration of glucose-6-P occurs in the liver, just as with diabetic animals after insulin injections. It can be concluded that, in the foregoing two metabolic states, increased glycogen synthesis is initiated by an increase in the amount of glucosyltransferase enzyme rather than by a rise in the steady-state level of glucose-6-P.

As shown by these experiments and others cited, insulin appears to be of critical importance for an integrated sequence of metabolic readjustments which occurs in the liver during the transition from a starved or diabetic state to the normal fed state. The role of insulin in such hepatic adaptations has often been ignored, while attention has been directed solely to dietary factors without due consideration of the endocrine consequences during re-feeding. To evaluate further the role of insulin, normal rats were fasted for three days, and just before re-feeding, some were given intraperitoneal injections of guinea-pig anti-insulin serum or non-immune serum. Those receiving the insulin anti-serum ingested food normally for about 1 h, but, as shown in Table 2, glycogen deposition and glucosyltransferase induction were abolished almost completely despite the development of significantly higher levels of blood glucose concentration in the acutely diabetic group. These results clearly demonstrate that insulin is essential for this phase of hepatic adaptation to re-feeding. However, in another experiment the administration of a small dose of insulin alone to animals fasted for three days resulted only in severe hypoglycæmia without any increase in glucosyltransferase activity. While this result suggests that some element(s) of the diet may also be indispensable for the induction of this enzyme, compensatory endocrine responses to hypoglycæmia which might inhibit the

Table 2. EFFECT OF ANTI-INSULIN SERUM ON THE RESPONSE TO RE-FEEDING OF HEPATIC UDPG-GLYCOGEN GLUCOSYLTRANSFERASE ACTIVITY, GLYCOGEN CONCENTRATION, AND BLOOD GLUCOSE CONCENTRATION IN RATS FASTED FOR 72 H

Group	No. of animals	Glucosyltransferase activity (μmoles glucose/g protein/h) −G-6-P	+G-6-P	Glycogen conc. (mg/g)	Blood glucose (mg/100 ml.)
Control	3	71 ± 8·8	480 ± 13	2·2 ± 0·6	76 ± 2·2
Re-fed	3	480 ± 107	700 ± 107	28 ± 1·4	113 ± 12
Re-fed + AIGPS	3	118 ± 14	450 ± 21	6·9 ± 1·0	275 ± 3·5
Re-fed + NGPS	3	430 ± 117	680 ± 56	28 ± 2·2	109 ± 7·8

Female Sprague–Dawley rats, fasted for 72 h, were divided into four equal groups. Two groups were given intraperitoneal injections of either 1·5 ml. of normal guinea-pig serum (NGPS) or 1·5 ml. of anti-insulin guinea-pig serum (AIGPS) 20 min before re-feeding, and an additional 1·0 ml. of the same serum 90 min after re-feeding. Control animals were not given food or injections. The animals were killed 3·5 h after re-feeding and the livers perfused with cold 0·3 M sucrose, 0·005 M EDTA, *p*H 7·4, via the portal vein before removal. The preparation of homogenates, assay and other analytical procedures have been described (refs. 7 and 8).

response cannot be excluded. Further investigation is required to clarify these points.

It has been reported recently that synthesis of glycogen in muscle is regulated by a mechanism which can be influenced by insulin and epinephrine, and also by muscle contraction[13]. The central feature of this mechanism is the circumvention of the normal allosteric mechanism for regulation of muscle glucosyltransferase, that is, glucose-6-P dependency, by the conversion of this enzyme to a glucose-6-P independent (I) form by means of a reversible dephosphorylation of the enzyme protein reminiscent of the transformations of muscle phosphorylase[14]. It is not clear at present whether this kind of mechanism, in addition to those discussed earlier in this report, participates in the regulation of glycogen synthesis in the liver, for, in contrast to the more stable muscle glucosyltransferase, the hepatic enzyme readily loses a large proportion of its glucose-6-P dependency on mild heating at 37° in the absence of glucose-6-P, and recovers it on further incubation in the presence of glucose-6-P and certain salts[15]. It is possible that this lability of the allosteric site of hepatic glucosyltransferase may explain the greater glucose-6-P independence of newly synthesized glucosyltransferase (note glucosyltransferase activity in absence of glucose-6-P in Figs. 1 and 2, and in Table 2).

If space allowed, many examples could be cited where selective control of the rate of synthesis of enzymes has been demonstrated by manipulation of the endocrine or nutritional state of animals. The inhibition of such enzyme adaptations by actinomycin *D* is regarded as presumptive evidence that the step in enzyme formation which is subject to control is that of transcription of information from DNA to messenger RNA. While control of transcription of a rapidly turned-over messenger RNA would seem to provide the necessary precision and flexibility of control, and is consistent with hypotheses of bacterial enzyme regulation[16], many problems, which are not yet resolved, have arisen in the application of this simple concept to enzyme synthesis in more complex organisms. For example, there is still considerable uncertainty regarding the stability of the total messenger RNA fraction in tissues such as mammalian liver[17], and, moreover, there are now indications that messenger RNA function may also be subject to regulation[18]. In the case of hormone-dependent acceleration of enzyme synthesis in mammalian tissues there is also considerable doubt that a hormone acts as an inducer or derepressor as defined in the more simple bacterial model, for although the hormone is essential to the process, it appears to be only one component among several which summate to modulate the rate of production of the enzyme protein. This is exemplified by the glucosyltransferase transitions which have been discussed here. The level of this enzyme is not significantly reduced by the insulin deficiency of severe diabetes, and it seems to require the presence of some unknown metabolite(s) in order to be induced by insulin or, conversely, to require insulin for its induction by some metabolite(s). Moreover, in contrast to the adult animal, accelerated synthesis of hepatic glucosyltransferase during the latter stages of gestation of the rat fœtus is dependent on the presence of cortisol rather than insulin[19].

This work was supported by U.S. Public Health Service grant *AM*–04931–04. I thank Miss Judith King for her assistance, and Mr. Lee Younger for preparation of insulin antisera.

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PROGNOSIS FOR INTERRUPTION OF MALARIA TRANSMISSION THROUGH ASSESSMENT OF THE MOSQUITO'S VECTORIAL CAPACITY

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WHEREVER malaria eradication relies on vector control, efforts should be made to assess the degree of control achieved and its effectiveness in producing a downward trend in malaria transmission.

I propose that 'vector control' should refer, in this context, to reduction of the mosquito population's vectorial capacity. The vectorial capacity of a population is defined as the average number of inoculations with a

specified parasite, originating from one case of malaria in unit time, that the population would distribute to man if all the vector females biting the case became infected. This definition, from Garrett-Jones and Grab (in the press), is an attempt to give more precision to one published by me in an earlier paper[1]. Vector control is epidemiologically effective when the vectorial capacity is such that a declining rate of transmission, leading to its interruption, can be predicted.

Vectorial capacity in this sense may be assessed as the product of three parameters: (*a*) *the man-biting rate*, representing the incidence of biting-contact between the mosquito and the people in terms of number of bites per person per night; this indicates the average number of vector females liable to become infected per case per day; (*b*) *the expectation of infective life*, or days of infective life per mosquito infected with the given parasite species; (*c*) *the man-biting habit*, or bites on man per day per individual female.

Table 1 illustrates degrees of control of a vector population having a natural man-biting rate of 10, man-biting habit of 0·25, female daily survival rate of 0·9 and transmitting *P. falciparum* with 12-day sporogony.

Table 1

	Proportion surviving per day	Man-biting rate (bites)	Expectation of infective life (days)	Man-biting habit (bites)	Vectorial capacity (inoculations)
Pre-attack	0·9	10·0	2·68	0·25	6·70
Attack:					
(*a*)	0·8	4·72	0·308	0·25	0·364
(*b*)	0·7	2·96	0·0388	0·25	0·0287
(*c*)	0·7	1·48	0·0388	0·125	0·0072

Three possible situations soon after spraying are shown, that is, before there has been time for output from the breeding-places to be affected. In lines (*a*) and (*b*) the indicated reductions in daily survival entail the reductions shown in the man-biting rate and expectation of infective life (life beyond 12 days from time of infection); hence the much sharper reductions in vectorial capacity. Line (*c*) postulates a halved man-biting habit (by deviation to animals, as may be common in sprayed areas) superimposed on reduced longevity. This, by halving also the man-biting rate, reduces vectorial capacity by a further 75 per cent. (From any given birth-rate the density of a population with constant mortality through life varies as its expectation of life. Hence, a mosquito's man-biting rate may be expected to vary directly as the female's expectation of life. For example, when the proportion surviving through one day is reduced from 0·9 to 0·8 the expectation of life is thereby reduced from 9·49 to 4·48 days[1] (Garrett-Jones and Grab, *Bulletin of the World Health Organization*, in the press), with the immediate result that the density and the man-biting rate are reduced proportionately. The relevant mathematical formulæ are given by Macdonald[3] and by Garrett-Jones[1].)

In terms of epidemiological effectiveness, in situation (*c*) the unit source (that is, the infective malaria case) could give rise to about one infective bite per 140 days (1/0·0072), which is probably below the critical malaria reproduction level even where most cases remain untreated. In (*b*) there could be one infective bite per case per 35 days, long enough to detect and treat most cases in a malaria-eradication programme. Ineffective vector control is represented by (*a*), where the source could give rise to an infective bite about once in three days.

In any given environment and situation (such as one of those postulated here), the vectorial capacity for *P. vivax* of a mosquito population fully receptive to infection is necessarily greater—and its degree of reduction by vector control is less—than for *P. falciparum*, owing to the quicker sporogony of *P. vivax*.

Where two or more vector species occur, their joint potential for transmitting a given parasite is equal to the sum of their vectorial capacities. This potential rate of transmission, which has been called the parasite's 'daily reproduction rate'[1], refers to the potential number of infections distributed *per case per day*, in contradistinction to the basic reproduction rate which refers to the potential number distributed *per case*[3,4]. (An example of how the basic reproduction rate may be calculated is cited from Macdonald[3] by Pampana[5]. The assumed situation is that postulated in line 1 of Table 1, except that the vector's man-biting habit is given as 0·5. In those circumstances the basic reproduction rate, from a case infective for 80 days, is shown to be about 1,120—thus: $10 \times 2{\cdot}68 \times 0{\cdot}5 \times 80 = 1{,}072$.)

Vectorial capacity is a characteristic of a mosquito population related to the ecological conditions, and may be assessed irrespective of whether any cases of malaria are present at the given place and time. It thus differs radically from the sporozoite rate, the 'average infective density', or any other index of vector efficiency as defined by the World Health Organization[4]. Obviously, in any malarious area the vector's vectorial capacity will be subject to natural variations (according to relative density, longevity and man-biting habit); before the start of an attack on malaria these variations might be assessed to reveal, not the month of maximal transmission, but the month of steepest upward trend in transmission (which must happen somewhat earlier in the season). Then, when the vector is subjected to a fixed régime of control, assessments of its vectorial capacity might indicate whether the trend of transmission is consistently downwards or takes an upward turn in certain localities and in certain months. Finally, when no known malaria cases remain, knowledge of the mosquito's vectorial capacity may give warning of where and when there is danger of transmission becoming re-established, in the event of the importation of one or more cases.

It is submitted, therefore, that the probability of achieving interruption of transmission in a given area by means of the anti-mosquito measures in force, and, afterwards, the risk of resumption of transmission in that area, should be judged by the same type of criterion—the daily reproduction rate, assessed through the mosquito's vectorial capacity. The criteria for assessing the probability of interruption and of resumption of transmission were discussed by the WHO Expert Committee on Malaria at its eleventh meeting (Geneva, 1964). It is anticipated that these matters will be dealt with in the Committee's report (World Health Organization, in the press).) The resulting advantage in the continuity of entomological approach to assessment, in the successive phases of eradication, needs no stressing.

The problems of assessing vectorial capacity in field practice are essentially ones of sampling and of scale. Direct biting-catches on human baits probably offer the best hope of estimating the average incidence of contact between the vector species and the people. But the great variability of that contact would seem to dictate that the sampling be repeated on several nights per month, using a large group of human baits, extending the catches through the whole of the mosquito's biting cycle and to those stations (indoor and/or outdoor) where the people are to be found at each period. These problems are discussed by me in an unpublished WHO document (*WHO/Mal/*450).

Estimation of the expectation of infective life involves observations on the proportion of a sample surviving in Nature over a known interval of days. As the technique of counting the dilatations in the ovariole is still too difficult for routine application to tropical vectors, it is more usual to determine the proportion of parous females. The technique of Detinova[6] is gaining increasingly wide application. However, it is important to sample the population in such a way that the mean difference in age between the nullipars and the primipars in the sample is ascertainable. If females caught biting man are used, as advocated by Hamon[7] and other workers, it often appears justifiable in warm climates

to take this interval as equivalent to three days. Samples thus caught in ovarian stages I and II are rapidly dissected to determine the proportion with uncoiled tracheolar skeins on the ovaries. The computation of the expectation of infective life from these data is discussed by Garrett-Jones and Grab (in the press).

Finally, the man-biting habit is itself compounded of the frequency of feeding and the human blood index of the mosquito[3]. The former has often been equated with the gonotrophic cycle, but this appears to need further study in many areas. Accurate assessment of the proportion of blood meals containing human blood in a given sample is carried out by the Lister Institute, England, in collaboration with the World Health Organization[5]. On the other hand, the unbiased sampling of blood meals in the field presents rather formidable difficulties, as I have already shown[1]. The samples should reflect the day-time distribution of the blood-fed females, but often we do not know the distribution of the types of resting place, let alone that of the mosquitoes. In sprayed areas the problem is still worse, since indoor-feeding mosquitoes may die before the daytime samples are collected, and then no form of diurnal sampling of survivors can fully represent the feeding population of the previous night. This problem merits urgent investigation in view of the probability that DDT and other insecticides may reduce vectorial capacity by diverting the mosquitoes to animals as well as by killing them.

In various malaria-eradication projects increasing attention is being given to the measurement of one or more of the factors determining vectorial capacity. Sustained attempts to evaluate all the factors together have been made at a few places, for example, in the Bobo-Dioulasso District of Haute Volta, where spraying with DDT failed to interrupt transmission of *P. falciparum* by *A. gambiae* and several associated vectors[2]. Yet it is true to say that nowhere, as yet, has an investigator combined his results with the view of estimating (without reliance on the sporozoite rate) the vectorial capacity of the mosquito populations under study. Neither has the daily reproduction rate of malaria—referring to the potential incidence of new infections per existing case per day—been considered in its bearing on the prognosis for interruption of transmission by the use of residual insecticides.

Despite the difficulties alluded to, it should be a primary concern of malaria entomologists to assess vectorial capacity and evaluate the resulting epidemiological trend. It is an assessment deserving priority as an operational criterion of insecticidal attack. Moreover, the problems involved constitute as strong a scientific challenge as may be encountered in most laboratory research. This fact, considered together with the epidemiological importance of the assessment, justifies the recruitment by malaria-eradication services of well-qualified scientists, and requires that they be carefully trained for these specialized entomological duties.

[1] Garrett-Jones, C., *Bull. World Health Org.*, **30**, 241 (1964).
[2] Choumara, R., Hamon, J., Ricosse, J., and Bailly, H., *Cahiers de l'Orstom*, **1**, 99 (1959).
[3] Macdonald, G., *The Epidemiology and Control of Malaria* (London, Oxf. Univ. Press, 1957).
[4] *Terminology of Malaria and of Malaria Eradication*, World Health Organization (Geneva, 1963).
[5] Pampana, E. J., *A Textbook of Malaria Eradication* (London, Oxf. Univ. Press, 1963).
[6] Detinova, T. S., *Age-grouping Methods in Diptera of Medical Importance* (WHO Mon. Ser. No. 47, 1962).
[7] Hamon, J., *Bull. World Health Org.*, **28**, 83 (1963) (for text in English, see unpubl. doc. *WHO/Mal/312*, 1961).
[8] *Bull. World Health Org.*, **22**, 685 (Division of Malaria Eradication, World Health Organization and Lister Institute of Preventive Medicine, 1960).

THE CHINESE WATER-BALANCE ESCAPEMENT

BURSTALL, Lansdale and Elliott[1] described the third of a laboratory series of water-wheel models of the mechanical escapement in Su Sung's clock, and expressed their belief that it represented the system used in the Chinese original of the eleventh century. From consideration of the nature and performance of the original I cannot agree that the representation is adequate.

Needham *et al.*[2,3] showed that the escapement wheel of A.D. 1088, 11 ft. in diameter, drove an assembly of heavy time-indicating and astronomical machinery. This comprised two vertical shafts about 15 ft. and 20 ft. long, six or eight pairs of gear-wheels including two or three separate solar-to-sidereal time-conversion trains, six jack-wheels over 6 ft. diameter carrying calendar-work and 150 time-indicating jacks, a 4½ ft. diameter celestial globe, and an observational armillary sphere with a 6 ft. diameter bronze rotating component which itself weighed over 150 lb.

It has been shown elsewhere[4] that there was one escapement action every 24 sec. At each action static friction had to be overcome, and the escapement wheel and machinery accelerated from rest; work had then to be done in movement against friction and the resistance of the striking mechanisms and other jack-work. Motive power was supplied by a regular flow of water at not more than 25 lb./min entering the buckets of the water wheel near its horizontal diameter. Even if a conversion efficiency of 100 per cent could be achieved in the water-wheel, an average power of only about 1/25 h.p.—28 watts—would be available for work. Yet the first laboratory model of Burstall *et al.* was designed to spill the water from each one of its eight fixed buckets as soon as the wheel moved[5,6]; and although not shown in their Fig. 1 a similar arrangement was evidently relied on in the third model to allow the 36-compartment wheel to reciprocate as described[1].

My assumption that in reality the 36 buckets emptied lower down the wheel is supported by the fact that a receiving vessel 11·4 ft. long[12] was provided to catch the water, and was positioned to extend below the entire lower half of the wheel.

The timekeeping of the water-balance escapement was such that Su Sung[7] was able in his astronomical chapter[8] to give data of the Sun's apparent position, in relation to the quasi-sidereal rotating co-ordinate system of the armillary sphere, with a precision of ± 1/16th of a Chinese degree of arc. This implies the maintenance, at least over the period between one sunset, sunrise, or noon observation and the next, of a time-keeping accuracy within ± 15 sec, which is consistent with the choice of a 24-sec time unit for the escapement, and with Su Sung's interest in the 'hurrying and dawdling' of the Sun in its annual circuit.

Good timekeeping depended on accurate determination of the time unit of 24 sec by division of the regular flow of water into equal portions of not more than 10 lb. successively in each bucket. The weighing operation must surely have been done under excellent conditions, but the models of Burstall *et al.* have provided for measuring only the variable small net force likely, under real conditions, to be exerted on an external balance-lever by a rigid water-wheel, while in the act of initial movement against the static friction of all the machinery. It seems to me that even if the original escapement could operate at all on this system, it would have been unlikely to keep good time.

A practical reconstruction, avoiding the difficulties of a rigid water-wheel and providing a truly chrono-

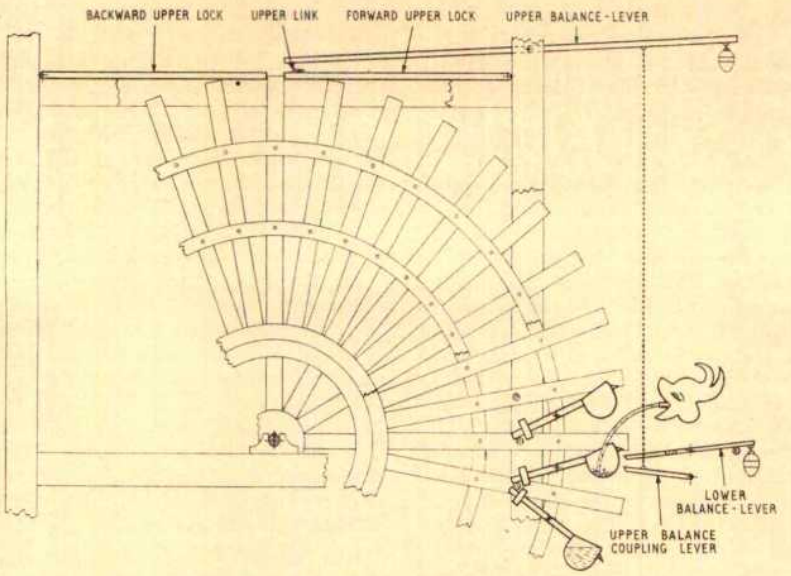


Fig. 1. Diagram of the escapement, as reconstructed

metric motion, was described[4] and demonstrated[9] in 1962. Fig. 1 is a diagram of the escapement mechanism, for comparison with that of Burstall *et al.* Fig. 2 is a photograph of a more recently constructed 1 : 6 scale working model of the original escapement, made, except for the outer framework, as nearly accurate as was conveniently possible on this scale. This model operates with the correct 24-sec time-unit cycle, by gravity feed of water from a true-to-scale constant-head tank fed by an electric pump. It would certainly be capable, when reproduced at full size, of doing all the work of the original while keeping time with an accuracy of at least about the right order. A model similar to this, but in more durable materials, is under construction for the new horological gallery of the Science Museum, South Kensington, and another, at 1 : 5 scale, for Historisches Uhren-Museum Wuppertal. This reconstruction has remained unchallenged by technical criticism since its publication, and has been stated by Dr. Needham to include nothing that the text does not authorize when fully understood[10].

According to the translation of Needham *et al.* the original escapement wheel had "seventy-two spokes and thirty-six scoop-holders mounted on the three reinforcing rings of the wheel"[11], and "each holder grasps a scoop, of which there are thirty-six in all"[12]. Needham *et al.* commented: "The scoop-holders are not described in detail but they were probably a simple shelf fixed to a spoke on either side"[13], but this did not appear to me to account for their being mounted on the reinforcing ring or rings. The 1962 publication[4] showed how practical consideration of the need to weigh each scoop separately from the wheel led to the suggestion that the scoop-holders, *hung* (radical 85 plus 6 strokes, signifying 'extensive' etc. in modern usage[14]), were supporting levers for the scoops, pivoted to the outer reinforcing ring, positioned by stops perhaps, as in the Fig. 2 model, fixed to an inner ring (not shown in Fig. 1), and counterweighted for slightly more than the tare weight of the scoops. This suggestion led, with a minimum of further experiment, directly to a successful reconstruction of the escapement. Dr. Needham tells me that a re-examination of the Chinese text, kindly carried out by him with the collaboration of Dr. Lu Gwei-Djen, has confirmed that it contains nothing which requires retention of the natural first assumption that the scoops and their holders were fixed rigidly to the wheel.

Burstall *et al.* do not mention scoop-holders, or spokes. The use of fixed water-compartments has led them to devise an 'interrupter cam' to permit a reciprocating motion of the wheel in their third laboratory model[1]. The textual difficulties which this 'interrupter cam' presents are mentioned later; apart from these it appears unlikely that the very light lower balance-weight, of say 5 lb. at most, which is all that a rigid wheel would possibly allow under real conditions, could have ensured backward rotation of the wheel against the resistance of the machinery, even if all the water-compartments were empty. Furthermore, Su Sung states that arrangements were made in the original to prevent any recoil of the wheel after the sudden arrest of its forward driving stroke. The relevant passage was translated by Needham *et al.* as follows: "After each scoop has so fallen, the stopping

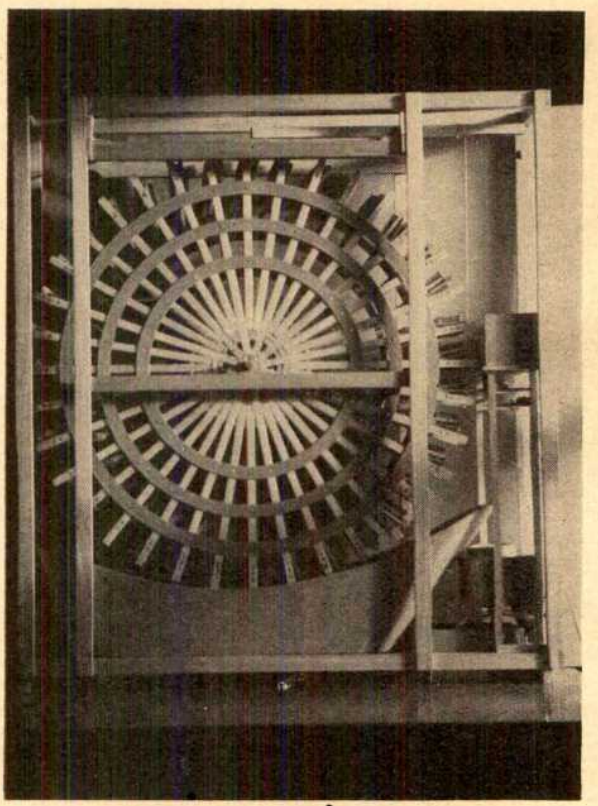


Fig. 2. Water-driven 1 : 6 scale model of the escapement

and locking device resists once again the next scoop, but since this might cause the wheel to recoil counter-clockwise, the right-hand (left as seen from the front) upper lock (that is, the ratchet action) acts to prevent any such movement"[15]. To avoid the seeming confusion of 'right' and 'left' in this account the part in question has been designated the 'backward upper lock' in Fig. 1.

Corresponding to the 'backward upper lock' but on the other side of the wheel, Su Sung's illustration[29] shows another 'upper lock' which has been designated 'forward upper lock' in Fig. 1. His text refers to both of the 'upper locks', and to their pivots on the escapement framework. Needham *et al.* mentioned that they had omitted the second 'upper lock' from their tentative theoretical reconstruction for simplicity. It has been omitted from the models of Burstall *et al.*, though their third model[1] shows a vestigial trace of it in the form of the 'limit stop H', which appears to have originated from a conjecture by Needham *et al.*, made before the real nature of the 'forward upper lock' had been established[16,17]. In reality, as explained by Su Sung, both 'upper locks' rested on the horizontal beam at the top of the escapement as shown in Fig. 1, and since the upper balance-lever could rest on the 'forward upper lock' there was no call for a separate 'limit stop H'. The relevant passages were translated by Needham *et al.* as follows: "Two upper locks, one on either side, are put to the left and right between the main posts, lying on the beam and controlling the even movement of the great wheel"[17] and, elsewhere, "There are two upper locks, one at the left, another at the right; at the end of each a shutting axle (discussed later) is attached. These two shutting axles are attached to the main posts (of the framework) at either side. They face each other on the left and right to stop the scoop-holders, *hung*, of the great wheel" (ref. 18). The text actually has not *hung*, but *fu* (spokes—radical 159 plus 9 strokes). It was the 'forward upper lock' which took the full impact of the wheel-spoke at the end of each driving stroke, and then held the load until lifted by the upper balance-lever at the correct instant for release 24 sec later. The 'backward upper lock' similarly had to take the impact of any recoil. This is why both 'upper locks' were pivoted to main vertical members of the framework.

As explained in 1962[4], it was found by experiment essential, for effective release of the 'forward upper lock' from the load of the wheel-spoke, to arrange for kinetic energy to be stored in the 'upper balance-lever' during the movement of the 'upper balance coupling lever' under the weight of the full scoop released by the 'lower balance-lever'. For this purpose, the 'upper balance-lever' was connected loosely to the 'forward upper lock' by the short length of chain designated 'upper link' in Fig. 1. In the absence of a 'forward upper lock' in their models, Burstall *et al.* followed the tentative reconstruction of Needham *et al.*[3] in the provision of a buffer or 'upper stop' fixed to the end of the 'upper balance-lever', where according to Su Sung's illustration[29] and text the 'upper link' hangs. This arrangement can now be shown to be the result of a pardonable misunderstanding of an eleventh-century Chinese technical term (see refs. 19 and 20).

In accordance with prevalent modern usage, Needham *et al.*[21] translated the character *kuan* (radical 169 plus 11 strokes) as 'stop' in *t'ien kuan* 'upper stop' and *t'ien hêng kuan* 'upper balance stop', as 'stopping' in *t'ien (hêng) kuan shê* 'upper (balance) stopping tongue', and as 'shutting' in *kuan chu* 'shutting axle'. This last example particularly aroused my technical curiosity and led eventually to the discovery of a previously untranslated passage in Su Sung's first chapter, in which he refers to his newly invented system for rotating the quasi-sidereal co-ordinate component of the armillary sphere by means of the *t'ien chu kuan lun* 'upper shaft coupling wheel'[22]. Clearly neither 'stop', 'stopping', nor 'shutting' will do for *kuan* here. With this clue, it became evident that amendment to 'coupling', or 'link' would at once make better sense where *kuan* appears elsewhere in illustration and text, and would agree with the practical need shown by experiment with the model escapement (see also refs. 23 and 24).

The suggested amendment may be considered to strengthen the slight textual evidence towards acceptance of practical arguments[25] for thinking that the water-balance escapement, substantially as now reconstructed, was that used by Chang Hêng for driving his astronomical apparatus in the second century[26].

Finally, it is to be noted that the 'star-shaped gadget'[16] which was elaborated by Burstall *et al.*[1] in their third laboratory model into an 'interrupter cam' for allowing the wheel to reciprocate, was identified and illustrated by me in 1962[4] as the horned dragon's head gargoyle (compare Fig. 1) traditionally used through many centuries to ornament the outflow pipes of Chinese water-clocks[27,28]. The illustration in the 1922 Chinese octavo reprint of Su Sung's book[29], and the better print in the Chinese quarto edition of 1844, reveal what—though at first sight designated 'checking fork'—may well be the outflow pipe itself, depicted as a square prism projecting from the dragon's mouth, on an alignment independent of that of the visually adjacent lower balance-lever, and corresponding to the form in which the artist depicted the outflow pipe from the upper flume of the water-raising machine in another illustration[30]. The (double) 'interrupter cam' of Burstall *et al.* needed additional features for which authority of both text and translation is lacking.

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[1] Burstall, A. F., Lansdale, W. E., and Elliott, P., *Nature*, **199**, 1242 (1963).
[2] Needham, J., Wang, L., and Price, D. J., *Heavenly Clockwork: the Great Astronomical Clocks of Medieval China* (Cambridge Univ. Press, 1960).
[3] Needham, J., *Science and Civilisation in China*, **3** (Cambridge Univ Press, 1959), and **4**, Pt. 2 (in the press).
[4] Combridge, J. H., *Horological J.*, **104**, 82 (1962).
[5] Needham, J., *Classical Chinese Contributions to Mechanical Engineering* (41st Earl Grey Memorial Lecture, King's College, Newcastle upon Tyne, 1961).
[6] Burstall, A. F., *A History of Mechanical Engineering* (London, 1963).
[7] Su Sung, *Hsin I Hsiang Fa Yao* (ed. Shih Yuan-Chih, 1172. Modern reprints in *Shou Shan Ko Ts'ung Shu*, 1922; and in *Ts'ung-Shu Chi-Ch'êng*, No. 1302, 1937).
[8] Needham, J. (unpublished).
[9] B. L. H., *Antiquarian Horology*, **3**, 297 (1962).
[10] Ref. 5, p. 34.
[11] Ref. 2, p. 31.
[12] Ref. 2, p. 39.
[13] Ref. 2, p. 31.
[14] Simon, W., *A Beginner's Chinese–English Dictionary* (London, 1958).
[15] Ref. 2, p. 45.
[16] Ref. 2, p. 58.
[17] Ref. 2, p. 33.
[18] Ref. 2, p. 42; and ref. 7, p. 119 (1937).
[19] Needham, J., *Babel*, **4** (1958).
[20] King, G. W., and Hsien-Wu, C., *Sci. Amer.*, **208**, 124 (1963).
[21] Ref. 2, pp. 33, 41, 42, 44, 45, 217–219.
[22] Ref. 7, p. 17, col. 9 (1937).
[23] Giles, H. A., *A Chinese–English Dictionary* (London, 1912). (See also ref. 2, p. 104.)
[24] Matthews, R. H., *Chinese–English Dictionary* (Harvard, 1952).
[25] Ref. 4, p. 86.
[26] Ref. 2, pp. 103, 104; and ref. 3, **4**, Pt. 2, pp. 485, 486.
[27] Maspero, H., *Mélanges chinois et bouddhiques*, **6**, 183 (1939).
[28] Ref. 2, p. 89; and ref. 3, **3**, pp. 320, 323, 324, 326.
[29] Ref. 4, Fig. 1. from ref. 7, Ch. 3, p. 18b (cf. ref. 2, p. 43, Fig. 18).
[30] Ref. 7, Ch. 3, p. 20b (cf. ref. 2, p. 43, Fig. 20).

We welcome the opportunity of commenting on that part of Mr. Combridge's paper concerned with our recent article[1], for he makes some astonishing assertions about the working models he has made to represent Su Sung's escapement, and they should not go unchallenged. We do not consider that his models represent the mechanism described in the original Chinese text, whereas the model we described gave what we believe was the first explanation (backed by experiment) of how a water-wheel

escapement with fixed scoops or buckets as described by Needham, Wang and Price[2] could have been made to operate.

It was Mr. Combridge's failure to see how this could be done that led him to mount the thirty-six scoops on thirty-six pivots around the wheel, each scoop having a separate counterweight and stop.

The important difference of principle between the Combridge models and ours can be summarized thus. In the Combridge models the great wheel is held rigidly by the two upper locks while each scoop is filled. The scoops moving on their individual pivots are the essential elements of the escapement, for the wheel is then being held stationary just as if it were part of the structure. Meanwhile, the lower balancing lever (which we regard as an essential element of the escapement) is doing nothing —just resting on a stop that is not mentioned in the original text. The scoop is positioned partly by the upper lock, against which a spoke of the great wheel must rest all the time because some water remains in all the scoops in the lower right-hand quadrant in the Combridge models. Accordingly it seems to us that the lower balancing lever in these models has become practically redundant; it does no more than break the fall of the scoops on their pivots and, if the various balance weights in the mechanism were suitably adjusted, it could be dispensed with altogether.

In our Mark III model, on the other hand, the great wheel itself is the scape wheel of the escapement, which is somewhat similar to a modern anchor escapement in that the forward movement of the wheel is restrained at all times by the lower balancing lever with which one of the scoops is always in contact, except at the moment of tipping. Forward over-run is prevented by the upper stop (or lock) and backward over-run by the back upper stop against which the wheel rests when the scoops are emptied.

In our model the weighing of the water in each scoop is effected, as it were, by two weighbridges—one the great wheel itself carrying the scoop being filled and the other the lower balance lever. The same two pivots are in use the whole time; they are (1) the bearings of the great wheel and (2) the pivot bearings of the lower balancing lever. In the Combridge model, thirty-six pivot bearings are involved in the weighing in turn, and it seems inconceivable that if they existed on the original they should never have been shown in the illustrations or mentioned in the text when every other bearing was described.

We were surprised to read that Mr. Combridge now tries to explain away the absence of an upper stop on his models by challenging the accuracy of the translation by Needham, Wang and Price of the word *kuan*, asserting that it means coupling or link and not 'stop' as given in *Heavenly Clockwork*. On this point we have consulted Mr. A. C. Barnes, lecturer in the Chinese Language in the University of Durham, who has authorized us to state that in his opinion there is no other way of translating the word *kuan* in this context except by the word 'stop'. On good authority he considers it would be un-Chinese to translate it in any other way. This opinion has also been confirmed by a Chinese engineer, Dr. Tao Ching Hsu, of the University of Edinburgh.

We concede the objections to the interrupter cam as shown in our earlier article, but since it was published we have realized that the function being performed by the cam, that is, lifting the chain at the correct point in the cycle, can be performed more simply by the checking fork itself. All that is required is the addition of a third central prong to the fork, and this prong could be the star-shaped gadget itself, since it is illustrated in the right place in the original woodcut. Alternatively, the star-shaped gadget may be the horned dragon's head gargoyle, as Mr. Combridge suggests. We do not know. This modification disposes of all the four features for which the authority of the text was lacking. We have also, following a comment by Dr. Needham, added, on the right-hand side of the wheel, a second upper lock that is lifted by the overhead stopping lever. While this is redundant and plays no part in the operation of the mechanism, it was described in the original text, and was presumably added as a safety device in case the buffer stop became worn or damaged. With these additions it appears to us that the method of operation of our model now meets in every respect the description given in *Heavenly Clockwork* for the escapement of Su Sung.

We have just learned[4] that a reconstruction of Su Sung's clock is now on display in the Historical Museum in Peking and we look forward to learning what form of escapement is portrayed there. The author, Li Jen-yi, describes it as "employing the escapement principle now used in modern timepieces".

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[1] Burstall, A. F., Lansdale, W. E., and Elliott, P., *Nature*, **199**, 1242 (1963).
[2] Needham, J., Wang, L., and Price, D. J., *Heavenly Clockwork: the Great Astronomical Clocks of Medieval China* (Cambridge Univ. Press, 1960).
[3] Combridge, J. H., *Horological J.*, **104**, 82 (1962).
[4] Li Jen-yi, *Horological J.*, 329 (October, 1963).

Burstall *et al.* and I were at one in seeing that the early theoretical reconstruction of Needham *et al.* would not work as it stood. We differ because our aims from there onwards have in fact not been the same.

Their aim has been to show that the reconstruction of Needham *et al.* can be made to work—under selected laboratory conditions and without a load—if various modifications and additions are made to it. Up to the present, they have produced in turn five different laboratory models. My aim was to deduce as accurately as possible from the published translation, considered as a whole, what the medieval Chinese engineers actually did and described in their own idiom. By a practical engineering approach, and some brief experiments, I arrived in 1961 at what I still believe to be a satisfactory conclusion, though without wishing to claim that it is incapable of further improvement.

It is not the fact that 'every other bearing was described'. There are almost no details, for example, of the internal mechanism of the jack-work and calendar-work. In assuming that the lower balance-lever in my reconstruction has become practically redundant and could be dispensed with altogether, Prof. Burstall has overlooked the explanation of the essential functions of the various parts. The function of the scoop-holder counter-weights (which might have been integral with the scoop-holders and so not mentioned separately) is to compensate for the *tare* weight of the scoops, so (*inter alia*) preventing them contacting the checking fork while being brought into position before filling. The function of the lower balance-weight was to support almost the whole net weight of the water in the scoop, and at the correct instant to release the full scoop suddenly on to the upper balance coupling tongue (or lever) for maximum speed and effectiveness of action of the release mechanism.

The backstops for the lower balance-lever and trip lever (upper balance coupling lever) are formed by the horizontal bars between the legs of the stand for the constant-head tank, which are illustrated and referred to by Su Sung. In my scale models they appear as functional members in the correct position as illustrated.

Dr. Needham, who has seen the Peking 1958 model recently, confirms my assumption[1] that it does not work.

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[1] Combridge, J. H., *Horological J.*, **105**, 347 (November, 1963).

LETTERS TO THE EDITOR

COSMOLOGY

Gravitational Collapse

RECENTLY there has been renewed interest in the ultimate fate of the gravitational collapse of large masses. From the early work of Chandrasekhar[1], Oppenheimer and Volkoff[2], and the more recent work of others[3], it is clear that for a relativistic gas there is a critical spherical mass of $M_c \sim 1\ M_\odot$, where $M_\odot$ is the mass of the Sun. In any relativistic degenerate mass of $M > M_c$, no matter what radius it has, the gravitational field everywhere exceeds the outward force exerted by the pressure gradient. No static equilibrium configuration is possible, and hence the mass continuously collapses.

There is nothing in the present theories of the structure of matter which can arrest the collapse of such a mass once it has started. This can be shown by comparing the relative strengths of the strong and gravitational interactions. If $g^2/\hbar c \sim 15$ and $Gm_n^2/\hbar c$ are the coupling constants for the strong and gravitational interactions ($\hbar$ is Planck's constant, c the velocity of light, G the gravitational constant, and m_n the nucleon mass), then:

$$\frac{g^2}{\hbar c} \sim 10^{39}\,\frac{Gm_n^2}{\hbar c} \qquad (1)$$

In a spherical mass consisting of N nucleons packed closely together the short-range strong interactions between the nucleons account for a total energy of the order Ng^2/λ, where λ is a characteristic distance over which these interactions are effective. The gravitational interactions, however, which are usually regarded as being very weak, are long-range and their total energy is of the order $GN^2m_n^2/R$, where R is the radius of the mass. In such a system the gravitational interactions predominate when:

$$GNm_n^2/R > g^2/\lambda \qquad (2)$$

Since $N \simeq R^3/l^3$, where l is the internucleon distance, it follows from (1) that this inequality is equivalent to:

$$N > 10N_\odot(l/\lambda)^{3/2} \qquad (3)$$

where $N_\odot \sim 10^{57}$ is the number of nucleons in a solar mass. This extremely crude calculation shows that in a dense and massive body it is possible for the strong interactions to be relatively weak compared with the gravitational interactions. At nuclear density: $l \sim 10^{-13}$ cm, and λ is of the same order for the pion Compton wavelength. Even if, at higher densities, the field quanta interacting with the baryons account for almost the entire mass of the system, the ratio l/λ cannot exceed unity, and once the collapse commences the nuclear repulsion is always crushed by gravitation.

The startling possibility of a complete gravitational collapse encounters an apparent dilemma when one considers the exterior space-time metric of such a mass. For a spherical body of mass M the exterior metric is given by Schwarzschild's solution:

$$ds^2 = (1 - r_s/r)c^2dt^2 - (1 - r_s/r)^{-1}dr^2 - r^2d\theta^2 - r^2\sin^2\theta d\varphi^2 \qquad (4a)$$

$$r_s = 2MG/c^2 \qquad (4b)$$

By Birkhoff's theorem[4] this metric applies independent of the rate of collapse of the spherical mass. The co-ordinate intervals dt and dr are the proper time and distance intervals for observers at very great distances. The radius r_s is the Schwarzschild singularity which, for an external observer, sets a lower limit on the size of the collapsing mass. For a massive body like the Sun this limiting radius is of the order 1 km.

Consider two separate systems of reference. The first or external system is at a co-ordinate distance r_1 where the proper intervals of time are:

$$dt_1 = (1 - r_s/r_1)^{1/2}dt \qquad (5a)$$

The second or co-moving system is at the surface of the contracting mass which has a diminishing co-ordinate radius r_2; in this system the proper intervals of time are:

$$dt_2 = \left(1 - \frac{r_s}{r_2}\right)^{\frac{1}{2}}\left(1 - \frac{v^2}{c^2}\right)^{\frac{1}{2}}dt \qquad (5b)$$

where v is the radial velocity of the surface relative to a local observer at rest. Thus as $r_2 \to r_s$, the time-interval dt_2 in the co-moving system becomes infinitely dilated compared with dt_1, in the external system, and vice versa, the time-interval dt_1 becomes infinitely contracted compared with dt_2. In the limit, a short moment of time in the history of the co-moving system is equivalent to an infinite duration in the history of the external system. For an observer in the external system the Schwarzschild singularity is undoubtedly real, since it requires an infinite amount of his time for the collapsing mass to attain the radius r_s. In the co-moving system this singularity does not exist. As an example, consider a free-fall collapse of a body in which the pressure is zero and the density is uniform. The internal metric is isotropic and homogeneous and it is found that it takes approximately 1 h in the co-moving system for a solar mass initially the size of the Sun to attain a radius of r_s. The time taken to collapse from the Schwarzschild radius to a point singularity is:

$$\tau_m = \frac{2r_s}{3c} = \frac{4GM}{3c^3} \qquad (6)$$

and for a solar mass this is of the order of 1 μsec.

The following question must therefore be asked. Since the time taken for collapse to the Schwarzschild radius is infinite in the external system, is it meaningful to consider the ultimate fate of gravitational collapse in massive bodies? It will now be shown that the answer depends on the nature of the universe.

As the radius r_2 of the collapsing mass approaches the Schwarzschild radius r_s, the entire future history of the distant system occurs during a short period of time in the co-moving system. In a universe which continuously expands this history contains, for the purpose of this discussion, nothing of any particular interest, and the answer to the foregoing question is that collapse beyond the Schwarzschild radius is meaningless for an observer in an external system. At present the universe is expanding and there is no need, therefore, to consider the ultimate fate of gravitational collapse in a continuously contracting universe. However, the universe may be oscillating, and in that case its present period of expansion will be followed by a period of contraction. In this type of universe the future history of the external system contains an event of profound importance. During the given short interval of time in the co-moving system the universe expands and then contracts. During the final state of its contraction the entire universe assembles around the collapsing body, and the collapsing body then falls into a point singularity in company with the rest of the universe. Thus the original external system and the original co-moving system both participate in the final collapse. A Schwarzschild singularity is physically real only for an external observer, but in the final state of collapse of the universe all observers are co-moving and are on an equal

footing, and there is no infinite dilation or contraction of one interval of proper time with respect to another. Consider, for example, an oscillating universe in which the pressure is negligibly small and the density is uniform. The time taken in the final stage to collapse from a density ρ_u to an infinite density is:

$$\tau_u \simeq \frac{2}{3}\left(\frac{3}{8\pi\rho_u G}\right)^{\frac{1}{2}} \quad (7)$$

By setting $M = 4\pi\rho_m r_s^3/3$ in (6), where ρ_m is the density of the collapsing body, it is seen that the time of collapse, τ_m, of the body and the time of collapse of the universe, τ_u, are equal when ρ_u becomes equal to ρ_m. This means that a solar mass in free-fall gravitational collapse becomes part of the collapsing universe in the last microsecond of its career. Such a universe can presumably oscillate indefinitely: it emerges from the singular state, innumerable bodies collapse asymptotically to their Schwarzschild radii, these singularities are swept up and smoothed out as the universe returns to its singular state, and finally it re-emerges to commence a fresh cycle.

To the question: is it meaningful in an external system to consider the ultimate fate of a body that is gravitationally collapsing, the answer is: yes, if the universe is oscillating. For the external system itself participates in the final stage of the gravitational collapse.

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Cosmological Red-shift

Hoyle[1] has shown that for a steady-state universe consisting of uniformly distributed matter of density ρ a metric of the form:

$$ds^2 = -dr^2\left(1 - \frac{8\pi}{3c^2}G\rho r^2\right)^{-1} - r^2 d\theta^2 - r^2\sin^2\theta d\psi^2 + c^2\left(1 - \frac{8\pi}{3c^2}G\rho r^2\right)dt^2$$

applies if it is assumed that a uniform creation of matter occurs.

From this metric Hawkins[2] has deduced an expression for a red-shift, arising in the absence of any galactic displacement, given by:

$$\frac{\Delta\lambda}{\lambda} = \frac{4\pi}{3c^2}G\rho r^2$$

I recently proposed[3] that a red-shift will occur in any model universe containing uniformly distributed matter if absorption or shielding of gravitational flux occurs on the cosmological scale, and will be given by:

$$\frac{\Delta\lambda}{\lambda} = \frac{2\pi}{3c^2}G\rho r^2$$

which differs by a factor of 2 from Hawkins's equation.

The purpose of this communication is to emphasize the fact that the very different initial assumptions of either (*a*) the continuous creation of matter, or (*b*) the shielding or absorption of gravitational flux, both lead to similar results. Of perhaps greater interest is the fact that in neither of these approaches is the red-shift expression related quantitatively with the initial assumption. This would indicate that a still more fundamental relationship exists.

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ASTROPHYSICS

Forbidden Lines from Atomic Sulphur

Forbidden lines from astrophysical and other sources are specially prominent from elements with an np^4 electron configuration. The best known are the auroral ($^1S_0$–$^1D_2$) and transauroral ($^1S_0$–$^3P_1$) lines of oxygen (OI). The present communication reports the laboratory excitation of the corresponding lines of sulphur (SI).

Conventional high voltage a.c. or d.c. discharges with positive columns were used. The tubes used varied in length from 12 to 200 cm, and in diameter from 1·5 to 3·0 cm. They were filled with xenon to a pressure of a few cm mercury after some sulphur had been distilled in, and were then sealed off and conditioned by passage of a current until impurities such as carbon monoxide had disappeared. With the smaller tubes local heating in the neighbourhood of the electrodes caused an increase in sulphur vapour pressure which tended to quench the forbidden lines, and limited the usable currents to less than about 1 m.amp. However, with the larger tube the intensity of emission was observed to be increasing even when currents as high as 15 m.amp were used. A detailed investigation of the excitation conditions is in progress.

Two spectrographs were used, a Recherches et Études d'Optique et de Sciences Connexes *HA* crossed grating instrument with a linear reciprocal dispersion of 3·5 Å/mm at 4600 Å and an aperture of *f*5, and a Huet *B*11 two-prism instrument which enabled the whole spectral range from 4000–8000 Å to be recorded on a single plate. The dispersion of the latter was 130 Å/mm at 7725 Å and the aperture *f*4. Kodak *OF*, II *L*, II *N* and 103 *aF* plates were used during the course of the investigation.

Using the large tube, with a current of 4 m.amp, it was found possible to record the lines in 4 min. The 4507 Å ($^1S_0$–$^3P_2$) line was not observed as it was obscured by a background of sulphur molecular radiation. It is hoped to record it using afterglow techniques.

Wave-length measurements were made using the xenon lines from the discharge as references. These measurements gave a wave-length of 4589·21 ± 0·05 Å for the $^1S_0$–$^3P_1$ transition and 7725·7 ± 1 Å for the $^1S_0$–$^1D_2$ transition. So far as we are aware, this is the first time the former line has been recorded.

These may be compared with the astrophysical value[1,2] of 7726·5 Å, and those computed from the accepted term values[3] of 4589·1 Å and 7724·4 Å.

We thank the Centre d'Études Nucléaires, Fontenay aux Roses, and the Observatoire de Paris, Meudon, for the facilities kindly granted during the course of the investigation. One of us (D. J. B.) also thanks the Ministry of Education, Government of Northern Ireland, for the award of a postgraduate studentship.

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GEOPHYSICS

A Smooth Type of Cosmic Noise Absorption

Riometer investigations on auroral absorption events have been carried out so far by various experimenters in Alaska[1], Canada[2] and Scandinavia[3]. The characteristics of the auroral type of riometer absorption are normally given as follows: Auroral absorption is associated with the occurrence of visible aurora and magnetic disturbances. It is normally highly irregular in character, and although it may continue for a number of hours the individual peaks last only a few minutes.

However, there exists a special type of absorption event occurring occasionally during the local morning hours which is characterized by its very smooth shape. A typical example of such an absorption event is given in Fig. 1. As can be seen, the absorption at Kiruna increases very smoothly from the zero level (ionospherically quiet conditions) to a few decibels within 30 min. After reaching its maximum value, the decrease starts, which again shows a remarkably smooth shape, lasting approximately 1 h, after which time the undisturbed level is reached again. Another feature characteristic for those events is the obvious absence of any remarkable magnetic activity at the station where the absorption event is observed.

These two facts, namely, the smooth characteristics of the events and the absence of magnetic disturbances, could easily lead to the conclusion that such events might be small-scale proton events, as has also been assumed by Ansari[4]. These events seem, in fact, to be very similar to polar cap absorption (PCA) events, when observed at a single station. However, Ansari implies that they could not be PCA proton events, but leaves unanswered the question of where the protons are coming from.

All such smooth type absorption events which have been observed at Kiruna (geomagnetic latitude 65·3° N.) in 1962 (23 altogether) have been analysed in detail. All these events occurred in the morning during 0600–1000 h local time. As indicated here, the absence of any remarkable magnetic activity was evident during this period, for each of the 23 events investigated.

Fig. 2 is the reproduction of the S_D current system of an average magnetic storm according to Silsbee and Vestine[5]. It shows that during the local morning hours the current system changes direction. This is obviously the period during which the smooth absorption events occur.

The duration of the morning transition period of currents at Kiruna has statistically been investigated for 170 magnetic disturbed days. It was found that the interval during which the geomagnetic X-component variations were less than 80γ was always at least 2 h long (0700–0900 h local time). Furthermore, it was found that in all cases when riometer absorption of any kind was observed during this morning period no remarkable magnetic disturbances could be correlated.

On the other hand, magnetic stations situated at the night side of the Earth observed remarkable activity simultaneously. This wide-scale correlation has been found already in connexion with balloon-measured auroral X-ray fluxes by Kremser[6].

Riometer data from Cape Jones have been examined, this station being situated on the night side of the Earth when the smooth absorption events were observed at Kiruna. It was found that in all 23 cases typical auroral type of absorption (changing rapidly with time) occurred at Cape Jones around the times when smooth events were observed at Kiruna. Thus it is evident that absorption can simultaneously be observed on a large scale around the auroral zone which is most pronounced at a station situated at the night side and possibly occurring as strong

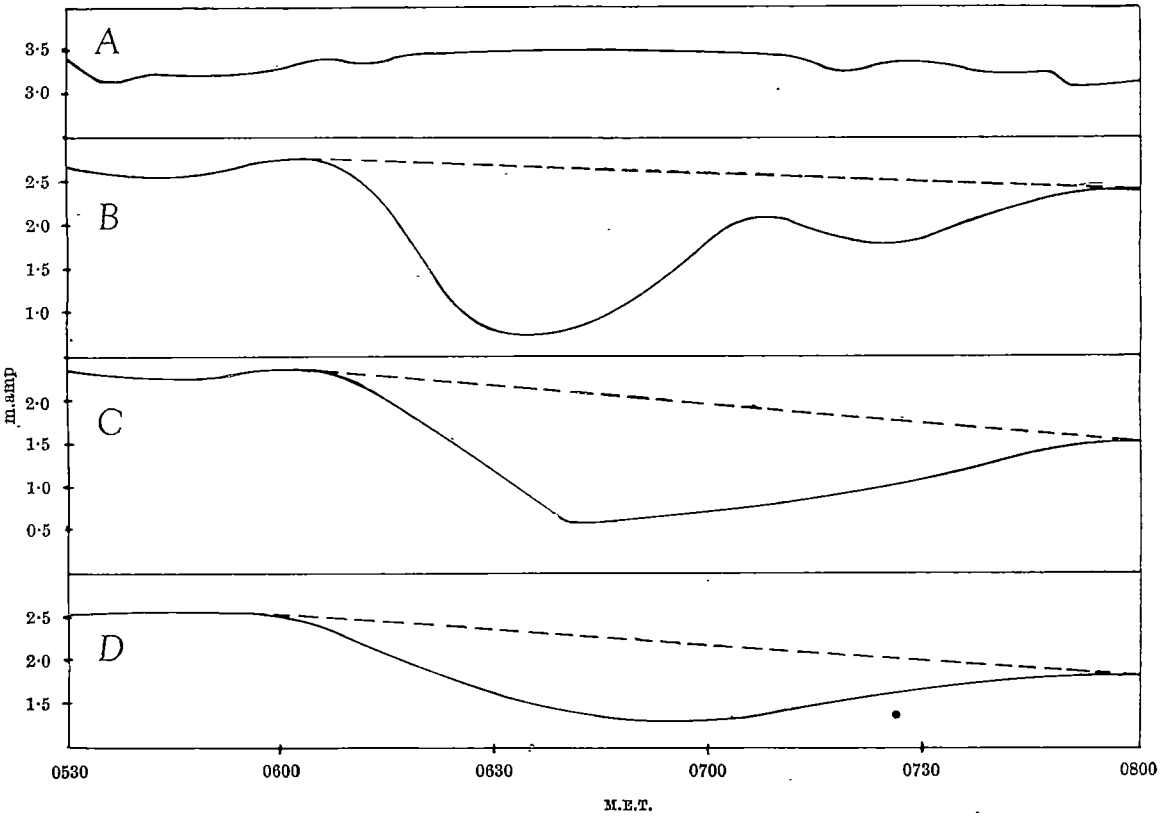


Fig. 1. Records of Scandinavian riometers for August 29, 1962. *A*, Longyearbyen (geom. lat. 74·1°); *B*, Tromsø (geom. lat. 67·0°); *C*, Kiruna (geom. at. 65·3°). Riometer frequencies all 27·6 Mc/s. *D*, Kronogård (geom. lat. 63·8°), riometer frequency 40·0 Mc/s. Broken rule indicates quiet day level

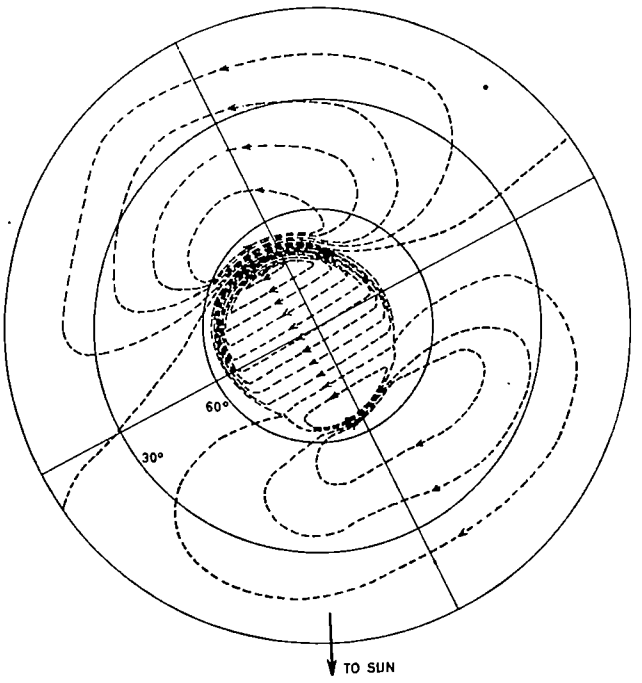


Fig. 2. S_D current system of an average magnetic storm (according to ref. 5)

absorption of smooth shape at a station situated on the morning side of the Earth.

Riometer data have been examined for the event of August 29, 1962, from Scandinavian stations situated at different latitudes, as shown in Fig. 1. It is evident that the smooth absorption event can only be observed at stations situated in or near the auroral zone. No absorption at all could be observed by the Spitsbergen riometers at Longyearbyen (geomagnetic latitude 74·1° N.). Unfortunately, there was no riometer operating south of Kronogård (geomagnetic latitude 63·8° N.) on this date. However, ionosonde data indicate that total blackouts were observed at auroral zone stations at 0700 and 0800 h local time. The f_{min} values were still increased at Lycksele (geomagnetic latitude 62·5° N.), whereas data at more southern stations did not differ from those observed on undisturbed days.

Another smooth absorption event, although much weaker in intensity, was observed on the following day, August 30, 1962. This event could only be recognized at Kiruna and Tromsø (geomagnetic latitude 67·0° N.), and not at Kronogård and Longyearbyen, that is, only at the stations situated well inside the auroral zone.

Thus, there seems to be very little evidence that the morning absorption events investigated for this work are caused by protons. Although these events show on riometer records a structure similar to PCA events, their occurrence is restricted to the auroral zone and to stations situated on the morning side of the Earth. The events analysed for this investigation did not occur during magnetically quiet periods, although no magnetic disturbances could be seen at Kiruna (the station where the smooth riometer absorption event was observed) due to the change-over of the S_D current system. Auroral (and magnetic) activity was observed simultaneously at stations located on the night side of the Earth. Balloon measurements by Brown and Barcus[7] seem also to indicate that electrons are causing the ionization responsible for this type of absorption. This is again emphasized in a recent publication by Brown[8].

Thus, it is concluded that these smooth events should be considered as belonging to the group of 'auroral type absorption events' caused by precipitation of energetic electrons.

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Evidence of Whistler Guidance by Magneto-ionic Ducts of Poorly-defined Structure

The highly localized character of multiple whistler signals suggests strongly (though indirectly) that columns of field aligned ionization must exist[1–4]. In addition, simple ray tracing calculations show quite clearly that without these hypothetical columns a rapid dispersion in latitude would be observed following successive reflexions at conjugate magnetic field regions[5]. There is some direct experimental evidence[6] for the existence of these columns; but by and large, only column fragments seem to have been observed. Indeed, recent top-side probe measurements show no evidence for continuous columns[7].

In order to understand this apparent paradox an attempt was made to deal exactly with this very complex electromagnetic field problem. The magneto-ionic duct was represented as a cylindrical plasma, of uniform density, aligned with its axis parallel to a constant, uniform magnetic field, and immersed in an infinite, uniform plasma. Thus, a lossless, cylindrical, gyrotropic medium was immersed in an infinite, lossless, gyrotropic medium of different density. The physical constants of the two media bore a realistic relationship to those of the magnetosphere. Using the exact equations of the problem, a search was made for those modes which corresponded to trapping of the whistler energy within the cylindrical region.

Most of the general details relating to the theoretical analysis of this problem have already been worked out by P. Epstein[8] and M. A. Gintzburg[9]. Turning to the numerical details, the ducts considered (2–20 km diam) were immersed in regions having of the order of 10^4–10^5 electrons/cm^3 and ambient fields of 0·1–0·5 gauss. Whistler frequencies were chosen to be a few kc/s. The calculations were performed using an IBM 7040 computer.

A number of interesting and surprising results emerged. For example, with the angular dependence of the fields described by exp. (inθ), where θ is the azimuthal angle, it was found that trapping in columns of enhanced ionization occurred only for positive n; trapping in columns of reduced ionization occurred only for negative n. No trapping was found for $n = 0$.

Other, additional information relevant to whistler propagation was obtained. The radial decay constants, which describe the decrement of the fields in the transverse direction, were computed and tabulated for cases typical of whistler propagation. The results suggested that, if several columns of enhanced ionization, not too far apart, were present, whistlers could be trapped in the regions between these columns (by the process corresponding to trapping in columns of reduced ionization) as well as in the columns of enhanced ionization.

An investigation of the difference in density between a magneto-ionic duct and its surroundings, that was suffi-

cient to achieve trapping, developed evidence that density differences as small as 0·05 per cent are capable of supporting, and guiding without loss, a large number of modes. Indeed, a figure several orders of magnitude less than this now seems reasonable.

This result suggests very strongly that well-defined ducts are not necessary for whistler propagation; even though the density difference between a duct and its surroundings varies very greatly along a given field line—and the duct therefore appears to be broken—such variations have only negligible effects on the guiding and propagation of whistler signals.

This work was supported under contract with Rome Air Development Center, Research and Technology Division, U.S. Air Force.

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Effect of Release of Compression on Magnetization of Rocks and Assemblies of Magnetic Minerals

Referring to the experimental results obtained by Kalashnikov[1,2] and Kapitsa[3] on reduction of magnetic susceptibility of rocks by their uniaxial compression, Stacey[4] has suggested that a possible local accumulation of mechanical stress within the Earth's crust in a pre-earthquake stage may result in an anomalous geomagnetic change of observable magnitude, which can serve as a pre-earthquake phenomenon for forecasting earthquakes.

The phenomenon of reduction of magnetic susceptibility by compression has been examined in more detail on assemblies of magnetites and titanomagnetites by Grabovsky and Parkhomenko[5], and these results have been theoretically justified by Kern[6] for the hydrostatic compression and by Stacey[7] for a special case. Experimentally, the fact has been confirmed as a part of results of our work on piezomagnetic phenomena of rocks and assemblies of magnetic minerals, as shown in Fig. 1.

It must be remarked, however, that the dependence of susceptibility on compression (P) obtained by Kalashnikov and Kapitsa, as well as by us, was measured under the condition that a magnetic field (H) is applied on a sample which has already been under the effect of compression. It may be considered, on the other hand, the local accumulation of mechanical stress within the Earth's crust occurs in the presence of the geomagnetic field (H). The magnetization in the former case is written here as $J(P,H)$ and that in the latter case as $J(H,P)$. Then our experimental results show that $J(H,P) > J(P,H)$.

Fig. 2 illustrates an example of magnetization curves of a sample of titaniferous magnetite under different experi-

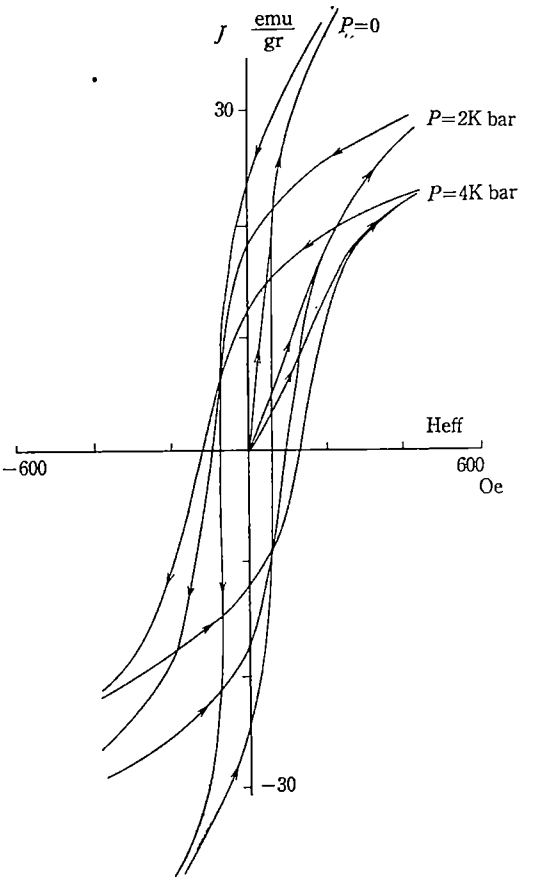


Fig. 1. Magnetic hysteresis curves of titaniferous magnetite under uniaxial compression P parallel to magnetic field H

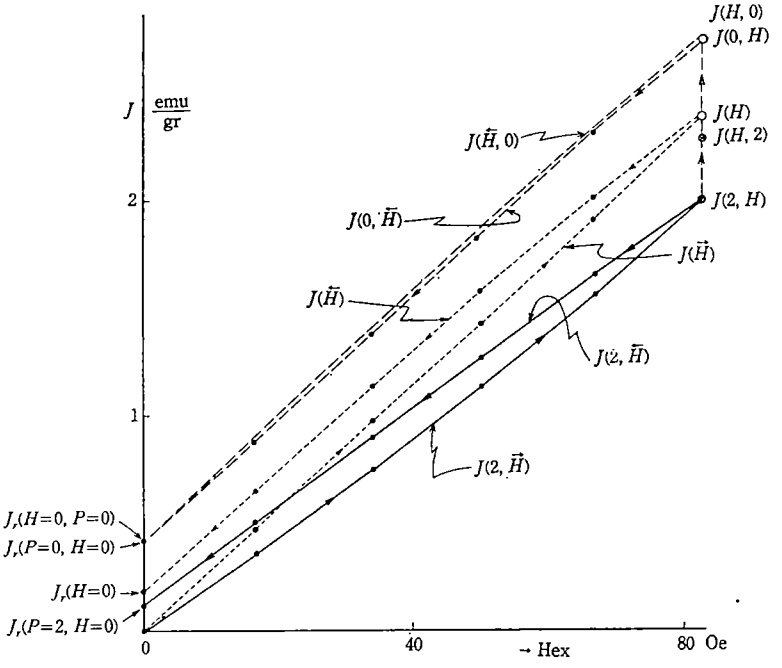


Fig. 2. Magnetization curves of titaniferous magnetite. $\vec{J(H)}$, magnetization with increasing H in $P=0$; $\overleftarrow{J(H)}$, magnetization with decreasing H in $P=0$; $\vec{J(2,H)}$, magnetization with increasing H in $P=2$ kbar; $\overleftarrow{J(2,H)}$, magnetization with decreasing H in $P=2$ kbar; $\overleftarrow{J(H,0)}$, magnetization with decreasing H after releasing P from $J(H,2)$ in $H=80$ œ; $\overleftarrow{J(0,H)}$, magnetization with decreasing H after releasing P from $J(2,H)$ in $H=80$ œ; $J(H,2)$, magnetization after compressing $J(H)$ in $H=80$ œ

mental conditions. The curves $J(\vec{H})$ and $J(\overleftarrow{H})$ represent respectively magnetization curves for increasing and decreasing values of H in case of no compression. The point indicated by $J(H,2)$ represents the value of $J(H,P)$ where P denotes a uniaxial compression of 2 kbar along H. The compression of a sample already magnetized in H results in a decrease of magnetization. The point indicated by $J(H,0)$ represents the magnetization after releasing P in H. By releasing P, the magnetization jumps up from $J(H,2)$ to $J(H,0)$. The curve $J(\overleftarrow{H},0)$ shows the magnetization curve with decreasing H after releasing P. It is seen that the remanent magnetization $Jr(H = 0, P = 0)$ in this case is larger than that of the ordinary isothermal remanent magnetization, $J(H = 0)$. The curve $J(2,H)$ represents the magnetization with increasing H under the effect of $P = 2$ kbar. The magnetization $J(2,H)$ is definitely smaller than $J(H,2)$ for the same field; namely, $J(H) > J(H,P) > J(P,H)$, and consequently $J(H)-J(H,P) < J(H)-J(P,H)$.

By releasing P in H, the magnetization jumps up from $J(2,H)$ to $J(0,H)$, which is approximately equal to $J(H,0)$. Therefore, $J(0,H)-J(P,H) > J(H,0)-J(H,P)$.

From these results, one may conclude that: (*a*) the reduction of magnetization by compression in a magnetic field, that is, $J(H)-J(H,P)$, is much smaller than the value expected from Kalashnikov–Kapitsa's experiment on $J(P,H)$; (*b*) a sudden release of compression causes an abrupt increase of definitely larger amount in magnetization. Therefore, we consider that the reported local geomagnetic change between before and after an earthquake[4,5] may be mostly caused by release of stress at the time of earthquake occurrence.

This work was partly supported by grant *NSF–G*21592 from the U.S. National Science Foundation.

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In the preceding communication Nagata and Kinoshita report that the effect of stress on rock magnetization is, in many cases, only partly reversible. It should be pointed out that susceptibility changes reversibly with stress and that the irreversibility is a property of remanent magnetization only. But any process by which a magnetic material acquires remanence must be irreversible in the thermodynamic sense. The vital question raised by Nagata and Kinoshita is: does this irreversibility invalidate simple calculations of the seismomagnetic effect which assume the established reversible piezomagnetic effect? On the evidence available at present I consider that it does not.

Graham *et al.*[1] reported that remanence was in some cases changed permanently by a temporary application of stress, and Powell[2] measured the remanence which could be produced by stress applied in a moderate ambient field. This effect has been termed 'stress-aided viscous magnetization'[3] but the term 'piezoremanent magnetization', or 'PRM', conforms better to conventional terminology. It occurs because stress modifies the potential barriers which restrict the movement of domain walls, making possible magnetic changes which would not otherwise occur. In a grain of demagnetizing factor N such changes tend towards the equilibrium magnetization H/N in a small ambient field H. Thus rocks are magnetized in a field by the application of stress and demagnetized in the absence of a field.

The magnitude of the remanence which a rock acquires by applying a field without stress is a strong function of the field, being negligible in fields comparable with that of the Earth (about 0·5 œrsted) but often quite appreciable in fields of the order 80 œrsteds, as used by Nagata and Kinoshita. Observations made in fields more than 100 times as strong as that of the Earth can scarcely be used to draw conclusions about the importance of irreversible effects in seismomagnetic calculations.

Another important problem raised by Nagata and Kinoshita and also by Kern[4] is the choice of experimental data on the piezomagnetic effect. They favour the results of Grabovsky and Parkhomenko[5], but these are irrelevant to a general calculation of the seismomagnetic effect because they refer to rocks with abnormally high concentrations of magnetic minerals (80–100 per cent), whereas common rocks do not contain more than about 10 per cent by volume and most contain much less. The stress-dependence of magnetic susceptibility $\chi(\sigma)$ of a rock in the direction of stress σ is given by[6]:

$$\frac{\chi(\sigma)}{\chi(0)} = \frac{1}{1 + s\sigma}$$

where:

$$s = \frac{3/2\lambda_s}{|\frac{2}{3}(K_1 + \frac{1}{3}K_2)| + \frac{1}{2}NI_s^2},$$

λ_s, K_1, K_2 and I_s are the saturation magnetostriction, the first and second anisotropy constants and the saturation magnetization, respectively, of its magnetic mineral. N is the demagnetizing factor of the individual magnetic grains so long as they are sufficiently widely separated for grain interactions to be neglected, that is, in rocks containing only a small per cent of magnetic minerals. In rocks with higher concentrations of magnetic mineral the self-demagnetizing field within each grain, due to its own magnetic moment, is partly compensated by the magnetizing field due to the moments of the surrounding grains and this reduces the effective value of N. For grains within a pure mineral $N = 0$ (although in some experiments the demagnetizing factor of the whole specimen must then be taken into account instead). For this reason, the specimens used by Grabovsky and Parkhomenko were much more stress-sensitive than any rocks likely to be of interest in seismomagnetic work. On the other hand, Kapitsa[7] experimented with common basic igneous rocks typical of those found in many seismic and volcanic areas. His average value of s is 1×10^{-4} cm²/kg, which further work is unlikely to revise drastically; this is the figure which I have used[8,9] for approximate calculations of the seismomagnetic effect.

In so far as it is reversible, the variation of remanent magnetization with stress is similar to the variation of susceptibility as given by the above equations, but irreversible changes may be superimposed. However, in seismically active areas where the stresses have been cycled many times during recent geological history and the rocks are therefore in a cyclic state, irreversible effects of the type considered by Nagata and Kinoshita can be disregarded.

For these reasons I consider the use of Kapitsa's data on the reversible piezomagnetic effect in seismomagnetic calculations to be satisfactory. The serious doubt which does arise concerns the seismic stresses which are not at all well understood, either as regards their extent and form or variation in time. If observations of the seismomagnetic effect differ substantially from the forms predicted, as the first reports[10,11] indicate that they do,

then erroneous assumptions in the theory must be sought first in the earthquake mechanism.

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PHYSICS

Apparent Anisotropy Field of Continuous Magnetic Films subjected to Inhomogeneous Drive Fields

Mr. J. G. Edwards[1] has attempted to explain the high values of anisotropy field measured under strip line conductors by equating the total anisotropy torque with the total applied field torque. He states that the total stray field torque is always zero. It is easy to show that this is, in fact, not the case. Using Mr. Edwards's notation, the stray field for $l \to \infty$ is:

$$H_s = \int_{-\infty}^{\infty} 2Mf \frac{\partial\,(\sin\theta)}{\partial x'} \frac{\mathrm{d}x'}{x' - x} \text{ in E.M.U.'s}$$

Hence the total or net torque:

$$T_s = Mfl \int_{-\infty}^{\infty} H_s \cos\theta \,\mathrm{d}x$$

The distribution of H_s and $\cos\theta$ are shown schematically in Fig. 1. As

$$\int_{-\infty}^{\infty} H_s \,\mathrm{d}x \propto \iint_{-\infty}^{\infty} \frac{\partial(\sin\theta)}{\partial x'} \frac{\mathrm{d}x'}{x' - x} \,\mathrm{d}x = \int_{-\infty}^{\infty} \frac{\partial(\sin\theta)}{\partial x'} \int_{-\infty}^{\infty} \frac{\mathrm{d}x}{x' - x} \mathrm{d}x'$$

is always zero, it is clear that $\int_{-\infty}^{\infty} H_s \cos\theta \,\mathrm{d}x$ will not be zero unless $\cos\theta$ is independent of x. This is approximately true for small values of θ and, therefore, the theory, developed by Mr. Edwards for small values of θ only, is also approximately correct. For larger values of θ, contrary to Mr. Edwards's assertion, the net torque is not zero. Consequently, his statement that, however large the applied field, the magnetization beneath the drive strip could never quite reach the hard direction, is also incorrect. There is nothing inconsistent about the distribution where the magnetization is in the hard direction under the influence of a large applied field for $|x| \ll w/2$ so that in this region the torque is zero, whereas for $|x| \geqslant w/2$ the anisotropy and stray field torques are equal. The coupling between the two regions is provided by exchange in two very narrow strips at $x = \pm w/2$. In this case, the net stray field torque, far from being zero, is equal to the net anisotropy torque.

Furthermore, Mr. Edwards's observations cannot be said to be an explanation for the anomalously high values of effective anisotropy field unless the coupling causing the observed values of p can be accounted for. As the p values observed are very much higher than those that could be due to macroscopic magnetostatic coupling alone,

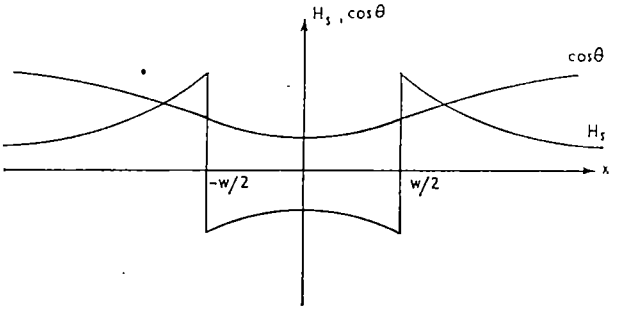


Fig. 1. The schematical distribution of H_s and $\cos\theta$ indicating that, if $\int_{-\infty}^{\infty} H_s \,\mathrm{d}x = 0$, then $\int_{-\infty}^{\infty} H_s \cos\theta \,\mathrm{d}x \neq 0$

it would be more correct to say that the anomalously high values of anisotropy field and the anomalously high values of p are corollary observations, both still awaiting explanation.

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I thank Dr. Bonyhard for his comments and agree that the net total torque caused by stray fields due to the film can never be zero. I was originally led to this erroneous conclusion in the search for a general theory independent of the nature of the coupling and by thinking that the dictum "action and reaction are equal and opposite" applied to any pair of torques as well as any pair of forces. However, the analysis of the increase in effective anisotropy field still holds: it is merely necessary to introduce the limitation that $\cos\theta \simeq \cos\theta_0 = 1$, already used to integrate equation (4), when equating equations (1) and (4). So long as changes in $\cos\theta$ are small over the range of the stray fields the balance of torques can still be used.

Whether the propagation provides an explanation of the anomalously high values of effective anisotropy field is a question of scientific philosophy. The experiments have shown that there is a strong correlation between the observed increase in anisotropy field and the propagation distance, and on the basis of a balance of torques one can be predicted from the other. At present, due to purely mathematical difficulties, we cannot be certain what values of propagation constant could be due to macroscopic magnetostatic coupling alone, but given that the propagation does occur we can 'explain' the anomalously high values of effective anisotropy field. This seems to be an advance in our understanding of the problem, albeit a small one.

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Radon Content of Rainwater

In recent years, interest has been renewed in the measurement of the radon content of the atmosphere[1-4]. In most cases, samples of air were drawn through fine grain filter papers and the activity of the filter-paper samples, corresponding to radium *A*, *B* and *C*, was afterwards estimated by a suitable Geiger–Müller counter and scaling system. It was, however, found that the filter

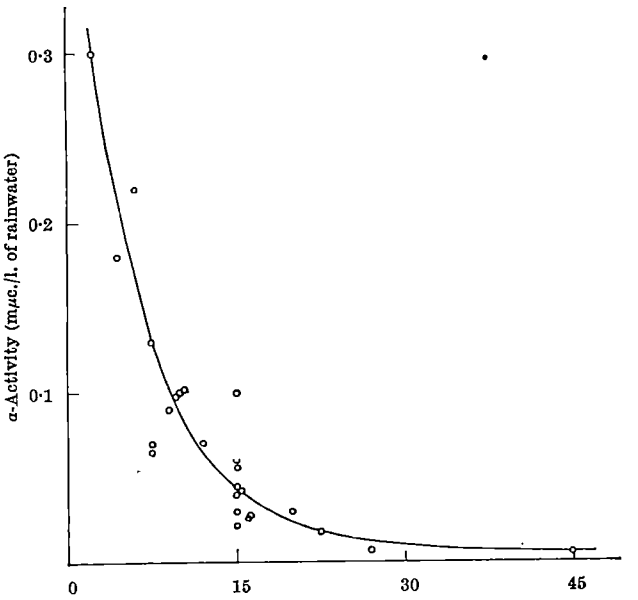


Fig. 1

paper method had a low collection efficiency of only 2–3 per cent as compared with the less equivocal ion chamber method[1]. Furthermore, Běhounek and Majerová[5] reported that there was no reason for assuming the ratio between the two measurements to be constant.

The natural radioactivity of rainwater was first reported by Wilson[6], with a half-life of about 30 min. Bumstead[7] identified the decay products captured by cloud droplets and brought to the surface by atmospheric precipitation to consist predominantly of radium $B + C$. More recently, Damon and Kuroda[8] reported that in their measurements on the natural radioactivity of rainfall the activity varied from 1 mμc. to 60 mμc./l. of rainwater and was due primarily to radium $B + C$. Since radon is present throughout the atmosphere, it continually supplies decay products which become attached to aerosols and condensation droplets. The equilibrium between radon and radium $A + B + C$ being established in about 4 h, the former should be essentially in equilibrium with the short-lived decay products in a certain volume of air, unless some disturbing force such as precipitation upsets this equilibrium. According to Damon and Kuroda[8], the decay products are swept downwards by growing raindrops, and if the rainfall is heavy, the atmospheric radioactivity will be essentially limited to the gas radon and the short-lived decay product radium A supplied directly by radon. Although the amount of radon in the atmosphere[9] is affected by meteorological conditions, it was postulated that owing to its long half-life and low concentration in rainwater a rapid depletion of radon is prevented during the course of a storm. It was therefore considered worth while to measure directly the radon content in rainwater.

During the most recent rainy season in Calcutta, the radon content of about forty separate samples of rainwater was measured. The rainwater was collected into a 2-l. flask, through a wide-mouthed glass funnel, the stem of which reached the bottom of the flask and was kept water-sealed. The separation coefficient of radon between water and air being 0·25, at 20° C, the air space in the flask retains four times as much radon as is contained in the same volume of water. Consequently, the air displaced by the collected rainwater was not allowed to escape but was trapped in a rubber balloon connected to the flask. The air laden with radon, together with the radon released by boiling rainwater, was flushed into a 3-l. evacuated ion chamber, with appropriate gas. Measurement was commenced after 3 h when the radon settled down into equilibrium with its decay products. The ion-chamber had been previously calibrated with a Hönigschmid radium substandard obtained from the Radium Institute, Vienna. The specific activity of radon varied from 5×10^{-12} c./l. to 3×10^{-10} c./l. It was noted that maximum specific activity could be associated with scant and isolated rainfalls. The specific activity rapidly diminished with the amount of rainfall as shown in Fig. 1. It was also noted that for equivalent rainfalls the specific activity of nocturnal rains was usually less than that of diurnal rains, indicating a confirmation of the diurnal variation of the radon content of the atmosphere[10].

We thank Prof. Berta Karlik and Dr. F. Hernegger of the Radium Institute, Vienna, for the gift of the radium substandard. We also thank Dr. F. Baumgärtner of the Institute of Radiochemistry, Technische Hochschule, Munich, for chemical treatment of the substandard.

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GEOLOGY

A Possible Western Continuation of the Scandinavian Caledonian Arc System

In a recent communication[1] an outline is given of the primary and secondary arcs[2] in the Scandinavian Caledonian chain. I should here like to direct attention to a possible western continuation of this arc system.

Dr. M. Lindström[3] has prepared a synopsis of axial strikes (lineations and sub-vertical s-surfaces) indicated on published maps of areas in the Scandinavian Caledonides. An important feature is the prominent west-north-westerly trend of the axial records in the western part of southern Norway.

I should also like to mention the gneiss area on the coast west of Bergen. Potassium/argon determinations of micas from pegmatites in this area gave the Caledonian ages 413 (ref. 4), 425, 434 and 450 million years[5]. The gneisses can be interpreted as being the core of an anticline. The lineations (axes of minor folds and stretching) have a west-north-westerly direction[6].

In my opinion this west-north-westerly trend of the axial records in the western part of southern Norway belongs to a primary arc west of the Möre arc. This western arc is supposed to continue to Scotland, and can be named the Scotland arc (Fig. 1). In actual fact the western part of southern Norway exhibits a corresponding junction of two primary arcs, as can be studied in central and in northern Norway.

The secondary arc formed at the junction of the Scotland arc and the Möre arc consists of two parts, an uplifted massif or welt of the Precambrian basement, and, farther from the primary arcs, a basin with thrust and folded Caledonian sediments. The Precambrian basement is exposed on the south and the south-east side of the Hardanger Fjord. A potassium/argon determination of mica from pegmatite on the south side of the fjord gave the pre-Caledonian age 904 million years[5]. A possible

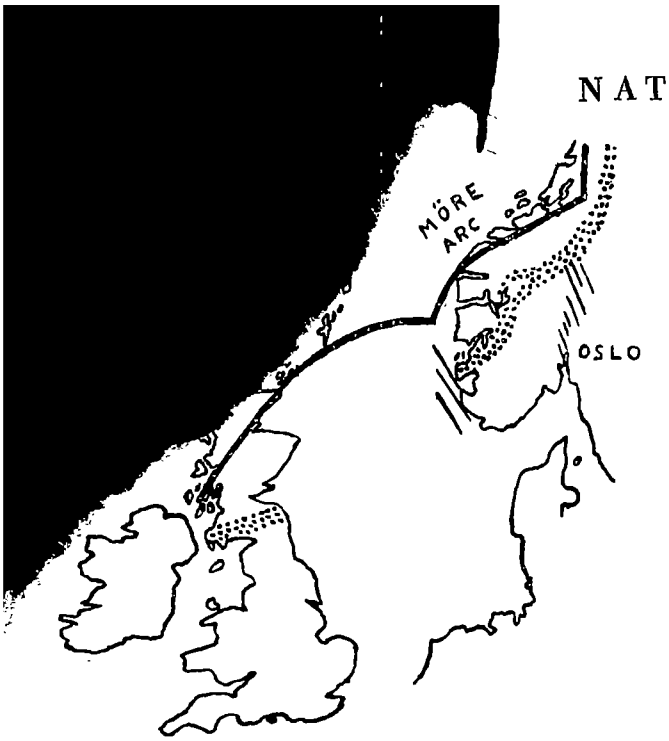


Fig. 1. Part of the British–Scandinavian Caledonian chain, an inactive narrow mountain system. Heavy lines represent axis of primary arcs, welts of secondary arcs are stippled, and graben are indicated by hatching

Caledonian origin has been discussed for this area, an interpretation which is contradicted by this figure. The Stavanger district is a preserved part of the basin.

The graben corresponding to this secondary arc is the south-western part of the Norwegian Channel.

According to Cloos[7], the origin of the Oslo region, the fault lines outside the west coast of southern Norway, and the Tertiary volcanic region of the British Isles can be explained as the result of movements between Grossblöcke of the crust of the Earth. This hypothesis seems to be in good accordance with the interpretation of the Scandinavian–British Caledonian chain as a narrow mountain system. The fault lines have repeatedly been exposed to fault movements; for example, some of the Permian fault breccias in the Oslo region are accompanied by older breccias.

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RADIATION CHEMISTRY

Pulsed Radiolysis of *Cyclo*hexane Solutions: Evidence for Reactions of Electrons

Recent investigations[1] of the effect of N_2O in the γ-radiolysis of liquid *cyclo*hexane have provided strong indirect evidence for reactions involving radiation-produced electrons. The results have confirmed the suggestion of Hamill *et al.*[2] that electron attachment can occur in irradiated liquid alkanes when suitable acceptors are present.

In this communication we report the results of some experiments carried out in *cyclo*hexane solutions using the technique of pulsed radiolysis, and these results provide corroborative evidence for the foregoing hypothesis.

*Cyclo*hexane solutions, de-aerated, were irradiated with 2-MeV electrons in single 2-μsec pulses. The irradiation assembly used was essentially as previously described[3], except that the method of kinetic spectroscopy was utilized. The mean dose delivered to the solution by one pulse was approximately 8 krads.

Diphenyl was selected as one of the solutes, since the diphenylide anion has a characteristic absorption spectrum[4], and, moreover, has already been observed in the radiolysis at −196° C of alkane glasses containing diphenyl[5]. Irradiation of solutions of diphenyl (10 mM) led to the formation of a transient absorbing in the visible, having a maximum around 6500 Å. This is shown by the results given in Fig. 1. Curve (*a*) shows the absorption of the irradiated solutions measured at the maximum of the oscillograph traces almost immediately (1–2 μsec) after the pulse of electrons was delivered. Curve (*b*) indicates the corresponding absorption after a period of 40 μsec. We attribute the absorption at 6500 Å to the diphenylide anion, which is known to have a wide absorption band in this spectral region[4]. (In the experiments described here the detailed band structure of the transient spectrum could not be ascertained, owing to the rather wide band-width used in the spectroscopic determination.) The results at the lower wave-lengths of Fig. 1, where the diphenylide ion also absorbs, are apparently complicated by the presence of more than one radiation-produced intermediate.

Further confirmation that the transient is the diphenylide anion follows from investigations of the effect of N_2O. Saturation of the diphenyl-*cyclo*hexane solutions with N_2O prior to irradiation led to a decrease in the yield of the transient, as is shown by curve (*c*). This observation is interpreted in terms of a competition between N_2O (an efficient electron scavenger[6]) and diphenyl for the available radiation-produced electrons.

Some preliminary pulse experiments have also been carried out using de-aerated solutions of benzophenone (10 mM) in *cyclo*hexane. As in the case of the pulsed radiolysis of aqueous and methanolic solutions of this solute[7], two radiation-produced transients absorbing in the visible region have been observed, one having an absorption maximum around 5300 Å, the other, which is much shorter lived, exhibiting a broad absorption maximum in the region 7000–7500 Å. The yield of both these transients is decreased if N_2O is present. On the basis of this and the previous experiments[7], we attribute the absorption at 5300 Å to the presence of the free radical $(C_6H_5)_2\dot{C}$—OH, and that at the longer wave-length to the

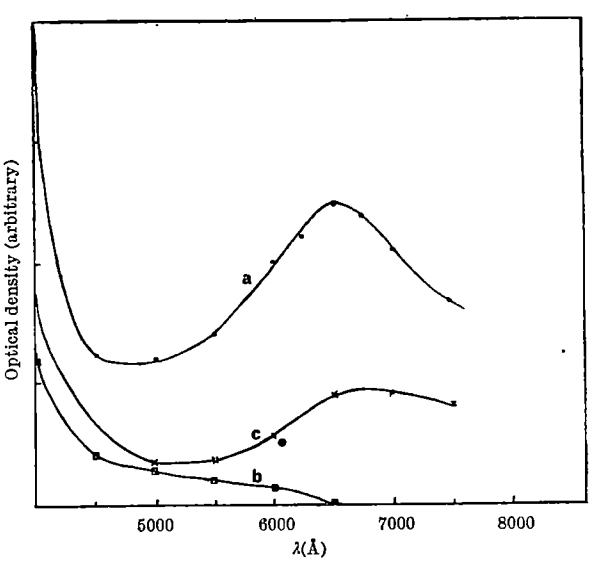


Fig. 1. Absorption spectra in solutions of diphenyl (10 mM) in *cyclo*hexane, (*a*), immediately after the pulse; (*b*), after 40 μsec; (*c*), in the presence of added N_2O (~50 mM), immediately after the pulse

presence of the radical-ion $(C_6H_5)_2\dot{C}—O^-$. (The radical ion absorbs at a somewhat higher wave-length in *cyclo*hexane than in the foregoing more polar liquids.)

Since these experiments indicate radical anion formation in irradiated alkane solutions, thereby substantiating the conclusions derived from chemical investigations, reactions of electrons should be taken into account in any interpretation of the radiation-induced decomposition of hydrocarbon systems.

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CHEMISTRY

Transport Processes in Low-melting Fused Salts

The experimental results of measurements of the viscosity and electrical conductance of molten salts have been expressed by many authors in the forms:

$$\eta = A_\eta e^{E_\eta/RT} \text{ and } \Lambda = A_\Lambda e^{-E_\Lambda/RT}$$

in which E_η and E_Λ are known as the activation energies for viscous flow and for equivalent conductance, respectively. The data frequently can be fitted with reasonable accuracy to equations of this type in which the pre-exponential factors (A_η, A_Λ) and the activation energies are independent of temperature in the temperature-range of the measurements, although there are exceptions.

It was observed by Heymann and Bloom[1] that for molten alkali halides, E_Λ is always greater than $E_\varkappa$, the activation energy for specific conductance. It has been demonstrated by Martin[2] that the latter is rather less than E_Λ, the difference being given by:

$$E_\Lambda - E_\varkappa = \alpha RT^2$$

in which α is the expansivity of the melt. Nevertheless, the ratio E_η/E_Λ, denoted by m, is found to lie between about 2 and 5 for the alkali halides when use is made of the best data at present available for the viscosities and equivalent conductances. The fact that E_η is considerably greater than E_Λ has been explained in terms of the configurational changes involved in the two transport processes. It is generally accepted that the molten alkali halides can be regarded as being completely dissociated into positive and negative ions[3]. Viscous flow presumably necessitates equal participation by the two types of ion, and hence is governed primarily by the larger species, while electrical conductance is associated predominantly with the motion of the smaller species, that is, the cations in the case of the alkali halides. It is of interest to apply these concepts to the transport properties of ionic melts in which the ions do not possess spherical symmetry. The quaternary ammonium salts are a convenient group in this category.

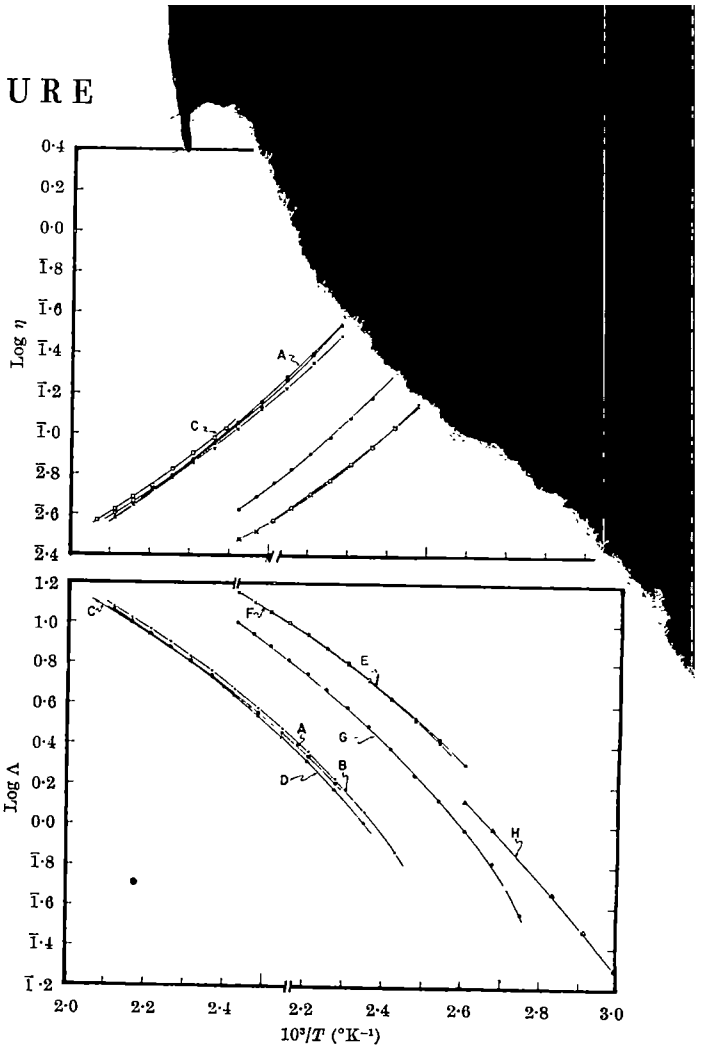


Fig. 1

Compared with most other ionic solids, the quaternary ammonium salts possess low melting points, generally in the range 50°–200° C. This fact may be connected with the relatively large separation of oppositely charged centres in the crystalline state, and with the dispersion of the total unit charge over a polyatomic species such as $\overset{+}{N}R_4$ or SCN^-. On account of the lower temperature range involved, the quaternary ammonium salts are more readily handled than most ionic melts, permitting greater accuracy in measurements of such properties as density, viscosity and conductance.

Results of the equivalent conductance and viscosity for seven tetraalkylammonium picrates have been given by Walden, Ulich and Birr[4], and for tetra-*n*-amylammonium thiocyanate by Kenausis, Evers and Kraus[5]. The graphs of log η and log Λ against $1/T$, shown in Fig. 1, are smooth curves; both E_η and E_Λ decrease with increasing temperature for each salt. The high precision of the data enables the temperature dependence of E_η and E_Λ, and of the ratio $E_\eta/E_\Lambda = m$, to be examined closely. Values of these quantities at a number of temperatures for the eight salts are given in Table 1.

Table 1. E_η AND E_Λ FOR QUATERNARY AMMONIUM SALTS

Salt	m.p. (° C)	Temperature (° C) 110° E_η	110° E_Λ	150° E_η	150° E_Λ	190° E_η	190° E_Λ
Me_3Pr_1NPic	93·0	$8·9_5$	$8·2_0$	$7·4_0$	$6·7_5$	$6·0_0$	$5·6_5$
Et_3Pr_1NPic	79·9	$8·6_0$	$8·4_5$	$6·9_0$	$6·5_5$	$6·0_0$	$5·5_5$
$Et_3PrNPic$	144·2	—	—	$7·1_5$	$6·7_5$	$6·0_5$	$5·5_5$
$MePr_3NPic$	82·2	$9·1_0$	$8·6_5$	$7·3_0$	$6·8_5$	$6·3_5$	$5·8_5$
$EtPr_3NPic$	107·2	$9·1_0$	$8·9_0$	$7·2_5$	$6·8_0$	$6·3_5$	$5·8_0$
Pr_4NPic	119·6	—	—	$7·5_5$	$6·9_5$	$6·3_0$	$5·7_0$
$(n\text{Am-})_4NSCN$	50·5	$9·5_5$	$8·7_5$	—	—	—	—
$(i\text{Am-})_4NPic$	87·0	$10·9_0$	$10·3_5$	$8·7_0$	$8·1_5$	$7·2_0$	$6·6_5$

Me, methyl; Et, ethyl; Pr, propyl; *n*Am, *n*-amyl; *i*Am, iso-amyl Pic, picrate.

For $(n\text{-amyl})_4$NSCN (m.p. 50·5° C) the E_η and E_Λ values at 60° are $11{\cdot}6_0$, $11{\cdot}4_5$ kcal/mole respectively, and at 90°, $10{\cdot}0_0$, $9{\cdot}4_0$ kcal/mole respectively.

The following points are noted: (1) E_η is always greater than E_Λ, but the values are similar, m falling between 1·0 and 1·1; values of m vary only slightly with increasing temperature, showing a small increase in most cases. (2) E_η and E_Λ at any given temperature show little dependence on the constitution of the cation when the alkyl groups are methyl, ethyl or propyl; slightly higher values are observed when R = n-amyl or iso-amyl.

It is inferred from the similarity between E_η and E_Λ that there is a close resemblance between the configurational changes involved in viscous flow and in conductance in these salts. It appears, therefore, that electrical conductance here requires in a high degree the co-operative movement of both anion and cation. Two possible reasons for this can be envisaged: (1) the existence in the melt (for times which are long compared with those involved in the relaxation of the applied electrical or mechanical stresses) of some form of ion association, which has been suggested by Davis, Rogers and Ubbelohde[6] as occurring in the nitrates of the alkali metals and of silver; (2) steric factors associated with the size, shape and relative orientation of the ions in the melt, such that neither species may be regarded as being kinetically 'free', in contrast to the small spherical alkali-metal ions in melts of the alkali halides.

In connexion with the question of association in quaternary ammonium salts, Walden *et al.*[7] suggested that these salts were completely dissociated in the liquid state. (It should be noted that interionic interactions of the type leading to partial covalency formation are precluded in the case of quaternary ammonium salts.) An important piece of evidence put forward by Walden *et al.* concerned the magnitude of the quantity $\eta\Lambda$ (the Walden product). The value of $\eta\Lambda$ for the molten picrates was found to be similar to that for infinitely dilute solutions of these salts in water and several other solvents, in which complete dissociation is assumed. Furthermore, optical investigations of the picrate ion indicated that the quaternary ammonium picrates behaved like typical strong electrolytes.

We therefore prefer to explain the observed similarity of E_η and E_Λ in these salts in terms of steric factors rather than in terms of ion-aggregate formation. The particular case of tetra-n-amylammonium thiocyanate has been examined in some detail. From the density data[5] it is clear that the average volume available to a 'molecule' of this salt in the liquid at 52° C is 656 Å^3. The $\overset{+}{N}(n\text{-amyl})_4$ group, with the four alkyl chains tetrahedrally disposed about the nitrogen atom, requires for 'free rotation' in the melt a volume of more than 1000 Å^3. Attempts to construct a model of the salt consistent with observed bond distances and with the density of the liquid show clearly that the $\overset{+}{N}(n\text{-amyl})_4$ groups must be relatively closely packed, in such a way that there is considerable interference between the alkyl groups of one ion and those of adjacent ions. This situation is shown schematically in Fig. 2. Such a closely packed assemblage of $\overset{+}{N}R_4$ ions does, however, possess interstices of sufficient size to accommodate the SCN^- ions, with length about 4·6 Å and radius 0·7–1·1 Å. According to this, free rotation of $\overset{+}{N}R_4$ ions is not possible at the temperatures involved here, and migration of SCN^- ions from one interstitial position to another is subject to considerable hindrance. This is borne out by the large activation energy for conductance, 11·6–8·75 kcal/mole between 52 and 110° C, compared with values of 2–4 kcal/mole for most molten salts with spherical ions. The reasons for the high viscosity (27–323 cP. in the range 52°–110° C) and the similarity of E_η and E_Λ are also apparent, as unit transport processes are considered to occur by means of a large number of co-operative rotational-vibrational movements of ions of opposite charge. The small increase in m with temperature generally observed in quaternary ammonium salts is explained in terms of greater freedom for the movement of the smaller ion at higher temperatures.

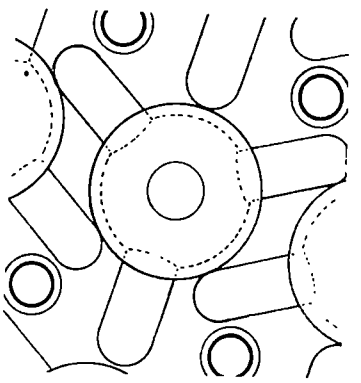


Fig. 2

In the case of the picrates, even when the alkyl chains have less than five carbon atoms, added interference and restriction on single-ion movement is provided by the nitro-groups of the picrate ion. It is concluded that gegen-ion co-operation in both transport processes for single salt melts does not necessarily indicate the presence of associated ionic species, but may arise in a fundamental way from the steric requirements of the ions and the available free space in the liquid.

Information on the crystal structure of the solid salts and on details of the fusion parameters such as $\left(\frac{\Delta V_f}{V \text{ solid}}\right)$ should prove interesting in the light of the comparative closeness of packing required in the liquid.

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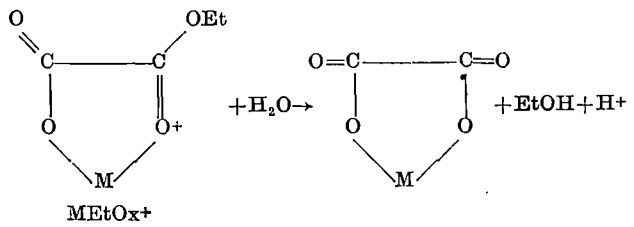
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Metal Ion Catalysis in the Hydrolysis of Potassium Ethyl Oxalate

Hoppé and Prue[1], during an investigation of kinetic salt effects in the hydrolysis of the half esters of dicarboxylic acids, found that calcium(II), barium(II) and hexammino cobalt(III) ions had a slight catalytic effect on the hydrolysis of potassium ethyl oxalate. The reaction was examined using equimolar quantities of sodium hydroxide and ester (0·01 M) in the presence of the various metal salts, conditions unsuitable for investigations of the effect of transition metal ions on the reaction. We wish to report catalysis by copper(II) and nickel(II) ions in this reaction.

The pH of solutions of potassium ethyl oxalate (0·005 M) decrease quite rapidly with time on addition of copper(II) or nickel(II) ions (0·011 M); but there is no observable effect with barium(II) ions, at this concentration.

The pH of the solution will decrease if hydrolysis occurs, as attack by a water molecule on the ester leads to the liberation of a proton:



The hydrolysis was followed by means of automatic titrator ('Radiometer TTT-1') as a *p*H-stat to monitor generation of acid in the solution. The reaction was found to obey first-order kinetics when the *p*H was held constant and the pseudo-first-order rate constants were obtained from data on the first 50 per cent reaction. In a number of runs the rate decreased after approximately 50 per cent reaction. At the metal ion concentrations used, the predominant complex in solution is $MEtOx^+$; the decrease in rate could be due to the initial formation of two different complexes such as $MEtOx^+$ and MEtOx(OH), which hydrolyse at different rates, or to the formation of a complex between the metal ion and the product such as MOx, which is more stable than $MEtOx^+$. That this second effect does, in fact, occur is supported by a number of observations. A kinetic run was attempted at *p*H 5·5 with 0·005 M potassium ethyl oxalate and 0·005 M copper(II) in the presence of 0·05 M potassium oxalate; during 60 min no hydrolysis was detected. A second run at *p*H 5·5 using *bis*(ethylene diamine) copper(II) perchlorate as catalyst also showed no hydrolysis. Presumably in this case $EtOx^-$ cannot compete with ethylene diamine for the metal ion.

The observed first-order rate constants for the copper(II) and nickel(II) catalysed hydrolysis carried out with an ester concentration of 0·005 M in 0·300 M potassium nitrate are listed in Table 1.

Table 1. Rate Constants for the Copper(II)- and Nickel(II)-catalysed Hydrolysis of Potassium Ethyl Oxalate at 25·06° C

Metal ion	[M(II)]	*p*H	$10^3 k_{obs}$ (min^{-1})
Cu(II)	0·005	5·0	0·445
Cu(II)	0·010	5·0	0·99
Cu(II)	0·015	5·0	1·54
Cu(II)	0·020	5·0	2·85
Cu(II)	0·005	5·5	1·10
Ni(II)	0·005	7·8	1·27
Ni(II)	0·010	7·8	2·60
Ni(II)	0·015	7·8	6·0

The reaction was also examined at a number of ionic strengths at *p*H 5·5 with a copper(II) concentration of 0·005 M. The following rate constants were obtained: $k_{obs} = 1{\cdot}08 \times 10^{-2}$ min^{-1} ($I = 0{\cdot}15$), $k_{obs} = 1{\cdot}08 \times 10^{-2}$ min^{-1} ($I = 0{\cdot}30$) and $k_{obs} = 1{\cdot}05 \times 10^{-2}$ min^{-1} ($I = 0{\cdot}45$). The rate of hydrolysis is, therefore, independent of ionic strength at *p*H 5·5, indicating that hydrolysis probably occurs by reaction of the complex with a water molecule rather than a hydroxide ion.

Measurements were made at three temperatures at *p*H 5·5 and a copper(II) concentration of 0·005 M, $k_{obs} = 1{\cdot}10 \times 10^{-2}$ min^{-1} (25·06°), $k_{obs} = 1{\cdot}84 \times 10^{-2}$ min^{-1} (30°), and $k_{obs} = 3{\cdot}50 \times 10^{-2}$ min^{-1} (37°), from which $\Delta H^+ = 17{\cdot}10$ kcal. mole^{-1} and $\Delta S^+ = -18{\cdot}3$ e.u.

Attempts were made to detect hydrolysis of potassium ethyl malonate and potassium ethyl succinate in the presence of copper(II) ions at *p*H 5·5 and with nickel(II) ions at *p*H 7·8. No hydrolysis was observed at 25·06° and at 30° but very slow hydrolysis appeared to occur at 37°. The greater effect with oxalate is in line with the expected greater stability of the oxalate complexes.

This work was supported by the University Grants Committee of New Zealand.

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Proton Magnetic Resonance of the Hydrogen Bond in Phenols

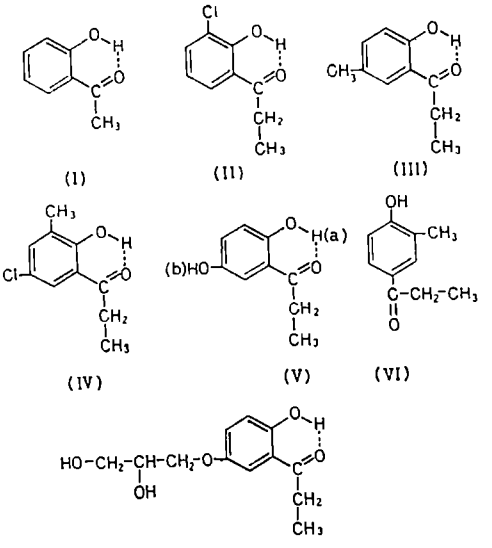
It is well known that the hydrogen bond gives rise in phenols to a large shift of the hydroxyl proton[1–6]. In a variety of compounds in which the OH groups are intermolecularly bonded, several workers have examined the chemical shifts of the hydroxyl proton in both the monomeric and polymeric compound, through the signal frequency variation which follows dilution in solvents.

A halogen atom *ortho* to the phenol OH forms an intramolecular hydrogen bond of medium strength with the hydroxyl group, giving rise to a remarkable low-field shift of the OH signal, while *ortho* substituents, containing a carbonyl or nitro group, give rise to considerably larger paramagnetic shifts, which are substantially independent of the concentration.

The phenols in which the internal hydrogen bond is formed by the OH with the donor oxygen atom of a carbonyl group are of particular interest. Batdorf[1] and, later, other workers[3,4] have examined salicylaldehyde and some salicylates in CCl_4 solution, with reference to *cyclo*hexane as an internal standard: the OH signal occurs at 9·53 and 9·13 p.p.m., respectively, for salicylaldehyde and methyl salicylate. Among the *ortho*-acylphenols, *o*-acetylphenol (I) only has been measured[4], which showed a chemical shift of 10·63 p.p.m. in CCl_4 solution and with reference to *cyclo*hexane.

It is the purpose of this communication to discuss the proton resonance of the OH group in acylphenols, and to compare the shifts found for these compounds with those of other bonded phenols.

The following compounds have been considered in my work: 2-acetylphenol (I), 2-propionyl-6-chlorophenol (II), 2-propionyl-4-methylphenol (III), 2-propionyl-4-chloro-6-methylphenol (IV), 2-propionyl-4-hydroxyphenol (V), 2-methyl-4-propionylphenol (VI), and 2-propionyl-4-hydroxyphenol α-glycerol ether (VII). While (I), (II), (III) and (IV) were measured in CCl_4 solution, the less-soluble (V), (VI) and (VII) were measured in $CDCl_3$. Tetramethylsilane (TMS) was used in each case as an internal standard. Measurements were made on a Varian *A*-60 spectrometer in a radio-frequency range of 0·15–0·25 milligauss.



Chelate acylphenols exhibit large paramagnetic shifts of the hydroxyl proton: 12·07 p.p.m. for (I) (at a 0·67 M concentration), 12·68 p.p.m. for (II) (at 0·33 M), 11·93 p.p.m. for (III) (at 0·56 M), 12·40 p.p.m. for (IV) (at 0·44 M), and 12·02 p.p.m. for (V) (at 0·36 M). These frequencies are substantially independent of the concen-

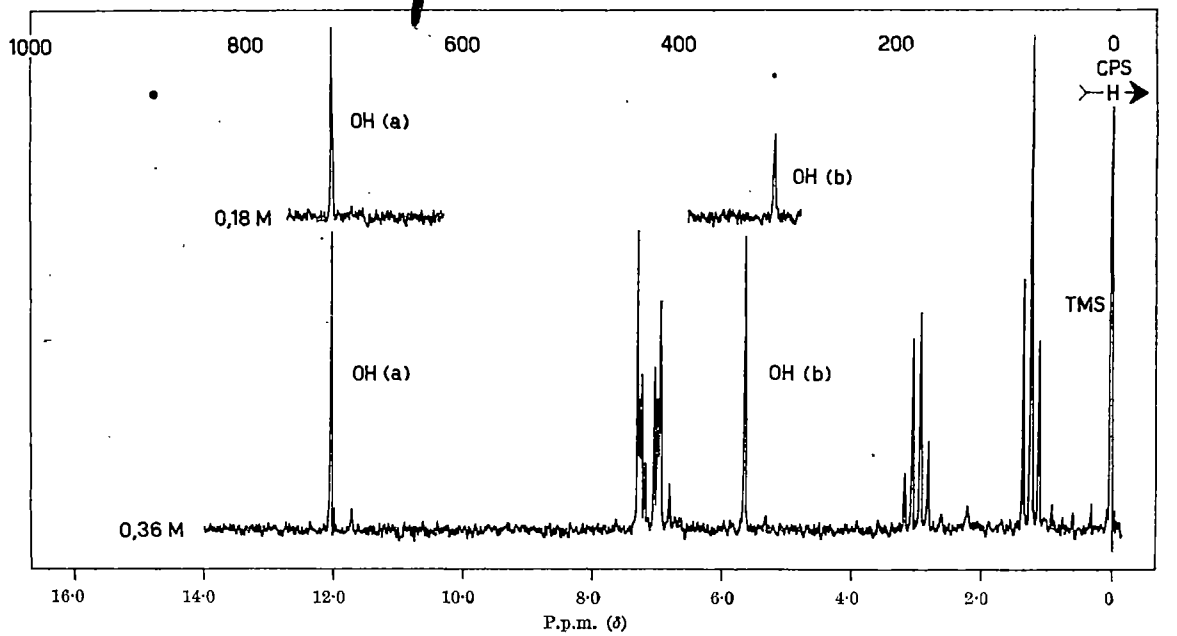


Fig. 1. Proton magnetic resonance spectrum of 2-propionyl-4-hydroxyphenol (V)

tration: the signal of (V), for example, shifts to 11·93 p.p.m. only at a 0·18 M concentration. The value found for 2-acetylphenol (I) is in close agreement with that found by Porte *et al.*[4], if their value is, of course, related to tetramethylsilane rather than to *cyclo*hexane. The frequencies are not remarkably affected by the solvent nature: the values found in CCl_4 show a difference of a few c.p.s. only from those measured in $CDCl_3$.

2-Propionyl-4-hydroxyphenol (V) is of considerable interest, and its spectrum is shown in Fig. 1. In fact, other than the signal at 12·02 p.p.m., it exhibits another signal at 5·63 p.p.m., owing to the hydroxyl (*b*) which is *meta* to the propionyl group. The difference in chemical shift between the two OH is clearly to be attributed to the fact that, while OH (*a*) is capable of giving an intramolecular hydrogen bond with the carbonyl–oxygen atom, OH (*b*) can give an intermolecular bond only. The signal of the OH (*b*) proton, therefore, shifts to 5·10 p.p.m. and tends to broaden on dilution (at a 0·18-M concentration), while the other signal shifts of a few c.p.s. only and remains sharp. Accordingly, the two hydroxyl groups show a different reactivity: by reacting the monosodium salt of (V) with glycerol α-chlorohydrin, (VII) is obtained, which exhibits the phenol OH signal at 12·00 p.p.m. at a 0·23 M concentration. The ether bond has thus been formed on the OH (*b*), and the fact that the chelate hydroxyl signal occurs at the same frequency as in (V) is evidence that this type of intramolecular bond is little affected.

(VI), in which the OH is *para* to the propionyl group, exhibits a sharp signal at 8·87 p.p.m., at a 4·20 M concentration. This signal shifts to a higher field on dilution, and becomes correspondingly broader and broader: at a 1 M concentration, it occurs at 7·93 p.p.m. and it is very broad. These data mean that the intermolecular hydrogen bond formed by the hydroxyl group in (VI) is stronger than the normal bond formed between phenol molecules: in fact, the δ_1 of the OH protons in intermolecularly bonded, non-hindered phenols are about 7 p.p.m. in CCl_4 with reference to tetramethylsilane. This may lead to the conclusion that the hydrogen bond in (VI) is formed by the hydroxyl group with the carbonyl-oxygen atom of another molecule and, taking into account that (VI) has been examined in $CDCl_3$ (which favours the relative concentration of the more polar monomer), one would think that such a bond is very strong.

Accordingly, one may consider it very likely that the intermolecular hydrogen bond formed by OH (*b*) in (V) is not of the same type as in (VI), but rather of the type of hydroxyl–hydroxyl bond normally occurring in phenols. In (V), OH (*b*) cannot form a hydrogen bond with the carbonyl group of another molecule, since the donor oxygen atom of this group is engaged in the internal bond with OH (*a*).

I have now measured the chemical shifts of the OH protons in other phenols in which an intramolecular hydrogen bond exists between the OH and a carbonyl group present in an *ortho* substituent. The results substantially support the findings of other workers[1,3,4]: the values measured in CCl_4 were 10·98, 10·84, 11·00 and 10·58 p.p.m., respectively, for salicylaldehyde, 5-bromosalicylaldehyde, 4-iso-propylsalicylaldehyde, and methyl salicylate.

It may be concluded from this work that chelate acylphenols exhibit the signal of the hydroxyl proton at 12–12·5 p.p.m., and this is substantially independent of the concentration and of the solvent. A comparison with similar chelate hydroxyl-bodies, such as salicylaldehydes and salicylates, in which the OH proton has a chemical shift of 10·5–11 p.p.m., reveals that the intramolecular hydrogen bond occurring in *ortho*-acylphenols is much stronger. When the carbonyl-bearing substituent is not *ortho* to the phenol hydroxyl group, the OH forms an intermolecular hydrogen bond with the carbonyl–oxygen atom of another molecule, being this bond stronger than the hydroxyl–hydroxyl bond normally occurring in phenols.

I thank Dr. E. Crescenzi for supplying the compounds used in this work.

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BIOPHYSICS

Differences observed between Electron Spin Resonance Signals from Surviving Tumour Tissues and from their Corresponding Normal Tissues

Commoner and Ternberg[1,2] have reported the presence of electron spin resonance signals from samples of normal rat, mouse, guinea-pig and human tissues. The samples were all surviving tissues measured quickly after the death of the animal and the signals remained for up to 100 min after death, disappearing more rapidly at the higher temperature at which the sample was maintained in the electron spin resonance spectrometer. The signals all occurred around $g = 2{\cdot}004$, indicating that free electron spins were present, perhaps in the form of free radicals, which were the origin of the resonant absorption signals. The size of signal was reasonably consistent for a given material but differed from tissue to tissue, and in the rat, mouse and guinea-pig were greatest for liver, with smaller signals from kidney and heart. Signals were also observed for intestines, adrenals and lung, but only low-intensity signals, if any, were reported for skeletal muscle and spleen.

Since then, Cook and Mallard[3] have shown a signal from normal surviving rat liver and Becker[4] has shown spectra obtained from amphibian and human whole cortical bone.

It seemed to us that one very interesting feature of Commoner's work was the observation that mouse and rat hepatoma gave scarcely any signal compared with the normal liver and we have examined a series of animal tumours and compared their signals with those from the corresponding normal tissue.

An X-band reflexion-type spectrometer developed during this project from the earlier one[3] (with a sensitivity of 10^{-6} M of Mn^{++}) was used. Small thin slices of tumour tissue (about 100 mg), which had been grafted and grown subcutaneously in otherwise healthy rats, were measured. The tumour was removed from the rat immediately after death and cut into slices, one sample being placed immediately in the spectrometer. Other samples for later use were kept in a saline solution below the freezing point. At the same time as the tumour was removed, normal tissue was removed from the same animal, the organ chosen being that type within which the tumour had originally been generated. This normal tissue was stored for later measurement.

The storage time for any tissue sample was in most cases less than a few hours and was never in excess of 1 day. Experiments were performed to confirm that this storage process did not give rise to any deterioration in the signal size measured for tissues from which signals could be obtained. Within the cavity of the spectrometer, however,

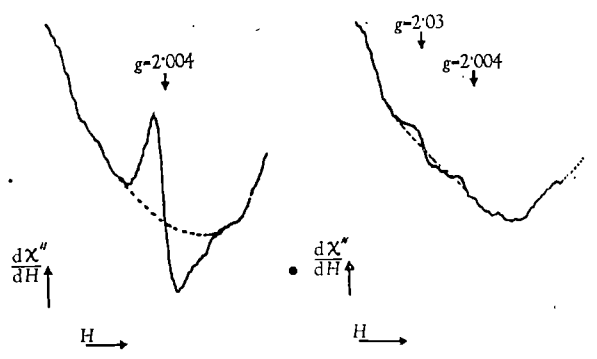


Fig. 1. Left, electron spin resonance spectrum from normal liver; right, electron spin resonance spectrum from liver carcinoma induced by butter yellow

Table 1

No. of rats from which tumours were measured	Tissue sample	Estimated concentration of spins (M)
2	Spontaneous carcinoma of renal collecting tubules	$\leqslant 10^{-6}$
	Normal kidney	$2\text{–}3 \times 10^{-4}$
2	Butter-yellow-induced carcinoma of liver	$\leqslant 10^{-6}$
	Normal liver	$5\text{–}6 \times 10^{-6}$
2	Methylcholanthrene-induced sarcoma	$\leqslant 10^{-6}$
	No normal tissue	—
2	Radiation-induced fibrosarcoma	$\leqslant 10^{-6}$
	No normal tissue	—

which is usually at about 30° C, the signals from normal liver were seen to decay slowly with a half period of about 30 min. This appears to be somewhat faster than that found by Commoner[1] in this temperature region, but he used a substrate of glucose.

The tumours used were all supplied by Dr. R. H. Thomlinson and, whatever their organ of origin, were transplanted subcutaneously through many generations. They included spontaneous, chemically-induced and radiation-induced tumours. Unfortunately no tumours were available which were growing within their organ of origin.

Table 1 shows the results on four different tumour types compared, where possible, with the nearest normal tissue. In all cases five samples were measured from each tumour. In the case of the tumours examined no definite observable signal could be detected greater than 10^{-6} M, which is our lowest limit of detection.

Table 1 also shows the number of spins observed in the signals from the normal tissue taken from the tumour-bearing rats. These agree well with those of Commoner[1]. Signals equal to these values, within a standard deviation of 30 per cent for liver and 45 per cent for kidney, have been obtained on a considerable number of samples of these tissues measured over many months before this tumour investigation was performed.

Fig. 1 shows the signals observed in the case of the rat liver carcinoma originally induced by butter yellow, and normal rat liver; the difference in size between the signals is clearly observed.

The very large background signal, on which the signals at $g = 2{\cdot}004$ are superimposed, arises from impurities in the quartz glass which is used for the sample cell; cells of pure silica will be used in future. It is possible that the same glass impurities may give rise to the small signals seen in the tumour samples at $g = 2{\cdot}004$; however, these signals may arise from the tumour itself.

A series of experiments is planned to confirm and explore these initial observations on four tumour types which show a striking difference between some malignant and normal tissues.

We thank Dr. R. H. Thomlinson of the Medical Research Council Experimental Radiopathology Research Unit at Hammersmith Hospital for the supply of tumours, and the Department of Scientific and Industrial Research for the research grant which has made this work possible.

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BIOCHEMISTRY

A Comparison of Free and Bound Amino-acids from Three Tissues of *Cancer antennarius*

THE relationship of free to bound amino-acids as well as their distribution in the various types of tissues is poorly understood in invertebrates. The variation in amino-acid content of mosquitoes was shown by Mick and Ellis[1] to correlate with species differences. Recent investigations of other invertebrates have shown that environmental differences will produce a change in amino-acid composition within the same species[2]. If amino-acid composition is to be used as a measure of species relationship or as a criterion of metabolic activity a better understanding of the relationship of free to bound amino-acids and of the variability of amino-acid content from one tissue to another within an animal would be desirable. The work recorded here was carried out with this purpose in mind.

A specimen of *Cancer antennarius* was collected in a trap in the Los Angeles Harbor area, returned to the laboratory alive, and quick-frozen. The tissues to be tested were removed from the animal and immediately lyophilized without intermittent thawing. Muscle tissue, digestive gland and excretory gland were selected for analysis. Muscle was chosen because it was believed that in this tissue a fairly stable equilibrium would be represented; digestive gland was used because this represented the most active metabolic area; excretory gland tissue was included for comparison as it represented a third and different metabolic phase.

Extracts of the free amino-acids present in these tissues were made after lyophilization by permitting the dried and powdered tissue to stand in two consecutive 50-ml. aliquot portions of 70 per cent cold ethanol, in excess of 12 h each. Dry weight quantities of tissues used were 0·7343 g of excretory gland, 0·2355 g of digestive gland and 2·2609 g of muscle. Hydrolysates were prepared by hydrolysing similar quantities of these tissues in 6 N hydrochloric acid for 30–36 h.

Chromatographic analysis was carried out on 18 in. × 22 in. Whatman No. 1 filter paper. Butanol, acetic acid and water (4 : 1 : 5) was used for the first direction while water-saturated phenol was used for the second. Development was brought about by dipping in a 0·2 per cent acetone solution of ninhydrin and drying in an oven at 37°. Various concentrations of extract were chromatographed to ensure the best possible identification. Although no exact quantitative measurements were made, visual comparisons, based on spot size and intensity, were noted. In Table 1 the figures 4, 3, 2 and 1 have been used to designate these relative concentrations with 4 representing the highest and 1 the least.

The chromatographic patterns obtained from the muscle tissue of one of the chelipeds showed the following free amino-acids to be present : alanine and glutamic acid in high concentrations; serine, glycine, valine, phenylalanine and leucines in slightly lighter concentrations; threonine, proline, hydroxyproline, aspartic acid, lysine, histidine and arginine in still lighter concentrations. A faint spot in the position of tyrosine indicated its presence in small quantity.

The bound amino-acids of the muscle tissue differed from the free amino-acids of this tissue both qualitatively and quantitatively. In addition to the amino-acids already mentioned, which of necessity were present because those in free form were not removed before hydrolysis, cystine, asparagine and/or taurine (in the solvent systems used, these latter two move to the same position), and three unidentified ninhydrin-sensitive spots occurred. The leucines, alanine, proline and hydroxyproline were noticeably heavier in relative concentration. Only in the chromatograms of muscle tissue were two distinct spots detectable for proline and hydroxyproline. Because these occur very closely together in the solvent systems used in this work, they will hereafter be reported as proline, which does not exclude the possibility of hydroxyproline at the same location.

Table 1. AMINO-ACID CONTENT OF THREE TISSUES OF *Cancer antennarius*

Amino-acid	Muscle (free)	Muscle (bound)	Digestive gl. (free)	Digestive gl. (bound)	Excretory gl. (free)	Excretory gl. (bound)
Alanine	4	4	3	3	4	4
Glycine	3	3	3	2	3	3
Glutamic	4	4	4	4	4	4
Serine	3	3	2	2	3	3
Aspartic	2	3	1	4	2	2
Threonine	2	3	1	2	2	2
Lysine	2	2	1	2	3	3
Histidine	2	2	2	2	3	3
Arginine	2	2	1	2	3	3
Valine	3	4	1	3	2	1
Phenyl-alanine	3	4	1	1	2	1
Leucine	3	4	3	3	4	3
Cystine	—	1	1	1	—	
Proline/ hydroxy-proline	2	4	2	1		
Tyrosine	1	2	1	1	2	2
Methio-nine	—	—		1	—	
Aspara-gine/tau-rine	—	2	—	—	—	
Unknowns	—	1,1,1	—	—	1,1	1,1

An analysis of the free and bound amino-acids of the excretory gland showed the same amino-acids to be present in both. In each form alanine and glutamic acid appeared in heavy concentration. In the free amino-acids of the excretory gland the proportion of leucine to the other amino-acids was higher than in the bound form in this tissue. Glycine, serine, lysine, histidine and arginine occurred in both forms in slightly lighter concentrations. Aspartic acid, threonine, tyrosine, glycine and an unidentified spot occurring near glutamic acid were present in even lesser amounts. In the free form, the amounts of valine and phenylalanine were slightly greater than from the combined form.

A comparison of the free and bound amino-acids of the digestive gland showed them to be qualitatively the same. Alanine, glycine, serine, valine, phenylalanine, the leucines, proline, tyrosine, cystine and a trace of methionine occurred in both cases. Differences occurred, however, in the relative concentrations (Table 1). The most notable of these differences was seen in the case of aspartic acid. In the hydrolysate the spot produced by this acid was very heavy whereas in the free form it appeared as only slightly more than a trace.

A comparison of the analyses of the three types of tissues showed that twelve amino-acids were held in common in both free and bound forms. These were alanine, glycine, glutamic acid, serine, aspartic acid, threonine, lysine, histidine, arginine, valine, phenylalanine, leucines and tyrosine. Asparagine/taurine occurred only in muscle hydrolysate, indicating that of the tissues tested in this animal this amino-acid is a component only of muscle tissue protein. Apparently no reserve quantity, or a quantity so small as not to be detectable, occurs in the tissue fluid of the muscle in free form. This seems also to be the case for cystine in muscle. The occurrence of this amino-acid, however, is not confined solely to muscle tissue but was found also in both forms of the digestive gland analysis. In the latter case it cannot be said with certainty whether the cystine detectable occurred only in free form or in the bound form as well.

Proline was detectable in both digestive gland analyses and muscle analyses. In the case of the muscle tissue the spot formed by this amino-acid from the hydrolysate was approximately twice the relative concentration as that of the free amino-acids of this tissue, indicating that proline occurs in both forms in muscle. In the digestive gland analyses no such sharp distinctions of concentrations of this amino-acid were seen between the two forms so that although it can definitely be said to be present in free form, it cannot be said with certainty to exist in bound form. No trace of this amino-acid was noted in the

excretory gland tissue, indicating that, if it is present, the quantity is extremely minute and thus not detectable by the method used. This situation differs from that of mammalian tissue where it has been shown by Scriver *et al.*[3] that renal tubules exhibit a reabsorption preference for proline and hydroxyproline over glycine, which would suggest their presence in free form in that tissue. Their absence here is emphasized by the fact that the quantity of excretory gland tissue used for analysis was approximately three times that of digestive gland, yet this amino-acid readily seen in the latter tissue was not observable in the excretory tissue. Thus, this amino-acid would seem to be absent from the protein comprising the excretory gland, from the tissue in free form and from the filtration products present in the gland at the time of free amino-acid extraction and of hydrolysis.

Methionine was found only in digestive gland tissue in a quantity so small that the spot formed by it in both the free and combined form was barely detectable. Its existence in a minute quantity here and its absence from the other tissues analysed would suggest that it may be rapidly used as a methyl donor at this site. A correlation may exist between the presence of cystine in the digestive gland and the presence of methionine, since the latter on demethylation becomes homocystine and this through a series of reactions can participate in the synthesis of cystine[4].

This investigation was part of the work supported by research grant *WP*-260 from the U.S. National Institutes of Health.

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Influence of Amino-acid Levels on Protein Synthesis *in vitro*

Although protein synthesis is obviously dependent on availability of amino-acids[1], the relationship of alterations in intracellular levels of amino-acids to rates of protein synthesis and breakdown remains obscure. Thus, enhanced protein synthesis has been observed in the presence of normal[2] as well as elevated[3] cellular levels of amino-acids. A maximum effective cellular concentration may exist for each amino-acid beyond which net protein synthesis cannot be further stimulated[4,5]. However, above this concentration alterations in gross protein synthesis may occur which are masked by concomitant variations in protein breakdown. The investigations recorded here were designed to investigate this possibility. The data indicate that a primary increase in extracellular concentration of one essential amino-acid may result in stimulation of protein synthesis and protein breakdown in surviving liver cells.

Hepatic tissue was obtained from adult male rats (160–170 g) of an inbred Sprague–Dawley strain. The animals were killed in the post-absorptive state by bleeding from the dorsal aorta under light 'Nembutal' (sodium pentobarbital) anæsthesia[6]. Slices obtained with the Stadie–Riggs slicer were 0·5 mm thick and weighed 100 mg each. Approximately 500 mg of liver slices were incubated in Krebs–Ringer bicarbonate buffer containing 0·5 μc. of uniformly-labelled ^{14}C-amino-acids and varying amounts of non-labelled L-amino-acids. Incubation was carried out at 37° in a Dubnoff metabolic shaker under a gas phase of 95 per cent oxygen–5 per cent carbon dioxide. After incubation, tissue and medium were separated by filtration through fine gauze and treated with trichloroacetic acid. Methods used for the extraction of amino-acids, the purification of liver protein, and the determination of radioactivity in these substances will be fully described in a subsequent report.

When liver slices were incubated in the presence of varying extracellular concentrations of one essential amino-acid, the rate of incorporation of another amino-acid into hepatic protein was altered (Table 1). Thus, as the initial extracellular concentration of phenylalanine was increased from subnormal plasma-levels (2 μM) to normal (50 μM) and supranormal (155 μM) values, radioactivity incorporated into protein from ^{14}C-leucine was significantly enhanced. Similar results were obtained when the initial extracellular concentration of threonine or valine was varied (Table 1). Column chromatographic separation of amino-acids in hydrolysates of hepatic protein revealed that radioactivity incorporated from ^{14}C-leucine was entirely in the form of this amino-acid.

Table 1. Effect of Initial Extracellular Level of One Amino-Acid on Uptake of ^{14}C-Leucine into Hepatic Protein

Amino-acid	Initial extracellular concentration (μM)	Incorporation c.p.m./mg protein	*P*
Phenylalanine	2	10·0 ± 0·8	
	50	11·4 ± 1·2	
	155	13·2 ± 0·8	< 0·01
Threonine	3	6·3 ± 0·2	
	228	8·2 ± 0·1	0·01
	500	9·5 ± 0·2	0·01
Valine	3	8·7 ± 0·1	
	157	10·2 ± 0·5	0·04
	338	16·3 ± 0·9	0·01

Liver slices were incubated for 15 min in the presence of 0·5 μc, (16 μM) of uniformly-labelled ^{14}C-leucine and varying amounts of another essential amino-acid. Normal plasma concentrations of the latter were as follows: phenylalanine, 50 μM; threonine, 228 μM; valine, 157 μM. After incubation, liver slices were homogenized in 5 per cent trichloroacetic acid. The precipitated protein was purified[6], air-dried, and counted at infinite thickness in Tracerlab '*TGC*-14' thin-window flow counter. Values are averages ± *S.E.M.* of 12 incubations with phenylalanine and 3 incubations each with threonine and valine.

The highest initial extracellular concentration of phenylalanine employed (155 μM) maintained normal or supranormal intracellular levels of this amino-acid over a 60-min incubation period (Table 2). Variations in phenylalanine levels appeared to have no effect on the distribution of leucine between cell and medium at the end of this period. However, the early flux[7] of leucine into the hepatic cell as well as the flux of phenylalanine were altered under these conditions. As anticipated[8], the highest concentration of phenylalanine greatly increased the flux of free phenylalanine (Table 2). In contrast, leucine exchange between extracellular and intracellular compartments was depressed. Therefore, the accentuated incorporation of leucine into protein under these conditions (Table 1) was apparently not due to increased availability of this amino-acid. Similarly, incorporation of ^{14}C-valine and ^{14}C-lysine into protein was increased at the highest concentration of phenylalanine even though the flux of these amino-acids into the cell was not significantly altered. Since alterations in the rate of incorporation of amino-acid into protein may not accurately reflect changes in protein synthesis or turn-over[9], measurements of the flux of leucine into hepatic protein were made. It was found that elevated levels of phenylalanine significantly enhanced the turn-over of hepatic protein. Thus, in a representative experiment the flux of leucine into hepatic protein during the 15- to 30-min interval of incubation was 22·2 μg/h/mg protein when the initial extracellular concentration of phenylalanine was 155 μM, but only 14·9 μg/h/mg protein with 50 μM phenylalanine.

Protein breakdown, as well as protein synthesis, was accentuated in the presence of supranormal levels of phenylalanine in medium and cell. For example, after incubation of hepatic slices for 60 min, 4·921 μmoles of essential amino-acids (excluding phenylalanine) were released from protein when the initial extracellular concentration of phenylalanine was 50 μM, whereas 5·342 μmoles were released when 155 μM phenylalanine was present. Larger increases in protein catabolism undoubtedly occurred at the supranormal level of phenylalanine (155 μM), since the accumulation of amino-acids was partially obscured by an increase in amino-acid

Table 2. Effect of Extracellular Level of L-Phenylalanine on Distribution and Flux of Free Phenylalanine and Leucine in Hepatic Tissue

Initial extracellular phenylalanine (μM)	Final phenylalanine concentration Liver (μmole/g)	Medium (μmole/ml.)	Final leucine concentration Liver (μmole/g)	Medium (μmole/ml.)	Flux Phenylalanine (mμmole/g/min)	Leucine
2	0·035	0·028	0·109	0·080	0·32	1·10
50	0·045	0·048	0·098	0·078		
155	0·141	0·115	0·122	0·082	14·34	0·75

Liver slices were incubated in the presence of 0·5 μc. each of uniformly labelled ^{14}C-phenylalanine and ^{14}C-leucine (16 μM) and varying amounts of non-labelled phenylalanine. Amino-acid content was determined in liver and medium after 60 min incubation using the Beckman model 120 automatic amino-acid analyser. Flux of free amino-acid was calculated over the 1·5–3 min period. Each value is a single determination on extracts combined from 3 incubations. Normal hepatic levels of amino-acid were 0·085 ± 0·005 μmole phenylalanine per g and 0·206 ± 0·017 μmole leucine per g of tissue. Radioactivity of amino-acids was measured directly on the column effluent of the amino-acid analyser employing a Packard scintillation flow detector.

utilization. Thus, the amount of ^{14}C-leucine remaining after incubation of hepatic tissue for 60 min in the presence of 155 μM phenylalanine was reduced about 15 per cent compared to that remaining in similar experiments with 50 μM phenylalanine.

These observations indicate that the degradative and synthetic aspects of protein metabolism were both stimulated in surviving hepatic tissue when the initial extracellular concentration of a single essential amino-acid was elevated. Secondary changes in uptake and distribution of other amino-acids between extracellular and intracellular liver compartments did not appear to be correlated with the alterations in protein turn-over. Information is not available to ascertain whether the primary effect of alterations in amino-acid levels on protein turn-over was on protein synthesis or protein breakdown.

This work was supported by research grants from the National Science Foundation (*G*–18487) and the National Institutes of Health, U.S. Public Health Service (*GM*–03869 and *GM*–12520), and from Cancer Research Funds of the University of California.

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Rapid Spectrophotometric Micromethod for Determination of Histaminase Activity

It is known that in biological systems histamine is broken down by histaminase[1]. In this reaction oxygen is consumed and hydrogen peroxide is formed. A manometric method of measurement[2] is based on the oxygen consumption and a chemical method[3,4] on the production of hydrogen peroxide. In the latter method indigo is added to the incubation mixture and the discoloration of this mixture by the hydrogen peroxide formed is a measure of the enzyme activity. This is due to the peroxidatic activity of catalase mostly present in the biological fluids or tissue extracts under test. The disadvantages of the chemical method are the long incubation period of 24 h and the necessity of at least 1 ml. of serum in the case of histaminase determinations in blood.

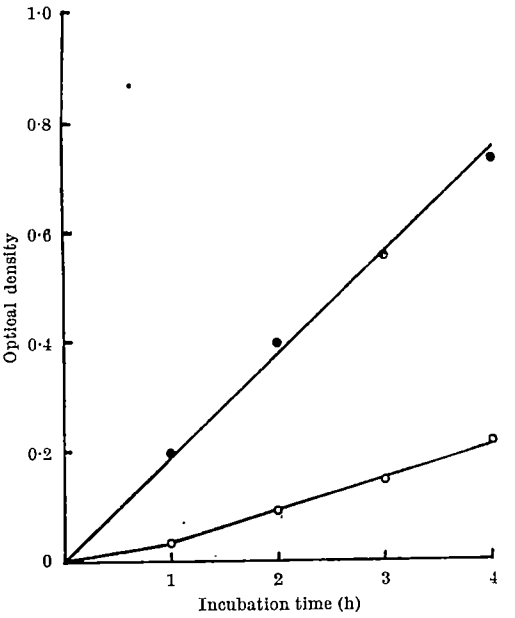


Fig. 1. Increase in optical density at 470 mμ of an incubation mixture of 1 ml. total volume, containing the phosphate buffer, *p*H 7·2, 0·1 ml. of rabbit plasma, 20 μg peroxidase, 50 μg ortho-dianisidine and 10^{-2} M histamine dihydrochloride (upper curve). The lower curve is related to a mixture without added histamine dihydrochloride

In our experiments with small animals this amount of serum makes it impossible to estimate histaminase activity repeatedly in the blood of the same animal. Therefore, we needed a more sensitive method, which was obtained by using ortho-dianisidine instead of indigo as a hydrogen donor. With this reagent it is possible to start the incubation with a colourless mixture. The increase in optical density during incubation is a measure of the histaminase activity. Moreover, we added peroxidase to the incubation mixture to be independent of the peroxidatic activity of the sample to be tested.

The reagents used were: (1) 0·1 M phosphate buffer, *p*H 7·2; (2) 2×10^{-1} M histamine dihydrochloride solution; (3) 4 mg of peroxidase (Boehringer) dissolved in 10 ml. of distilled water; (4) a solution of ortho-dianisidine in 96 per cent alcohol (1 mg/ml.).

An incubation mixture of 1 ml. containing the buffer, 0·05–0·1 ml. of the test sample and 0·05 ml. of the reagents 2, 3 and 4 was prepared on ice in a 10-mm light pass cell of a Beckman spectrophotometer. After measuring the optical density at 470 mμ the mixture was incubated at 25° C. The intensity of the resulting colour was determined at intervals of 1 h.

With this method it is possible to determine histaminase activity in samples of 0·05–0·1 ml. of plasma within a period of 4 h. The slope of the upper curve in Fig. 1 shows the increase in optical density of an incubation mixture to which 0·1 ml. of rabbit plasma had been added. The lower curve relates to a mixture with the same amount of rabbit plasma but without added histamine (reagent 2) and demonstrates that there is still some increase in optical density. This is most likely due to the presence of histamine in the sample of plasma. This may indicate that this method is also suitable for determining histamine. In this case the substrate instead of the enzyme will be the limiting factor in the reaction.

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Prevention of Fatty Liver by Administration of Adenosine Triphosphate

It has recently been shown that the fatty liver produced in rats by ethionine administration is associated with a decrease in hepatic adenosine triphosphate (ATP) levels and that injections of ATP are able to prevent both the fatty liver and the drop in ATP[1,2]. We have observed that a variety of agents which produce a fatty liver lead to decreased ATP concentrations in the liver and that the protective effects of ATP administration are not limited to ethionine toxicity.

Investigations were conducted with three hepatotoxic drugs in addition to ethionine, namely: ethanol, carbon tetrachloride and azaserine. It is well known that the administration of ethanol and carbon tetrachloride to animals leads to the production of a fatty liver; the antibiotic azaserine (*o*-diazoacetyl-L-serine) is able to produce a fatty liver as well as liver cell necrosis[3,4].

Female albino Sprague–Dawley rats weighing 150–200 g were fasted overnight and divided into four different groups: (1) control; (2) control plus ATP; (3) hepatotoxic drug; (4) hepatotoxic drug plus ATP. Details of the dosage schedules and routes of administration are outlined in Table 1. The animals were killed after times which were sufficient to lead to accumulation of fat in the liver—namely, 3 h for azaserine, 5 h for carbon tetrachloride, 6 h for ethionine and 10 h for ethanol. Liver tissue was assayed for ATP by the luciferin-luciferase method[5] and for triglycerides (after extraction and fractionation of lipids) by the method of Van Handel and Zilversmit[6].

Table 1 shows the hepatic levels of ATP and triglyceride under the various experimental conditions. It will be seen that a fatty liver was produced by each agent. Ethionine treatment produced the highest hepatic triglyceride levels (21 mg/g), while the degree of lipid accumulation produced by the other agents was somewhat less (approximately 17 mg/g). In all cases, hepatic levels of ATP decreased. This decrease was 82 per cent with ethionine, 52 per cent with carbon tetrachloride and approximately 37 per cent with ethanol and azaserine. Injections of ATP together with these agents prevented both fat accumulation and decreases in hepatic ATP levels. The results (Table 1) were all highly significant statistically.

It will also be noted that in control animals the administration of ATP served to elevate hepatic ATP concentrations with a concomitant decrease in hepatic triglyceride content. Animals fed mineral oil showed a definite increase in liver triglycerides compared to saline ($P < 0.005$) or glucose ($P < 0.005$) controls. The mechanism of this latter effect, observed on previous occasions in this laboratory, is obscure.

The question arises as to the manner whereby the injected ATP raises hepatic ATP levels. Since it is generally believed that ATP as such does not cross cell membranes, it is likely that the ATP is first hydrolysed, after which the nucleoside derivatives enter the cell and are then converted back to ATP. Whatever the mechanism whereby the cellular ATP levels are elevated by ATP administration, we cannot yet conclude that such cellular levels *per se* are directly related to the prevention or amelioration of the fatty infiltration by the agents used in the work recorded here.

We have indeed obtained evidence in carbon tetrachloride- and azaserine-treated animals that precursors of ATP, such as adenosine-5′-monophosphate and inosine, can raise hepatic ATP levels without protecting against the development of fatty liver. Furthermore, azaserine-treated rats given nicotinamide injections do not develop fatty livers and yet the hepatic ATP levels remain subnormal.

The effects of ATP administration on hepatic lipid metabolism could be mediated by a variety of mechanisms. In view of the importance of synthesis and secretion of lipoproteins in the transport of lipids out of the liver, interference with hepatic protein synthesis could readily lead to the development of a fatty liver[7]. Indeed, in the case of carbon tetrachloride[8] and puromycin[9], this is believed to be the major mechanism for production of the fatty liver by these agents. Farber *et al.*[1,2] likewise have suggested that the ethionine-induced fatty liver is due to impaired protein synthesis secondary to the depletion of ATP levels by the formation of *S*-adenosyl-ethionine. They postulate therefore that injections of ATP prevent the cellular depletion of ATP produced by ethionine and thus prevent the fatty liver. However, in the case of ethanol and azaserine there is no direct evidence implicating impairment of protein synthesis as the major mechanism for the fatty liver produced by these agents. While ethanol can be shown to inhibit hepatic secretion of lipoproteins, this occurs only at very high concentrations[10] and hence this is unlikely to be a phenomenon of major importance.

It is quite possible that ATP administration to animals may exert protective effects by indirect means. ATP injections may shock the animal and predispose to the

Table 1. Effects of ATP Injections on Hepatic ATP and Triglyceride Levels in Rats treated with Hepatotoxic Agents and in Control Rats

Exp.	Agents administered*	ATP injections	ATP (mean hepatic level) μmole/g liver ± S.D.	Difference from controls (per cent)	P	Triglyceride (mean hepatic level) mg/g liver ± S.D.	Difference from controls (per cent)	P
CCl_4	CCl_4	−	0·509 ± 0·150(9)†	−52·1	< 0·001	17·21 ± 3·89(10)	+122·4	< 0·001
	CCl_4	+	0·870 ± 0·063(6)	−18·2		6·82 ± 2·18 (7)	− 11·9	
	Mineral oil	−	1·063 ± 0·086(6)	—		7·74 ± 2·08 (5)	—	< 0·02
	Mineral oil	+	1·502 ± 0·405(5)	+41·3		4·06 ± 2·11 (5)	−47·3	
Ethanol	Ethanol	−	0·808 ± 0·110(8)	−36·8	< 0·01	17·61 ± 5·52(13)	+403·1	< 0·001
	Ethanol	+	1·099 ± 0·238(7)	−14		4·30 ± 1·41(11)	+22·9	
	Glucose	−	1·278 ± 0·160(5)	—		3·50 ± 1·20 (5)	—	
	Glucose	+	1·491 ± 0·333(5)	+16·7		2·17 ± 1·26 (6)	−38·0	
Azaserine	Azaserine	−	0·545 ± 0·101(7)	−37·2	< 0·001	16·75 ± 3·45 (8)	+483·6	< 0·001
	Azaserine	+	1·051 ± 0·470(7)	+21·1		6·82 ± 2·17 (6)	+137·6	
	Saline	−	0·868 ± 0·171(8)	—		2·87 ± 1·42 (7)	—	
	Saline	+	1·133 ± 0·177(7)	+30·5		2·10 ± 1·13 (5)	−26·8	
Ethionine	Ethionine	−	0·119 ± 0·038(5)	−86·1	< 0·001	21·06 ± 4·20 (5)	+592·8	< 0·001
	Ethionine	+	0·860 ± 0·250(4)	+0·6		3·32 ± 1·32 (4)	+9·2	
	Saline	−	0·855 ± 0·145(5)	—		3·04 ± 0·94 (4)	—	
	Saline	+	1·166 ± 0·195(5)	+36·4		2·04 ± 0·56 (5)	−32·9	

*Dosage and route of administration: (*A*) Toxic agents: CCl_4, 1 : 1 v/v in mineral oil, 1 ml. mixture per 100 g body-weight, by stomach tube; ethanol, 47%, 2 ml. (= 0·75 g) per 100 g body-weight, by stomach tube; azaserine, 10 mg/ml. water, 10 mg/100 g body-weight, intraperitoneally; ethionine, 25 mg/ml. water, 100 mg/100 g body-weight, intraperitoneally, in two equal doses 2 h apart; (*B*) Control agents: Mineral oil, 0·5 ml./100 g body-weight, by stomach tube; glucose, 65·5 per cent w/v, 2 ml./100 g body-weight (= isocaloric with dose of ethanol), by stomach tube; saline, 0·9 per cent, volume equivalent to that of azaserine or ethionine respectively, intraperitoneally; (*C*) ATP: Neutralized aqueous solution 100 mg/ml., 1-ml. doses subcutaneously 3 or 4 times during experiment.

† Numbers in brackets indicate number of animals in each group.

development of hypothermia[11]. The possibility that hypothermia might play an important part in protecting the liver against carbon tetrachloride was suggested by observations on chordotomized[12] and adrenalectomized[13] animals. We have found that animals treated with carbon tetrachloride or ethanol, together with ATP injections, become hypothermic, and this may be a factor in the protection afforded by ATP in these animals. However, hypothermia has not occurred in our experiments in which ethionine and ATP, or azaserine and ATP, were given. Work is now in progress to elucidate further the mechanisms for the protective action of ATP administration on the production of fatty livers in the experimental animal.

This work was supported in part by grants from the John A. Hartford Foundation Inc., and the U.S. National Institutes of Health (*AM*-01392). One of us (D. E. H.) held the Comyns Berkeley fellowship for 1963 during part of this work.

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Differential Analysis by Various Staining Techniques of Structures present in Developing Spermatids of the Silkworm

In the course of an electron microscope investigation[1] of spermiogenesis in the silkworm, *Bombyx mori* Linné, the formation of a tubular structure and two bands have been detected in the developing spermatids fixed with potassium permanganate or osmium tetroxide, which have not, to our knowledge, been reported previously from light microscopic investigations[2–7]. The two bands, which run parallel with the developing spermatid nuclei, were found to be of equal density when the material was fixed with osmium tetroxide, while one of them was less dense when the material was fixed with potassium permanganate.

Testes of the fifth larval instar of silkworm were immersed for 1–2 h in 1 per cent osmium tetroxide or potassium permanganate buffered to *p*H 7·4 with veronal–acetate buffer. When the specimens were fixed, they were dehydrated in a series of increasing concentrations of ethyl alcohol, and embedded in a mixture of methyl and *n*-butyl methacrylates or in 'Epon' epoxy resin. Sections were cut with the Porter–Blum's microtome equipped with a glass knife. They were examined in *JEM-T6* or *TRS-E*$_1$ electron microscopes.

Fig. 1 shows a cross-section of a developing spermatid, fixed with potassium permanganate, at the level of the nucleus, in which the fine granular nucleus (*N*) enveloped with the dense nuclear membrane is found to be almost completely surrounded by the elongated endoplasmic

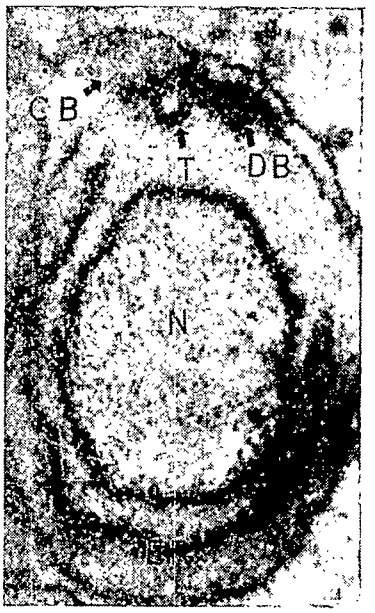


Fig. 1. ×45,000

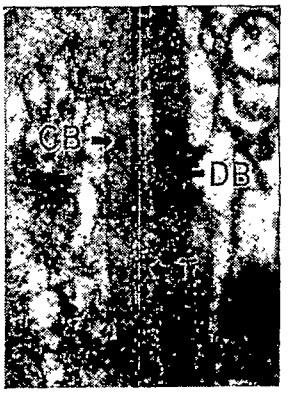


Fig. 2. ×23,000

reticulum (*ER*). A tubular structure (*T*) is attached to the plasma membrane, and a dense amorphous substance (*DB*) can be seen at the periphery of the cytoplasm. The less-dense amorphous substance (*CB*) appears to be attached to the plasma membrane, but is situated outside the cell. Fig. 2 shows a longitudinal section of the developing spermatid, fixed with potassium permanganate, through the tubular structure (*T*) to which belong the dense (*DB*) and the less-dense bands (*CB*). These bands correspond, respectively, to the dense and less-dense substances which can be seen in Fig. 1. Fig. 3 shows a cross-section of the developing spermatid nucleus fixed with osmium tetroxide at the level higher than that in Fig. 1. The nucleus (*N*) is filled with dense filamentous elements approximately 100 Å in diameter. The tubular structure (*T*) is clearly visible, but the clear band (*CB*) outside the cell cannot be distinguished from the dense band (*DB*). Fig. 4 shows an oblique longitudinal section of the developing spermatids fixed with osmium tetroxide, showing a small part of the nucleus (*N*). The clear band (*CB*) being outside the cell appears to be too dense to identify it from the dense band (*DB*). The tubular structure (*T*) can be seen here as a spindle-shaped structure. The clear and dense bands are always identifiable in cross-sections of developing spermatids but cannot always be seen in longitudinal sections. However, serial sections reveal that the bands run parallel with the developing spermatids from the base to the top of the conical head.

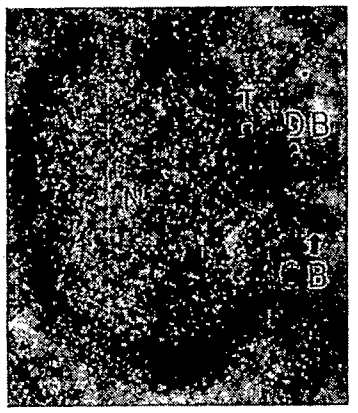


Fig. 3. ×40,000



Fig. 4. ×37,000

The present investigations emphasize the point that the ultra-structure of cellular components should be observed with various fixatives. In the present case, two structures which at first appeared to be similar due to their density being the same on osmium tetroxide-fixation appeared differently on potassium permanganate-fixation.

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Catalase Activity of a Crude Enzyme Preparation from Iron-chlorotic Barley (*Hordeum vulgaris*) Seedlings

We have reported that air ions of either charge accelerate the development of iron chlorosis in barley seedlings (*Hordeum vulgaris* var. California 'Mariout') cultivated in an iron-free nutrient solution[1–3]. During the course of experiments undertaken to explore the mechanisms involved in this phenomenon, we observed that the addition of iron in the form of iron–potassium–ethylene-tetraacetate (Fe-EDTA) to cell-free extracts prepared from barley seedlings which had developed chlorosis as the result of growth in an iron-free culture medium in a normal atmosphere produced a marked increase in the catalase activity of the extracts. The results presented here indicate that the pattern of increase in catalase activity is related to the extent of chlorosis.

Seedlings of barley were raised in an iron-free chemically-defined nutrient solution under greenhouse conditions. The details of the plant growth system are described in earlier papers[1–4], and we have used these same methods here. The essentials are: germinated seeds raised in a nutrient solution without an iron source for 7 days. By this time the seedlings attained a length of about 12 cm without developing visible signs of chlorosis. One third of the seedlings designated 'non-chlorotic seedlings' were collected at this time. By the 15th day severe chlorosis appeared in the remaining seedlings, and half of them—'severe chlorotic seedlings'—were gathered. At this point 5 p.p.m. of iron in the form of Fe-EDTA[5] was added to the culture medium. By the 30th day the seedlings had recovered completely from iron chlorosis. All of them were collected and were designated 'recovered seedlings'.

Because of their more abundant content of catalase, only the aerial parts of the collected seedlings were used in the present experiments. The extraction procedures used were modifications of those developed by Kotaka[6]. The aerial parts were washed thoroughly with distilled water; excess water on the surface of the seedlings was removed with tissue paper and the fresh weight was determined. The plant tissue was cut into small pieces with scissors and ground in a mortar with a small amount of quartz sand and a small volume of M/15 phosphate buffer at *p*H 6·8. The homogenate was squeezed through 8 layers of cheese-cloth and the pulp was ground again with a small amount of phosphate buffer. This process was repeated until the pulp was free from chlorophyll. The total volume of filtrate was adjusted to 10 times its fresh weight, allowed to stand at 4° overnight and then was centrifuged at 3,000*g* for 10 min. The supernatant obtained was used as the enzyme solution.

Enzyme protein was determined by the method of Sutherland *et al.*[7]. Catalase activity was determined as follows: to 9 ml. of substrate (0·01 N H_2O_2 in M/15 phosphate buffer, *p*H 6·8) 1 ml. of enzyme preparation was added. The reaction mixture was incubated at 25° for exactly 5 min. At the end of this time, 10 ml. of 2 N H_2SO_4 was added in order to stop the reaction. For a blank control, 1 ml. of enzyme preparation was added to a flask containing 9 ml. of 0·01 N H_2O_2 in M/15 phosphate buffer, *p*H 6·8, and 10 ml. of 2 N H_2SO_4. In both solutions the residual H_2O_2 was titrated with 0·05 N $KMnO_4$. The amount of H_2O_2 decomposed was calculated by the subtraction of the value at 5 min from that of the blank control. The catalase activity was expressed as mg of H_2O_2 decomposed/mg enzyme protein/5 min. The catalase assay was performed in duplicate.

Fe-EDTA prepared by the method of Jacobson[5] was so diluted with the phosphate buffer that it provided a final iron concentration of 50 p.p.m. when added to an equal volume of test solution. Twenty ml. of enzyme preparation, which was diluted with the phosphate buffer if necessary, was incubated in a water bath at 25° for 10 min, then 20 ml. of the diluted Fe-EDTA was added to the enzyme solution. This mixture was designated 'Fe-enzyme mixture'. Immediately after mixing, 1 ml. of the iron-enzyme mixture was pipetted into 9 ml. of the substrate solution and the titration of H_2O_2 was conducted as already outlined here. This value of H_2O_2 decomposed was designated 't_0 activity'. A blank reading was also taken at this time. Thereafter the catalase activity of the iron-enzyme mixture was determined at stated intervals. From each reading at a given incubation time, the t_0 reading was subtracted. The differences obtained were defined as the increment in catalase activity.

As a control an equal volume of M/15 phosphate buffer, *p*H 6·8, was added to 20 ml. of enzyme preparation after 10 min pre-incubation. In order to test H_2O_2 decomposition by Fe-EDTA, another control was provided consisting of 20 ml. of the diluted Fe-EDTA added to 20 ml. of M/15 phosphate buffer, *p*H 6·8. The catalase activity of this mixture was determined at intervals by the method already described here. No decomposition of H_2O_2 was detected throughout the period of incubation.

The activity of the enzyme preparation made from non-chlorotic barley was not increased by adding 50 p.p.m. iron (Fig. 1), although its initial catalase activity was the highest of those tested. The extract from severe chlorotic barley showed a marked increase in catalase activity after the addition of Fe-EDTA (Fig. 2). The peak of activity appeared at 120 min and was followed by a well-defined decrease. The initial catalase activity of this preparation was the lowest of those tested. In the case of recovered seedlings the increase in catalase activity reached its maximum after 120 min incubation and remained at this level for the entire 6-h period of observation (Fig. 3). Presumably the increased activity may last beyond that period. The catalase activity of this preparation was slightly lower than that made from non-chlorotic seedlings.

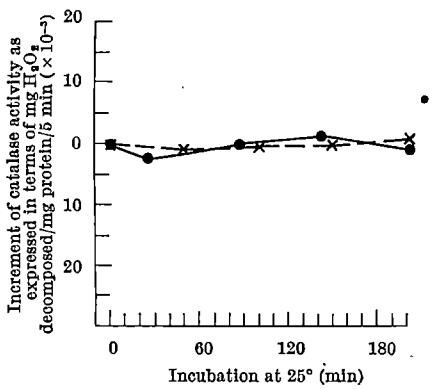


Fig. 1. Effect of Fe-EDTA on catalase activity of enzyme preparation from non-chlorotic barley seedlings
●, Enzyme preparation + Fe-EDTA in phosphate buffer; ×, enzyme preparation + phosphate buffer

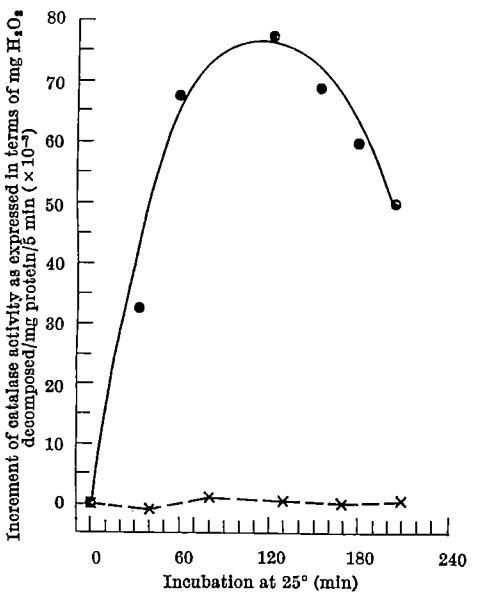


Fig. 2. Effect of Fe-EDTA on catalase activity of enzyme preparation from severe chlorotic barley seedlings
●, Enzyme preparation + Fe-EDTA in phosphate buffer; ×, enzyme preparation + phosphate buffer

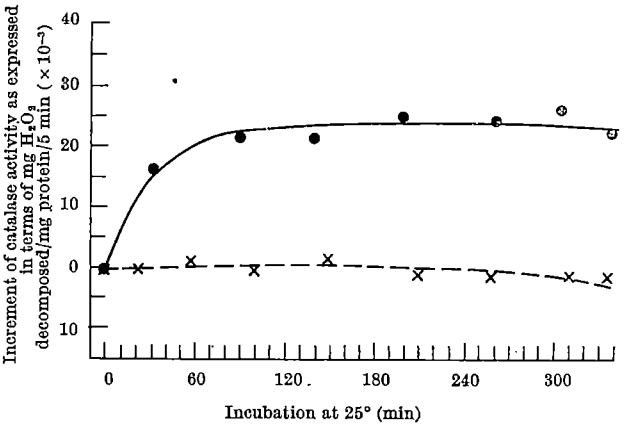


Fig. 3. Effect of Fe-EDTA on catalase activity of enzyme preparation from recovered barley seedlings
●, Enzyme preparation + Fe-EDTA in phosphate buffer; ×, enzyme preparation + phosphate buffer

It is curious that extracts prepared from chlorotic barley seedlings react with Fe-EDTA to produce a large increase in catalase activity while those made from normal seedlings fail to do so. This property of reactability seems to decline as the plants recover from chlorosis (Fig. 3). The simplest explanation of the phenomenon would be to assume that chlorotic plants develop an incomplete catalase which reacts with Fe-EDTA to form catalase. However, the results are equally explicable on the assumption that some sort of inhibitor is involved, for example, crude enzyme preparations from normal plants contain catalase + inhibitor while chlorotic plants in addition produce a substance which promotes a reaction between Fe-EDTA and the inhibitor, thereby releasing catalase. Various other possibilities exist but, until we have more experimental data, serious speculation about which one is correct appears unwarranted.

This work was supported by contract *Nonr* 3656(06), (*NR*102587) between the Office of Naval Research, Department of the Navy, and the Regents of the University of California; research grant *AP*00002–06 from the Division of Air Pollution, Bureau of State Service, U.S. Public Health Service; and a grant from the Committee on Research of the University of California.

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Treatment of Seeds with Ethylmethansulphonate and Diethylsulphate

Following Heslot's discovery of the strong mutagenic action of diethylsulphate (DS) and ethylmethansulphonate (EMS) in barley[1], techniques for the use of aqueous solutions of both compounds in mutagenic experiments have been investigated by some workers[2,3].

Table 1

	Ki, 20° C (l.mole^{-1} min^{-1})	*Ki*, 10° C (l.mole^{-1} min^{-1})	*Ki*, 5° C (l. mole^{-1} min^{-1})	*E* (kcal/ mole^{-1})
Diethylsulphate	$3{\cdot}600 \times 10^{-5}$	$1{\cdot}102 \times 10^{-3}$	$0{\cdot}625 \times 10^{-3}$	18·87
Ethylmethansulphonate	$0{\cdot}110 \times 10^{-3}$	$0{\cdot}035 \times 10^{-3}$	$0{\cdot}015 \times 10^{-3}$	21·32

tion depends only on the amount of ester absorbed and not on the treatment technique, a series of tests were carried out. The results are shown in Table 4.

It is concluded that it is possible to compare the effects of two different substances on the same material, as well as the sensitivity of different biological materials with respect to the same agent, only when the treatment is carried out in controlled conditions and the amount of the active substance absorbed is measured.

Table 2. Absorption of DS and of $C_2H_5OSO_3H$ into Seeds of *durum* Wheat, var. 'Cappelli', and of Fodder Pea, var. 'Parvus'

DS $\times 10^{-5}$ M	Hours of treatment	Amounts absorbed at 20° C (moles/seed $\times 10^{-8}$) Wheat DS	Wheat $C_2H_5OSO_3H$	Pea DS	Pea $C_2H_5OSO_3H$	Amounts absorbed at 25° C (moles/seed $\times 10^{-8}$) Wheat DS	Wheat $C_2H_5OSO_3H$	Pea DS	Pea $C_2H_5OSO_3H$
7·67	3	2·50	3·40	13·9	126·0	4·30	4·30	39·6	136·0
	5	3·65	5·70	55·2	108·0	5·82	6·55	42·5	166·0
	7	4·20	6·35	164·2	230·0	7·60	8·20	65·0	295·0
	15	5·00	9·10						
15·34	3	3·20	5·08	22·4	140·0	6·10	8·30	47·8	168·0
	5	6·38	7·40	91·5	298·0	10·20	10·40	78·0	318·0
	7	9·18	8·45	253·0	423·0	13·10	11·60	143·0	443·0
	15	11·00	10·20						
23·01	3	3·10	6·58	25·5	200·0	8·70	10·00	49·6	212·0
	5	8·50	8·70	156·5	562·0	12·25	11·20	137·0	600·0
	7	12·82	11·30	270·0	600·0	17·30	18·90	278·0	680·0
	15	14·80	13·20						
30·68	3					10·50	10·30	75·6	307·0
	5					13·60	12·26	276·0	694·0
	7					17·80	14·20	300·0	765·0

Table 3. Absorption of EMS and of Methansulphonic Acid into Seeds of *durum* Wheat, var. 'Cappelli'

EMS $\times 10^{-3}$ M	Treatment (h)	Amounts absorbed at 20° C (moles/seed $\times 10^{-8}$) Acid	EMS	Amounts absorbed at 25° C (moles/seed $\times 10^{-8}$) Acid	EMS
9·7	4	3·00	4·60	3·20	5·70
	7	6·25	6·35	7·10	9·00
	24	8·20	12·50	9·00	23·00
29	4	4·10	8·70	4·60	9·40
	7	9·32	10·00	4·40	16·20
	24	11·50	13·60	12·60	26·50
58	4	5·50	10·00	7·90	15·90
	7	10·08	13·40	11·50	22·50
	24	13·00	23·60	17·40	53·50
97	4	7·00	12·00	9·20	18·20
	7	11·60	19·60	13·00	26·50
	24	17·20	32·50	22·50	58·00
140	4	8·50	15·00	10·70	21·30
	7	13·80	20·80	16·60	28·50
	24	27·50	42·20	36·00	71·00

Table 4. Germination of *durum* Wheat, var 'Cappelli', treated under Different Experimental Conditions leading to the Absorption of the Same Amount of Ester (5×10^{-5} moles/seed)
(The absorption of the acid is below the toxicity threshold)

Temperature	Treatment Time	DS moles/litre	Germination (control = 100)
15° C	4 h	$23{\cdot}01 \times 10^{-3}$	$64{\cdot}3 \pm 2{\cdot}2$
20° C	12 h	$7{\cdot}67 \times 10^{-3}$	$67{\cdot}4 \pm 1{\cdot}1$
25° C	4 h	$7{\cdot}67 \times 10^{-3}$	$67{\cdot}1 \pm 2{\cdot}1$
30° C	2 h 50′	$7{\cdot}67 \times 10^{-3}$	$66{\cdot}6 \pm 1{\cdot}2$

It is well known that these alkylating substances, in solution, hydrolyse. We have measured the hydrolysis constants and the activation energy of EMS and DS, and the results are shown in Table 1: it can be seen that the stability of the solutions of the two substances is sharply different. This difference must necessarily affect the process of absorption into the seeds of the compounds and of their hydrolysis products.

Measurements of absorption into the seeds of the non-hydrolysed substances and of the hydrolysis products were carried out as described in a previous paper[4]. The results obtained for the absorption of DS on fodder pea, var. 'Parvus', and on *durum* wheat, var. 'Cappelli', and for EMS on *durum* wheat, var. 'Cappelli', are shown in Tables 2 and 3.

Since the acidic products of hydrolysis can have a negative effect on germination, it was necessary to ascertain the minimum amount of acid which could be absorbed by seeds without damage. This threshold, measured by the method described in our previous work[4] for *durum* wheat, var. 'Cappelli', amounts to 8×10^{-8}, and to 13×10^{-8} absorbed moles/seed for $C_2H_5OSO_3H$ and CH_3SO_3H, respectively. On fodder pea, var. 'Parvus', the threshold was measured only for $C_2H_5OSO_3H$ and was found to amount to 580×10^{-8} absorbed moles/seed.

In order to demonstrate that, when the acid is absorbed below the threshold concentration, the effect on germina-

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Auxin Activity of Certain Oximes

In auxin studies on compounds not possessing a carboxyl group Wain found, in this laboratory, that 2 : 3 : 6-trichlorobenzaldoxime exhibits activity as a plant growth regulating substance. At his suggestion other oximes were investigated by the writer and Mr. P. Cozens, and 3-indolylacetaldoxime and 2 : 4-dichlorophenoxy-acetaldoxime were also found to be active (Table 1). Further work was then undertaken to determine whether these aldoximes are active *per se* or if they undergo metabolism in plant tissues to yield active substances.

While this study was in progress, chromatographic evidence was reported for the conversion of 3-indolylacetaldoxime to 3-indolylacetonitrile and 3-indolylacetic acid (IAA) in fungi and banana leaf tissue[1], results which are of interest with respect to the 'Indole Cycle'[2]. The conversion of 2 : 6-dichlorobenzaldoxime to 2 : 6-dichlorobenzonitrile by soil micro-organisms has also been reported; such conversion in higher plants, however, was not established[3].

The metabolism of certain aldoximes in plant tissue has now been studied by exposing solutions of each compound to wheat coleoptile or pea stem segments followed by chromatographic analysis of the products, using chromogenic reagents and standard bioassay techniques[4].

The aldoximes were readily separated from their corresponding carboxylic acids by chromatography using Whatman No. 1 paper and *n*-butanol : ammonium hydroxide : water (100 : 3 : 18) as solvent. 3-Indolylacetaldoxime ($R_F = 0{\cdot}89$), after metabolism in wheat or pea tissue for 48 h, yielded an acidic product that gave marked auxin activity in the chromatogram segments which corresponded with the position of IAA ($R_F = 0{\cdot}28$).

Table 1. PLANT GROWTH-REGULATING ACTIVITY OF OXIMES AND THEIR CORRESPONDING ACIDS IN THE WHEAT CYLINDER, PEA SEGMENT AND PEA CURVATURE TESTS

Compound	Wheat cylinder test				Pea segment test				Pea curvature test			
	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
3-Indolylacetaldoxime	98	105	137*	152*	100	110†	125*	127*	0	1	2	3
3-Indolealdoxime	98	98	98	99	99	100	101	99	0	0	0	0
2 : 4-Dichlorophenoxyacetaldoxime	103	114‡	150*	137*	101	104	108†	121*	0	0	1	3
2 : 3 : 6-Trichlorobenzaldoxime	100	98	110‡	120*	100	101	101	104	0	0	0	0
3-Indolylacetic acid	117*	139*	157*	160*	106†	121*	127*	130*	1	2	3	5
3-Indolecarboxylic acid	101	100	99	98	101	101	102	103	0	0	0	0
2 : 4-Dichlorophenoxyacetic acid	106	118*	153*	159*	105†	114*	124*	125*	1	4	6	6
2 : 3 : 6-Trichlorobenzoic acid	99	103	121*	150*	100	109†	124*	128*	0	1	4	5

Control measurements: wheat test 100; pea segment test 100; pea curvature test 0. For details of these tests see ref. 4.
Results in wheat cylinder and pea segment tests significantly different from water control: * at 0·1 per cent level; ‡ at 1 per cent level; † at 5 per cent level.

Furthermore, the chromogenic reactions with Ehrlich, Salkowski and nitric/nitrous acid reagents were identical to those obtained with IAA.

Similar results were also obtained when 2 : 4-dichlorophenoxy-acetaldoxime ($R_F = 0{\cdot}96$) was treated with wheat and pea tissue, the highly active acidic product being identified as 2 : 4-dichlorophenoxyacetic acid by bioassay and chromogenic reagents ($R_F = 0{\cdot}52$).

The above results therefore establish the conversion of these aldoximes to the corresponding carboxylic acids in wheat and pea tissue metabolism. It is also most likely that the auxin activity shown by these oximes is dependent on this conversion within the cells of the test material. When the auxin activity of the respective oximes and acids is considered, the inactivity of 3-indole-aldoxime is not surprising since the expected product of metabolism, 3-indolecarboxylic acid, is inactive (Table 1).

The significant auxin activity of 2 : 3 : 6-trichlorobenzaldoxime in the wheat cylinder test contrasts with its inactivity in the pea tests. Evidence was obtained in metabolism experiments confirming that wheat tissue converts 2 : 3 : 6-trichlorobenzaldoxime to 2 : 3 : 6-trichlorobenzoic acid, whereas with pea tissue this was not the case. Further examination of this interesting difference in the behaviour of wheat and pea tissue with 2 : 3 : 6-trichlorobenzaldoxime is proceeding.

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Volatile Amines as Components of Toasted Oat Flakes

DURING the toasting process of oat flakes a number of components influencing their organoleptic properties are developed. Some of them escape with the steam; others are non-volatile. Among the non-volatile components the carbonyl compounds predominate[1]. It is, however, interesting that during the toasting process amines, a number of sulphur compounds and other so far unidentified compounds are also formed. For isolation, the volatile compounds obtained in processing were transferred to corresponding chlorides. On purifying the samples to be examined, the amines were separated by means of paper chromatography and identified by their standards, R_F values and by means of the necessary agents.

Table 1. AMINES AS COMPONENTS PRESENT IN TOASTED OAT FLAKES

Spot	Amine	R_F value	Relative amount of component	Colour
1	*NN'*-di-1,4-diaminobutane	0·03	+	red-violet
2	1,3-Diaminopropane	0·10	+	red-violet
3	1,4-Diaminobutane	0·12	+	red-violet
4	1,5-Diaminopentane	0·15	+	violet
5	Unidentified	0·18	+ + +	yellow
6	Ethanolamine	0·15	+	violet
7	Methylamine	0·30	+ +	violet
8	Unidentified	0·32	+	violet
9	Dimethylamine	0·33	+	brown-violet
10	Ethylamine	0·39	+ + +	violet
11	iso-Propylamine	0·45	+ +	grey-violet
12	Propylamine	0·50	+	violet
13	iso-Butylamine	0·58	+	violet
14	Butylamine	0·61	+ +	violet
15	Amylamine	0·71	+ + +	violet

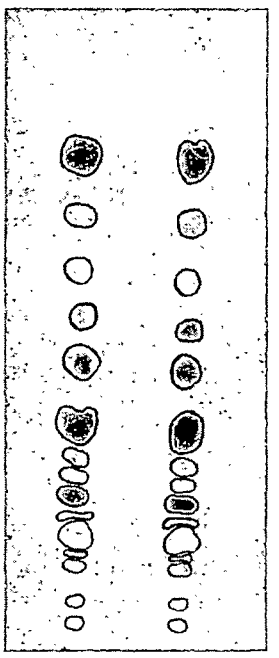


Fig. 1. Chromatogram of volatile amines present in toasted oat flakes. Solvent system, *n*-butanol–glacial acetic acid–water (4 : 1 : 5); developing time 24 h; detection by ninhydrin

Analytical results showed that during the toasting process of flakes a variety of amines was formed. In accord with the supposed formation the aliphatic amines C_1–C_5 are present, constituting the major part of the total amount. The diamines are represented in relatively small amounts by putrescine, cadaverine and 1,3-diaminopropane. Of the polyamines, *NN'*-di-1,4-diaminobutane is present in traces. The small amount of ethanolamine apparently originates by the splitting of phospholipid, probably cephalin. Of the secondary amines, dimethylamine present in traces was identified.

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PHYSIOLOGY

Minimal Salt Load required to induce Secretion from the Nasal Salt Glands of Sea Gulls

Most investigators[1–3] examining the function of the avian nasal salt gland have used a salt load consisting of 1 g NaCl/kg body-wt. administered either intravenously or orally. This form of osmotic stimulation will promptly induce secretion of a hypertonic solution of NaCl, ranging in concentration from 400 to 1,200 m.equiv. NaCl/l., from the salt gland. Nechay, Larimer and Maren[4] were able to induce secretion from the salt glands of ringbilled (*Larus delawarensis*) and herring (*Larus argentatus*) gulls by constantly infusing at the rate of 0·6 ml./kg/min a salt load of 1·8–7·2 m.mol. This work is reported here since so far there has been no other information concerning the interrelationship of body-weight and the minimum amount of salt required to induce secretion from the salt gland.

A group of 17 gulls captured along the northern California coast were used. Five glaucous-winged (*Larus glaucescens*), six western (*Larus occidentalis*), three herring (*Larus argentatus*), and three California (*Larus californicus*) gulls were in the group. The birds were maintained in a flight cage and given canned dog food periodically supplemented with frozen white bait to eat and fresh water to drink. The wing vein was cannulated with a short piece (about 8 in.) of 'Teflon' tubing (A.W.G. No. 22). The gulls were gently restrained by securing the wings with masking tape, supporting the body on a V-shaped wooden trough, and clamping the head to prevent head-shaking. An initial blood sample was drawn into a heparinized syringe, and the 'Teflon' tubing was then connected to a burette containing a 10 per cent NaCl solution. The salt solution was infused at rates varying from 0·15 to 0·65 ml./min, stopped with the first drip of nasal fluid and the volume infused recorded. The drips of nasal fluids were counted and collected for analysis into a 50-ml. conical plastic test tube placed just under the tip of the bill. A second heparinized blood sample was taken after stopping the infusion, and both blood samples were centrifuged and the plasma taken for analysis. All fluid voided from the cloaca during the experimental period was collected for analysis. The concentration of sodium and potassium in the plasma, nasal fluid and cloacal fluid was determined with a Baird-Atomic internal standard flame photometer and that of chlorine using a Buchler–Cotlove chloridometer.

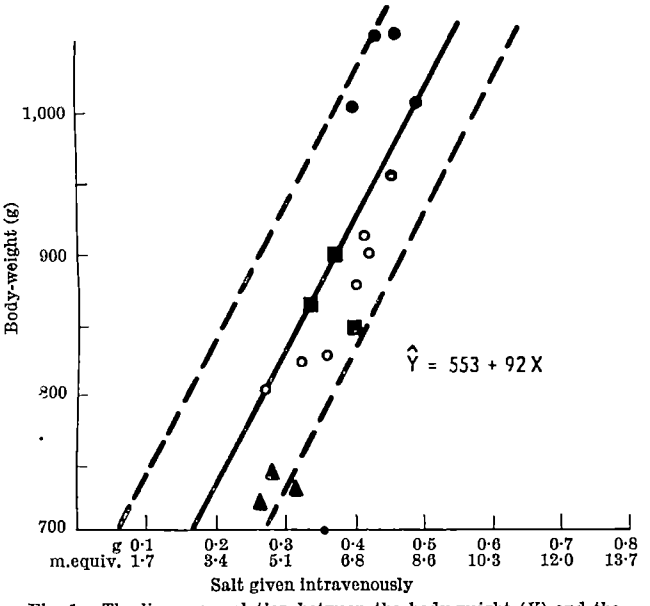


Fig. 1. The linear correlation between the body-weight (Y) and the minimum amount of salt (X) necessary to stimulate salt gland secretion in gulls. The scatter of the data is shown by the dotted lines. Solid circles, glaucous-winged gulls; open circles, western gulls; solid squares, herring gulls; solid triangles, California gulls

A striking linear correlation was found between the body-weight of the gulls studied and the minimum volume of 10 per cent NaCl solution required to initiate salt gland activity (Fig. 1). There was a tendency for the larger, more marine glaucous-winged gulls to require slightly less salt to induce secretion while the smaller, more freshwater California gulls required slightly more than average. The insignificant differences, however, between the responses of the various species of gulls justified considering them as a single group.

The data indicate, for example, that a 1-kg gull would require a minimum of 0·49 g of NaCl (with a scatter of 0·36–0·58 g) to stimulate secretion from the salt gland, or approximately half the 1-g level used by the other workers cited[1–3].

The secretory pattern of the salt glands activated by a minimum salt load showed some variation between gulls, the first drip occurring 5–24 min after starting the infusion and seemingly directly dependent on the rate of infusion. The maximum number of drips/min varied from 1·2 to 5·8, and the duration of secretion lasted 60–120 min. A typical secretory pattern is shown in Fig. 2. Notice that the maximum drip rate is reached rapidly, in all cases within 2–10 min after secretion commenced. The drip rate then falls off rather acutely during the next 10–20 min, levelling off and eventually stopping.

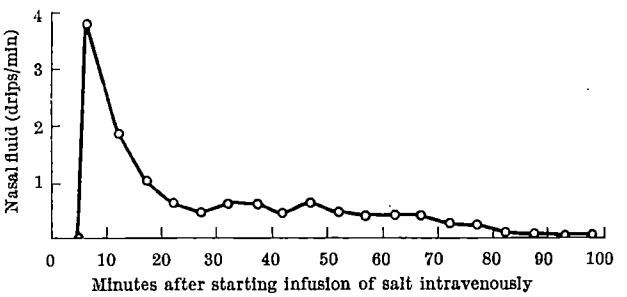


Fig. 2. Typical secretory pattern observed from a western gull (*SG* 243) receiving a minimal salt load

Following the minimal salt load the mean concentration of plasma sodium was elevated 21 m.equiv./l., the chlorine concentration was elevated 30 m.equiv./l., whereas the concentration of potassium was decreased 1 m.equiv./l. (Table 1). The concentrations of sodium, chlorine and potassium in the nasal fluid was approximately 4–5 times that noted in the plasma (Table 1) and are similar to values noted in gulls by other workers[1,3–6]. The gulls discharged the cloaca infrequently during the experiment and usually only once during the period of infusion. The electrolyte concentrations found in the cloacal discharge (Table 1) do not indicate that the kidney is concentrating at a maximum capacity, since the gull's kidney is able to eliminate about 300 m.equiv. NaCl/l. (ref. 5).

Table 1. Electrolyte Concentrations in Plasma, Nasal Fluid and Cloacal Discharge following a Minimal Salt Load in Sea Gulls which stimulates the Activity of the Salt Gland

Electrolyte	Plasma during salt gland inactivity	Plasma during salt gland activity	Nasal fluid	Cloacal discharge
Na	169 ± 4	190 ± 3	783 ± 63	184 ± 14
Cl	124 ± 1	154 ± 5	690 ± 36	191 ± 8
K	5·5 ± 0·8	4·5 ± 0·6	32·8 ± 2·6	18·2 ± 4·8

Values are expressed as the mean m.equiv./l. ± *S.E.*

It was not surprising to find a linear correlation between the body-weight of a gull and the minimum amount of salt required to induce salt gland activity, since food and water intake and metabolism are dependent on the size of an animal, and the primary function of the salt gland

is to eliminate the excess salt accumulated in these processes. How the salt load stimulates salt gland activity is uncertain. It is thought that osmoreceptors located probably in the central nervous system respond to the increased salt concentration[5], and this in turn causes the parasympathetic fibres innervating the salt gland to release acetylcholine, which is primarily responsible for stimulating salt gland secretion[1].

In the work recorded here an average increase in osmotic tension in the plasma of 21 m.equiv. Na/l., the difference between plasma sodium before and after salt loading (Table 1), was required to stimulate the central nervous system and salt gland activity. In the experiment of Nechay, Larimer and Maren[4] an increased osmotic load of 27 m.equiv. Na/l. was necessary to stimulate the glands. Wolf[7], investigating the stimulation of thirst in man and dogs by infusing hypertonic NaCl, concluded that a cellular shrinkage of 1–2 per cent resulting from the elevated osmolarity was required to initiate drinking. Later, Fitzsimons[8] infused rats not only with hypertonic NaCl but also sucrose, Na_2SO_4 and urea, and found that an increased osmotic tension of about 2 per cent was needed to initiate drinking. Therefore, the initial stimulus for the osmoreceptors of the central nervous system regulating the secretion of the salt gland is probably brought about by cellular shrinkage caused by the increased osmolarity associated with the elevated intravascular NaCl concentration which approximated 10 per cent in this investigation. Furthermore, the minimal stimulus required to induce the elimination of salt from the sea-gull is approximately 5 times that required to induce drinking in mammals.

A dramatic change in blood volume is also associated with the intravascular injection of salt. Assuming the blood volume of these gulls approximates 8·3 per cent of their body-weight and their hæmatocrit is 49 per cent[9], a 1-kg gull would have 83 ml. of blood and 40 ml. of plasma. The addition of 8·4 m.equiv. NaCl (0·5 g NaCl) to the blood would increase the plasma concentration of sodium by 210 m.equiv./l. since an average of only 21 m.equiv. Na/l. (or 10 per cent) was noticed in the plasma after salt loading; 90 per cent of the sodium was either lost by diffusion or the blood volume increased in order to dilute the added salt. A change in blood volume by as much as 80 per cent has been observed within 15 min after injecting a salt load of 0·5 g into gulls[9].

This work was supported by research grant *GM*–08538 from the U.S. National Institutes of Health.

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Rhythmic Urinary Excretion of Amphetamine in Man

The urinary excretion of amphetamine in man has been previously examined, both with regard to the elimination kinetics[1,2] and the total amount of unchanged drug excreted[1–9]. However, the majority of assay procedures adopted were non-specific, and no detailed investigation on the excretion kinetics of amphetamine, using a specific method of assay, appears to have been published.

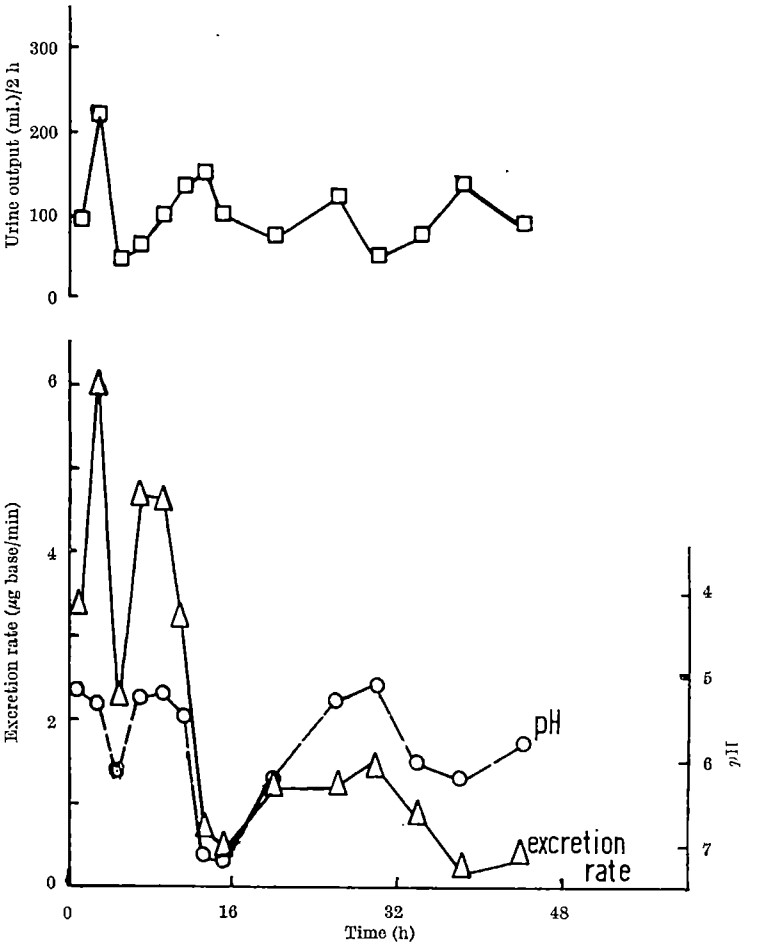


Fig. 1. Effect of urine *p*H and urine output on the urinary excretion of amphetamine in man, after oral administration of 10 mg (+)-amphetamine sulphate

This communication gives results obtained when 5–15 mg amphetamine sulphate were administered orally to male subjects. The elimination of the unchanged drug in the urine was determined by a specific assay procedure, based on gas-liquid chromatography[10]. Large fluctuations in the excretion rate of (+)- or (−)-amphetamine were observed, for example, Fig. 1, during drug excretion in all subjects examined. Different diets did not affect the pattern substantially. The total amount of unchanged amphetamine excreted in 48 h was 15–40 per cent or 30–66 per cent on giving (+)- or (−)-amphetamine respectively.

The variation in the excretion rate pattern of amphetamine was found not to be influenced significantly by changes in urine volume output, but rather appeared to be related to changes in urine *p*H (Fig. 1). A low urine *p*H resulted in a high excretion rate, whereas alkaline urine gave a low excretion rate. These results would appear to agree with the theory of non-ionic diffusion of bases in the distal portion of the kidney tubules[11,12]. The higher the urine *p*H, the higher will be the ratio of un-ionized amphetamine in the luminar fluid of the kidney tubules

relative to that in the plasma. This, in turn, will result in more drug being reabsorbed into the body and hence reduce the excretion rate of amphetamine.

Further trials in which the urine was kept acidic (pH 5·0 ± 0·2) by administering ammonium chloride showed that about 60 per cent of the drug was excreted unchanged over a 48-h period using a dose of 10 mg (+)-amphetamine sulphate. When the urine was kept alkaline (pH 8·0 ± 0·2) by the administration of sodium bicarbonate, only 5 per cent of the drug was eliminated over the same period. The fluctuations in the excretion rate of amphetamine were abolished by the control of urine pH at these relatively constant acidic and alkaline values.

It is known that diurnal changes in urinary pH occur[13–17] even under controlled conditions[18]; the rhythmic pattern of the amphetamine excretion rate under normal conditions is thus probably caused by this urinary pH rhythm.

One of us (M. R.) thanks the Medical Research Council for a grant.

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PHARMACOLOGY

Effect of Altered Thyroid Status on Desmethylimipramine Mortality in Mice

In previous papers we have directed attention to the enhancement of imipramine mortality in mice by hyperthyroidism and its diminution by propylthiouracil pretreatment[1,2]. One of the major metabolites of imipramine is its demethylated product, desmethylimipramine (DMI)[3], and this compound is said to be responsible for the antidepressant effect of the drug[4,5]. DMI is more potent than imipramine in antagonizing reserpine sedation in rats[4] and in potentiating the reaction of cat nictitating membrane to norepinephrine[6]. For these reasons it seemed of interest to ascertain the effect of thyroid status on DMI mortality according to the previous technique.

Young (25–30 g) male Swiss–Webster white mice were given 0·2 per cent propylthiouracil in their drinking water for 30 days. At the end of this time they had gained less weight than controls, their coats were dingy, and they appeared somewhat sluggish. Work with other mice has shown that this treatment produces decreased oxygen utilization per unit body weight, and prolongs survival in closed jars. These mice were termed propylthiouracil-treated (PTU) rather than hypothyroid, since PTU is known to produce extra-thyroidal changes[7]. Another group of similar mice were fed 2 per cent desiccated thyroid in their food for 96 h. These mice were termed hyperthyroid (HR), as this treatment produces behavioural and metabolic changes characteristic of the disorder[1]. Control mice were treated in an identical way except that neither their food nor water was medicated.

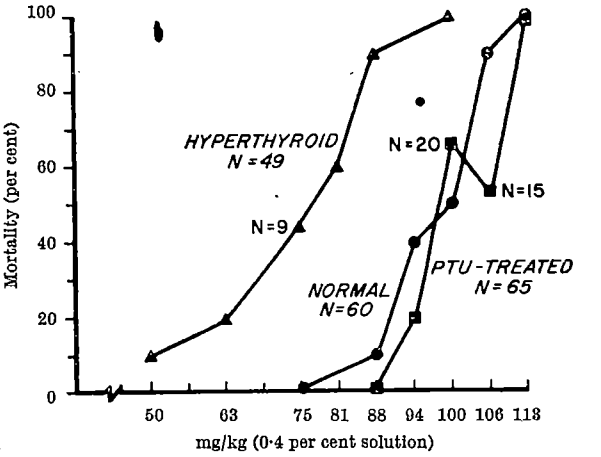


Fig. 1. Intraperitoneal desmethylimipramine acute (1 h) mortality in adult male Swiss-Webster white mice

On the same day in random sequence all mice were injected intraperitoneally with a 0·4 per cent saline solution of DMI. They were observed for 1 h, though the vast majority were dead or appeared to have recovered after 20 min. The mortality results are shown in Fig. 1.

DMI, like imipramine, shows enhanced mortality in HR mice though the magnitude of the effect is somewhat less. Increased rate of absorption from increased peritoneal vascularity in hyperthyroidism may partly account for this effect, but direct evidence is lacking. In any case, it is unlikely to be a sufficient explanation, since we have found that HR mice are more sensitive than controls to the mortal effects of pentobarbital, and also to sleep *longer* after a sub-lethal dose. Both imipramine and thyroid hormones interfere with the storage of administered norepinephrine, and their effects are additive[8], so that this, too, may account for the increased mortality in HR mice.

DMI mortality is not reduced by PTU. DMI is more potent than imipramine (LD_{50} = 100 mg/kg versus 115). It produces a steeper dose-mortality curve in all thyroid groups. It acts more quickly and its gross effects are dissipated more quickly. Mice given DMI appear stimulated and the occasional mouse may show opisthotonos, and even convulse, but the degree of stimulation is only a fraction of that seen after comparable doses of imipramine. These comments apply equally to all three experimental groups.

We were intrigued by the finding that PTU pretreatment spared imipramine-injected mice[2] but not DMI-injected mice. This finding, together with our other observations regarding rate of action and degree of stimulation, suggests that, while imipramine has stimulating properties of its own, DMI is the active metabolite, that demethylation occurs *in vivo*, and that PTU pretreatment interferes with the rate of demethylation, thus delaying the formation of the more potent DMI when mice are injected with imipramine.

To test the latter hypothesis, a group of mice were pretreated with PTU for 30 days and injected with an LD_{50} of either DMI or of imipramine. As expected, the pretreatment had no effect on DMI mortality rate. There was a tendency toward PTU sparing of imipramine-injected mice but this was statistically insignificant. Therefore, another group of mice were PTU treated for 60 days. Again the pretreatment had no effect on DMI mortality. The tendency toward sparing of imipramine-injected mice was slightly greater than at 30 days but still not statistically significant. Fourteen of 24 control mice died; 11 of 25 PTU mice. As in previous experiments the PTU sur-

vivors seemed to recover and to resume their usual habits more promptly than control survivors.

We have been unable to replicate to our satisfaction a PTU sparing effect on imipramine-injected mice. There is no evidence for such an effect in the case of DMI. More refined studies are in progress to determine whether PTU interferes with *in vivo* demethylation of imipramine. Both DMI and imipramine show enhanced lethal effects in HR mice. DMI is more potent, acts more quickly, and yields steeper dose-mortality curves than its methylated parent compound.

This work was supported in part by 325–*NIH*–290 (A. J. P.), and 325–*NIH*–304 (M. A. L.). Geigy, Inc., kindly furnished imipramine and DMI; and Lederle, Inc., supplied PTU.

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Salicylate, a Powerful Inhibitor of Free Fatty Acid Release

THE level of utilizable glucose represents a control of the release of free fatty acids (FFA) and, therefore, of the level of serum FFA[1]. Drugs acting on glucose utilization are therefore likely to be effective in changing the level of FFA. The observation that salicylate increases oxidative reactions[2,3] led us to the finding that this drug is a powerful inhibitor of FFA release. Previous investigations carried out in man were contradictory. While Carlson[4,5] found a decrease of plasma FFA in normal and diabetic subjects, Gilgore *et al.*[6] did not confirm these results. However, it is likely that in the latter data there was an interference of salicylate in the determinations of FFA carried out according to Dole's method.

Male Sprague–Dawley rats of the average weight of 150 g were used for all experiments. Sodium salicylate was given intraperitoneally in resting animals as well as in animals submitted to treatments designed to increase the level of FFA.

FFA were determined according to the method of Trout[8] to avoid interference of salicylic acid with the titration. The inhibitory effect of salicylate is very clear in suckling, adult or adrenalectomized animals and in rats with an increased level of FFA induced by fasting, cold, noradrenaline, ACTH or chlorpromazine. The blockade of thyroid activity did not affect the inhibition exerted by salicylate.

Table 1

Treatment or experimental condition	FFA μequiv./1,000 ml. plasma	
	Controls	Salicylate 300 mg/kg i.p.
Saline	215 ± 21	137 ± 6
Fasting 18 h*	671 ± 64	341 ± 11
Cold, 2° C 4 h*	765 ± 60	111 ± 3
Norepinephrine s.c. 1 mg/kg †	723 ± 54	252 ± 36
ACTH 20 units* ¶	957 ± 37	201 ± 22
Chlorpromazine, i.v. 1,5 mg/kg‡	505 ± 22	138 ± 29
Adrenalectomy*	380 ± 60	143 ± 22
Suckling rats*	540 ± 77	308 ± 40
Suckling rats on propylthiouracil diet§	648 ± 36	326 ± 20

* Salicylate given 30 min before rat killed.
† Norepinephrine given in oil suspension; both drugs administered at the same time. Time between treatment and killing 60 min.
‡ Salicylate given 10 min before chlorpromazine injection. Rats killed 30 min after chlorpromazine injection.
§ Propylthiouracil 0·4 per cent in the diet given to the mothers (ref. 7) during 17 days before salicylate administration.
¶ ACTH was given 120 min before rat killed.

By using the test of the increased level of FFA after cold it was observed that quite small amounts (25 mg/kg) of salicylate exerted an effect, and the effect of a large dose (300 mg/kg) lasted about 2 h. Orally administered salicylate was also effective in a dose of 50 or 100 mg/kg. Acetylsalicylic acid and benzoic acid shared the effect of salicylate, while *p*-aminosalicylic acid and salicylamide were ineffective up to a dose of 300 mg/kg.

Preliminary experiments show that salicylate may act directly on the adipose tissue because it slightly inhibits the release of FFA by the epididymal fat pad *in vitro*. Large doses are necessary, however, so it cannot yet be said that there is a causal relationship between the inhibition of FFA release *in vitro* and the decrease of plasma FFA *in vivo*.

The effect of salicylate on the FFA is obtained without significant changes of blood glucose, but this should not be considered to contradict the hypothesis previously mentioned because a small increase in glucose oxidation would not necessarily be reflected in the level of blood glucose.

These results are relevant to the interpretation of the reported hypoglycæmic and hypocholesteræmic effects of salicylate[3].

This work was supported in part by the U.S. Army European Research Office, contract *DA*–91–591 *EUC*-2685.

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Effect of Drugs on the Amount of Substance P in Rabbit Brain

THE content of 'Substance P' (SP) in the central nervous system under the influence of drugs has been studied repeatedly. Paasonen and Vogt[1] did not find an influence of D,L-amphetamine, ephedrine, insulin, caffeine, β-tetrahydronaphthylamine, and reserpine on the SP content of the hypothalamus and nucleus caudatus of anæsthetized dogs. Zetler and Ohnesorge[2] estimated the SP content of mouse brain after treatment with various centrally-acting substances, and found that substances which are centrally exciting in mice (benzedrine and morphine) increased the total content of SP in the brain, whereas chloroform, urethane and phenobarbital lowered it. Stern and Kocić-Mitrović[3] found an increase in SP content in rabbit brain after treatment with reserpine, but Laszlo[4] did not find any striking change of SP content in rabbit brain after reserpine and numerous other drugs.

The present experiments were made in order to obtain further data, using the purification technique of Pernow[5] for SP. Whole rabbit brain except cerebellum was used. The brain was ground, and boiled in acidified distilled water. After precipitation with ammonium sulphate SP was adsorbed on aluminium oxide and eluted with distilled water as described in detail by Pernow[5], Euler[6] and Radmanović[7].

Bioassay was performed on the isolated guinea-pig ileum with addition of atropine, promethazine and

d-lysergic acid diethylamide (LSD). All drugs were given to rabbits subcutaneously once a day for 3 days. Drugs used were : physostigmine salicylate (4 mg/kg), reserpine 'Serpasil-Ciba' (0·5 mg/kg), chlorpromazine chloride 'Hibernal-Leo' (5 mg/kg) and *p*-hydroxy mercuribenzoate (10 mg/kg).

The results are shown in Table 1. Only physostigmine salicylate had an effect on SP content in rabbit's brain. After 3 days administration of this drug concentration of SP in rabbit's brain decreased from 19 ± 1·5 u./g to 7 ± 0·73 u./g. The other drugs administered to the rabbits did not influence change of SP content in the brain.

Table 1. Amount of SP u./g in the Rabbit's Brain after Administration of Drugs

Drug	No. experiments	SP (u./g)	Significance
Control	5	19 ± 1·5	
Physostigmine salicylate	5	7 ± 0·73	$P < 0·01$
Reserpine	5	16 ± 1·3	Not sign.
Chlorpromazine	5	19 ± 2·1	,,
p-Hydroxy mercuribenzoate	5	16 ± 1·6	,,

These results are in accordance with those obtained from rabbit small intestine the SP content of which was also influenced only by physostigmine[7]. Similar results were obtained with reserpine and chlorpromazine by Paasonen and Vogt[1] and by Laszlo[4]. One possible explanation for the disagreement with results of other authors is the interference produced by nucleotides present in the SP extracts if they are not purified[4].

The physostigmine effect on SP content in rabbit brain, in conjunction with previous results[7], suggests a relation between central cholinergic mechanisms and SP.

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Postnatal Behavioural Effects of Meprobamate injected into the Gravid Rat

Werboff and Kesner[1] have claimed that administration of meprobamate to gravid rats for four successive days during any period of pregnancy resulted in offspring that, compared with the controls, were lighter in body-weight and significantly poorer at learning a Lashley III maze. The latter deficiency was attributed to some unspecified effect of the drug on the developing brain. Since previous experience with meprobamate had not revealed any deleterious effects on body-weight or size among the progeny of meprobamate-treated females[2], and since it seemed unlikely that a cerebral impairment due to prenatal drug exposure should be unrelated to the embryo's age at the time of insult[3], we repeated the Werboff and Kesner experiment. This replication also provided the opportunity for verifying an earlier report by Werboff and Havlena[4] that prenatal exposure to meprobamate altered performance on the inclined plane and in the open-field test.

The experiments were carried out exactly as described by Werboff *et al.*[1,4]. Pregnant female rats were injected three times a day at approximately 8-h intervals for four days. One group received distilled water subcutaneously, the other group received meprobamate, 20 mg/kg dissolved in distilled water. They were injected during gestation on days 5–9, 10–14 or 14–18. The offspring were weighed twice-weekly. At 25 days of age they were given three trials on an inclined wire screen, but no more than 120 sec were allowed per trial. The shortest time required to reach the top was selected for calculating the time of climbing. At about 55 days of age the rats were placed in the open field (a 5-ft. × 3-ft. grid of 12-in. squares) for 120 sec once a day for five consecutive days. The number of squares crossed and the activities (number of fæcal boli, etc.) were counted. At 75 days of age the rats were placed on a 23-h food deprivation schedule and began seven days of preliminary training on a 36-in. straight alley. On the eighth day of training, practice on the Lashley III maze was started with the following schedule: days 8, 9, 10—2 trials per day; days 11, 12, 13—3 trials per day; days 14, 15—5 trials per day. The criterion of learning was two out of three correct trials run in 20 sec or less per trial.

No measure revealed any significant difference between the offspring of meprobamate-treated and those of water-treated rats. Body-weights at 75 days of age, for example, averaged 288·3 ± 38·6 g for control males as compared with 290·4 ± 36·7 g for experimental males, while female offspring weighed 186·2 ± 22·4 g and 190·1 ± 36·0 g for control and experimental groups, respectively. Median inclined plane scores were 13·5 (range 2–120) sec for control and 16·5 (range 2–120) sec for animals of the experimental groups. The difference was not statistically significant at the 0·05 level. Furthermore, open-field scores were not significant for the number of squares traversed nor was emotionality significantly different between the two groups at the 0·05 level. There was no significant difference between the drug-tested animals and the controls in learning the Lashley III maze (Table 1). The learning scores of the offspring were also analysed according to the stages of pregnancy during which their mothers had been injected. No significant differences were found relating either to the substance injected or to the fœtal age at which injections were made.

Table 1. Trials to Criterion on Lashley III Maze of Progeny of Rats Administered Meprobamate during Various Stages of Pregnancy

Group	No. rats	Total stages	Stage First (3–9 days)	Second (8–12 days)	Third (13–18 days)
			(Mean number of trials ± *S.E.*)		
Control	81	14·2 ± 0·6	14·5 ± 1·2	12·9 ± 2·3	14·2 ± 0·7
Meprobamate	83	12·8 ± 0·6	15·0 ± 3·6	13·0 ± 0·8	12·1 ± 0·9

Our experiments have failed to verify the reports by Werboff *et al.*[1,4] of behavioural alterations subsequent to prenatal exposure to meprobamate. Furthermore, neither these rats nor those previously described by Berger have shown the weight loss which was noted in Werboff's rats and attributed by him to meprobamate. It appears likely that the behavioural data presented by Werboff are merely a reflexion of the poor physical status of his rats. Our results clearly show that the physical status of the offspring of meprobamate-treated rats should be in no way inferior to the offspring of the untreated animals. Treatment with meprobamate during *pregnancy* did not cause any impairment of performance on an inclined plane, behaviour in the open-field test, or in ability to learn a maze by the offspring.

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HÆMATOLOGY

Abnormal Transport of Vitamin B_{12} in Plasma in Chronic Myelogenous Leukæmia

RECENTLY[1] we demonstrated that two substances which we believe to be proteins carry the vitamin B_{12} in the plasma of normal subjects. These will be referred to as transcobalamin I and II. We then investigated their behaviour in the plasma of patients with chronic myelogenous leukæmia (CML) and found a major abnormality.

In the investigation of the control subject and of those with leukæmia, plasma which was taken at various times after intake of $^{57}CoB_{12}$ was fractionated by DEAE-cellulose anion-exchange column chromatography. Normally[1], almost all of the $^{57}CoB_{12}$ as it is taken into the plasma from injection, or from the intestine, is bound to a protein which was eluted pre-albumin, transcobalamin II. The B_{12} is rapidly lost from this binding site and little remains after the first day. A small amount of the B_{12} recently taken into the body and all the endogenous B_{12} which has been present in plasma for some time is bound to an alpha-1 globulin, transcobalamin I, the first binding substance to be described[2,3]. The loss from this binding site is quite slow.

When a patient with chronic myelogenous leukæmia was given 0·8 μg of $^{57}CoB_{12}$ by mouth, almost all the radioactive B_{12} was taken up by transcobalamin I, a complete reversal of the normal pattern. The plasma peak was not reached until 24 h in contrast to a peak at 8 h in normal subjects. There was little loss from this binding site during the next 24 h, and more than 50 per cent remained at 13 days. Two other subjects with CML were given 1·0 μg intravenously. Here, too (Fig. 1), almost all the $^{57}CoB_{12}$ was bound to transcobalamin I. In a normal subject almost all would have been bound to transcobalamin II. The investigation was repeated 3½ months later when the patient was in a good remission induced by 'Busulfan'. As shown in Fig. 2, transcobalamin II participated to a much greater extent than previously, an apparent return towards the normal. Usually we have found a sharp peak of $^{57}CoB_{12}$ in the transcobalamin II region, and while the peak in this case was in the expected position, it was unusually broad. The *in vitro* binding of 300 μμg/ml. of plasma was examined in 4 patients with CML in relapse. An abnormal binding pattern was found which was identical to that found when the $^{57}CoB_{12}$ was added *in vivo*.

The abnormality of the binding of B_{12} in the plasma in CML could be due to an increase in transcobalamin I, to a decrease in transcobalamin II, to a change which alters the binding capacity of either, or to some chemical change in the plasma which alters the union of B_{12} and transcobalamins which carry it. Because they give up their B_{12} at very different rates, the two transcobalamins appear to have different functions. The abnormality of plasma transport of B_{12} in CML could therefore alter the distribution of B_{12} within the body.

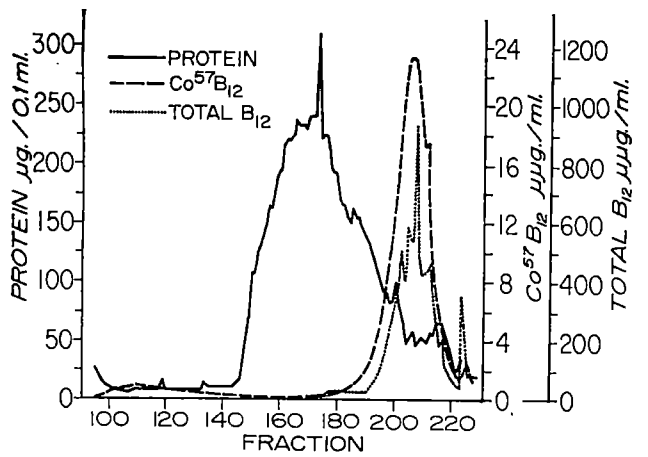


Fig. 1. Fractionation of plasma taken 1·5 min after i.v. $^{57}CoB_{12}$ from a subject with chronic myelogenous leukæmia in relapse. Total B_{12} includes both endogenous and recently injected B_{12}. Both are bound to transcobalamin I (peak in fractions 207–208) with only a trace of $^{57}CoB_{12}$ in transcobalamin II (peak in fraction 109). Fractions 0–95 contained γ and β globulin but no B_{12}

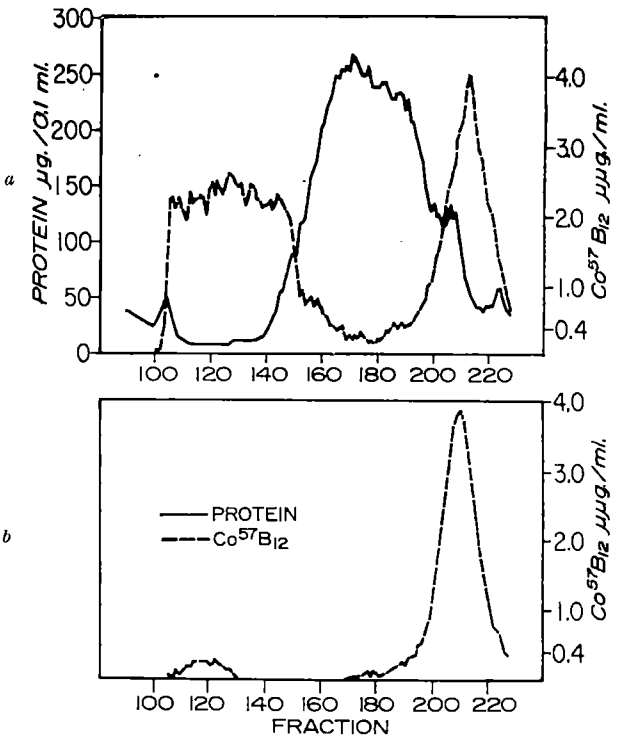


Fig. 2. A study of the same patient as Fig. 1 during remission. Note the rapid loss from transcobalamin II and the slow loss from I. (*a*) Plasma taken 1·5 min after injection, (*b*) plasma taken 90 min after injection

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In vivo Maturation of Immature Reticulocytes transfused into a Normal Rabbit

WE have recently published the results of an investigation of polyribosomes and the loss of synthesis of hæmoglobin in maturing rabbit reticulocytes fractionated by buoyant density centrifugation in an albumin gradient[1]. Several cytological and biochemical criteria were applied to show that this procedure fractionates the reticulocyte population according to their degree of physiological maturity. This communication presents additional proof, using *in vivo* maturation of a fraction of the youngest reticulocytes, that the position of the cells in the albumin gradient is a function of their age. In addition, these investigations provide an estimate of the life-span of the reticulocytes produced in phenylhydrazine-induced anæmia.

Reticulocyte fractionation by buoyant density centrifugation in bovine serum albumin (BSA) gradients and *in*

vitro incubations were performed as previously described[1]: 1·75 ml. of the most immature cells were isolated from 9·7 ml. of reticulocytes from a phenylhydrazine anæmic rabbit and incubated in the complete reaction mixture with 2 mc. of L-leucine-4,5-^{3}H, 3·37 mc./μmole (Nuclear Chicago), for 1 h at 37° C. These tritium-labelled cells were washed, suspended in 2 vol. of rabbit serum containing 5 mg/ml. sodium citrate and injected into the marginal ear vein of a normal rabbit. Blood samples of 10 ml. each were taken from the ear of the recipient after 1 h and at various intervals thereafter for 15 days (Fig. 1). After measuring blood and cell volumes, the cells were washed in NKM and suspended in 0·5 vol. of NKM. Five to six ml. of this cell suspension were fractionated on a 25-ml. BSA gradient, and collected in 1-ml. fractions. Cells from each fraction were washed with NKM and lysed with 0·8–1·6 ml. of lysing solution[1]. Stroma pellets were washed with 0·2–0·4 ml. of 0·1 M KCl, 0·1 M *tris*, *p*H 7·5, and the washings added to the stroma-free lysate.

Radioactive protein was determined by treating 0·1–0·5 ml. of lysate with 5 ml. of 1 per cent HCl in acetone to remove the hæm. The protein precipitate was centrifuged, washed with acetone, dried, and dissolved in 3 ml. water. One-tenth-ml. aliquots of these final protein solutions were added to 10 ml. of Bray's solution[2] for liquid scintillation counting.

The distribution of radioactivity in the blood samples analysed on BSA gradients is shown in Fig. 1. The time of sampling, given in hours after the initial injection of labelled cells, is indicated for each curve. In the first sample, taken 1 h after injection, the labelled cells reband at almost the same position as on the original gradient from which they were isolated. In the blood samples taken during the first two days following injection, the tritiated cells banded in successively lower (denser) positions in the gradient as they matured *in vivo*. During this time the position of the peak of radioactivity on the gradients moved linearly with time toward more dense fractions (Fig. 2). A similar but slower increase in density with age has been observed in normal rabbit erythrocytes by Leif and Vinograd[3]. The results presented here confirm our earlier report based on cytological and biochemical evidence that the position of the cells in the gradient is a function of their age.

After the first two days, the banding position of the tritiated cells rapidly levels off two-thirds of the way down the gradient at fraction 9. This final banding position corresponds in density to that of the lightest normal erythrocytes. We have previously shown that the least dense cell fractions consist of the most immature, macrocytic reticulocytes[1]. The present findings show that such cells mature to erythrocytes which are less dense than the majority of normal erythrocytes. This maturation, in terms of buoyant density changes, occurs in about 48 h. Since the injected reticulocytes band well above the normal erythrocytes during the first day, this method offers the possibility of studying the biochemical events of maturation *in vivo*. Such studies have been precluded in

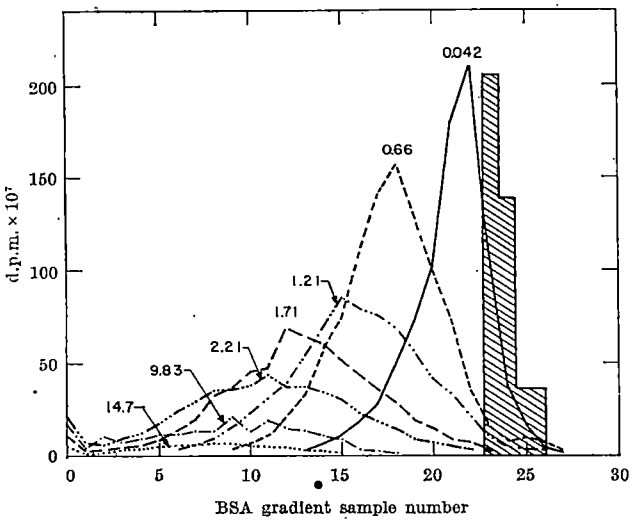


Fig. 1. Distribution of radioactivity in the blood cell samples as analysed on BSA gradients. Time, in days, after injection of tritium-labelled reticulocytes is indicated for each curve. Gradient samples are numbered from the densest (1·090 g cm^{-3}, 24° C) to the lightest (1·065 g cm^{-3}, 24° C). Cross-hatched area represents the position of the initial band of young reticulocytes as isolated from a phenylhydrazine anæmic rabbit

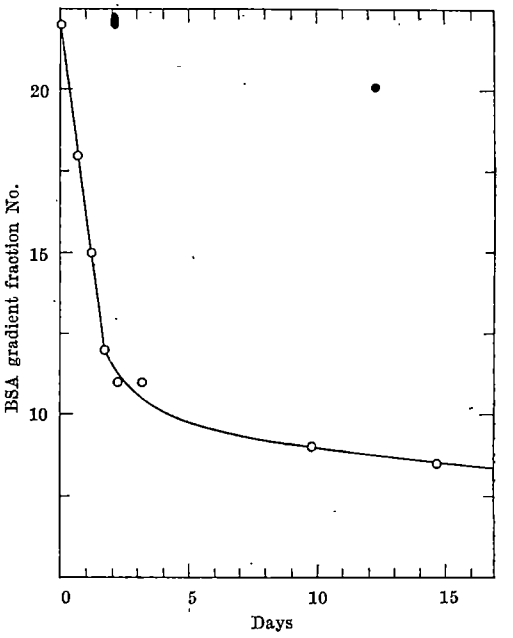


Fig. 2. Position of peak of radioactive cells as analysed on BSA gradients, as a function of time after transfusion

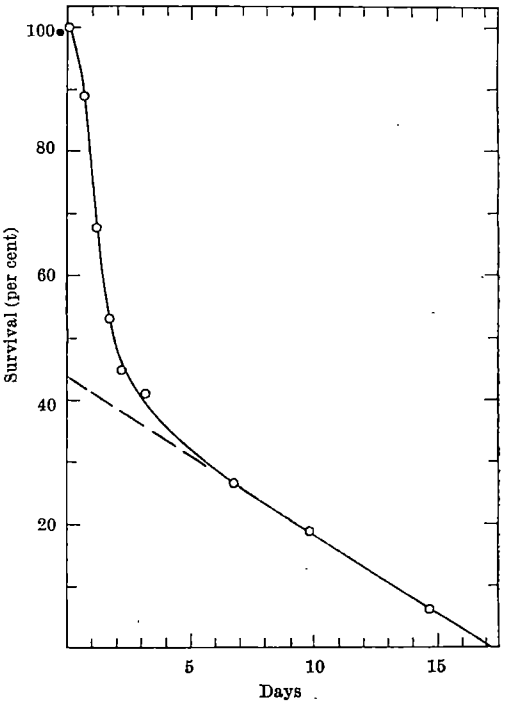


Fig. 3. Per cent survival of tritium-labelled reticulocytes as function of time after transfusion. Values represent per cent of initial radioactivity injected, corrected for cell samples removed

anæmic animals due to the constant influx of new reticulocytes from the bone marrow.

A graph of the per cent survival of the labelled cells versus time (Fig. 3) shows two distinct populations. One population, approximately 55 per cent of the injected cells, shows a life-span of about two days. The remaining 45 per cent of the cells disappear from the circulation after 17 days. A similar survival curve of sickle cells transfused into normal human beings has been reported by Singer and Fisher[4]. This is evidence for the extremely short life-time of the red blood cells which arise in response to a hæmolytic crisis. Previous reports, based on *in vivo* incorporation of ^{14}C-glycine[5] and incorporation of iron-59 (ref. 6), have indicated considerably long half-lives, of about 19 days, for such reticulocytes. However, the present findings are not only consistent with the results of *in vivo* ^{14}C-valine labelling of reticulocytes reported by Borsook *et al.*[7], but are in excellent agreement with the survival times observed by Mazur and Carleton for ^{14}C-glycine labelled reticulocytes transfused into normal recipients[8]. Such extremely short life-spans are not unexpected since survival times as short as four days have also been observed in several hæmolytic anæmias of human beings[9–11].

We thank Prof. Henry Borsook for his advice.

This work was supported in part by a U.S. Public Health Service predoctoral fellowship (for R. L. M.) (1–*F*1–*GM*19, 333–01) and by a U.S. Public Health Service training grant (for E. R. G.) (5–*T*1–*GM*–86).

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Chemical Reactivity of Avian and Mammalian Hæmoglobins of Different Ages

THE structural alteration of mammalian hæmoglobin on ageing *in vivo* is evidenced by the accelerated electrophoretic mobility of older hæmoglobin[1] and its changed chromatographic behaviour[2]. Hybridization investigations have shown the variant chain in old hæmoglobin to be the β-chain[3]. A number of suggestions have been made as to the possible chemical nature of the modification[4], the main one being that mixed disulphide formation with glutathione occurs[5,6] as a consequence of the diminished enzyme activities present in older red blood cells.

The development of methods for the separation of young and old mammalian red blood cells by centrifugation[7] or by counter-current distribution[8] or of their lysates by a serial osmotic hæmolysis procedure[9] has led to a probing of the enzymological and morphological differences of these cells and also to an examination of the relative reactivities of hæmoglobin as a function of its age. The latter investigations may possibly provide some information on the conformational differences between young and old hæmoglobin. Thus, Edwards *et al.*[10] reported that the dialysed macromolecular fractions derived from young and old erythrocytes have a different oxyhæmoglobin saturation; and Walter[11] found that young mammalian hæmoglobin binds more chromium-51 than its older counterpart. Since ^{51}Cr is known to be bound by the β-chain of mammalian hæmoglobin[12], the reported alterations in structure and chromium binding on hæmoglobin ageing occur on the same chain.

Although mammalian hæmoglobins show great structural similarities, as revealed by their peptide maps ('fingerprinting')[13], chicken hæmoglobin is completely different. Our interest, therefore, turned to an examination of the behaviour of young and old chicken hæmoglobins with respect to chromium binding.

We have recently reported that young and old chicken erythrocytes, like their mammalian counterparts, exhibit a correlation between their age and their resistance to hypotonic lysis[14]. It was further found that young and old chicken erythrocytes take up ^{51}Cr to the same extent. This finding differs from the preferential uptake of ^{51}Cr by young mammalian red blood cells[15]. In the latter case it was shown that the increased uptake of ^{51}Cr by young mammalian erythrocytes is due to the increased ^{51}Cr binding by young hæmoglobin[11]. This communication presents results showing that the equal labelling of young and old chicken erythrocytes with ^{51}Cr is due to the corresponding equal labelling of chicken hæmoglobin derived from these cells rather than to an alteration of red cell permeability to chromium as a function of age or to some other factor[15] unrelated to hæmoglobin structure or reactivity.

White Leghorn hens were injected intravenously via the wing vein with 50–75 μc. of ^{59}Fe in the form of ferrous citrate. The blood was collected one (chickens Nos. 20, 22, 23) or two (chicken No. 19) days later in acid–citrate–dextrose (ACD) solution. The red cells were washed five times at room temperature with isotonic saline, and the serial osmotic hæmolysis procedure of Simon and Topper[9] was used to obtain separately lysates enriched with respect to young or old cells. Lysates corresponding to younger cells have higher specific activities than those from older cells, enabling us to verify the age of cells giving rise to a particular lysate by measuring its specific activity (that is, the number of ^{59}Fe counts/min/absorbancy unit at 540 mμ). The hæmoglobin in the most concentrated lysate derived from the younger cells or of the older cells was purified by precipitating most of the non-hæmoglobin proteins by half saturation with neutralized ammonium sulphate. After removal of the precipitate, the hæmoglobin solution was made saturated with respect to ammonium sulphate, and the hæmoglobin was centrifuged off. The hæmoglobin was dissolved in water and dialysed. Solutions containing identical amounts of younger or older chicken hæmoglobin were diluted to equal volumes and the hæmoglobin labelled by standard methods with the same amount of ^{51}Cr (about 0·5 μc./absorbancy unit) and then treated with sodium ascorbate[16]. Hæmoglobin was precipitated by adding solid ammonium sulphate to the saturation point and allowed to stand in the cold overnight; it was centrifuged, dissolved in water and dialysed overnight. The specific activity of hæmoglobin with respect to ^{51}Cr was determined using a scintillation well-counter with a Baird-atomic pulse-height analyser and a spectrophotometer.

The results presented in Table 1 show that young and old chicken hæmoglobins take up ^{51}Cr at the same rate and to the same extent. This behaviour of chicken hæmoglobin thus differs from that of mammalian hæmoglobin where the younger hæmoglobin is found to take up appreciably more ^{51}Cr than the older hæmoglobin[11].

Although the internal environment of the chicken erythrocyte appears to change on ageing in a way similar to that observed in the mammalian red blood cell[14], this altered environment (that is, the diminution of enzyme activities on erythrocyte ageing) is obviously not all that is required for a structural alteration in hæmoglobin, detectable by a decrease in ^{51}Cr binding, to occur. The

Table 1. Specific Activity of Younger and Older Chicken Hæmoglobins labelled with ^{59}Fe *in vivo* and with ^{51}Cr *in vitro**

Specific activities given are counts/min of the isotope in question per absorbancy unit (at 540 mμ). See text for details

Chicken	Age of Hb	^{59}Fe Spec. act.	Ratio of ^{59}Fe spec. act. of younger/older Hb	^{51}Cr Spec. act.	Ratio of ^{51}Cr spec. act. of younger/older Hb
No. 20	Younger	55		584	
	Older	3	18·3	523	1·1
No. 22	Younger	23		768	
	Older	5	4·6	745	1·0
No. 23	Younger	3		584	
	Older	1	app. 3	560	1·0
No. 19	Younger	201		865	
	Older	99	2·0	858	1·0

*For data on a comparable experiment with young and old mammalian hæmoglobin see ref. 11, in which the average ratio of the specific activity of younger/older hæmoglobin with respect to ^{51}Cr was found to be 1·4.

detection of the possible occurrence of a change in avian hæmoglobin on ageing (which is not of the type that is reflected by a commensurate, lower ^{51}Cr uptake as in the mammalian system) awaits further investigation.

This work was supported in part by research grant *HE*–08304 from the National Heart Institute, U.S. Public Health Service.

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PATHOLOGY

Uptake of Protein by Regenerating Liver Cells

Using an immunofluorescent method, Cohen and his colleagues[1] reported that hepatomas induced in rats by *p*-dimethylaminoazobenzene could take up plasma proteins without prior extracellular hydrolysis. Later we showed[2] that intact plasma proteins could similarly be demonstrated within the cells of benzpyrene-induced primary fibrosarcomas in rats, and several other transplanted mouse tumours, both in solid and in ascites forms. However, the question whether this capacity of taking up intact protein molecules is associated with malignancy or is a property of all rapidly growing tissues remained unanswered. The present investigation was designed to study the uptake of intact protein molecules in a rapidly growing tissue *in vivo*, namely in regenerating rat liver cells after partial hepatectomy.

Lister hooded rats (180–220 g) of either sex were partially hepatectomized—approximately half to two-thirds of the liver parenchyma was removed. Protein uptake was investigated both by direct fluorescent protein tracing and by the fluorescent antibody method. For direct tracing, normal human serum globulin was conjugated with lissamine rhodamine *B* (*RB* 200) and was injected into the jugular vein of rats. The dose injected was approximately 60 mg protein/200 g of body-weight. For the immunofluorescence method a γ-globulin fraction was injected in the same dose and by the same route.

Pairs of rats were killed 1, 2, 3, 6, 12, and 24 h after hepatectomy and then once every 24 h until the 20th day. Protein uptake was studied in one of the two rats by the immunofluorescent method and by direct fluorescent protein tracing method in the other. After the 20th day only one rat was killed daily until the 35th day, and protein uptake was studied by immunofluorescent and direct fluorescent protein tracing methods on alternate days. Some parts of this serial study were repeated and protein uptake was investigated in about 150 hepatectomized rats. The rats received the appropriate proteins intravenously at least 1 h before death. Small blocks of liver tissue were snap-frozen immediately after killing and were then sectioned at 5μ in a cryostat. In the direct tracing experiments, the sections were fixed in 95 per cent ethanol for 30 sec, and were then rinsed in phosphate-buffered saline (*p*H 7·1) and mounted in buffered glycerol. In the immunofluorescence experiments, the unlabelled human γ-globulin was identified in ethanol-fixed sections, after treating for 30 min with goat anti-human-γ-globulin conjugated with *RB* 200. Liver from normal and hepatectomized rats not injected with proteins served as controls. The specificity of immunofluorescent staining was assessed by treating parallel sections with labelled anti-rabbit-globulin which, of course, failed to stain.

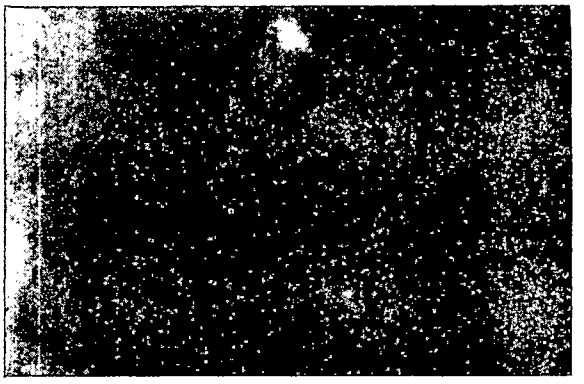


Fig. 1. Frozen section of a rat liver on the sixth day after hepatectomy. The liver was removed 2 h after intravenous injection of human serum globulin conjugated with *RB* 200. The fluorescent protein aggregates can be distinguished as droplets in the cytoplasm of the hepatic cells

Examination of preparations with the fluorescence microscope showed distinct fluorescent protein aggregates in hepatic cells by the end of the fifth day: very small amounts were perhaps present in the hepatic cells on the second, third and fourth days but the appearance was not conclusive. Between the fifth and tenth days after hepatectomy uptake was maximal; afterwards it gradually fell, and virtually no protein could be demonstrated within hepatic cells after the fourth week of hepatectomy.

The proteins were distributed as droplets within the cytoplasm of the occasional single hepatic cell or in small groups of cells (Fig. 1), and these positive cells were found near the line of section as well as far removed from it. In the days immediately after hepatectomy a few necrotic areas could be seen near the line of section, and these showed an intense green autofluorescence, although no *RB* 200 orange fluorescence could be demonstrated. Küpffer cells always took up protein in normal[2] as well as in regenerating livers, but they can be easily distinguished from the hepatic parenchymal cells.

Regenerating hepatic tissue *in vivo*, therefore, shares with malignant cells the capacity to take up intact protein molecules. It has to be remembered, however, that hepatic parenchymal cells, even after minor injury, are known to be permeable to serum proteins[3], and further studies must be made on other rapidly growing *in-vivo* systems before any generalization can be made. Moreover, we have yet to learn whether the uptake of intact protein molecules is attributable to increased permeability of the cell membranes of rapidly growing tissues or to a more active process, and also how such intracellular serum proteins contribute to the increased metabolic demands of rapidly growing systems.

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Existence in the Thymus of a Factor protecting the Skin of the Mouse against the Induction of Skin Cancers induced by 20-Methylcholanthrene

MILLER[1] has shown that thymectomy in the neonatal period of life favours the development of skin benzpyrene cancer in mice.

Vandeputte and De Somer[2] have shown that rats thymectomized during the first days after birth are still susceptible to the induction of cancers by polyoma virus during several weeks after birth.

We have shown[3] that subcutaneous isologous grafts of neonatal thymus repeated every 14 days protect the mice, to a certain extent, against the induction of cancer by 20-methylcholanthrene. Furthermore, we have shown that repeated injections of homogenized neonatal thymus also protect the mice against the same type of cancer, suggesting the possibility of the existence in the thymus of an extracellular factor or factors protecting the skin of the mouse against induced cancer. However, the existence of such factor or factors has not been demonstrated in this last experiment[4].

Therefore, we have undertaken another experiment in order to investigate the possible existence of such a factor or factors in neonatal thymus of the mouse. We have selected 3 groups of 60 mice of the same age and the same sex (50 per cent of each sex in each group) belonging to an isologous strain, the *L* strain of the Cancer Institute of Louvain. One group has been kept as control and painted 3 times a week with two drops of a 1/400 20-methylcholanthrene solution in ether, up to a total of 23 paintings. The two other groups have been painted similarly, but in addition have been injected every 14 days with thymus extracts, prepared as follows : every week, during the whole course of the painting period, 60 neonatal thymuses from young mice, aged 2–5 days, have been thoroughly homogenized in a Potter tube and then suspended in normal saline. This suspension has then been centrifuged for 30 min at a speed of 40,000 r.p.m. The supernatant, after this centrifugation, has been injected to the second group of our experimental animals. The sediment has then been re-suspended in normal saline and injected to the third group of our animals. Each animal of those two groups received thus every 14 days the corresponding amount of more or less one neonatal thymus, either in the form of the sediment or as supernatant. After the painting period the same injections were repeated every 14 days until the end of the experiment. There is no doubt that the supernatant represents an extract of thymus in normal saline devoid of cells. The results of the experiments are presented in Table 1.

Table 1

	No. of animals alive after 60 days	No. of tumours after (days) 60	90	120	150	180*	No. of animals alive at 180 days	No. of animals without tumour at 180 days†
Controls	63	2	7	20	24	32	23	7
Supernatant	58	4	4	6	10	15	33	21
Sediment	39	1	2	2	6	10	21	7

* $\chi^2_{1a} = 6.89$ $(1/2)P < 0.01 = P < 0.005$
$\chi^2_{1b} = 5.29$ $(1/2)P < 0.05 = P < 0.025$
† $\chi^2_{1a} = 4.72$ $(1/2)P < 0.05 = P < 0.025$
$\chi^2_{1b} = 0.015$ $(1/2)P < 0.70 = P < 0.35$

The differences between the results of the three groups are marked. Both groups injected with thymus show, during the whole course of the experiment, a marked delay in the development of cancer. At the end of the period of observation (180 days), there is still a marked difference in cancer incidence among the three groups. Moreover, the number of animals surviving at the end of the experiment without tumours, benign or malignant, is markedly higher in the group injected with the thymus supernatant. On the other hand, there is no marked difference in cancer incidence between the group injected with the supernatant and the group injected with the sediment after centrifugation. This, obviously, shows that only part of the active factor or factors, soluble in normal saline, has been extracted through this fractionation and that an important fraction of those factors remains in the sediment. We know nothing yet about the chemical nature of this factor or factors. We do not know either if the factor or factors are heat-resistant or not. Nor do we know if a heterologous extract of thymus would be active.

From the results of this experiment, it may be concluded that there exists, in the neonatal thymus of the mouse of strain *L*, a factor or a group of factors soluble in normal saline protecting the skin of the mouse of the same strain against the incidence of cancers induced by 20-methylcholanthrene.

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Blastomyces (Paracoccidioides) brasiliensis in Africa

SINCE the discovery of "South American Blastomycosis" by Adolfo Lutz in 1908, it has been generally accepted that the disease is confined to South America. The few cases reported outside this region are of patients who lived for years in South American countries and probably contracted the "Lutz disease" there[1–8].

A sample of spinal fluid taken from a four-year-old Ghanaian girl living in Accra was sent to my laboratory. The clinical diagnosis was miliary tuberculosis. A few hours later the child died. Specimens of heart and liver tissue were taken. The spinal fluid was clear and apparently normal. The fragments of heart and liver tissue were covered with miliary abscesses. After centrifugation the spinal fluid was examined by routine procedures in dry and wet mount preparations. Spheres with refractile walls ranging from 5 to 30μ diameter were seen. In indian ink preparations no capsules were found. The heart tissue was ground with saline and the liquid phase was examined in wet mount preparations. In addition to the

spheres described above, large spheres ranging from 10 to 30μ appeared covered with small buds. These multiple budding cells strongly suggest the tissue forms of *B. brasiliensis*. Histological sections from post-mortem material revealed single and multiple budding spheres stained deep red by periodic acid–Schiff.

The liquid phase extracted from heart tissue was inoculated in Sabouraud glucose agar and in nutrient broth-serum agar. In Sabouraud glucose agar, growth is extremely slow and no fully developed colony has yet been obtained.

The liquid phase extracted from heart tissue was mixed with antibiotics and inoculated intra-peritoneally in mice (strain 'N.I.H.', U.S.A.). Four weeks later the animals, all in apparently good health, were killed. No macroscopic lesions were visible. In histological sections there were no noticeable lesions.

However, in histological section stained by the periodic acid–Schiff stain, multiple-budding cells like those seen in the patient's tissues were seen.

The diagnosis of *B. brasiliensis* in this case is based on: (*a*) The presence of multiple budding cells in the liquid phase extracted from heart tissue. (*b*) The presence of multiple, budding, mulberry-like cells stained deep red by periodic acid–Schiff in the patient's tissues.

From the evidence it appears that I have found a case of 'South American Blastomycosis' which must have been contracted in Africa. This case is unique so far as I know and unfortunately has not been fully documented. I have practically no clinical observation and no information on the environmental conditions in which the disease developed. The case cannot be evaluated properly until others have been described.

I thank Prof. C. da Silva Lacaz for his interest and advice, Prof. J. Gilman for facilities, and Drs. G. Lythcott and J. Edgecomb for the collection and preparation of material.

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Role of Bradykinin in the Development of Acute Pancreatitis

The ability of bradykinin or kallidin to cause hypotension, pain, vasodilatation, increased vascular permeability, smooth muscle stimulation, leucocyte migration, and leucocyte accumulation suggests the possibility that bradykinin may play a part in a disease process, such as acute hæmorrhagic pancreatitis, characterized by hypotension, pain, hæmoconcentration, dynamic fluid shifts with the accumulation of ascitic fluid, leucocytosis, and œdema and leucocytic infiltration of the gland[1,2]. The attractiveness of this hypothesis is enhanced by the knowledge that the pancreas contains large quantities of trypsinogen and kallikreinogen, both of which, in active form, are capable of releasing bradykinin from its inactive precursor, bradykininogen. Bradykininogen, as a normal pseudoglobulin of plasma, lymph, and interstitial fluid, is readily available to the pancreas[1].

This tempting circumstantial evidence notwithstanding, attempts at demonstrating a role of bradykinin or kallidin in the pathogenesis of acute pancreatitis have been inconclusive[3]. No small part of the frustration has been the problem of obtaining a quantitative measurement of the active bradykinin of the body fluids[4]. However, a reliable method for the measurement of the bradykinin precursor, bradykininogen, has been described[5]. By determining the disappearance of precursor over time, one can estimate the bradykinin release. In work recorded here, because of the dynamic fluid shifts and colloid concentration that occur in severe acute pancreatitis, values for bradykininogen in weight per unit volume were corrected to weight per total plasma volume. The latter value was termed 'total circulating bradykininogen'.

Adult dogs of either sex were splenectomized two weeks before experiment. The animals were anæsthetized with sodium pentobarbital, 30 mg/kg. Pancreatitis was induced by a modification of the method of Elliott[6,7]. The animals were divided into three groups: Group 1, laparotomy only; Group 2, laparotomy followed by the induction of pancreatitis; Group 3, laparotomy followed by the intravenous injection of 'Trasylol', 25,000 units, followed by the induction of pancreatitis. 'Trasylol' was administered to the last group as a potent trypsin-kallikrein inhibitor capable of inhibiting the release of bradykinin-like polypeptides[8].

Mean femoral arterial pressure was monitored continuously. Blood samples were taken through a polyethylene cannula inserted into a femoral vein with the cannula tip advanced into the right atrium. Blood was collected in siliconed glassware and anticoagulated with balanced oxalate, 2 mg/ml. A control blood volume measurement was obtained and determinations were repeated once-hourly for 5 h. Hæmatocrits were determined at each blood volume measurement. At the end of each experiment (4 h after the induction of pancreatitis) the animal was killed, the volume of the peritoneal fluid was measured, and an estimation was made on the increase in size of the pancreas.

All animals of Groups 2 and 3 developed an acute hæmorrhagic pancreatitis. The mean arterial blood pressures of these animals dropped below 80 mm mercury within 3–14 min after the induction of pancreatitis. Three animals of Group 3 showed return of mean arterial pressure above 90 mm mercury. The pancreas became swollen, developed areas of interstitial hæmorrhage, and could be seen to exude a dark red fluid, containing fat droplets, within 15 min of the onset of pancreatitis.

Table 1

Parameter	Group 1 (3 animals)	Group 2 (5 animals)	Group 3 (6 animals)	*P* (<)
Blood volume loss (%)	3·77 ± 3·53	22·84 ± 2·00		0·01
	3·77 ± 3·53		16·18 ± 0·55	0·01
		22·84 ± 2·00	16·18 ± 0·55	0·02
Plasma volume loss (%)	5·17 ± 3·20	31·52 ± 3·98		0·01
	5·17 ± 3·20		26·00 ± 2·90	0·002
		31·52 ± 3·98	26·00 ± 2·90	> 0·10
Red cell mass loss (%)	3·90 ± 2·41	11·1 ± 2·60		> 0·05
	3·90 ± 2·41		4·12 ± 2·07	> 0·10
		11·1 ± 2·60	4·12 ± 2·07	> 0·05
Hæmatocrit	0·00 ± 0·56	7·20 ± 1·66		0·01
	0·00 ± 0·56		6·83 ± 1·54	0·01
		7·20 ± 1·66	6·83 ± 1·54	> 0·10

Group values include ± *S.E.M.*
'Per cent loss' and 'change' parameters denote differences between control measurements and the terminal measurements, four hours after the onset of pancreatitis.

Table 1 demonstrates the profound fluid shifts that occur in acute pancreatitis. Almost all the measured blood volume losses could be accounted for by the fluid found in the peritoneal cavity plus the gain in volume of the pancreas itself.

The total circulating bradykininogen loss, percentage change of the total circulating bradykininogen, and the calculated bradykinin release are seen in Table 2. No significant change in bradykininogen concentration (μg/ml. plasma) was evident during the period of observation.

A role of bradykinin in the pathogenesis of acute pancreatitis is probable in view of the following findings.

Table 2

Parameter	Group 1 (3 animals)	Group 2 (5 animals)	Group 3 (6 animals)	P (<)
Total circulating bradykininogen loss (mg)	0·097 ± 0·055	0·683 ± 0·130	0·385 ± 0·067	0·01
	0·097 ± 0·055		0·385 ± 0·067	0·02
		0·683 ± 0·130	0·385 ± 0·067	> 0·05
Total circulating bradykininogen loss (%)	8·30 ± 4·58	47·12 ± 6·65		0·01
	8·30 ± 4·58		24·55 ± 2·59	0·001
		47·12 ± 6·65	24·55 ± 2·59	0·02
Bradykinin release (μg/kg)	9·20 ± 4·74	73·14 ± 12·57		0·01
	9·20 ± 4·74		39·32 ± 7·36	0·02
		73·14 ± 12·57	39·32 ± 7·36	0·05

Group values include ± the *S.E.M.*

Among the animals of Group 2, the calculated bradykinin release was of sufficient magnitude to account for sustained hypotension. Bradykinin release was seen to correlate with severity of disease as manifest by hæmatocrit change ($r = 0{\cdot}719$, $P < 0{\cdot}01$) and peritoneal fluid accumulation ml./kg ($r = 0{\cdot}812$, $P < 0{\cdot}001$).

'Trasylol' given immediately before the onset of pancreatitis resulted in amelioration of the disease experienced by those animals composing Group 3. The amelioration was evident in terms of duration of hypotension, per cent blood volume loss, per cent total bradykininogen loss, and bradykinin release. The ability of 'Trasylol', an agent capable of inhibiting bradykinin release, to lessen the severity of pancreatitis buttresses the hypothesis that bradykinin is active in the development of acute pancreatitis.

This investigation was supported by FBA Pharmaceuticals, Canada, which also made a gift of 'Trasylol'. Synthetic bradykinin was the gift of Dr. K. Royal Stewart, Sandoz Pharmaceuticals, Canada.

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IMMUNOLOGY

Strain Immunity in Babesiosis

The importance of immunological differences between strains in the epidemiology of human malaria is well known[1]. A disease comparable in some respects with malaria is bovine babesiosis. It is transmitted by a tick, and the protozoan parasites which cause the disease invade the red blood cell.

The possibility of the existence of differences between strains of *Babesia* was investigated by inoculating calves with one of two strains of *Babesia bigemina*. The origin of the two strains ("Y" and "R") has been described previously[2]. The calves were then splenectomized to amplify differences in acquired immunity and, at varying periods after splenectomy, they were challenged with either the homologous or the heterologous strain. Before most challenges, sub-inoculations were made to determine whether the calves were still carrying the original infection. In several instances, this information was obtained after splenectomy, a procedure which provokes relapse in infected animals[3].

Challenge of carriers. A total of nine calves was shown by sub-inoculation to be carrying *B. bigemina* at the time of challenge. When the challenging dose of approximately 10^9 organisms was inoculated intravenously, all developed parasitæmias. In the two receiving a homologous challenge, fewer than 1 per cent of the red blood cells became infected, whereas five of the other seven, which were challenged heterologously, developed parasitæmias within the range of 1–5 per cent. All animals, except one which was anæmic before challenge, controlled their infections without treatment. None of the nine appeared clinically sick during the reaction. As controls, eight splenectomized calves which had not been exposed to *B. bigemina* previously were inoculated with blood used in challenges. Irrespective of the strain used, these calves experienced fulminating infections. Parasitæmias were high, in several instances of the order of 50 per cent. Every calf was treated, but despite this three died.

These results show that splenectomized calves carrying *B. bigemina* are relatively resistant to superinfection with both homologous and heterologous strains, and suggest that a greater degree of resistance is possessed by cattle challenged homologously.

Challenge of animals no longer carrying B. bigemina. Of considerably greater significance were the findings when calves in which the infection had been eliminated were challenged in the same way as the carrier and the control animals. Twenty-nine animals were used in all, and it was possible to make several observations on many of these by repeatedly challenging them homologously before performing a final heterologous challenge (see Table 1 for two examples). In most of these animals, *B. bigemina* had been eliminated naturally some months after the infecting inoculation. Treatment with quinuronium sulphate contributed to the elimination of infection in a few instances. The results of homologous challenges with the "Y" and "R" strains are summarized in Tables 2 and 3, respectively, the results of heterologous challenges in Table 4.

It can be seen that a marked residual resistance was present against homologous challenge with either strain. For the "Y" strain, resistance had developed to a high degree after only one prior exposure to *B. bigemina*, and

Table 1. Repeated Challenges of Splenectomized Cattle which had ceased to carry *B. bigemina* (Two Examples from Series which gave Similar Results)

Animal	Challenge: No. and type	Challenge: Time since last inoc. or challenge (weeks)	Results of challenge: Parasitæmia: Days of	Parasitæmia: Degree of	Clinical effects and treatments	Sub-inoculations: Time after challenge (weeks)	Sub-inoculations: Transmission
578*	1, homol.	16	2	<1%	–	10	–
	1, heterol.	5	3	5%	+, treated second day of reaction	Not done	
341	1, homol.	11	–	–	–	2	+
						19	–
						22	–
	2, homol.	22	–	–	–	2	+
						8·5	–
	3, homol.	12	–	–	–	2	–
	1, heterol.	2	5	15%	+, died despite treatment fifth day of reaction	Not done	

* No reaction to a second homologous inoculation 5 weeks after first homologous challenge, but carrier status unknown at this time.

Table 2. Effects of Homologous Challenge of Animals no longer infected with "Y" Strain *B. bigemina* (after 1 to 6 Prior Exposures)

No. of previous homologous exposures		1	2	3	4	5	6	Totals
Pathological effects from challenge	Parasitæmia with clinical effects	2		1				3
	Parasitæmia without clinical effects	1			3	1		5
	No parasitæmia	6	3	3	3	4	2	21
Carrier status after challenge	Not carrier two weeks after challenge		2	1	1	2	1	7
	Carrier two weeks after challenge			1	4	2	1	8
	No test for carrier two weeks aftet challenge	9	1	2	1	1		14

Table 3. Effects of Homologous Challenge of Animals no longer infected with "R" Strain *B. bigemina* (after 1 to 6 Prior Exposures)

No. of previous homologous exposures		1	2	3	4	5	6	Totals
Pathological effects from challenge	Parasitæmia with clinical effects	2				1		3
	Parasitæmia without clinical effects	1	1		4			6
	No parasitæmia					1	1	2
Carrier status after challenge	Not carrier two weeks after challenge					1		1
	Carrier two weeks after challenge		1				1	2
	No test for carrier two weeks after challenge	3			4	1		8

Table 4. Effects of Heterologous Challenge of Animals no longer infected with Either "Y" or "R" Strain *B. bigemina*

No. of previous homologous exposures	1	2	3	4	5	6	7	Totals
No. challenged	3	1	6	2	1	4	1	18
Parasitæmia with clinical effects	3	1	6	2	1	3	1	17
Parasitæmia without clinical effects						1		1

did not diminish thereafter. Resistance to homologous challenge with the "Y" strain was sufficient to prevent detectable parasitæmia in a total of 21 instances. Further investigations (by sub-inoculation and heterologous challenge) of 15 of these showed that in 7 the animal was not carrying *B. bigemina* two weeks after its homologous challenge. In other words, the homologous challenge had failed to set up a new infection. Results were similar but not so spectacular for the "R" strain.

From Table 4 it can be seen that there was a completely different response to heterologous challenge. These animals reacted severely, and several died despite treatment. The reactions resembled those of the splenectomized controls.

Hence, a sterile immunity has been demonstrated in an infection which has always been presented as a classic example of "prémunition"[4] (co-infectious immunity). It must be pointed out, however, that it is a limited sterile immunity in that it is only effective when the animal has been exposed previously to the challenging strain, and even then not 100 per cent of the animals possess it. This sterile immunity does not exist when a heterologous challenge is performed.

The results also indicate an immunological difference between the two strains of *Babesia* used. A more detailed account of these investigations, the extension of the work to *Babesia argentina* and the epidemiological significance of the findings will be presented elsewhere[5].

This work was begun in the Department of Parasitology, University of Queensland, and continued at the Animal Research Institute, Brisbane, where I am now employed.

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ANATOMY

Possible Explosions in Absorptive Cells of the Jejunum

During the course of a long-term investigation of isolated, fresh, absorptive cells of the rat's jejunum suspended in physiological saline, prepared by the method described by Mohiuddin and Ramsbotham[1], we have occasionally come across cells showing a particular abnormality. This consists of a dome-like elevation of the plasmalemma over an accumulation of clear fluid covering one surface of the cell or, at the most, two adjacent surfaces. When this change affects the apex the striated border becomes unrecognizable, probably because the microvilli are taken in by the ballooning plasmalemma. In this respect the condition differs markedly from the transient apical blistering due to rapid pinocytosis reported by Mohiuddin[2].

In cells isolated from animals engaged in digesting fat, the abnormal cells contained fat globules in the cytoplasm, indicating the recent presence of functional capacity. We are satisfied that extraneous factors such as change in tonicity of the saline or pressure of the covership were not responsible for the abnormality, because such cells were usually dispersed among normal cells. We suspect that cells showing such an abnormality might have been observed more frequently if the technique of isolation, which is based on differential centrifugation, was not biased towards elimination of cells of a density differing materially from that of normal absorptive cells.

The appearance of the abnormal cells suggests the imminence of rupture of the ballooned part, although we have not actually witnessed such an end. We are struck by the similarity of appearance between this ballooning and the changes immediately preceding the 'explosions' which seem to occur with such dramatic speed in time-lapse cinemicrographs of cells in cultures.

It is possible that cells as short-lived as the intestinal epithelium (Leblond[3]) are lost not only through the process of shedding, but also in other ways, including 'explosion'.

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Location of Renshaw Cells

Ever since their discovery[1], the mammalian spinal cord interneurones known as 'Renshaw cells'[2] have been the object of extensive physiological and pharmacological research[3]. The recurrent inhibitory pathway from motor axon collaterals via Renshaw cells back to motoneurones serves as one of the best-documented examples of a negative feed-back mechanism in the central nervous system. Furthermore, the cholinergic synapses made by the motor axon collaterals on Renshaw cells are the most thoroughly analysed central synapses from the point of view of their pharmacology.

The precise location of Renshaw cells is of considerable interest for the understanding of the functional anatomy of the spinal cord. The electrophysiological investigations of Eccles's group have shown that most Renshaw cells are to be found medial to the motor nuclei[2]. Several investigators have described interneurones just medial to the motor nuclei which might qualify as Renshaw cells[4]. However, recently, interneurones in a still more medial position in the ventral horn have been thought to correspond to the Renshaw cells[5].

In the experiments recorded here, Renshaw cells were located by recording their electrical activity and then marking the position of the tips of the recording electrodes. This provided a physiological control for the histological observations. The preparations used were cats anæsthetized by sodium pentobarbital. The spinal cord was exposed and several ventral roots, usually *L6* and *L7*, were cut peripherally and the central ends mounted on platinum electrodes for stimulation. The recording electrodes were glass micropipettes filled with a solution containing a copper complex, prepared according to the method of Galifret and Szabo[6]. The resistances of the microelectrodes were initially 10–20 megohms, and they were found to be suitable for either extra- or intra-cellular recording.

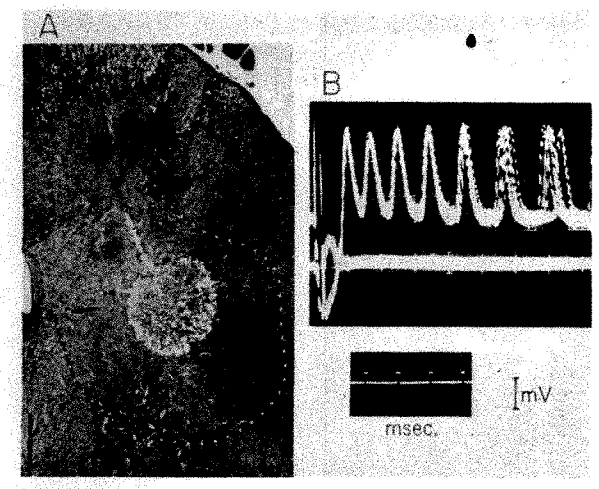


Fig. 1. *A*, Low-power photomicrograph of a transverse section through the lumbosacral spinal cord of a cat. The grey matter has been outlined by a dotted line. In the dorsal part of the ventral horn there is a circular profile corresponding to a lesion produced by copper complex ions which were electrophoretically ejected from a microelectrode, the track of which is seen entering the lesion from its upper left border. *B*, The upper trace shows the characteristic burst discharge of a Renshaw cell in response to ventral root stimulation. The recording was made in a partially intracellular position, positivity being upwards. The stimuli were repeated at 10/sec, and several sweeps of the oscilloscope beam have been superimposed in the photograph. The lower trace was recorded by a monitoring electrode on the cord dorsum. Note the time and potential scales

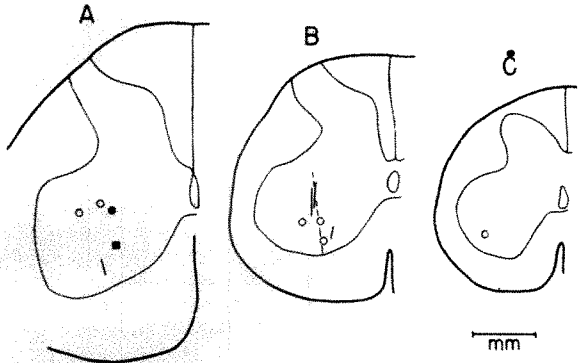


Fig. 2, *A*, *B* and *C* are outline drawings of transverse sections of the lumbosacral spinal cord at three segmental levels. The approximate positions of the various Renshaw cells studied have been marked on these drawings. The filled circle and square in *A* are the locations of two Renshaw cells found in the experiment illustrated in Fig. 1, the circle being the position of the centre of the lesion in Fig. 1*A*. The short lines in *A* and *B* represent the ventralmost extent of microelectrode tracks in those cases in which no lesion was found; the Renshaw cells examined in these instances were presumably just below the ventral tips of these lines. The dashed line in *B* shows the direction of a track in one experiment in which the electrode was partially withdrawn following recording from a Renshaw cell; the neurone was located somewhere along the line and was in the most ventral part of the ventral horn according to the depth readings on the micromanipulator. Note the distance scale

Renshaw cells were sought by systematic tracking within the appropriate segments of the cord. Their characteristic burst discharges in response to ventral root stimulation served to identify them (Fig. 1*B*). Often, recording was at first intracellular. After the electrical activity of a Renshaw cell was observed, the position of the tip of the microelectrode, and presumably of the Renshaw cell, was marked by electrophoretically ejecting copper complex ions from the microelectrode[6]. The microelectrode tip was cut off with a pair of fine scissors and left *in situ* during fixation of the cord with neutral formalin. The microelectrode was then removed, the cord sectioned, embedded and stained. The approximate location of the Renshaw cell was determined by following the microelectrode track into the centre of the large spherical lesion made by the copper complex (Fig. 1*A*). The neurone was assumed to lie at or near the centre of the lesion. Sometimes, no lesion was found, although the microelectrode track was easily traced. Apparently, in these cases, the current passed through the microelectrode was insufficient to allow enough copper complex to leave the microelectrode. Nevertheless, the position of the tip of the microelectrode was noted, since the Renshaw cell must have been located nearby.

Fig. 2 shows the approximate positions of 12 Renshaw cells at three levels of the lumbosacral spinal cord. In addition, the track along which another was found is indicated (Fig. 2*B*, dashed line). The area in which these Renshaw cells were located corresponds to the lateral and ventral parts of Rexed's lamina VII (ref. 7). The Renshaw cells would thus be located near groups of motor axons as they course medially from the motorneurones and then ventrally to join the emerging ventral root filaments. Such a position is obviously a strategic one for interneurones receiving synaptic connexions from collaterals of these motor axons.

This work was supported by U.S. Public Health Service Research grant *NB* 04779–01, from the National Institute of Neurological Diseases and Blindness.

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RADIOBIOLOGY

Manganese-54 Concentration in Fall-out, Water, and *Unio* Mussels of Lake Maggiore, 1960–63

The ability of *Unio* (Mollusca, Lamellibranchia) to concentrate manganese has been known for more than fifty years[1]. High concentrations of this element in Unionidae were also reported more recently by Vinogradov[2] and by Merlini[3]. In September 1960, manganese-54 was found in *Unio mancus* var. *elongatulus* (Pfeiffer) collected in Lake Maggiore and in nearby Lake Varese. It could not be detected in fall-out, lake water, sediment, rooted aquatic plants, gastropods or fish. After having ascertained that the ^{54}Mn present in *Unio* was not due to local contamination we supposed that this radionuclide must have been present also in the fall-out over northern Italy, but only in very low, undetectable concentrations[4].

^{54}Mn is presumed to originate from the activation of ^{54}Fe and ^{55}Mn present in nuclear weapons[5].

It would seem that because of its ability to accumulate manganese, *Unio* might be used to detect ^{54}Mn contamination of the environment, especially at very low levels which would not be measurable otherwise. To check this, ^{54}Mn was measured in *Unio* as well as in fall-out and lake water during 1960–63.

Lake water and fall-out were sampled as described before[6]. Large samples, at least 100 individuals, of *Unio* were collected at random from the lake bottom. Radionuclide separation of ^{54}Mn and its determination by γ-spectrometry were carried out as reported elsewhere[7]. The standard error of the ^{54}Mn determination was 2–5 per cent for *Unio*, about 10 per cent for fall-out and 10–20 per cent for lake water. ^{54}Mn fall-out increased from 1961 to 1963 (Fig. 1): maximum, monthly ^{54}Mn fall-out (dry + wet), amounting to 22 mc./km^2, occurred in June 1963.

Table 1. Concentration, Content, Percentage and Specific Activity of ^{54}Mn calculated for the Different Parts of *Unio mancus* var. *elongatulus* (Angera, December, 1963)

Stable manganese data are from Merlini[3]

Part	μμc. ^{54}Mn/g Dry wt.	μμc. ^{54}Mn/g Wet wt.	μμc. ^{54}Mn Part	^{54}Mn as % of total	μμc. ^{54}Mn mg Mn
Total body	34·9	13·3	338·0	101	39
Extrapalleal liquid	137·8	0·5	4·6	1	—
Shell	13·4	12·2	117·5	35	40
Total soft tissues	223·4	31·3	215·9	65	30
Gills	366·9	44·0	59·9	18	23
Mantle	280·1	44·0	127·1	38	31
Visceral sac	84·5	13·0	26·8	8	
Foot	32·9	4·0	2·1	1	21

Concentration of ^{54}Mn in surface water of the lake increased from less than 0·01 μμc./l. in September 1960 to a maximum value of 0·60 μμc./l. in June 1963. It decreased thereafter to 0·14 μμc./l. in December 1963. This might be explained by precipitation of insoluble oxidized manganese compounds, absorption by plankton, adsorption by seston, and subsequent settling in the lake bottom[8].

During September 1960–September 1961 ^{54}Mn in *Unio* decreased from 520 to 169 μμc./kg of wet soft tissue, which is 8·5 per cent more than could be expected on the basis of physical decay. This may correspond to a decrease in the specific activity of ^{54}Mn in lake water due to fixation by the sediment. From September 1961 onwards, the concentration of ^{54}Mn increased about thirty-fold to reach 14,000 μμc./kg wet soft tissue in September 1963, which corresponds to a dose to the organism of about 2·3 μrad/h. Calculated fall-out data corrected for decay are reported together with the concentrations in water and *Unio* in Fig. 1. From this, it would appear that *Unio* is a valid bio-index for ^{54}Mn contamination.

Since it was previously found[9] that ^{54}Mn concentration tends to increase with age and size in *Unio*, measurements of length were made for each sample. They revealed a change in population structure from 1960 to 1963, there being an increase in the upper-length classes. On this basis alone, as found by calculating weighed mean concentrations, ^{54}Mn content in *Unio* would have increased by about 11 per cent from 1960 to 1963 if the level of the nuclide in the environment had remained constant. Such an increase is very small compared with the observed increase resulting from changes in concentration of ^{54}Mn in the lake water, but such corrections might be significant under other circumstances and for other species.

The distribution of ^{54}Mn in the body of *Unio* is given in Table 1. The highest concentrations were found in the mantle and gills, the lowest in the extrapalleal liquid.

The distribution of ^{54}Mn in the body was compared with that of stable manganese measured by radioactivation by Merlini[3] (Table 1). The two distributions are strikingly similar although measurements were made on different samples collected at different times. This would indicate that ^{54}Mn was introduced in the lake environment in a form similar to that for stable manganese, from the point of view of availability to *Unio*.

Measurements of ^{54}Mn made on *Anodonta cygnea* indicate that this other genus of Unionidae is also strongly able to accumulate manganese, although the highest value found was one-third that of *Unio*.

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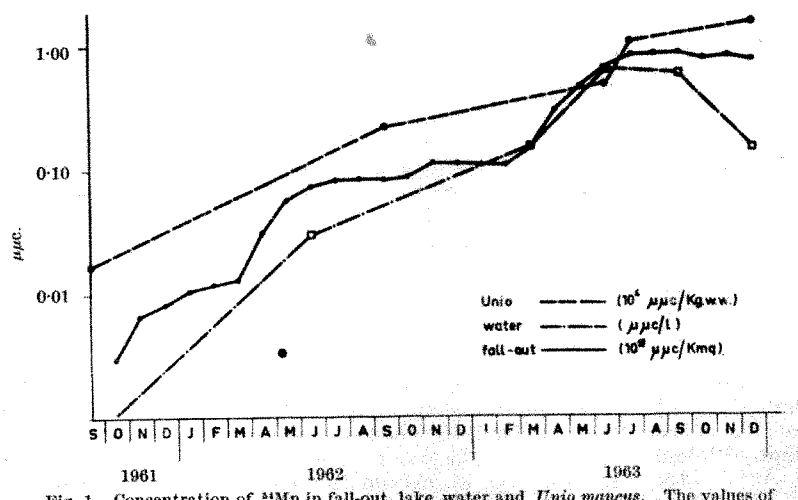


Fig. 1. Concentration of ^{54}Mn in fall-out, lake water and *Unio mancus*. The values of fall-out, corrected for radioactive decay, are cumulative

BIOLOGY

Axial Organ and Fluid Circulation in Echinoids

The axial organ has long been one of the most enigmatical structures in echinoderms. That of echinoids has been considered excretory[1–4], genital[5], vestigial[6], as producing pigment[7] and as a circulatory organ[8–17].

The idea that the organ functions as a heart is time-honoured and stems from observations of its slow pulsations[17]. Such observations have recently been strikingly confirmed by Boolootian and Campbell[18], who photographed pulsations in the axial organ of *Strongylocentrotus purpuratus*. Contraction, however, is not confined to the aboral extremity of the organ, as sometimes stated for other echinoids, but involves a vessel extending internally along its length.

We have found what appears to be the anatomical counterpart of this vessel in the axial organs of *Arbacia lixula*, *Diadema antillarum* and *Echinus esculentus*. It is not always prominent, however, and it is sometimes difficult to trace through the whole organ.

In the past much misapprehension and argument have arisen from attempts to categorize the axial organ in a simple fashion. In particular, the use of the term 'heart' has been the cause of much contention[7]. It is therefore unfortunate that Boolootian and Campbell report their valuable findings under the somewhat arresting caption of 'a primitive heart' without making it altogether clear whether they are labelling the whole organ or only a part of it. In the context of bygone arguments, such an element of ambiguity can be misleading. Setting aside the more general and fundamental question of the suitability, on embryological grounds, of terms such as 'heart' and 'hæmal' in echinoids, our work on the axial organs of such diverse species as *Arbacia lixula*, *Diadema antillarum* and *Paracentrotus lividus* dispels ambiguity on this particular point, for whatever may be urged concerning the head process, the organ as a whole is pre-eminently glandular. Muscle fibres exist, but apart from those clustered around the stone canal and what appears to represent the pulsating vessel, they are a minor constituent, and over much of the



Fig. 1

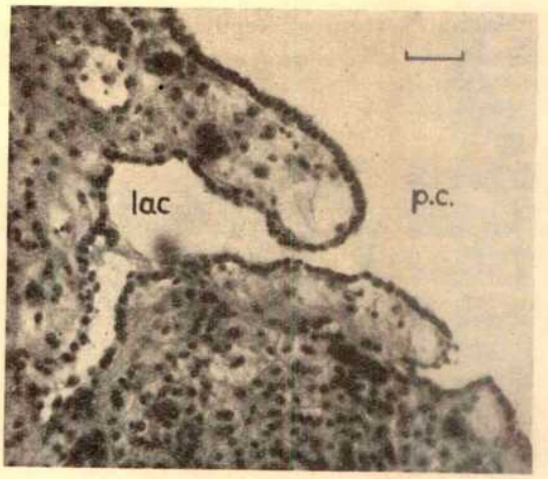


Fig. 2

Fig. 1. *Arbacia lixula*, portion of the axial organ showing an opening into the perivisceral cœlom (*p.c.*) and secretion (*sec.*) which is periodic acid–Schiff-positive, arising from the walls of the glandular recesses of the organ. Rossmann fixation followed by staining with periodic acid–Schiff and hæmatoxylin. Scale as in Fig. 2

Fig. 2. *Paracentrotus lividus*, portion of the peripheral region of the axial organ, showing an opening from the perivisceral cœlom (*p.c.*) into one of the so-called 'blood-lacunæ' (*lac.*), which contains secretion. Rossmann fixation followed by staining with Ehrlich's haematoxylin and eosin. Scale 15μ

organ they are completely lacking. A comparison of the whole organ with a heart is therefore inappropriate.

There is room for second thoughts, however, in making more restricted comparisons with specialized hearts. The capacity to move fluid within vessels of the so-called hæmal system does not endow the axial organ with the status of a heart. No evidence cited as yet indicates that the propulsive agent is localized, or even pre-eminently developed, in the axial organ of echinoids in general. On the contrary, power to pulsate is shared with other vessels, as has long been known[8] and is now re-affirmed (see following).

Boolootian and Campbell claim to have shown the existence of 'a true circulatory system' in *Strongylocentrotus*, which is presumably to be correlated with their description of the uni-directional propagation of contraction through the pulsating vessel of the axial organ. Unfortunately they do not quote details, so that their precise meaning is not clear. We should point out, however, that any view which might attempt to assess the significance of fluid movement in echinoids in general must eventually come to terms with some peculiar features. Thus, as reported in the succeeding communication, some of the so-called hæmal vessels are partially occluded; indeed, those investing the axial organ of *Diadema* are usually completely blocked by dense deposits of melanin and lipofuscin[19]. Again (see following communication) the movement of fluid in parts of the system is of the 'ebb and flow' type[1]. Finally, cardinal regions of the 'circulatory system', including the supposedly central propulsive structures such as the axial organ (with its contained vessels) and stone canal, together with the head process and neighbouring structures such as the madreporite, can be removed completely from living *Paracentrotus*, yet a considerable number of urchins survive the treatment for some weeks, a few for five months. The most consistent and obvious immediate outcome is a local effect on the gut, the inferior loop of which soon begins to degenerate.

It is unfortunate that recent accounts of the axial organ[4,20] have paid little or no attention to a most significant structural feature reported long ago[15], namely, the system of deeply penetrating canals which arise as infoldings of the external surface. Our investigations have shown that their inner end may sometimes be traced into the central lumen of the organ (Fig. 1) or into the network of so-called hæmal vessels that invests it (Fig. 2). Thus, there are a number of direct connexions between the perivisceral cœlom and both hæmal vessels and glandular lumen. In addition, the existence of a communication between the lumen of the axial organ and the stone canal has been re-affirmed[18]. It is now possible to grasp a most essential character of the organ, namely, that it is a central, glandular, point of confluence of perivisceral cœlom, 'hæmal' system and water vascular system. Apart from the controversial relation with the gonads[5,21], it therefore has at least an eminently suitable position to function as an endocrine organ. Sections reveal that considerable quantities of secretion may leave glandular recesses of the organ and pass into the perivisceral cœlom by the canals just mentioned (Fig. 1). Histochemical tests indicate that it is periodic acid–Schiff positive and contains 3-indolyl compounds (possibly tryptophan), together with acid mucopolysaccharide, which also appears in the stone canal and hæmal vessels.

Although it is still uncertain whether the ubiquitous amœbocytes arise in the axial organ, there is abundant evidence that they egest and degenerate within it.

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Hæmal System of Regular Echinoids

Examination of accounts of the echinoid hæmal system shows that the term 'hæmal' has never been really justified: its function has not been conclusively demonstrated, and the precise nature of the movement of fluid within the system as a whole has never been satisfactorily established.

Uncertainty even as to the gross anatomy of the system is well illustrated in the instance of the radial hæmal sinuses. Thus Perrier[1] did not describe them, Chadwick[2] could not demonstrate them and doubted their existence. Cuénot[3] affirmed their existence, but admitted that they could not be injected.

Tiedemann[4] first noted the contractile nature of the hæmal vessels. Perrier[1] noted that some vessels of *Echinus esculentus* showed non-rhythmical contraction and thought that the direction of the resultant hæmal flow was not constant. Farmanfarmaian and Phillips[5] could not detect any fluid movement in the hæmal system of *Strongylocentrotus purpuratus*, although a rhythmic beat was observed in the outer sinus (external marginal vessel) and in the collateral sinus of the stomach. Stott[6] considered that, in the absence of a pumping mechanism, distribution of nutrients was effected by migration of amœbocytes within the hæmal system. Boolootian and Campbell[7] have described a contractile chamber and pulsating vessel associated with the axial gland of *S. purpuratus*. They consider that fluid can enter the contractile chamber by two routes, the inner hæmal sinus and also by microscopic ostia. The abstract of the paper states that a pulsating vessel and a compartmented contractile chamber were found to move cœlomic fluid from the perivisceral cavity into and throughout the hæmal system of the urchin. This statement is not fully justified in the account. The authors also state that the rhythmic contraction of the pulsating vessel together with the pulsations of the stone canal may be important in moving fluids through the entire water vascular system.

I have examined the so-called hæmal system of *Echinus esculentus* and *Psammechinus miliaris*. Sea-water contamination was minimized as this causes rapid deterioration of the internal tissues. I can confirm that the collateral and internal marginal vessels of the stomach of both species are contractile, rates of 10 contractions per 60–90 sec being recorded for the internal marginal vessel and 10 contractions per 45–180 sec for the collateral vessel in *P. miliaris*. Particulate matter, principally clumped amœbocytes, was observed through the walls of the vessels. The particles are small and difficult to observe in fresh preparations.

Despite evidence of contractility its significance is not yet clear. First, although some vessels showed a patent lumen (for example, collateral, internal and external marginal vessels of the stomach region), some, such as the peri-œsophageal hæmal ring and the œsophageal vessel, did not, being largely occluded by spongy tissue. Secondly, the particulate contents of the vessels showed ebb-and-flow rather than uni-directional movement, although a net transfer in their position was sometimes achieved. The most usual pathway was as follows: material passed from the œsophageal to the intestinal end of the internal marginal vessel at a rate of about 15 mm/min, and passed along the collateral vessel towards the œsophagus at a similar rate. Net transfer of material from the intestinal to the œsophageal end of the internal marginal vessel simultaneous with collateral flow towards the intestine was also observed but simultaneous flow in both vessels towards the œsophagus or the intestine was not observed.

Pathways available to injected dyes were investigated: it was not possible to inject the œsophageal vessel or the peri-œsophageal hæmal ring as a lumen could not be detected. Neither was it possible to inject the collateral vessel due to its delicate suspension and extreme contractility. Injection into the internal marginal vessel showed that dye passes readily into the collateral vessel when injected from either end of the vessel towards the other, but there is resistance to injection towards the œsophagus. Material injected towards the œsophagus may pass into the origin of the œsophageal vessel, and, similarly, material injected towards the intestine may pass a short way along the external margin of the intestine.

It is concluded that it is unlikely that in these species the so-called hæmal system functions as a true circulatory system. However, there is undoubtedly active movement, and possibly a more restricted circulation, within the vessels of the stomach region. Recent work[5,6] has suggested that absorbed nutrients pass rapidly to the cœlomic fluid. Preliminary experiments, in which the presence of radioactive material was sought in the hæmal vessels after oral injection of isotope-labelled substances in *P. miliaris*, suggest that the hæmal vessels around the stomach may function in absorption, as previously surmised[1,9]. Perhaps they may assist passage of extracted nutrients to the cœlomic fluid, both 'extraction' and diffusion being assisted by the oscillation of fluid within these vessels.

This investigation was conducted, under the supervision of Prof. N. Millott, during the tenure of a Department of Scientific and Industrial Research studentship.

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Effects of Histones on Embryonic Development

I have recently examined in detail the effects of actinomycin *D*, an inhibitor of RNA polymerase and of messenger RNA synthesis, on the development of amphibian eggs[1,2]. It is now known that histones also inhibit RNA polymerase[3,4] and it has been suggested that they could act as the natural regulators of genetic activity[5,6]; by combining with specific segments of DNA molecules, they would block the production of messenger RNAs by these segments. It has been suggested[7–9] that such a mechanism could account for the 'switching on and off' of gene activity during embryonic development. It was of interest to compare the effects of exogenous, abnormal histones on embryonic development with those of actinomycin *D*. The latter are quite different from those of another basic protein, ribonuclease, which quickly stops cleavage in amphibian eggs[10], while actinomycin *D* has no effect prior to gastrulation.

The lysine-rich fraction of thymus histones, prepared by the method of Allfrey *et al.*[4], was used in the present work. Three types of experiments were performed on eggs of *Pleurodeles* at various stages of their development: addition of histones to the medium, replacement of the blastocele fluid by a histone-containing solution and micro-injection of histones in just fertilized eggs.

(1) *Addition of histones (3 to 100 μg/ml.) to external medium.* No immediate effect on cleavage could be detected, even at the highest concentrations: when early morulæ or late blastulæ are treated with histones (10–90 μg), development stops at the gastrula stage. A very conspicuous effect of histones (10–30 μg/ml.) is

an intensive contraction of the embryo at the gastrula stage: it leads to exogastrulation and even complete inversion of the cell layers. While the eggs are very sensitive to histones at this critical stage (late blastula–early gastrula), they quickly become insensitive again: late gastrulæ react to histones by a contraction of the neural plate (similar to that observed by Ambellan[11] on addition of ATP to the eggs), while neurulæ are practically insensitive. There is partial reversibility when the embryos are replaced in normal medium: but developmental abnormalities such as microcephaly, cyclopia, complete absence of the nervous system in the extreme cases, *spina bifida*, etc., are frequent in these embryos.

The strong contraction produced by the histones at the critical stage suggests an effect on the cortical layer of the gastrulæ (the absence of ATP in our preparations was of course checked). That histones do not penetrate very deep in the treated embryos was demonstrated by staining slides with fast green, according to the method of Moore[9] for the detection of histones: the vitelline membrane and the external layer of cells stain strongly and a definite gradient in the penetration of the histones can often be observed. Later on, the cells of the superficial layer undergo cytolysis; it is noteworthy that the nuclei and yolk platelets of the dead cells stain much more heavily than those of the normal cells: the latter must, somehow, be protected against the penetration of the histones.

When late blastulæ or early gastrulæ are treated during 5–20 h by concentrated (50–100 μg/ml.) solutions of histones, many cytological abnormalities are seen after staining with Unna or Feulgen. The contraction of the individual cells is visible, with a distortion of the normal gradient in the distribution of RNA. Many cells are multinucleate; all the nuclei are very irregular in shape and size. They contain nucleoli, but their chromatin stains green instead of blue with Unna: it seems that the histones inhibit the synthesis of chromosomal RNA rather than that of nucleolar RNA. The mitoses are usually numerous, with a tendency towards a block in metaphase. It is likely that multinucleate cells result from two different processes: amitosis and lack of cytodieresis due to the strong contraction of the cells, and lack of fusion of the chromosomes at telophase. When the blocked gastrulæ are replaced in normal medium the nuclei soon resume their normal staining (blue chromatin, red nucleoli) characteristics.

(2) *Injection of histones in the blastocele fluid.* The effects depend very much on the concentration of histones used, all the embryos reacting in almost the same manner to a given concentration. At the 1 μg/ml. level, all embryos[9] formed abnormal tadpoles. After injection of histones at a 2 μg/ml. concentration, 7 abnormal neurulæ (out of 7 injected blastulæ) were obtained; with 4 μg/ml., the block of development was practically immediate in all[9] the injected blastulæ.

Cytological investigations showed that, after the injection of histones (2–4 μg/μl.), the cells surrounding the blastocele have large nuclei, which stain green with Unna and have no visible nucleoli: obviously, the block in DNA and nuclear RNA synthesis is complete in the cells which have been directly exposed to the histones. In the outer layers, one can find a mosaic of normal and abnormal (irregular shape, larger size) nuclei.

(3) *Injection of histones* (1 μg/ml.) *at the one-cell stage.* Out of 13 injected eggs, 8 failed to divide, probably as a result of the injury. The 5 others cleaved normally, but stopped developing at the late blastula stage. They displayed the usual nuclear abnormalities. In one case, an egg was injected in one of the blastomeres at the 4-cell stage and fixed 8 h later, as a normal morula. It was cytologically normal, except for the two blastomeres corresponding to the injection point: they contained huge, swollen nuclei, apparently blocked in interphase, which remained unstained with Unna.

In conclusion, it can be said that the effects of histones present, as one would expect, great similarities with those of actinomycin *D* (refs. 1, 2, 12): cleavage—but not gastrulation—is possible after injection into fertilized eggs, and it is not affected by histones added from the outside. Exogastrulation, as found by Wallace and Elsdale[12] in the case of *Rana pipiens* eggs treated with actinomycin *D*, is very frequent. Abnormalities, such as microcephaly, become conspicuous at later stages: they are identical with, but not so extensive as, those produced by actinomycin *D*. The difference is probably due to a poorer penetration of the histones. Regarding the effects on cleavage, histones are much less effective than ribonuclease[10]: it is therefore probable that the inhibition of cleavage by ribonuclease is due to its enzymatic activity and not to its basic character.

I thank Miss E. Van Stevens for making the microinjections, and Prof. E. Schram, who prepared and analysed the histones. This work was supported by Euratom (contract 016–61–10 *ABIB*).

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Light as an Inhibitor of Strobilation in *Aurelia aurita*

The environmental conditions under which strobilation of *Aurelia* scyphistomæ occurs are incompletely understood. Berrill[1] in a review suggests three factors as being of particular significance: a heavy diet of suitable food prior to metamorphosis; a definite, low critical temperature, usually the winter minimum; and a good supply of food and oxygen at the critical period. Thiel[2], as a result of observations on naturally-occurring populations of scyphistomæ, largely supports these suggestions and places particular emphasis on the food supply prior to strobilation. Laboratory experiments have produced conflicting results[3].

A series of experiments was arranged to investigate the effects of these environmental conditions on strobilation in the laboratory. Scyphistoma larvæ were selected from stocks reared from planulæ released by adult *Aurelia aurita* collected at Whitstable. These larvæ were put in vessels which in turn were placed in a low-temperature water bath. The temperature of the bath was gradually lowered from 11° C to 5·25° C during a period of 2·5 months. The larvæ were fed twice a week on *Artemia* nauplii throughout the experiments. Initially all the larvæ were under 6 h daily illumination from a 3-ft. Osram 30 W warm white fluorescent tube at a distance of 26 in. The results of transferring some larvæ into the dark while leaving others in the light as control are shown in Table 1.

These results indicate that the presence of light of too high an intensity inhibits strobilation. With the exception of '63/*A*, strobilation only occurred in the absence of light. This interpretation does not necessitate that all larvæ in the dark should strobilate, and therefore experiments '63/*E*, '63/*H* and '63/*I* are not necessarily unsatisfactory. The apparently anomalous result in '63/*A* can perhaps be explained by the fact that all specimens

which strobilated belonged to the same group of larvæ which were attached to the underside of a shell and were therefore shielded from direct light. The failure of strobilation to take place in '63/*E*, *H* and *I* could be attributed to fundamental differences in the tissues of the larvæ. Two such factors which could account for these results are the size of the food reserves and the age of the individuals. Although specimens were selected from a stock population which had been derived from the same batch of planulæ, there was no way of identifying those scyphistomæ which had developed directly without budding from those which had resulted from asexual reproduction. Again, although specimens were selected which were initially the same size, it was noticeable that strobilation ultimately occurred most readily among the larger individuals.

Table 1

Exp.	Lighting conditions Dark	Light	Aeration	Temp.* (° C)	No. of larvæ Total	Strobilating	Av. time† (days)	Av. No. ephyræ
'62/*J*	+		+	8·25	3	3	39 ± 9	8·6
'62/*K*	+		−	8·25	3	3		16·3
'63/*J*	+		−	7·75	6	6		19·3
'63/*O*	+		+	7·75	7	7		17·7
'63/*M*	+		+	7·75	6	5		17·8
'63/*P*	+		−	7·75	7	6	79 ± 22	16·2
'63/*F*	+		−	9·75	6	3		22·6
'63/*L*	+		−	7·75	6	3		2·6
'63/*G*	+		+	8·75	7	3		13·3
'63/*K*	+		+	7·75	6	2		3·5
'63/*A*		+	+	−	*c* 75	15	112 ± 14	*c* 6
'61/all (5 exp.)		+	+/−	−	*c* 100	0	−	−
'62/*I*		+	+	−	*c* 50	0	−	−
'63/*C*		+	−	−	3	0	−	−
'63/*D*		+	+	−	6	0	−	−
'63/*E*	+		+	9·75	6	0	−	−
'63/*H*	+		+	8·75	6	0	−	−
'63/*I*	+		+	7·75	6	0		−
'63/*R*	+		−	Temp. maint. at 11–13°	6	0	−	−
'63/*S*		+	−		6	0	−	−

*The temperature listed in each case is the point during the gradual lowering to 5·25° C at which specimens were transferred from the light into the dark. The exceptions are Exps. *R* and *S* which were maintained at 11°–13° C as a control.

† These figures show the extreme range of intervals in days between the start of the experiment and the onset of strobilation in those specimens which metamorphosed.

If light is an inhibitory factor, then the progressive fall in illumination[4] which occurs in late autumn might be a positive stimulus for the onset of strobilation. Such a fall occurs simultaneously with the lowering of temperature at that time of the year[5]. Although there is a record of the appearance of ephyræ larvæ in the plankton at various stations along the coast of northern Europe during January–April[6], there are no direct observations on the onset of strobilation at these points. This suggests that strobilation must have started some 3–6 weeks earlier than the appearance of these ephyræ, that is, late November–March—a time when values of illumination and temperature are both low. It should be noted further that scyphistoma larvæ tend to settle on the underside of rocks and shells where there is a low level of illumination; and that the sediment in coastal waters is often responsible for blocking the light to a greater or lesser degree. It is suggested that light of too high an intensity may have contributed to the failure of earlier experiments designed to induce strobilation under laboratory conditions.

This work was supported by a grant from the Royal Society's Fund for Scientific Research in Schools.

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Growth of Embryos of *Amyema*, *Amylotheca*, and *Scurrula* on Synthetic Media

We have already reported[1] the growth responses of globular and complete embryos of *Dendrophthoe falcata* (L.f.) Ettings. These investigations have now been extended to *Amyema pendula*[2] (Sieb. and Spreng.) van Tiegh., *Amylotheca dictyophleba*[2] van Tiegh., and *Scurrula philippensis*[2] (Cham. and Schlecht.) G. Don.—all members of the Loranthaceae.

The mature embryo has a broad hypocotyl but has no radicle. The cotyledons are closely appressed to each other giving a pseudo-monocotyledonous appearance in *Amyema* and *Scurrula*, but they are mostly free in *Amylotheca*. The hypocotyl of *Amyema* is studded with papillate outgrowths (Fig. 1,*A–C*), while that of *Amylotheca* shows finger-like projections (Fig.1,*D*).

The fruits were surface sterilized with chlorine water for 10–15 min and washed thoroughly with sterile water. The embryos were excised and sown on sterile modified White's medium[3] under aseptic conditions. The *p*H of the medium was adjusted to 5·6. The cultures were maintained at 25° ± 2° C.

For purposes of comparison we may briefly recapitulate the results previously obtained with the mature embryos of *Dendrophthoe*[1]. The embryos (4–8 mm long) planted on modified White's medium supplemented with indolyl-3-acetic acid (IAA) (0·5 p.p.m.) + casein hydrolysate (500 p.p.m.) developed a superficial meristem around the hypocotyl and produced a discoid holdfast within five weeks. Eight haustorial disks developed in succession during the next 10–15 weeks. The plumular leaves emerged 4–5 weeks after inoculation and 25-week-old seedlings were about 3·5 cm long with three pairs of leaves. When the embryos were cultured with intact endosperm, the unfolding of the plumular leaves was delayed or altogether inhibited.

The embryos of *Amyema* (2·5–4 mm long) were sown on White's medium + IAA (0·5 p.p.m.) + casein hydrolysate (500 p.p.m.). Within a week the embryo turned dark-green, and the hypocotyl elongated and swelled appreciably. In 3–5 weeks after inoculation the hypocotyl developed a marginal meristem which gave rise to an irregular, massive, discoid holdfast (Fig. 1,*B*). The first pair of plumular leaves also appeared at this time, and in another 10–15 weeks three pairs of leaves were formed. In nearly 10 per cent of the cultures the papillate outgrowths became very prominent and during the next 15 weeks they simulated the later stages of embryonic development in Nature. Finally, they produced multiple shoots (Fig. 1,*C*). When the nutrient medium was supplemented with autoclaved coconut milk (10 per cent, v/v), the basal end of the hypocotyl (radicular end) and localized areas of cotyledons produced friable callus masses.

On White's medium + IAA (0·5 p.p.m.) + casein hydrolysate (500 p.p.m.) the embryos of *Amylotheca* (4–6 mm long) attained almost double the length in 3-week-old cultures. The finger-like processes (Fig. 1,*D*) elongated considerably but failed to produce shoots. The cotyledons mostly withered and abscissed in eight weeks. 25-week-old seedlings showed a pair of leaves, a holdfast and 2–5 haustorial disks.

The embryos (2–3 mm long) of *Scurrula* were also cultured on White's medium + IAA (0·5 p.p.m.) + casein hydrolysate (500 p.p.m.). The first pair of plumular leaves was noticed in 5-week-old cultures and three pairs of plumular leaves developed within the next 15 weeks. In one instance a sucker also developed from the holdfast (Fig. 1,*E*). If cultured with intact endosperm, the embryos germinated only rarely.

It is concluded that the embryos of the semi-parasitic mistletoes can be easily grown on synthetic media. With the endosperm intact, the appearance of plumular leaves

is usually delayed or inhibited. However, the holdfast and the haustorial disks develop normally. The papillate outgrowths on the hypocotyl of *Amyema* represent vegetative buds and differentiate into multiple shoots. Probably this is also true of the finger-like outgrowths on the hypocotyl of *Amylotheca*.

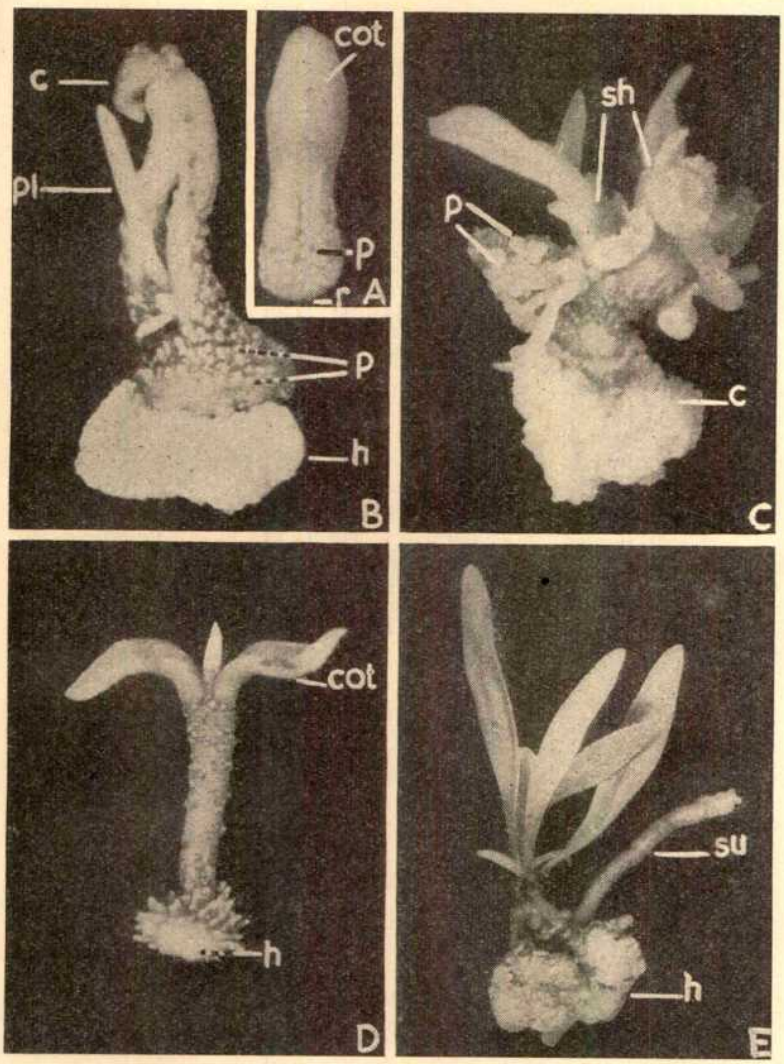


Fig. 1. *A–C, Amyema pendula: A*, mature embryo; note the papillate outgrowths on the hypocotyl (× *c.* 11); *B*, 5-week-old seedling showing a holdfast and plumular leaves, on White's medium + IAA (0·5 p.p.m.) + casein hydrolysate (500 p.p.m.) (× *c.* 3·6); *C*, 15-week-old proliferated embryo on White's medium + coconut milk (10 per cent) showing various stages of differentiation of the papillæ into shoots (× *c.* 3); *D*, 5-week-old seedling of *Amylotheca dictyophleba*; note the free condition of cotyledons and the elongation of the finger-like outgrowths (× *c.* 5·3); *E*, 20-week-old seedling of *Scurrula philippensis* with a massive holdfast, sucker and leaves (× *c.* 2). Key: *c*, callus; *cot*, cotyledon; *h*, holdfast; *p*, papillate outgrowth; *pl*, plumular leaf; *r*, radicular end; *sh*, shoots; *su*, sucker

We thank Prof. P. Maheshwari for his advice, and the Council of Scientific and Industrial Research, Government of India, for financial support.

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ENTOMOLOGY

Effects of High Temperature in *Rhodnius prolixus* (Stål).

Rhodnius fourth instar larvæ fail to moult at a temperature higher than 35° C; and at this temperature, or slightly lower, there is a disturbance in the hormone balance[1]. This communication deals mainly with the results of exposing the different larval stages, directly after feeding, to 36·5° C for different periods, and then transferring them to 28° C, the optimum temperature. Such treatment results in delayed moulting in all the larval stages, and in a reduction in the numbers of sensory bristles and plaques which are differentiated and in their malformation. If the insects are kept at the high temperature before feeding, the same symptoms appear; there is also inhibition of the diuresis which normally takes place during the first 4 h after feeding[2], despite the apparently normal functioning of the Malpighian tubules.

Wigglesworth states that, although high temperature (36° C) may have an adverse effect on the secretion of the brain factor, since the thoracic glands show no signs of activity, it may also arrest moulting by interfering with the secretion of the thoracic gland hormone or its action on the epidermis[3]. By combining heat treatment, transfer experiments and decapitation experiments with histological investigations, it has been shown that the brain hormone is not secreted at high temperature and that, after transfer to the optimum temperature, its secretion is delayed. This results in a delay in the activation of the thoracic gland, which is the main target of the brain hormone, and in the mitotic activity of the epidermal cells; hence moulting is temporarily arrested. High temperature was found to interfere with wound healing at the stage of mitosis. This would explain the cessation of moulting, at 36° C, in larvæ injected with ecdysone, or in larvæ supplied with the hormone in parabiotic experiments[3]. Thus it is concluded that, even in the presence of the moulting hormone, moulting does not occur at high temperature, because of the effects of the latter on cell division. Moreover, at 28° C, the oxygen consumption of decapitated injured larvæ shows a maximum increase of about two-fold at five days after injury—a time corresponding to the maximum growth activity in the epidermis during wound healing[4], whereas at 36·5° C there is no increase in respiration.

Examination of proteolytic activity in the midgut of fifth instar larvæ showed that the optimum *p*H is 3·55 and that 84 per cent of the total activity is found in the lumen contents. At high temperature, proteolytic activity is normal until 2 days after feeding; it then decreases to reach a very low level 7 days after feeding. In the insect decapitated 4 h after feeding and kept at 28° C, the level is normal 2 days after feeding, remains constant, and by 8 days after feeding it decreases. This agrees with Thomsen and Møller's results on the influence of the median neurosecretory cells of the brain on protease activity in *Calliphora erythrocephala* Meig[5].

Seven days after feeding, the concentration of total free amino-acids in the hæmolymph at 36·5° C is approximately twice that at 28° C. Conversely, the abdominal fat body

protein content and the hæmolymph protein concentration are much less at high temperature. Directly after transfer to the lower temperature, the amino-acid concentration decreases, while the fat body and hæmolymph proteins and the protease activity in the midgut start to increase. If the insects are decapitated immediately after transfer, the hæmolymph amino-acid concentration does not decrease to the normal level, even 13 days after transfer. However, even after transfer to normal temperature, the levels of these metabolites and of oxygen consumption do not revert to normal; this is correlated with the longer period required for development under these conditions.

It is concluded that high temperature interferes mainly with protein synthesis. This is reflected in the inhibition of cell division, the impairment of differentiation, the reduction in midgut protease activity and the cessation of secretion of the brain hormone which may prove to be a protein or a proteinaceous compound. This compares with Wigglesworth's suggestion that the characteristic defect in the resting or dormant insect, where no moulting hormone is being secreted, is a failure of protein synthesis[6].

Preliminary investigations on the inhibition of reproduction in both sexes at high temperature revealed signs of inactivity of the corpus allatum.

I thank Sir Vincent Wigglesworth for facilities and guidance and the University of Assuit, Egypt, U.A.R., for financial support.

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A Large Amount of Trehalose in a Frost-resistant Insect

With a few exceptions[1], almost all the remarkably frost-resistant insects have been shown to contain glycerol or sorbitol[2]. The overwintering pre-pupal larvæ of a sawfly, *Trichiocampus populi* Okamoto, however, were found to survive freezing at a liquid gas temperature without any polyhydric alcohol. An extraordinarily large amount of a sugar, trehalose, was detected in this insect.

T. populi is one of the common pests of poplar trees. The full grown larvæ of this insect leave the food-plant in autumn and pass the winter as prepupæ in withered plants. The body weights of the prepupæ were more than 30 and 80 mg in male and in female respectively. Since some of them are found in small holes or slits in the twigs of some trees or shrubs, and remain uncovered by snow during the coldest season, they are exposed to severe cold. In fact the overwintering prepupæ were found to survive freezing at −30° C for many days. As the supercooling point in the prepupæ is −8·6° ± 0·4° C, and the highest freezing temperature is about −3° C, they conceivably freeze and thaw many times during the winter.

Our earlier work showed that an animal could survive freezing even at liquid gas temperatures provided it could withstand prefreezing at −30° C (refs. 3 and 4). Our prefreezing method was also applied to the overwintering prepupa of *T. populi*. Four groups of ten prepupæ were subjected to freezing in air at −30°, −25°, −20° and −15° C respectively for 1·5 h. One other group was also frozen at −30° C as control. All groups of the insects, except the control, were then immersed directly in liquid nitrogen. After being kept there for 2 h they were rewarmed in air at −30° C for 30 min, and transferred into air at room temperature. As shown in Table 1, the prefreezing treatment at temperatures below −20° C were sufficiently effective to enable the insect to withstand a very low temperature. It is of interest that pre-freezing proved to be as effective at −20° in the sawfly prepupa as at −30° C, because in extremely frost-resistant insects so far known, pre-freezing has been found to be far less effective at a temperature above −25° C than at −30° C. After re-warming from the liquid nitrogen temperature, most of the prepupæ survived for more than 100 days. Many of them were able to resume development even up to the formation of the imago, but could not shed their pupal skins. This was also the case in other insects in which our pre-freezing method had been successfully applied.

Table 1. Prefreezing Temperature and Survival in the Sawfly Larvæ after Cooling to and Thawing from the Liquid Nitrogen Temperature

	Prefreezing at (° C)	Prefreezing for (h)	No. insects used	No. surviving insects
Control*	−30	4·0	10	9
1	−30	1·5	10	9
2	−25	1·5	10	10
3	−20	1·5	10	10
4	−15	1·5	10	0

* Prefreezing only.

The possession of small-molecule neutral substances has been thought to favour survival during freezing[2,5]. The protective substance has been shown to be sugar, mainly sucrose, in higher plants, and polyhydric alcohol, mainly glycerol, in insects. No glycerol was found in the prepupa of *T. populi*, in which a remarkably large amount of sugar was detected. The total sugar content of the overwintering prepupæ ranged from 5 to about 9 per cent of the fresh body-weight (average more than 6·3 per cent). It was nearly constant throughout the 4 months cold season. The termination of diapause, therefore, seems to have no direct effect on the high sugar content in this insect, because the prepupæ were generally released from diapause by the beginning of January.

The sugar in the prepupæ was identified by a chromatographic method[6]. In midwinter about 97 per cent of the sugar content was the non-reducing disaccharide, trehalose, which has recently been shown to be the major blood sugar in a variety of insects[7]. As the water content of the prepupa of *T. populi* was found to be about 65 per cent, the concentration of sugar in solution in the insect must be about 9 per cent. So far as we are aware, this is a higher concentration of trehalose than has previously been recorded in any insect. The concentration of sugar in these sawfly larvæ is comparable with that of the sum of polyhydric alcohol and sugar in very frost-hardy plants[8].

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MICROBIOLOGY

Lysogenic Conversion in *Staphylococcus aureus*, to a Change in the Production of Extracellular 'Tween'-splitting Enzyme

Observations on death or survival of 554 patients with staphylococcal bacteræmia[1,2] indicated a correlation between virulence in this situation and the lack of diffusible extracellular lipase as investigated on 'Tween'/calcium agar plates. Therefore an attempt was made to discover whether the negative lipase reaction was a

marker for other properties of the strains, for example, a lowered membrane permeability or a factor inhibiting the lipase or blocking the production of this enzyme.

The approach to this problem included a survey of 10,000 strains from 10,000 persons, 1,507 of which were phage type 80/81, and 235 lysed by typing phage 80 only. In the former group 15 per cent were 'Tween' negative (*TW*−), as against 80 per cent in the latter group, which suggested a connexion between the negative 'Tween' reaction and a loss of sensitivity to phage 81, possibly due to lysogenization[3,4].

In order to investigate this possibility we isolated, using the chloroform method described by Kjems[5], from a *TW*-type 80 strain, a phage (24724′) which lysed a *TW*+ type 80/81 strain. All 40 colonies from surviving bacteria of the latter strain after exposure to the filtrate of phage 24724′ (titre 10^9/ml.) were by means of replica tests[6], demonstrated to be lysogenic for phage 24724′, resistant to phage 81, and to be *TW*−. In repeated experiments 84–87 per cent of the cells from the receptor strain cells were lysed, and the remaining colonies were all lysogenic and *TW*−.

A phage with converting properties similar to that of phage 24724′ was isolated from each of a further four *TW*− type 80 strains examined. A phage isolated from a *TW*+ type 80 strain was able to lysogenize the receptor strain and change the type from 80/81 to 80, but—as expected—did not cause any change of the 'Tween' splitting ability.

Conversion experiments with phage 24724′ were made using 20 *TW*+ type 80/81 receptor strains. In all cases the phage-resistant colonies were lysogenic for 24724′, had lost sensitivity to typing phage 81, and were 'Tween'-negative.

Furthermore, in several instances it was possible to detect *TW*+ mutants in those *TW*− type 80 strains carrying converting phages; on examination these proved to be non-lysogenic phage type 80/81.

It was concluded that the negative 'Tween' reaction in the type 80 strains investigated was due to lysogenic conversion, as: (1) all lysogenic colonies were *TW*−; (2) the conversion occurred even when the converting phages were propagated on a *TW*+ strain; (3) loss of the converting phage caused a reversion to *TW*+.

This evidence suggests that the presence of a prophage may influence virulence in *Staphylococcus aureus*. However, it has not been shown whether changes in the bacterial membrane or factors altering the enzyme production are responsible for the negative 'Tween' reaction.

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Production of Aposymbiotic Cockroaches by means of Lysozyme

IT has long been known that special cells (bacteriocytes) within the abdominal fat body of the cockroach are packed with rod-shaped bodies presumed to be symbionts[1]. This intracellular symbiosis, obligatory in Blattariae, is unusual in that cockroaches are omnivorous and symbionts occur predominantly in insects the diet of which is deficient in certain essential nutrients. The production of symbiont-free cockroaches, so-called aposymbiotic insects, is an aid to the investigation of the significance of the symbiosis. Several authors[2–5] have reported the elimination of the cockroach symbionts by feeding or injecting antibiotics and bactericidal drugs.

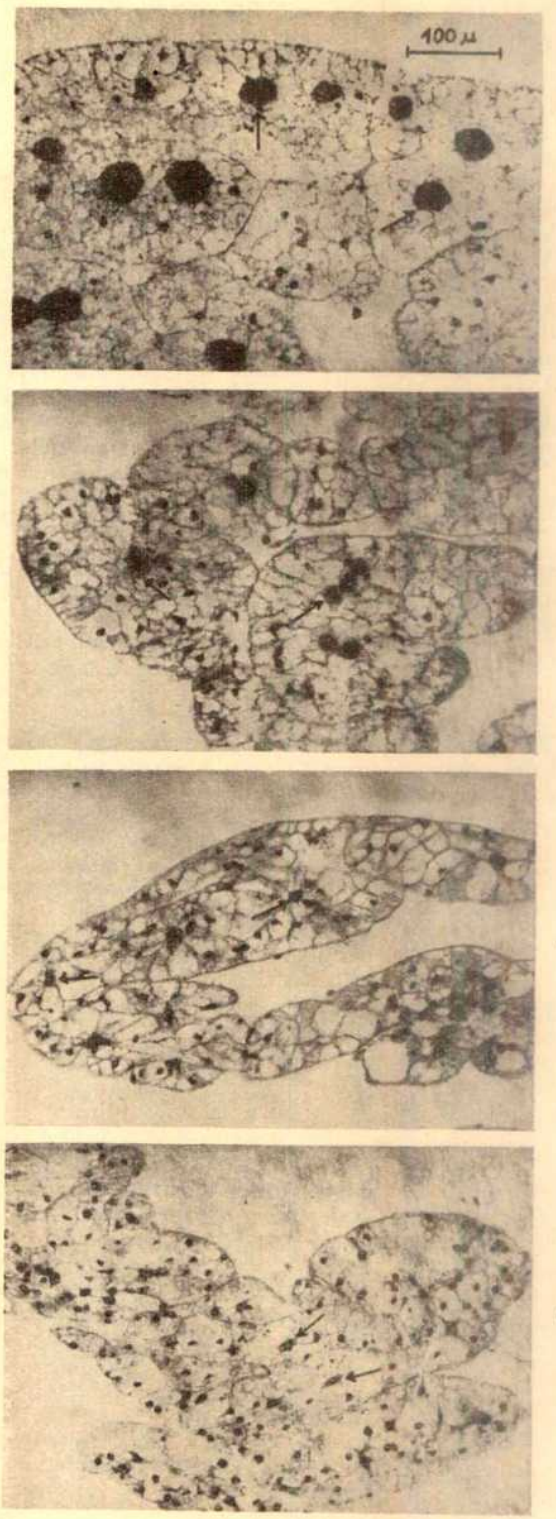


Fig. 1, *a–d*. Photomicrographs of sections through the abdominal fat body of *Periplaneta americana* L. nymphs. Fixed in Bouin's fluid and stained with Giemsa's solution; cut at 8μ. Photographs were made with objective 10, eyepiece 6·3, and a green VG 4 filter (Carl Zeiss, Jena). The magnification is the same throughout. Arrows point to some of many bacteriocytes in the fat body. (*a*) Normal NaCl-treated control. (*b*) 25 days after the first lysozyme injection. (*c*) 70 days after the first lysozyme injection. (*d*) 123 days after the first lysozyme injection

However, it is always difficult to say whether this treatment produces deleterious effects on the insects or whether abnormal reactions by the host are due to loss

of symbionts. Furthermore, Brooks[6] emphasized that complete elimination of symbionts is out of reach unless the dose is high enough to kill the host, and concluded that "the only way to obtain aposymbiotic insects is by preventing the original infection in the offspring".

In a previous paper[7], I have suggested that the symbionts of the cockroaches are lysozyme-sensitive and therefore are lysed by the enzyme. In the present experiments, 1 per cent lysozyme from egg white in 0·5 per cent NaCl solution was injected directly into the body cavity of the nymphs in three 0·05 ml. doses at 3-day intervals. Control animals received only 0·5 per cent NaCl solution. The condition of the bacteriocytes and their symbionts was examined by phase contrast observation of smear slides of the fat body and, after fixation in Bouin's fluid, sectioning in paraffin and staining in Giemsa's solution.

Fig. 1*a–d* shows the results. It can be seen that the symbionts disappear under the influence of lysozyme. The bacteriocytes become depopulated and smaller in size, but their nuclei remain clearly visible and do not shrink. As a rule, one injection is sufficient to disintegrate all the symbionts within a few days, but cell débris is still seen months afterwards. Normal bacteriocytes become deep blue in Giemsa's solution whereas lysozyme-treated bacteriocytes get a reddish colour and only their nuclei remain blue-stained.

The method has the following advantages: (*a*) lysozyme is not toxic; the mortality is as low as among the NaCl controls. Even in extremely high concentrations (22 injections) the insects tolerate this agent; (*b*) unlike antibiotics, lysozyme affects resting cells, and so eliminates the symbionts more rapidly; (*c*) lysozyme resistance has not been observed; this implies that all the bacteria are apparently killed, leaving no residue to repopulate the insect; (*d*) the loss of the symbionts is demonstrable in the treated generation and not only in their offspring.

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Isolation and Significance of *Streptococcus faecalis sensu strictu*

In an earlier communication[1] a medium was briefly described which had been developed at the Laboratories of the Metropolitan Water Board for the isolation of certain strains of *Streptococcus faecalis* with a combination of attributes which offered the possibility of distinguishing fæcal pollution of predominantly human origin in some climatic regions. Strains which possess the distinctive characteristics of simultaneous high tyrosine decarboxylase activity on a sorbitol substrate and the ability to reduce tetrazolium chloride to formazan may be isolated on the thallous acetate medium which selectively demonstrates the presence of these organisms.

The possible use of the characteristics of high tyrosine decarboxylase activity for distinguishing strains of *Strep. faecalis* from other Group D streptococci was apparent from a consideration of the findings of Sharpe[2]. In an analysis of human-derived strains of fæcal streptococci Sharpe revealed that strains of *Strep. faecalis* var. *faecalis* and the varieties *zymogenes* and *liquefaciens* could be grouped together on the basis of a high tyrosine decarboxylase activity, and that other species and unclassifiable intermediate strains of Group D streptococci possessed little or no tyrosine decarboxylase activity. Moreover, the activity of the enzyme was clearly associated with other biochemical characteristics, and no raffinose-fermenting strains were encountered which produced this enzyme. Hence, high tyrosine decarboxylase activity was associated with biochemically more 'typical' strains which have been referred to in the present investigation as *Strep. faecalis sensu strictu*, and since Sharpe did not further sub-divide her strains to distinguish *Strep. faecium* from *Strep. faecalis* it was necessary to include the criterion of tetrazolium chloride reduction for this purpose in the tyrosine isolation medium since it is known that some strains of *Strep. faecium* possess the ability to decarboxylate tyrosine.

Investigation of the extent to which strains of *Strep. faecalis sensu strictu* are restricted to the human alimentary tract, and the implications of this restriction, in assessing the origin of fæcal pollution, is continuing. As the relative predominance of *Strep. faecalis* in the human gut is probably related to diet[3] it is highly probable that the incidence of strains with high tyrosine decarboxylase activity is also affected by the constitution of the diet, so that the significance of *Strep. faecalis sensu strictu* in assessing pollution may differ in different countries. This fact does not suggest a hard-and-fast yardstick by which all human-derived strains of *Strep. faecalis* may be separated from all animal-derived strains. This is a possibility which may be realized in the heat tellurite test[4,5] for those strains which are heat-resistant, though for the purpose of an ecological survey of bacteria it is a considerable advantage to have the use of a direct isolation medium for selecting certain organisms by easily recognizable characteristics. The heat-tellurite test is a relatively lengthy procedure demanding pure cultures and particular attention to certain technical details[6,7].

A recent communication by Mundt[8] prompts me to reiterate in greater detail some of the points made in my original contribution together with a reference to some supporting experimental results which will be published more fully at a later date. Mundt has equated the use of the term *Strep. faecalis sensu strictu* with the variety *Strep. faecalis* var. *faecalis*, although this was not implied in the original usage which is independent of varietal status. In fact it has been suggested that the designation of varieties within this species be discontinued following the recent detection of a more widespread proteolytic ability among strains of *Strep. faecalis* and *Strep. faecium*[10], and the long-recognized instability of the property of hæmolysis in certain strains. These findings are contrary to the arguments in favour of attempting to recognize consistently definable varieties on the basis of only two unreliable characteristics. Thus, it is surprising that Mundt distinguishes *Strep. faecalis* var. *liquefaciens* as a separate species.

There is scope for a new approach to the problem of separating human- from animal-derived strains. For this purpose there are some possibilities arising from the results concerning *Strep. faecalis sensu strictu* presented in Table 1 in which the percentage distribution of the organisms has been determined in the fæces of 50 individuals from each of a number of animal species and man. The animals are those species which are most likely to pollute water or milk supplies[4], including wild birds. A significantly higher incidence in man of *Strep. faecalis sensu strictu* and in fact of all strains showing high tyrosine

Table 1. Isolation of *Streptococcus faecalis* from 50 Individuals from Each Host

Host	No. of individuals with *Strep. faecalis sensu strictu*	No. of individuals with colonies of all types showing high tyrosine decarboxylase	No. showing no growth	% of Individuals with *Strep. faecalis sensu strictu*
Man	26	26	4	52
Cattle	2	3	8	4
Sheep	4	8	2	8
Deer	6	16	4	12
Rabbit	5	12	23	10
Bird	4	8	15	8

decarboxylase activity on the tyrosine medium may be noted. Quantitative results for *Strep. faecalis sensu strictu* have also been obtained varying from 0 to $18{\cdot}4 \times 10^4$ per g of fresh fæces for human material and 0 to $7{\cdot}46 \times 10^2$ per g for sheep and cattle.

The finding of Mundt that not all human-derived strains of *Strep. faecalis* are able to produce high tyrosine decarboxylase activity is in agreement with the findings in the present investigation, and seemed likely from an examination of Sharpe's earlier results[2]. However, it is suggested that the greater frequency of *Strep. faecalis sensu strictu* in human fæces than in that of most animals may be best exploited in quantitative determinations, the possibilities for which have been referred to elsewhere[11]. The results so far obtained apply to the distribution of *Strep. faecalis sensu strictu* in this country only, which may be different from other regions with a more widely-ranging fauna and/or different dietary habits among the human population.

Mundt[8] has also raised some criticisms relating to the use of the tyrosine medium for the quantitative recovery of strains of *Strep. faecalis* when compared with the medium of de Man, Rogosa and Sharpe (MRS) and using pure cultures of the test organisms. The anomalous results obtained suggest that a 1 per cent concentration of thallous acetate may have been employed instead of 0·1 per cent, owing to an error in the published formulation of the medium which was subsequently corrected (in reprint copies of the communication). A comparison between the highly selective tyrosine medium (thallous acetate + sorbitol) and the rich and completely non-selective MRS medium designed for the cultivation of lactobacilli is of little practical value. In the present investigation a comparison has been made between the tyrosine medium and that of Barnes[12], which is used at the same *p*H and includes the same selective inhibitor as the tyrosine medium. From a range of 20 fæcal samples from various sources the results obtained were as follows: in 9 samples a significantly higher total yield of colonies was isolated on the tyrosine medium; in 4 samples Barnes's medium produced a greater yield, while the remaining 7 samples gave comparable results by both methods. However, it is possible that subsequent improvements may be made in the quantitative recovery of *Strep. faecalis* by the tyrosine medium. The necessity for resuscitation at 37° C prior to incubation at 45° C has been emphasized already[13], particularly when attenuated strains are to be isolated from water.

The isolation of *Strep. faecalis sensu strictu* is not an exclusive index of human fæcal contamination in all situations, but in certain instances it can yield valuable evidence in assessing the origin of pollution. The necessity for future investigations in this field is pointed to in the words of a recent review[9]: " . . . continued study with the streptococci is necessary in regard to the development of specific media for species isolation, and their incidence under field conditions in various water supplies. . . ."

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CYTOLOGY

Sedimentation Rate of Cells in Metaphase as compared with Cells in Interphase

In recent years there has been considerable interest in the study of the chromosome complement of malignant tumours. In most of these investigations culture techniques have been utilized. Direct methods of study have an advantage in that there can be no questions of selection of particular clones that happen to proliferate better in *in vitro* conditions. Direct methods have worked well in examination of bone marrow and neoplastic effusions. However, study of solid neoplasms has been hampered by the fact that usually only a few metaphases suitable for karyotyping can be obtained from any given tumour.

During the course of investigations of solid neoplasms we noted considerable variation in the number of metaphases from slide to slide prepared from the same suspension, and decided to see if such variation might be due to a difference in sedimentation rate of cells in interphase and metaphase. If such a difference existed, advantage could be taken of this to concentrate cells in metaphase for examination. The following experiments were designed to detect any such difference.

Leucocyte cultures were prepared from peripheral blood by standard techniques. (We thank Dr. William Mellman of the Department of Pediatrics, Hospital of the University of Pennsylvania, for providing us with many of the chromosome preparations used in these experiments.) All glassware used in the experiments was siliconized. Heavy cell suspensions of cultured leucocytes in Carnoy's fixative were thoroughly mixed to obtain uniform distribution of cells. Five capillary blood coagulation tubes (Precision grade, Drummond Scientific Co., Cat. No. 1200) were partly filled with the cell suspension by capillary action. The capillary tubes were pre-calibrated so that the volume of any given column of liquid could readily be calculated from the formula:

$$V = 3{\cdot}1416\, r^2 \times L$$

where V = volume of fluid (ml.),
r = internal radius of the tube (cm)
L = length of liquid column (cm)

One of the five tubes served as a control and the remainder were placed in a vertical position in 'Seal-Tube Paste' (Clay-Adams, New York) and allowed to sediment for 5, 10, 15 and 20 min.

At the end of each period the tube was removed from the paste and placed in a horizontal position, and the sealed end of the capillary together with any sediment that may have been present was discarded. The length of the remaining liquid column was measured, the volume calculated, and the entire contents were expelled on to a wet, pre-cooled slide with the aid of small rubber bulb. The cell preparation was spread on the slide by the 'flame' technique making certain no significant quantity of material was lost through spillage and that the cell suspension was spread over a relatively small area of the slide to make counting easier. All slides were stained for 20 min in 10 per cent Giemsa, decolorized through successive changes of acetone, acetone-xylene, cleared with xylene and mounted with 'Permount'. All the metaphases on each slide were counted and the number of metaphases per millilitre of suspension was calculated. In addition, relative counts of the number of metaphases per 1,000 interphases were performed.

In a second experiment, a Leffman-Beam type of centrifuge tube was used (Arthur H. Thomas, Phila., Cat. No. 2998–*G*–65) in order to test the same principle. 0·12 millilitre of a thick cell suspension of cultured leucocytes was allowed to stand vertically in the centrifuge tube for 20 min. At this time, with the aid of a siliconized, capillary pipette (Pasteur type) with a very

fine tip, the supernatant and the upper and lower portions of the sediment were sampled and slide preparations were made as above. In this case only relative counts of the number of metaphases per 1,000 interphases were performed.

Table 1. Rate of Settling of Metaphases as compared with Interphases as indicated by the Number of Metaphases per Millilitre and the Number of Metaphases per 1,000 Interphases

Tube	Sed. time (min)	Length of column (cm)	Volume (ml.)	Total No. metaphases	No. metaphases per ml.	No. metaphases per 1,000 interphases
A	0	2·0	$1·60 \times 10^{-2}$	165	10,312	20
B	5	1·5	$1·20 \times 10^{-2}$	74	6,167	24
C	10	2·8	$2·24 \times 10^{-2}$	83	3,733	20
D	15	2·1	$1·76 \times 10^{-2}$	49	2,882	18
E	20	1·8	$1·44 \times 10^{-2}$	44	3,142	19

Table 1 summarizes one set of values obtained in the first experiment and is representative of the results obtained with ten cell suspensions from different leucocyte pools.

These findings indicate that the ratio of metaphases to interphases remains essentially constant throughout the process of sedimentation. The total number of metaphases/ml. of supernatant in each capillary tube fell progressively with time. Both findings strongly suggest that cells in metaphase sediment at the same rate as cells in interphase.

Table 2. Relative Counts (Metaphases/1,000 Interphases)

Exp. No.	Supernatant	Upper level of sediment	Lower level of sediment
1	12	14	15
2	18	22	23
3	27	23	24
4	18	18	20
5	6	8	5
6	8	6	10

Table 2 shows the results of the second experiment. Since the total volume of cell suspension used for the preparation of each slide in this case was not accurately known, only relative counts were recorded. A total of six separate determinations were carried out.

These results confirm the findings of the first experiment, that is, they strongly suggest that with the methods employed no difference in the sedimentation rates of cells in interphase and cells in metaphase exists. Whether the same holds true for tumour cells in suspension was not determined.

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GENETICS

Mutants of *Salmonella typhimurium* induced by 5-Bromodeoxyuridine

Freese has shown in a series of papers[1–3] that most mutants induced by 5-bromouracil or 5-bromodeoxyuridine in bacteriophage *T*4 can be induced to back-mutate by these same base analogues. This important observation led him to propose as one type of mutation the substitution of a G-C pair in the DNA for an A-T pair by a mechanism that necessarily could work in reverse. We have carried out similar experiments in the course of a study of the mutagenic action of nucleotide base analogues in *Salmonella typhimurium* and the results are somewhat different from those obtained with the bacteriophage.

Auxotrophs were induced by incubating an aerated culture of the wild type strain of *Salmonella typhimurium* known as 'LT-2' in 1 per cent 'Difco' nutrient broth supplemented with 200 μg/ml. of 5-bromodeoxyuridine, and the same concentration of aminopterin (to suppress the formation of thymine), for 18 h. After screening by the standard penicillin technique[4] 83 auxotrophs were isolated and characterized. Twenty-four were shown to be genetically different and the induction of reversions in 13 of these was examined (Table 1). These data were obtained by growing each auxotrophic strain to saturation in broth containing the base analogue and aminopterin. The bacteria were centrifuged and washed with saline before plating about 10^8 cells on each of 3 Petri dishes with enriched minimal media. The data are the average number of fast-growing colonies per plate that appear; slow-growing colonies are not included as they are unlikely to be back-mutations. Some of the fast-growing colonies also may not be back-mutations, but our experience with *Salmonella typhimurium* shows that this will probably be true of very few of them. The data on the frequency of spontaneous mutants were obtained similarly; the broth cultures contained no base analogue or aminopterin and the bacteria were plated when the population of cells had reached the same density as that of the saturated cultures with the base analogues.

Reconstruction experiments showed that prototrophic cells were not at a selective advantage or disadvantage in the media with or without base analogues. We were also able to show, by sampling the broth cultures at different times, that in the presence of bromouracil and aminopterin the induction of mutations occurred mainly in the last two cell generations of the culture whereas with bromodeoxyuridine and aminopterin the induction started somewhat earlier. Aminopterin without a base analogue does not induce mutations. Bromouracil and bromodeoxyuridine, but not aminopurine, require aminopterin to become mutagenic.

Table 1. Frequencies of Fast-growing Reversions of 13 5-bromodeoxyuridine-induced Auxotrophs of *Salmonella typhimurium* in the Presence of 2-aminopurine (AP), 5-bromouracil (BU), 5-bromodeoxyuridine and in the Absence of any Base Analogues (C). (The Range indicates Extreme Frequencies in 2 or more Repeat Experiments)

Auxotrophs	Reversions/10^8 cells			
	C	AP	BU	BD
cys-1053	0·0–0·3	40·3–77·7	0·5–1·7	1·0–3·0
cys-1054	0·0–0·3	27·0–31·3	0·0–0·3	0·3–0·7
cys-1055	0·0–0·3	22·0	59·5	c.700
cys-1056	0·3	23·7	3·3	19·7
arg-149	0·3–1·3	10·0–33·0	0·5–0·7	0·3–0·7
ser-703	0·0–0·3	0·7–1·3	8·3–27·0	15·0–15·7
ser-704	0·3	107·3–129·3	9·7–24·3	110·0–309·0
ser-705	1·5–4·5	57·0–106·3	98·7–115·3	c.3000
ser-706	0·0–1·7	28·7–46·0	0·0–8·7	2·0–8·3
pyr-190	0·3–0·7	7·3–119·3	0·0	11·0–29·3
gua-14	0·0–0·3	15·0	0·0	3·3
gua-15	0·0	0·0–19·0	0·0	0·0–0·3
nic-7	0·0	0·0	0·0	0·0

From Table 1 the order of decreasing effectiveness of the base analogues is bromodeoxyuridine, bromouracil and aminopurine on mutants *cys*-1055, *cys*-1056, *ser*-703, *ser*-704, *ser*-705 and possibly *pyr*-190. This was the most common order of effectiveness found on 9 spontaneous and 18 aminopurine-induced mutants. Omitting the completely stable *nic*-7 auxotroph all the remaining 6 mutants were most sensitive to aminopurine while they responded little, if at all, to the pyrimidine analogues; a relative effectiveness that we have not found in any spontaneous or aminopurine-induced mutant. Four of the 6 gave even higher frequencies of reversions with aminopurine when thymine, and to a lesser extent adenine, was added to the broth. Although the reversion of some of the other 5-bromodeoxyuridine-induced auxotrophs by aminopurine was also enhanced by thymine this effect was absent in all of four spontaneous and five aminopurine-induced mutants that were tested.

It appears that, in *Salmonella typhimurium*, bromodeoxyuridine induces mainly two types of mutation

which are distinguishable by their sensitivity to aminopurine on one hand, and to bromodeoxyuridine and bromouracil on the other, in inducing back-mutations. One type can be reverted more readily by the pyrimidine analogues than by aminopurine. The other type responds poorly, if at all, to the pyrimidine analogues but responds well to aminopurine; the effectiveness of aminopurine on most of these mutants is enhanced by thymine. We have not found this second class among spontaneous or aminopurine-induced mutants; it appears to be one of the two main classes among bromodeoxyuridine-induced mutants. As it is induced by a base analogue it is likely to be a base-pair change—a change that cannot be readily reverted by the base analogue that induced it.

It seems from this and other work that the pattern of induction and reversion of mutations in *Salmonella typhimurium* is more complex than the patterns described in bacteriophage.

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SOIL SCIENCE

Resistance of Tea Plants to Blister Blight in Soils augmented with Nickel

In 1961, protective spraying of tea plants with nickel chloride hexahydrate gave dramatic control of the blister blight disease[1], and later certain salts of nickel were shown to possess eradicative control properties when applied in a dilute aqueous solution to tea foliage 3–5 days after inoculation with *Exobasidium vexans* Massee, the causal pathogen[2]. Very recently it has been observed that, in soils augmented with nickel ammonium sulphate and nickel nitrate, tea plants developed disease resistance.

Potted tea plants of susceptible monoclonal selection, 12 months from cutting, were pruned back to 6 in., incubated in a plastic house and used for the experiment when they had produced 3–4 shoots. The 5 lb. of soil in each pot was treated with 1, 2 or 3 g of nickel ammonium sulphate, applied on the surface and watered down, untreated controls being maintained. The young leaves in four plants in each treatment, and the control, were artificially inoculated with spores of *E. vexans* by the usual method[2], 5, 8, 15 or 20 days after the nickel was applied to the soil. After inoculation the plants were incubated at room temperature (20°–30° C), protected against natural infection from air-borne spores. Observations were made on the number of lesions that developed, and the number of sporulating blisters, up to 14 and 21 days, respectively, after inoculation.

Results (Table 1) showed that the number of lesions developing on plants in soil treated with nickel ammonium sulphate 5 days before inoculation was markedly lower when compared with the control. In the lots inoculated 8, 15 and 20 days after treatment, no lesions formed on plants treated with nickel ammonium sulphate at 3 g/pot; at the lower dosage of 2 and 1 g the plants developed only a few lesions whereas control plants were heavily infected. Similar results were obtained when nickel nitrate was used. No specific influence of nickel application on sporulation was noticed; lesions which formed on treated plants sporulated in the same proportion as those in the untreated ones. Symptoms of iron chlorosis developed in the second week in the treated plants and growth was slightly retarded.

Table 1

Nickel ammonium sulphate treatment	Number of lesions on 4 plants maintained for 5, 8, 15 and 20 days in treated soil							
	5		8		15		20	
1 g/pot	87 (10)	**52***	14 (12)	**14**	5 (14)	**4**	23 (13)	**19**
2 g/pot	126 (19)	**91**	33 (10)	**22**	2 (12)	**2**	8 (11)	**6**
3 g/pot	44 (18)	**30**	0 (13)		0 (18)		0 (14)	
Control	261 (13)	**225**	201 (14)	**180**	141 (14)	**114**	256 (14)	**219**

* Figures in parentheses give the number of leaves examined, those in bold type the number of lesions which sporulated.

In preliminary experiments in the field, when 4-year-old clonal plants were each given 4, 8 and 12 g nickel ammonium sulphate, dibbered into the soil around the collar and watered down, they seemed to develop resistance to blister blight for 4 weeks following treatment, but mature bushes which received 15 g showed no such resistance. No increase in nickel level was observed in the leaf from these mature bushes when analysed by dry ashing polarographic technique 2, 3 and 4 weeks after soil treatment. Symptoms of iron chlorosis were not seen in either the 4-year-old clonal plants, or the mature bushes, at these rates of nickel application. Leaves from different clones growing in the same locality were analysed, when it was seen that one which is immune to blister blight contained 14–25 p.p.m. nickel, as opposed to 6–10 p.p.m. in two susceptible clones, indicating that in tea plants nickel may have an important role in host resistance to blister blight.

Help from the International Nickel Company (Mond), Ltd., London, in the analysis of leaf samples for nickel content is gratefully acknowledged.

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Simple Estimation of Soluble Salts in Glasshouse Soils

For a number of years the National Agricultural Advisory Service has been carrying out routine determinations of electrical conductivity on aqueous extracts of glass-house soils as an advisory service to horticulturists. Extracts are usually prepared at a water : soil ratio of 2·5 : 1 by shaking 16 g of soil with distilled water for 15 min. After settling, conductivity determinations on the clear extract are carried out at a temperature of 20° C and the results are usually expressed as pC or Cf values, pC being the negative logarithm of the specific conductivity and Cf being the specific conductivity multiplied by 10^4. These values have been classified on the basis of soluble salt content of soils and effect on plant growth[2], and are often used to predict harmful effects to plants due to accumulation of fertilizer residues.

It has been recognized for some time that the interpretation of pC values has certain difficulties caused by the properties of calcium sulphate which tends to accumulate in glasshouse soils where sulphate fertilizers are used or where the water is hard. Calcium sulphate cannot reach a high concentration in the soil solution owing to its low solubility, and it has been shown by experimental work (ref. 3, confirmed in this laboratory) that calcium sulphate is harmless to lettuce and tomato plants even when present in large excess. It can, however, greatly affect laboratory measurements of conductivity when carried out at a high solvent : soil ratio[4,5] for convenience in routine analysis. This is because at a high solvent : soil ratio much more calcium sulphate can dissolve, if a considerable amount is present in the soil, than would be in solution when plants were growing in the soil.

Winsor *et al.*[3] found that the difficulties due to calcium sulphate could be eliminated simply by determining the conductivity in a soil extract obtained by using saturated calcium sulphate solution instead of water[5], thereby eliminating calcium sulphate as a variable in the extract.

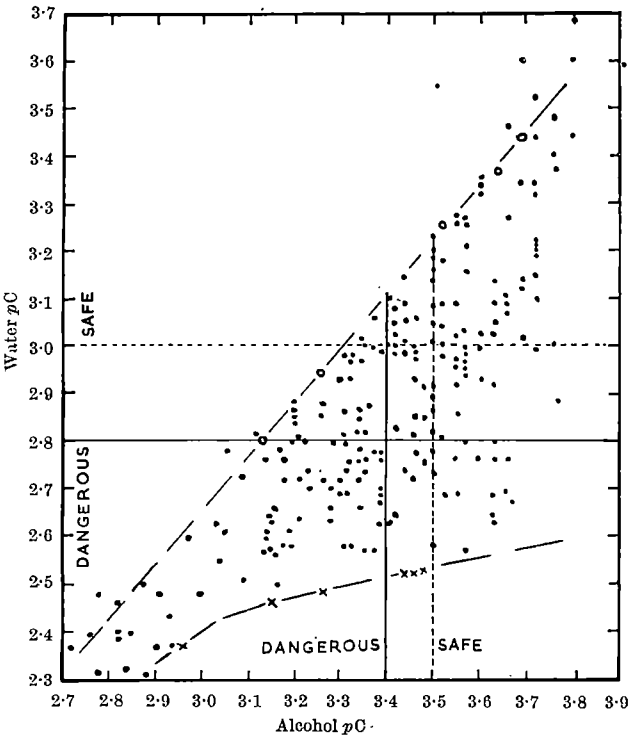


Fig. 1. The relation between *p*C readings, determined in water and in 25 per cent alcohol saturated with calcium sulphate, of soils with various calcium sulphate contents. ○ Soils known to contain no more than a trace of calcium sulphate. ● Routine 'advisory' soils. × Experimental soils to which excess calcium sulphate has been added

They pointed out, however, that the range of conductivity is restricted in this way and measurements must be precise. As the conductivity due to harmful salts will nearly always be much less than the conductivity due to calcium sulphate, when this is present in excess, small analytical errors may be important. In this paper a method is described which overcomes the difficulties due to calcium sulphate without restricting the range of values obtained.

Water–alcohol mixtures for preparation of extracts. The solubility of calcium sulphate in water (0·22 g/100 ml. at 20° C) was found to decrease sharply when ethyl alcohol was added until at 25 per cent by volume the solubility was reduced to approximately 0·01 g/100 ml. Further addition of alcohol had little effect, so the use of 25 per cent alcohol instead of water appeared to be a possible way of minimizing the effects of calcium sulphate on *p*C readings.

Salts which commonly occur in glasshouse soils were found to be sufficiently soluble in 25 per cent alcohol for complete solution of the amounts likely to be present to take place at a solvent : soil ratio of 2·5 : 1. The ratio of conductivity in 25 per cent alcohol to that in water was 0·5 for all salts tested except for magnesium sulphate for which the ratio was 0·4.

In 1961, 25 per cent alcohol, saturated with calcium sulphate to eliminate this salt as a variable, was introduced as an additional solvent for *p*C determinations in the West Midland Region of the N.A.A.S. Apart from the change of solvent the technique used was identical to the water *p*C method. As the solubility of calcium sulphate in 25 per cent alcohol is very small, the conductivity due to calcium sulphate is much less than that due to the highly soluble salts present, except in soils containing very small amounts of the latter.

The relation between water and alcohol pC readings. Since 1961 a considerable amount of information has accumulated on the relation between *p*C determinations on routine advisory soils from commercial glasshouses in the West Midlands using water and 25 per cent alcohol saturated with calcium sulphate as solvents. These data are summarized in Fig. 1, which also includes some data from soils known to contain no more than a trace, and some containing an excess, of calcium sulphate. The calibrations of the water *p*C method used in the West Midland Region before 1961, and of the alcohol *p*C method described here, are shown.

In Fig. 1 almost all the points from routine soils lie within the area bounded by broken lines through the points representing soils free of calcium sulphate and soils with excess calcium sulphate. Thus it is evident that the calcium sulphate content of soils from commercial glasshouses varies from no more than a trace to sufficient to saturate the water extract obtained at a solvent : soil ratio of 2·5 : 1.

The use of alcohol pC readings in advisory work. Since the introduction of the alcohol *p*C method, experience has shown that the readings obtained reflect the harmful effects of excessive fertilizer on plant growth. A tentative calibration of the method was obtained from experimental work on tomato plants and chrysanthemums. On the basis of this experimental work and advisory experience, alcohol *p*C values are at present classified as follows:

3·50 and above — safe
3·40–3·49 — doubtful
3·39 and below — dangerous

The alcohol *p*C method overcomes the difficulties due to the presence of calcium sulphate in the soil in a simple way and is suitable for routine laboratory work. Interpretation of the values obtained will be affected to some extent by variations of moisture-holding capacity, with texture and organic-matter content, as the determinations are made on a constant weight of soil. However, the calibration reported here has been found to be generally applicable in the West Midlands. It should be possible to make a more detailed calibration for different plants when further experimental work has been done.

I am indebted to Mr. G. H. A. Edwards, who initiated the investigation on the effects of calcium sulphate on plant growth and *p*C values, and to Mr. A. J. Cavell, who suggested the use of ethyl alcohol to minimize the solubility of calcium sulphate and supervised the laboratory work.

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Quantitative Analysis of Clay Mineral Mixtures by X-ray Diffraction

The basic relation underlying all quantitative diffraction analyses with the X-ray diffractometer is[1]:

$$I_1 = \frac{K_1 X_1}{\rho_1 (X_1(\mu_1^* - \mu_M^*) + \mu_M^*)} \quad (1)$$

From this it can be established mathematically that[2]:

$$\frac{I_A}{I_B} = \frac{K_A X_A \rho_B}{K_B X_B \rho_A} \quad (2)$$

where I_A and I_B represent peak intensities, K_A and K_B specific mineral constants, X_A and X_B the weight fractions and ρ_A and ρ_B the densities of components A and B in a diffracting mixture. This relationship holds whatever the amount or number of components. In equation (2) K_A, K_B, ρ_A, ρ_B are constants for minerals A and B. The equation thus reduces to the linear relationship:

$$\frac{I_A}{I_B} = \omega \times \frac{X_A}{X_B} \quad (3)$$

where I_A/I_B is independent of specific mineral as well as of matrix mass absorption coefficients (μ_A^*, μ_B^* and μ_M^*).

If the value of ω for mixtures of two diffracting components can be established by observations on mixtures containing these two components in known proportions, then the relative fractions of these two components in a mixture of unknown composition can readily be determined. Likewise, in a mixture of more than two diffracting components, the weight fractions of components in pairs can be estimated and, hence, using the relation:

$$X_A + X_B \ldots X_N = 1 \qquad (4)$$

an estimate of each weight fraction of each constituent can be obtained. Thus a simple procedure for the quantitative estimation of diffracting constituents in clay mixtures and other materials becomes available.

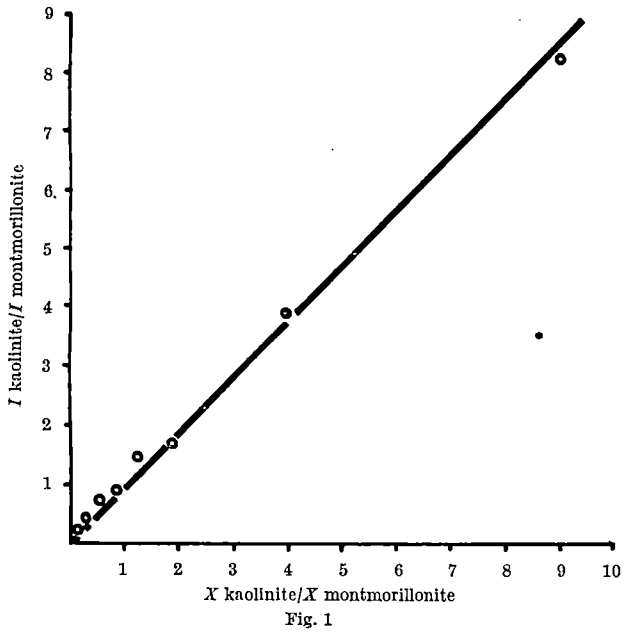


Fig. 1

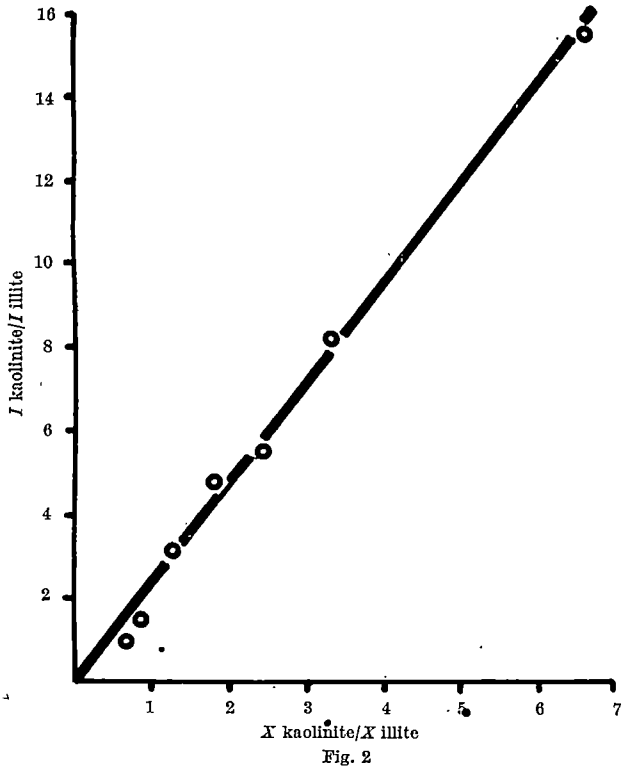


Fig. 2

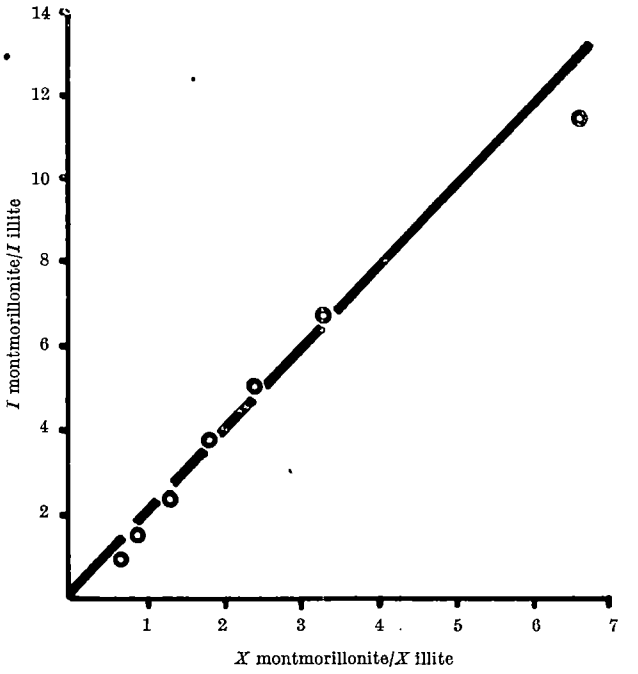


Fig. 3

The validity of relationship (3), and hence the validity of the estimation procedure based on it, has been confirmed for binary mixtures of kaolinite and montmorillonite, montmorillonite and illite, and kaolinite and illite, respectively, using type specimens of these minerals obtained from Wards Natural Science Establishment Inc., Rochester, New York. The samples were prepared for diffractometry by spreading small quantities of magnesium-saturated 2–0·2μ clay from the deposit at the bottom of a centrifuge tube evenly with unidirectional strokes on to petrographic microscope slides[3]. After air- and oven-drying, each sample was examined with a Philips direct-recording X-ray diffractometer, using copper $K\alpha$ radiation. Measurements of first order peak intensities were obtained by a 'fixed time' counting method. The linear relationship between I_A/I_B and X_A/X_B for each of the three mixtures is shown in Figs. 1–3.

Furthermore, the method was tested for mixtures containing three components. Using the straight-line relationships illustrated in Figs. 1–3, 15 different ternary mixtures were estimated by considering the intensity ratios of the kaolinite, montmorillonite and illite components in pairs. The final estimates, together with the actual compositions, are illustrated in Table 1. Nine of the 15 samples were estimated with an accuracy of ±3 per cent or better, 13 with an accuracy of ±4 per cent or better, and all 15 with an accuracy of ±4·5 per cent or better. If mixtures of extreme composition (10 per cent or less) of montmorillonite, illite and kaolinite are separated from those of

Table 1

Sample No.	Actual composition: Montmorillonite	Illite	Kaolinite	Estimated composition: Montmorillonite	Illite	Kaolinite	Deviation
3	83·0	6·5	10·5	78·5	10·0	11·5	4·5
1	78·0	16·5	5·5	75·0	17·5	7·5	3·0
5	63·5	10·0	26·5	59·5	12·0	28·5	4·0
8	50·0	16·5	33·5	50·5	14·5	35·0	2·0
9	41·5	23·0	35·5	40·0	23·0	37·0	1·5
15	40·0	20·0	40·0	42·0	17·0	41·0	3·0
6	38·5	29·5	32·0	40·0	27·5	32·5	2·0
4	38·0	39·5	22·5	38·0	38·0	24·0	1·5
14	35·5	23·0	41·5	36·0	21·0	43·0	2·0
10	28·5	20·0	51·5	26·5	18·5	55·0	3·5
12	25·5	3·5	71·0	22·5	7·0	70·5	3·5
2	23·5	53·0	23·5	21·0	54·5	24·5	2·5
11	16·5	16·5	67·0	19·5	13·5	67·0	3·0
13	10·5	6·5	83·0	12·5	8·5	79·0	4·0
7	9·0	46·0	45·0	13·5	41·5	45·0	4·5
				Total deviation			44·5
				Average deviation			2·97

more balanced composition, the average deviation of the latter is reduced to ±2·33 per cent.

Where an accuracy of ±10 per cent is adequate, on the basis of equation (1), the K constants for the individual minerals can be estimated using peak intensities of type specimens. The lower accuracy of this direct calculation procedure arises from the inherent inaccuracies attached to the estimation of specific mineral density and of mass absorption coefficients.

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FORESTRY

Field Technique for the Concentration of Rainfall within the Individual Seedling Root Range

Forestry nursery practice provides young seedlings, particularly during the critical period following germination, with conditions in which the necessary protection and adequate soil moisture are ensured. In the field, direct seeding in partially buried bamboo sleeves will ameliorate the seedling environment. However, under field conditions it is not feasible to augment soil moisture by watering, and the plant is therefore dependent on rainfall, which in many cases is unreliable and insufficient. Recent work by Glover and Gwynne[1] has demonstrated that the interception of rain by the aerial parts of established plants can concentrate otherwise ineffectual light showers of rain into useful quantities of water within the root range of the plant. An experiment on the direct seeding of three commonly grown exotic species *Pinus radiata*, *P. patula* and *Cupressus lusitanica* at Muguga, Kenya (1° S., 37° E., altitude 6,800 ft.), has shown that a rainfall conductor stick implanted in the soil adjacent to the sown seed increases the seedling survival rate, when used in conjunction with the bamboo sleeve technique (which will be described elsewhere).

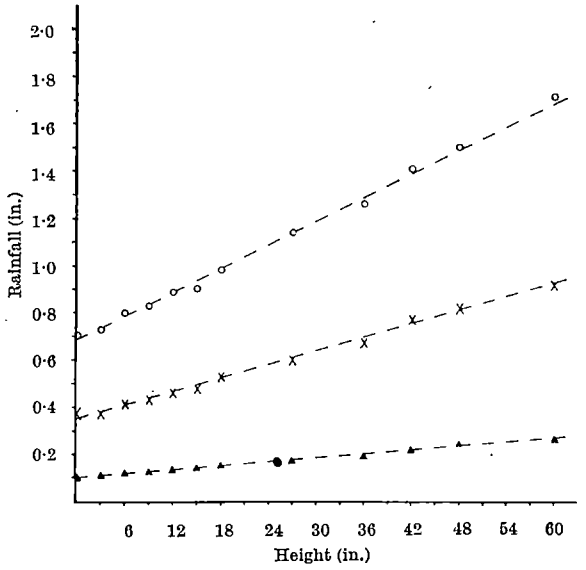


Fig. 1. The mean increase for 1-in. conductors, classified by three storm sizes. ○, rainfall class 0·50–1·00; ×, rainfall class 0·25–0·50; ▲, rainfall class 0·00–0·25

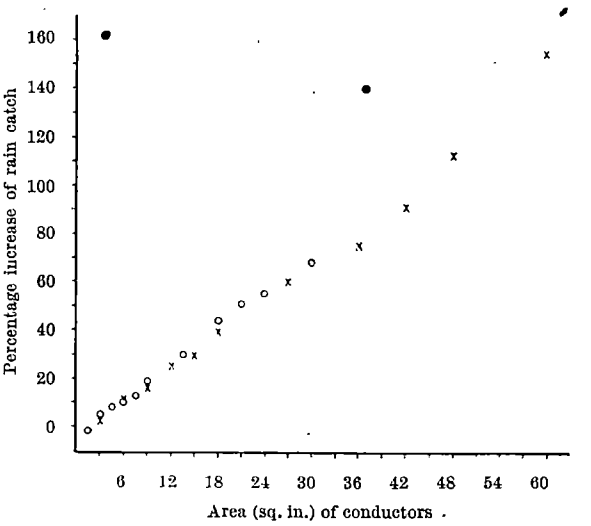


Fig. 2. Percentage increase of rainfall catch by conductors for all storms

To measure the catchment effect of these conductors, bamboo splits, 1 in. wide and 3 in. to 60 in. long, were mounted vertically in standard Snowdon-type rain-gauges and placed in line at right angles to the prevailing wind, with a similar gauge used as the control. After the first rainy season these conductor sticks were halved in area by reducing their width to 0·5 in. The daily rainfall catch was measured for two rainy seasons in 1963; it totalled 17 in. in 46 wet days and 13 in. in 43 wet days respectively. The rainfall pattern was similar for both seasons, with about half of the total rainfall in each period occurring as light showers of less than 0·25 in. These light showers would, under the evaporation conditions obtaining at Muguga, contribute little or nothing to the soil moisture build-up[2]. The measured mean daily wind speed at the site ranged from 5–9 m.p.h., and as few rainstorms were accompanied by strong winds the angle of rainfall for the vast majority of the storms was near the vertical.

The mean daily rainfall totals during the first season, when the 1-in. conductor sticks were in operation, have been classified by storm size into three groups as shown in Fig. 1; the efficiency of the conductor is maintained in all three classes. Fig. 2 illustrates the effect of surface area on rainfall catch for both widths of stick measured over the two rainy seasons. Both figures show that for each class of rainfall the gain in water is consistently proportional to the surface area of the conductor sticks. A depression of the order of 2 per cent occurred in all catches due to the reduction in receiving area of the rain-gauge consequent on the installation of the bamboo sticks; this cancelled out the gain in rainfall from the 0·5 in. × 3 in. stick, but for larger conductors the effect was negligible compared with the increased rain catch. A conductor stick of practical size for field work, say, 30 sq. in., will increase the quantity of water arriving at the base of the stick by more than 60 per cent. Thus, rainfall conductor sticks effectively increase the soil moisture within a given area and thereby enhance the conditions for plant survival and growth. In areas where the daily loss by evaporation is high, the conductors can turn ineffectual light showers into beneficial concentrations of water.

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East African Agriculture and
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APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

EXPERIMENTAL OFFICER (with a degree or diploma in chemistry and appropriate experience) IN THE CHEMISTRY DEPARTMENT, for handling and analysis of crop and soil samples and to develop analytical methods for nitrogen—The Secretary, Rothamsted Experimental Station, Harpenden, Hertfordshire (December 24).

LECTURER IN CHEMISTRY to teach in O.N.C. and G.C.E. "A" level courses and to assist in the organization of the Science Department; and an ASSISTANT LECTURER, Grade B, FOR BIOLOGY, to teach G.C.E. "O" and "A" level biology mainly to full-time courses—The Principal, North-East Essex Technical College and School of Art, Sheepen Road, Colchester, Essex (December 26).

ADMINISTRATIVE SECRETARY (man or woman, with administrative experience, and preferably a degree or qualification in geography or education) to take general charge of the headquarters in Sheffield—The Honorary Secretary, The Geographical Association, 343 Fulwood Road, Sheffield, 10 (December 30).

ENTOMOLOGIST to the Citrus Research Unit, University of the West Indies, Kingston, Jamaica—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (December 30).

LECTURER or ASSISTANT LECTURER IN LOGIC AND METAPHYSICS; and a LECTURER or ASSISTANT LECTURER IN MORAL PHILOSOPHY—The Secretary, The University, Aberdeen (December 30).

D.S.I.R. RESEARCH ASSISTANT (honours graduate in engineering or physics) IN THE DEPARTMENT OF MECHANICAL ENGINEERING, to carry out investigations in the field of electrostatic precipitation—Prof. H. E. Rose, King's College (University of London), Strand, London, W.C.2 (December 31).

LECTURER or ASSISTANT LECTURER IN THE SCHOOL OF BIOLOGICAL SCIENCES—The Registrar, University of East Anglia, Earlham Hall, Norwich, NOR 05G (December 31).

LECTURER or ASSISTANT LECTURER (or, exceptionally, Senior Lecturer) IN SOCIOLOGY—The Assistant Registrar, University of Sussex, Stanmer House, Stanmer, Brighton, Sussex (December 31).

LECTURER (with an honours degree with specialization in modern algebra or analysis, and three years teaching experience, preferably at university level) IN MATHEMATICS at the University of Nigeria, Nsukka, Eastern Nigeria—The London Representative, University of Nigeria, 33 Craven Terrace, London, W.2 (December 31).

SENIOR LECTURER (with high academic qualifications and relevant teaching or industrial or research experience) IN MAMMALIAN PHYSIOLOGY IN THE DEPARTMENT OF BIOLOGICAL STUDIES—The Principal, Lanchester College of Technology, Priory Street, Coventry (December 31).

SENIOR LECTURERS, LECTURERS or ASSISTANT LECTURERS IN MATHEMATICS—The Secretary, University of Lancaster, Bailrigg House, Lancaster (December 31).

CHAIR OF PHYSICS—The Registrar and Secretary, University of Sussex, Stanmer House, Stanmer, Brighton, Sussex (January 1).

UNIVERSITY LECTURER IN SOIL SCIENCE—The Secretary of Faculties, University Registry, Oxford (January 1).

LECTURER IN CLINICAL PSYCHOLOGY IN THE DEPARTMENT OF PSYCHOLOGICAL MEDICINE—The Secretary of the University Court, The University, Glasgow (January 4).

LECTURERS (3) (with a degree in physiology, biochemistry, pharmacology, chemistry, medicine or zoology and postgraduate research experience of Ph.D. or equivalent standard) IN PHYSIOLOGY or BIOCHEMISTRY—The Deputy Secretary, The University, Southampton (January 8).

SENIOR LIBRARY ASSISTANTS (2), one to take charge of the Library of the Faculty of Agriculture, and one for duties in the David Keir Science Library—The Secretary, The University, Belfast, Northern Ireland (January 8).

ENGINEER or PHYSICIST (with a good honours degree in engineering or physics and either research experience of Ph.D. standard or good practical civil engineering experience) IN THE BUILDING SCIENCE LABORATORIES, to work under the direction of Dr. G. H. Tattersall, on work connected with industrialized building and compaction of concrete by vibration—The Registrar, The University, Sheffield (January 9).

LECTURER or ASSISTANT LECTURER IN MATHEMATICAL STATISTICS, to work with an existing group of five members of staff—The Registrar, The University, Manchester, 13 (January 9).

LECTURER (preferably with main interests in the field of inorganic or theoretical chemistry) IN THE DEPARTMENT OF CHEMISTRY—The Assistant Registrar (Science), The University of Birmingham, Birmingham, 15 (January 11).

UNIVERSITY LECTURER IN GEOCHEMISTRY IN THE DEPARTMENT OF GEOLOGY AND MINERALOGY—The Professor of Geology, Department of Geology and Mineralogy, University Museum, Oxford (January 11).

ASSISTANT LECTURER IN PHYSIOLOGY at the University of Hong Kong—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Hong Kong and London, January 15).

ASSISTANT LECTURERS (3) IN PHILOSOPHY—The Registrar, Room (T), The University, Hull (January 15).

CHAIR OF CROP HUSBANDRY AND HORTICULTURE at the University of Ghana—The Assistant Registrar, University of Ghana Overseas Office, 15 Gordon Square, London, W.C.1; or The Registrar, University of Ghana, P.O. Box 25, Legon, Accra, Ghana (January 15).

LECTURER IN ENGINEERING SCIENCE—The Registrar and Secretary, University of Durham, Old Shire Hall, Durham (January 15).

LECTURERS or ASSISTANT LECTURERS (with a recognized honours degree in civil, mechanical or electrical engineering, experience in research, and preferably practical experience and experience of teaching at degree level) IN THE DEPARTMENT OF ENGINEERING, University of Malaya—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Kuala Lumpur and London, January 15).

SENIOR LECTURER IN THE DEPARTMENT OF ANATOMY at the University of the Witwatersrand, Johannesburg, South Africa—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, January 15).

SENIOR LECTURER or LECTURER IN THE DEPARTMENT OF ZOOLOGY, University of Khartoum—The Registrar, University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (January 15).

SENIOR LECTURER or LECTURER (qualified veterinarian with postgraduate clinical teaching and research experience) IN THE DEPARTMENT OF VETERINARY MEDICINE, TOXICOLOGY AND THERAPEUTICS, at the University of Khartoum—The Registrar, University of Khartoum, c/o The Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (January 15).

SENIOR TECHNICIAN or TECHNICIAN IN THE DEPARTMENT OF BIOCHEMISTRY AND SOIL SCIENCE, University of Khartoum—The Registrar, University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (January 15).

LECTURER IN PHILOSOPHY—The College Secretary, Balliol College, Oxford (January 16).

ASSISTANT RESEARCH FELLOW IN SOCIOLOGY, ANTHROPOLOGY or SOCIAL PSYCHOLOGY at Makerere University College, Uganda—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (January 20).

LECTURER IN PSYCHOLOGY at the University of Cape Town—The Secretary-General, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1; and The Registrar, University of Cape Town, Private Bag, Rondebosch, Cape Town, South Africa (January 20).

READER IN EXTRACTION METALLURGY at Imperial College—The Academic Registrar, University of London, Senate House, London, W.C.1 (January 20).

LECTURER (with an honours degree in pharmacy, and preferably Registered Pharmacist with a higher degree and teaching or industrial experience) IN PHARMACY at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, January 22).

SENIOR LECTURER or LECTURER IN APPLIED MATHEMATICS—The Registrar, Room (T), The University, Hull (January 26).

LECTURER or ASSISTANT LECTURER (with interests in soil mechanics) IN ENGINEERING—The Registrar, The University, Manchester, 13 (January 30).

LECTURER (preferably interested in numerical analysis or a related field) IN PURE MATHEMATICS in the Faculty of Science—The Registrar, The University, Manchester, 13 (January 30).

RESEARCH FELLOW IN MATHEMATICS—The Secretary of the University Court, The University, Glasgow (January 30).

TUTORIAL FELLOW IN BIOCHEMISTRY—The Senior Tutor, University College, Oxford (January 30).

UNIVERSITY LECTURER IN BIOCHEMISTRY—Professor Sir Hans Krebs, F.R.S., Department of Biochemistry, South Parks Road, Oxford (January 30).

AGRICULTURAL ENGINEER (with a degree in agricultural engineering or mechanical engineering with postgraduate qualifications in agricultural engineering, and some practical experience) in Mauritius, to design or modify machinery and equipment for agricultural use in the Colony, to design and install pilot mechanical plants relating to indigenous agricultural development and for general consulting and engineering work—The Appointments Officer, Room 301, Ministry of Overseas Development, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 213/111/01.

AGRICULTURAL OFFICERS (national of the United Kingdom or the Republic of Ireland, with a degree in agriculture or natural science, plus at least two years experience of tropical agriculture) in Sarawak, for the administration of an Agricultural Division, comprehensive advisory and supervisory duties and supervision of experiment stations and staff training—The Appointments Officer, Room 301, Ministry of Overseas Development, Eland House, Stag Place, London, S.W.1, quoting RC 213/155/04.

ASSISTANT LECTURER (with a good honours degree in biology or zoology) IN BIOLOGY—The Registrar, University of Strathclyde, George Street, Glasgow, C.1.

CHAIR OF PHYSIOLOGY—The Registrar and Secretary, The University, Bristol.

LECTURER IN OPERATIONAL RESEARCH AND APPLIED STATISTICS—The Academic Registrar, Loughborough College of Technology, Loughborough, Leicestershire, quoting Ref. 27/G.

LECTURER IN THE DEPARTMENT OF PHYSICS, University of the Witwatersrand, Johannesburg, South Africa—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1.

LECTURERS or SENIOR LECTURERS (with a first- or second-class honours degree or equivalent) IN PHYSICS—The Registrar, Royal Military College of Science, Shrivenham, Swindon, Wilts.

MASTER to take charge of Biology; and a CHEMISTRY MASTER to teach up to G.C.E. "A" level and scholarship—The Rector, The Edinburgh Academy, Edinburgh, 3.

PHYSICISTS and PHYSICAL CHEMISTS (preferably with a minimum of three years research experience) to work on various problems in the fields of membrane permeation, polymer structure and its relation to electrical behaviour; and ORGANIC CHEMISTS to work on interesting problems in the carbohydrate field in connexion with the utilization of natural products—The Director, Arthur D. Little Research Institute, Inveresk, Musselburgh, Midlothian, Scotland.

PHYSICS TECHNICIAN (with G.C.E. "A" levels in mathematics or physics), or a RADIOGRAPHER IN THE RADIOTHERAPY DEPARTMENT for work on clinical application of isotope techniques—The Assistant Secretary, University College Hospital, Gower Street, London, W.C.1.

PLANT PHYSIOLOGIST (national of the United Kingdom or the Republic of Ireland, with a good second-class honours degree in botany, with particular emphasis on physiology, ecology, and preferably postgraduate experience in plant physiology research, with particular reference to water shortage on plant growths) with the East African Common Services Organization, to carry out research on agreed problems in the physiology of root development and growth in some East African economic plants, with particular reference to the effects of water stress—The Appointments Officer, Room 301, Ministry of Overseas Development, Eland House, Stag Place, London, S.W.1, quoting RC 213/214/010.

RESEARCH ASSISTANT (with an honours degree or a Diploma in Technology, and preferably some familiarity with semi-conductor circuits, optics or the design of small instruments) IN THE DEPARTMENT OF MATHEMATICS for daylight star detection—The Secretary and Registrar, Bristol College of Science and Technology, Ashley Down, Bristol, 7, quoting Ref. CST 64/111.

RESEARCH FELLOW (with the degree of Ph.D. or about to submit a Ph.D. thesis in organic chemistry) IN THE DEPARTMENT OF CHEMISTRY for an investigation concerned with the synthesis of heterocyclic compounds of potential therapeutic interest—The Professor of Chemistry, Room (T), The University, Hull.

SENIOR LABORATORY TECHNICIAN (with specialized skill in either botany or zoology and a general familiarity with the techniques in the other subject for supervision purposes) IN THE DEPARTMENT OF BOTANY AND ZOOLOGY—The Secretary, Sir John Cass College, Jewry Street, London, E.C.3.

SENIOR TECHNICIAN (with extensive experience of machine tools) to assist in construction of a vacuum system, magnets and associated equipment for a 15 MeV Linear Accelerator—The Secretary, The Medical College of St. Bartholomew's Hospital, West Smithfield, London, E.C.1.

TEACHING and RESEARCH ASSISTANTSHIPS leading to M.Sc. and Ph.D. degrees—Head, Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada.

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Editorial and Publishing Offices of "NATURE"
MACMILLAN (JOURNALS) LIMITED
ST. MARTIN'S STREET, LONDON, W.C.2
Telephone Number: Whitehall 0081. Telegrams: Phusis London W.C.2

Annual subscription: Inland £13, Overseas £13 15s., payable in advance, postage paid to any part of the world.

Advertisements only should be addressed to
T. G. Scott & Son, Ltd., 1 Clement's Inn, London, W.C.2.
Telephone Number: Holborn 4743

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DISARMAMENT AND THE ROLE OF THE SCIENTIST

IN his Essex Hall Lecture, "Scientists' Responsibility in the Atomic Age" (now published*), Prof. J. Rotblat urges that progress in science and technology has not been matched by corresponding progress in problems of relations between communities and nations, or in strengthening moral principles and adhering to standards of ethics, and claims that this divergence can be expressed quantitatively. While the growth of scientific and technological activities has a doubling time of about ten years, corresponding to an annual growth of about 7 per cent, for non-scientific activities the doubling time is about forty years, with an annual rate of growth roughly proportional to that of the world's population. This divergence is dangerous because of the immense destructive potentialities of the new powers if they are misused. The danger is the greater because of the lack of progress in international relations, the neglect of moral ideals and the prevalent disregard for the value and sanctity of human life.

Prof. Rotblat considers that the most immediate danger arises from the presence of large stockpiles of nuclear weapons, which increase in size exponentially as the delivery time of such weapons is continually shortened. He is sceptical as to the real deterrent value of such weapons and comments on the depressing effect that such a situation has on moral values, especially among the younger generation. The Commonwealth Prime Ministers in 1961 accepted the need for a disarmament agreement, the aim of which must be to achieve total world-wide disarmament subject to effective inspection and control; and in view of the slaughter and destruction experienced in so-called 'conventional' wars and the difficulty of preventing such a war, once started, from developing into a nuclear war, our aim must be nothing less than the abolition of the means of war of any kind. Similar ideas have been expressed by the leaders of Governments of the United States and the U.S.S.R., but so far the only worth-while achievement has been the agreement signed in Moscow in 1963 between the United Kingdom, the United States and the U.S.S.R. to cease tests of nuclear weapons in the air, under water and in the upper regions of the atmosphere.

While this will mean the end to further poisoning of the atmosphere with radioactive materials from fall-out, Prof. Rotblat points out that this is not enough, and, since his lecture was delivered, his warning has been substantiated by the French and Chinese nuclear tests. Indeed, he visualizes new and much more simplified means of wholesale destruction becoming accessible even to small and less-developed nations, and maintains that the world will not be a safe place to live in so long as mistrust exists between the nations. One cause of this mistrust is the enormous disparity in the standards of living, which is increasing rather than decreasing, and while Prof. Rotblat recognizes that aid to the underdeveloped countries could be expanded and organized to diminish this danger, he does not think that this alone will suffice. An entirely new approach is needed to the fundamental problems of society.

First, Prof. Rotblat insists once again that the change resulting from the advance of science and technology necessitates a radical change in our attitudes to major issues. Ultimately a world authority must be established, but this is a long way ahead and in the meantime it is no longer tolerable that international disputes should be settled by force. If we are to survive we must develop a community of interest for all mankind, irrespective of national boundaries or differences in economic or political systems.

This is largely common ground, differences of opinion lying rather in the means by which progress is to be achieved. Prof. Rotblat points out, moreover, that to achieve our purpose we must change completely the aims of education. Without abandoning loyalties to the national heritage we must emphasize the permanent community of interest of mankind and seek to develop a sense of responsibility for mankind as a whole. Admittedly, this is a long-term process and some effective measures are required immediately. For these Prof. Rotblat looks especially to the scientist who, besides sharing the common duty of mankind, has more specific obligations.

Before discussing this special role, Prof. Rotblat refers to the responsibility of both public and Government as expressed by the Atomic Scientists' Association. The right use of science is a joint responsibility of scientists, governments and public; and abuse arises from the neglect of this shared responsibility. Clear recognition by any government of its own responsibilities—including that of ensuring full freedom of discussion as well as of investigation by the scientists—and public understanding of the implications, will go far to create better understanding between public and scientists, as well as more effective utilization of scientific results.

The scientist, however, must recognize that science is a natural, if not the best, medium for fostering international understanding, as well as a most important force in the development of society. Moreover, it can make vital contributions to the solution of social and political problems, and the Pugwash Movement is one of the most important ways in which scientists themselves have sought in recent years to fulfil their social obligations. From the outset the Movement gave high priority to the problem of disarmament, of finding means of halting the arms race and destroying the stockpile of weapons, and the measures emphasized at its first conference in July 1957 still hold. These include the lessening of tensions among nations and the promotion of mutual understanding among their peoples; the ending of the arms race; the provision of reasonable safeguards in the arms control system to give substantial protection and to build up mutual confidence; the negotiation of a step-by-step process to develop as satisfactory a set of controls and safeguards as practicable.

The Pugwash Conferences have become established as an important and effective channel of communication between East and West for constructive thought and the pursuit of peace. They have demonstrated the practicability of successful international debate on controversial issues, and conferences of this type may well play an

* *Scientists' Responsibility in the Atomic Age* (The Essex Hall Lecture, 1964). By Prof. J. Rotblat. Pp. 28. (London: The Lindsey Press.) 2s. 6d.

increasingly important part in world affairs. Prof. Rotblat does not, however, indicate exactly how the way forward lies, but seems to suggest that some more formal organization may be desirable with perhaps the institution of an international council of scientists to keep the political situation continually under review. On that there may well be two opinions, particularly if the further development of the social sciences leads to the establishment of an international union in that field.

What is clear is that the position offers a great challenge to clear and constructive thinking. When Earl Russell addressed a meeting organized by the Bertrand Russell Peace Foundation in Manchester in April he expressed the opinion that the task had very largely been accomplished of inculcating on Governments and peoples the calamitous nature of nuclear war, and went on to suggest that the time had come for a new approach. He believed that one of the most urgent tasks was to devise some scheme of disarmament to which both the United States and U.S.S.R. could agree.

Earl Russell is also among the signatories to a statement addressed in July to scientists and engineers in Great Britain which urged that the social responsibility of engineers and scientists was to concentrate their energies on the exploration and development of the world's resources to meet the problems of peace, and, secondly, to impress on their colleagues or students, when seeking a job, that there are as many opportunities for constructive as for potentially destructive work, even though the latter may be better paid. The statement urges that a real way to peace would be to divert much of the money now being spent on armaments to agricultural research and education and the equipment needed to make them effective. In Britain much scientific and engineering work is urgently needed to make it a more pleasant place to live in.

One of the most impressive features of recent months has been the way in which technical assistance and economic help to the developing countries, quite apart from the campaign against hunger, have been seen as a positive measure towards world peace and order. Nor is recognition of their social responsibilities confined to Western scientists: the Science Council of Japan has prepared a statement on such responsibilities for issue when a proposed Fundamental Law on Scientific Research is enacted, and independently of this has expressed the opinion that the principles outlined in that Law and this statement should guide both scientists and governments.

Too much, however, must not be expected of scientists alone. What is required above all is some effective means by which they may discharge their responsibilities in co-operation and partnership with both government and community, and this involves some clear understanding of the function of politics as well as of science, and their respective limits. On this, Mrs. M. Gowing's recent book, *Britain and Atomic Energy, 1939–1945* †, is full of suggestions. Even the scientist cannot always foresee the possible consequences of a particular development far ahead. Mrs. Gowing notes that Dr. C. G. Darwin, as he then was, had some appreciation of the implications of exploding a nuclear bomb when the Maud Committee reported in 1941, and wrote to Lord Hankey on the point. Another scientist closely concerned in the work reflected that perhaps the Committee should have studied the moral and political implications and considered its use more carefully.

† *Britain and Atomic Energy, 1939–1945*. By Margaret Gowing. With an introductory chapter by Kenneth Jay. Pp. xvi + 464 + 14 plates. (London: Macmillan and Co., Ltd.; New York: St. Martin's Press, Inc., 1964.) 55s. net.

The whole energies of the Committee, however, were directed to answering two questions: Was a uranium bomb scientifically possible ? and, if so, Could the British and their allies make one before the Germans ? At that early stage there was no dilemma for British scientists. The heartsearchings, described so vividly by Dr. A. H. Compton in *Atomic Quest*, came much later, and to expect more of the scientists at the early stage would be to confuse the issue, as well as to exercise hindsight. The scientist works not only within the framework set by his own scientific discipline but also within that set by the society within which he lives and the insight and traditions of that society.

In this context it is worth recalling some words of Sir Henry Tizard who, in his A. D. Little Memorial Lecture in 1951, referred to the deplorable intellectual gap between the students of science and the students of the so-called 'humanities' long before Sir Charles Snow (now Lord Snow) had given prominence to the term "the two cultures". That gap Sir Henry attributed to the failure of the humanists to move with the times. Even then, however, he thought humanists and scientists were coming much closer together. In that lecture he was concerned more particularly with the anxiety of scientists and technologists about the social implications of their work—anxiety which derived from the haunting fear that war on a large scale in future would altogether destroy civilization as we know it, and also from the fact that the application of scientific knowledge had so far brought only a fraction of the benefits to mankind which should have been possible.

Sir Henry does not countenance any dichotomy between scientist and politician. He reminds us pertinently that at one time it was quite normal for a man to gain a degree in science as a preparation for a career in law, in business or in public service, and he points out that scientists should study the political experience of other democracies with the object of adopting what is good and rejecting what is bad. The art of government rests on being in constant touch with the best advice available, and then making the experiment so that in the event of failure the *status quo ante* can be restored.

That may not always be possible, but Sir Henry was clearly right in stressing the importance of real teamwork in the social sciences. He holds also that if science is to have any real influence on national policy, some scientists must be prepared to spend part of their lives in the closest daily touch with men who formulate the policy. They must enjoy a reasonable measure of the confidence of their fellow scientists, who may be more gifted than themselves: but what is necessary is that they should study and appreciate all the ingredients of political decisions, including the ingredient of human nature—the most refractory material known to science.

There can thus be only welcome for the appointment by the British Government of a Minister of State in the Foreign Office to deal expressly with disarmament. The Prime Minister on November 19 said that the appointment gave practical expression to the importance attached to a fresh initiative by Britain. Mr. A. Gwynne-Jones would be advised on scientific aspects of his work by Sir Solly Zuckerman, who has been advising successive Governments on the scientific aspects of war and defence since 1939 and is still chief scientific adviser to the Ministry of Defence. How far the Government proposes to go remains to be seen. In his Guildhall speech on November 16, the Prime Minister was resolute as to the Government's

keep Britain's defence strong, and also to the Atlantic Alliance and to endeavour to e effective means by which the United Nations in to keep the peace and prevent small clashes lating into big ones. Equally it was its intention initiatives in disarmament whenever the opportrose or could be created.

clear intention to keep disarmament in the foreimplies some consideration of the consequences of rmament as well as of the means by which it can be ieved. The increased attention which is now being rected generally to those economic and social consequences may indicate that, despite all the technical and political difficulties, practical steps towards disarmament are not so remote as would at first appear. Taken with the Prime Minister's firm assertions on defence, the organization which the Government is establishing should help to ensure that when the time is ripe Britain is prepared with the economic and social measures which must be the counterpart of any reduction in armaments. One preparatory step must be the education of public opinion for the necessary changes, and in this connexion it is significant as well as welcome that the November 1964 issue of *The Unesco Courier*, which contains the text of the Nuclear Test Ban Treaty of August 1963, includes also several articles on these social and economic aspects of disarmament.

THE FOUNDER OF MODERN ATOMIC PHYSICS

J. J. Thomson and the Cavendish Laboratory in his Day

By Sir George Paget Thomson. (British Men of Science.) Pp. xi + 186 + 20 plates. (London and Edinburgh: Thomas Nelson and Sons, Ltd., 1964.) 25*s*. net.

THIS short biography of Sir J. J. Thomson by his son is one of a series on *British Men of Science* intended for the general reader.

'J.J.' entered Owens College, soon to become the Victoria University of Manchester, at the early age of fourteen and studied engineering, mathematics, physics and chemistry—Owens being one of the few Colleges with a physics laboratory at that time. At the age of nineteen he tried for a scholarship at Trinity College, Cambridge, but did not even qualify for an Exhibition. He tried again the following year and obtained a minor scholarship and sizarship and entered Trinity College to take the Mathematical Tripos at the age of twenty. At this time Clerk Maxwell was Cavendish professor. J.J. became second Wrangler and second Smith's prizeman, with Joseph Larmor in the lead. At the early age of twenty-eight, he succeeded Rayleigh as Cavendish professor and began work on the discharge of electricity through rarefied gases. This was a time when Hertz, Lenard, Perrin, Schuster and Wiechert were experimenting with the cathode rays. The discovery of X-rays by Roentgen in 1895 led J.J. to work on the ionizing properties of the X-rays. He then moved on to his successful measurement of e/m for the electron. Hertz had tried this experiment but failed, and J.J. was successful entirely because of the improved vacuum he was able to produce by using the better vacuum pumps then available due to the development of the electric lamp industry—an interesting example of feed-back from technology. Working in the Cavendish in those days were Townsend, C. T. R. Wilson and Rutherford, newly arrived from New Zealand and soon to go on to McGill University. The Laboratory staff included such characters as W. G. Pye, later to found Pye Instrument Company, Everett—J.J.'s assistant—Hayles, who in later years became Rutherford's book-keeper/secretary in the Cavendish of the 1920's, and Lincoln, famous for his moustaches, the laboratory steward of the 1920's. F. W. Aston, who worked with J. J. Thomson in later years, said: "Working under him never lacked thrills. When results were coming out well, his boundless, indeed childlike, enthusiasm was contagious and occasionally embarrassing. Negatives just developed had actually to be hidden away for fear he would handle them while they were still wet, yet when hitches occurred and the exasperating vagaries of an apparatus had reduced a man who designed, built and worked with it to baffled despair, along would shuffle this remarkable being, who, after cogitating in the characteristic attitude over his funny old desk in the corner and jotting down a few figures and formula in his tiny handwriting on the back of somebody's Fellowship thesis or an old envelope or even the laboratory cheque book, would produce a luminous suggestion like a rabbit out of a hat, not only revealing the cause of the trouble but also the means of a cure."

Another chapter describes J.J.'s work on positive rays and isotopes, which was started in 1905, following Wien's work on the measurement of e/m for the 'canal rays' discovered by Goldstein in 1866. J.J.'s improved vacuum technique led to his well-known parabolas and to the separation of the neon isotopes and thence to the mass spectrograph.

The author refers to the discussions at Farnborough—now the Royal Aircraft Establishment, Farnborough—in mid-1915 between Aston and Lindemann on whether the 22 parabola was due to $NeH_2.CO_2$. Lindemann always won the argument but "Aston had faith and was unbeatable". He returned to the Cavendish after the First World War to prove this by his mass spectrograph.

J.J. founded the Cavendish Physical Society for weekly discussions of laboratory experimental work, interspersed with lectures by visiting scientists; also the Cavendish tea-hour which was and is an occasion for general conversation rather than scientific discussion.

J.J. was interested in cricket, especially Lancashire cricket, and was also a keen supporter of the University Rugby Club. His greatest hobby was flowers, both wild and cultivated, and he made yearly pilgrimages to places near Cambridge when favourite flowers were in bloom.

In 1918 J.J. became Master of Trinity and handed over the Cavendish professorship to Rutherford, but continued to work in the part of the laboratory known as "the Garage". Here his assistant Everett presided over and erected variations of the maze of glass tubing where his experiments were carried out and J.J. appeared punctually at 1.00 p.m. every day to hear about the results of the experiments. He continued to lecture in the Cavendish, but his views of physics were by now rather conservative.

During the First World War he was chairman of a Royal Commission set up to report on the position of Natural Science in the educational system of Britain. Many of his views are still relevant to-day. He felt that the famous schools were too apt to tempt boys into classics at an early age by their system of scholarships. He valued an education which retained the boys' interest and freshness. Evidence was presented to the Royal Commission that boys who were to compete for entrance scholarships did little in the two years before the examination other than write out answers to papers set in previous examinations. Under this system the boys became more and more fed up with mathematics the more they persevered with it. He believed that some teaching was a positive advantage to a man's research and that interruptions at the right time helped original thought.

Sir George Thomson's biography gives a good account of a very great scientist.

J. COCKCROFT

BIOGEOGRAPHY OF THE PACIFIC BASIN

Pacific Basin Biogeography

A Symposium. General Editor: J. Linsley Gressitt. (Tenth Pacific Science Congress, Honolulu, Hawaii, 1961.) Pp. ix+563. (Honolulu, Hawaii: Bishop Museum Press, 1963.) n.p.

RECENT geophysical and geological investigations of the Pacific basin have done much to elucidate the past configuration of land and sea, and so define for biogeographers the conditions under which observed distribution patterns of animals and plants arose. This new information, even though swamped at times by more traditional inferences from biotic distribution, lends especial interest to this latest discussion of the biogeography of the region, the proceedings of a symposium on "Pacific Basin Biogeography" organized during the tenth Pacific Science Congress.

Because the Pacific basin is flanked by five out of the six great biogeographical regions and spans all climatic zones, the symposium was wisely subdivided. The first section considered relationships between nearctic and palæarctic plants, insects, birds and mammals across Bering Strait, and revealed a remarkable agreement in evidence and deduction. At various periods in the Tertiary and Quaternary Periods, continuous land linking Asia and North America permitted floral and faunal exchange, especially and most recently of tundra and taiga species. An unglaciated refugium in this area during glacial maximum appears to have been a centre for subsequent dispersal. In contrast, the Aleutian arc seems to have been of minor significance as a 'stepping stone' dispersal route.

The second section of the symposium, concerned with the richer, more complex and perhaps older biotas of tropical regions, revealed less unanimity. Geological evidence points to the persistence of the Pacific basin at least since the mid-Cretaceous, and to many of the guyots in mid-ocean having formerly emerged as high islands available as distributional stepping stones. A pre-Cretaceous trans-Pacific tropical land bridge was, however, invoked by two eminent phytogeographers, although other botanists, ornithologists and entomologists united in regarding it as unnecessary and in ascribing a larger role to direct dispersal. In most respects the mid-Pacific archipelagoes are a part of the Oriental region and there is a progressive biotic attenuation as one passes eastwards from the putative source area of the Indo–Malay island chain. Eastward spread along present and past island groups may account for this pattern, and a longer time-scale than has sometimes been assumed and a greater density of islands in the past may explain also the more puzzling botanical anomalies. It is refreshing to find that the familiar and sterile controversies based on diverse subjective estimates of dispersal capacity in various taxonomic groups are at last being supplemented by direct evidence based on insect trapping from aircraft, and it is evident that such research, together with further taxonomic, palæogeographical and palæontological investigations are urgently needed.

In the Antarctic region problems characteristic of both tropical and northern Pacific are combined. Each major land mass projecting into the southern temperate zone has three biotic elements. One (including all land vertebrates) has northward affinities in each case, and appears to have dispersed mainly in the Tertiary when continents were arranged as they are to-day. The second element is one peculiar to each region. The third, numerically smallest, is the austral element, which shows a markedly disjunct distribution between the southern continents and poses major biogeographical problems. It may be subdivided into a neoaustral element reaching recent volcanic oceanic islands and showing evident dispersal capacity, and a larger palæoaustral element of continental [illegible] perhaps requires contiguous land for dispersal. [illegible] group includes ancient conifers such as the Pod[illegible] the cytotaxonomic examination of which is c[illegible] stimulating new lines of research brought ou[illegible] symposium. A valuable geological paper acc[illegible] biological evidence by suggesting that, whil[illegible] Cretaceous biogeographical distributions mu[illegible] considered on a basis of present continental po[illegible] pre-Cretaceous ones must take into account [illegible] approximation of southern land masses. The palæoau[illegible] biota may therefore provide a genuine example of a [illegible] and fauna dispersed overland before the disruption [illegible] great southern continent. But if this is so, it is necessar[illegible] to explain how land vertebrates (such as snakes, dinosaurs and early mammals) failed to accompany the forest trees from South America or Africa to Antarctica, New Zealand and Kerguelen.

As a whole, this symposium volume highlights the advances and defects of biogeography in recent years. There is still much subjective speculation on dispersal capacity, where direct experiment would be more valuable. There is still a tendency among biologists to regard the distribution of species as more permanent than that of continents. Insufficient attention is still being directed to geological and geophysical evidence, which can alone define for biologists the framework on which their material must be arranged. Relationships between taxa now spatially separated still need critical assessment using modern cytotaxonomic techniques. But it is encouraging, in a symposium of this kind, to find all these lines of thought given prominence in an appraisal of the distribution of so many groups of plant and animal over so vast an area.

M. W. HOLDGATE

AEROSOL BEHAVIOUR

The Mechanics of Aerosols

By N. A. Fuchs. Translated from the Russian by R. E. Daisley and Marina Fuchs. Translation edited by C. N. Davies. Revised and enlarged edition. Pp. xiii+408. (London and New York: Pergamon Press, 1964.) 120*s*. net.

THIS edition of *The Mechanics of Aerosols* is completely revised and enlarged, incorporating the author's original Russian publication of 1955 and an addendum of 1961. The main object of the book is to collect and examine critically both theoretical and experimental material relating to the mechanics of aerosols. This latter term is one chosen by N. A. Fuchs to embrace the movement of aerosol particles and clouds, the collision of particles and the hydrodynamic disintegration of aggregates.

The major topics covered embrace the classification of aerosols, including size and shape; steady rectilinear motion including non-sphericity, electric fields, radiometric forces, thermophoresis, photophoresis, and diffusiophoresis; non-uniform rectilinear motion including acoustic, hydrodynamic and electrostatic scattering; curvilinear motion including a detailed discussion of the theory of aerosol sampling; Brownian motion and diffusion including filtration and respiratory deposition; convective and turbulent diffusion including atmospheric dispersion and deposition; coagulation including thermal, polarization, ultrasonic and gravitational modes; and finally the dispersal of powder as aerosols. While existing experimental data are critically reviewed in relation to relevant basic theories, detailed information on measuring techniques is not included. Reference is made to no less than 886 reports published up to 1960.

The work is intended as a unifying theoretical source for a subject covered in a diversity of journals devoted to widely differing branches of science and technology. The

translators and the English editor are to be congratulated on producing a final work which scarcely ever reveals that English is not the original language of composition. The whole is a most scholarly and satisfying work, rigorous and yet imaginative in its analytical treatment of aerosol behaviour. Unlike some highly specialized technical works, this book is eminently readable. It is logical in presentation and full of incisive and perceptive comment on where future investigations might be directed.

The somewhat long delay in producing this English edition is presumably due to the complicated checking procedure adopted to ensure a rigorous translation. A good deal of work has naturally been published in this field since 1960, and in some topics, therefore, experimental data discussed are already incomplete. For example, the assessment of atmospheric dispersion using Pasquill's treatment, published in 1961, has been shown to be more accurate over long distances than Sutton's formulæ. Its use is preferred, for example, in most present-day reactor safety calculations. Furthermore, some previous work was reported in sources not available to the author. A future edition might include an analysis of the global atmospheric dispersion of aerosols together with a collation of those topics which have sufficient relevance to radioactive wastes.

There are, however, no major omissions in the subject dealt with, while the general treatment is so authoritative and rigorous as to guarantee this book as a standard reference work of lasting value. It will be of unquestionable worth to research workers in university, industrial and relevant applied research departments.

J. F. CROFT

MASS SPECTROMETRY IN NATURAL PRODUCT CHEMISTRY

Structure Elucidation of Natural Products by Mass Spectrometry

By Dr. Herbert Budzikiewicz, Dr. Carl Djerassi and Dr. Dudley Williams. Vol. 1: Alkaloids. (Holden-Day Series in Physical Techniques in Chemistry.) Pp. xi+233. (San Francisco and London: Holden-Day, Inc., 1964.) 10.50 dollars.

THE most striking development in post-War organic chemistry has been the intensive application of physical methods to the elucidation of molecular structure. The various spectroscopic techniques, ultra-violet, infra-red and nuclear magnetic resonance, can all give important information very quickly; X-ray crystal analysis still remains the ultimate and final arbiter of molecular architecture, but it is unfortunately very time-consuming. Now another physical weapon has been added to the armoury of the chemist, high resolution mass-spectrometry, and, although this technique has been in general use only since 1960, the results achieved have been spectacular.

In Volume 1 of *Structure Elucidation of Natural Products by Mass Spectrometry*, the three authors—all pioneers in this field—confine their attention to alkaloids. This group of natural products presents a very favourable situation for the interpretation of cracking-patterns, since the first effect of electron impact is to remove an electron from the basic nitrogen atom leaving an ion-radical, the following homolytic fragmentation of which can usually be fairly easily predicted. Examples of various types of fragmentation are given, with particular reference to the various groups of indole alkaloids. Indeed, it is in this particular field that mass spectrometry has scored some of its most striking successes—complete structure determination of very complex molecules using only a few milligrams of material and with an absolute minimal recourse to 'chemical' operations. Among other alkaloids discussed are the isoquinoline, emetine, colchicine, lycopodium and quinazolone groups.

This is an admirable book which every practising organic chemist concerned with natural products will need to read: the immense power of the techniques described is fast changing the whole face of the subject. A further work, covering steroids and terpenoids, is promised shortly. At the end of their preface, the authors thank their publisher "for sharing our feeling that, if newspapers can be produced from one day to the next, books—which are generally shorter than the Sunday Edition of the New York Times—need not take more than ninety days". Other publishers of similar urgently awaited material, please note.

J. HARLEY-MASON

EXPANDING HORIZONS IN PHARMACY

Advances in Pharmaceutical Sciences

Vol. 1. Edited by H. S. Bean, A. H. Beckett and J. E. Carless. Pp. xi+334. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc., 1964.) 75*s*.

"THE aim of this new series is to concentrate on areas of specific interest to the pharmaceutist . . .". For the uninitiated, pharmaceutist and pharmacist are synonymous in the Pharmacy Acts. Further, "The articles will be an appraisal of a subject rather than a mere factual abstraction of the literature".

The aims are praiseworthy, and although the four subjects chosen for this volume are of wide interest, the authors have included pharmaceutical applications.

The chapter on rheology is well written and is aimed at the undergraduate level. The theoretical section is brief, and the simpler concepts are dealt with in an explicit manner. The section on instruments is mainly descriptive and may be improved by a greater appraisal of the limitations and use of the instruments described.

The review is most concerned with solid/liquid and liquid/liquid systems, so that the perfunctory treatment of powders and the omission of the compression of powders is understandable. The systems and processes in which rheological properties are important are listed and should be useful in directing the attention of a pharmacist to the need for extensive work. This last section is good, but the factors affecting the viscosity of suspensions should also be included under emulsions, with the exception of the 'shape factor'. The use of Stokes's equation, which does not include a volume fraction term, for describing the rate of creaming of an emulsion is best avoided as the impression remains that the viscosity of the continuous phase, rather than that of the system, is important.

The first three sections of "Solubility in Systems Containing Surface Active Agents" gives a good review of the relevant ideas on micelle formation and solubilization. The last two sections, too, are very well done and contain an admirable summary of published information which is probably of greater value to the postgraduate worker.

Unfortunately, in the section on phase equilibria, the systems are not adequately described, most of the phase diagrams included are not even mentioned in the text, and the symbols used are not consistent in all the diagrams. If this section could be improved to the level of the rest of the chapter it would be a valuable summary for pharmacy students.

Mrs. Wedderburn has produced a very carefully written appraisal of a difficult subject in the chapter on "Preservation of Emulsions against Microbial Attack". It was enjoyable to read and easy to understand, since the problems involved were well discussed. Apart from those for internal use, the difference between cosmetic and pharmaceutical emulsions is one of degree, since cosmetics

are meant to be used on healthy skin, and this could have been emphasized a little more, particularly with reference to irritancy. On p. 260 the use of "soil or other solid matter" as a test inoculum is not recommended, but in view of her experience of a laboratory contaminant, lower down the page, I feel that soil, shelf dust, hay dust, etc., may be a wise adjunct to the more systematic testing.

The article "Contemporary Trends in Heat Sterilisation" is not so good as the rest of the book. The first eight pages consist of snippets of information that are inadequately digested, and considerable reliance on a review by Rahn (1945) seems to imply that Rahn was responsible for the work cited. The reference to super-saturated steam (p. 277) is incorrect, so is the reference to metals having a high specific heat (p. 294), and a number of erroneous impressions are also conveyed which may be the result of poor phrasing, as with the dryness fraction which would not normally have a value greater than one if (1 − wetness fraction) is intended.

On p. 278, Fig. 3 gives no units for the abscissa and, if these are lb./in.^{2} in excess of atmospheric pressure, the temperatures and pressures are not correlated. Certainly this figure does not appear to refer to dryness fractions as implied in the text.

The occasional inclusion of some elementary practical detail, such as the description of calorimetry (p. 279), is unnecessary and out of keeping with the book as a whole. The main part of the chapter is a detailed description of the design of autoclaves and controls which are now available, and as such scarcely justifies the word 'trends' in the title. Section XXXIII, entitled "Heat Output", rather surprisingly refers to lagging, ventilation and servicing.

The editors must accept some responsibility for not maintaining the standard set by the preceding chapters, and it is to be hoped that in future volumes we shall see more 'appraisals' of the standard set by the authors of the three other sections.

E. Shotton

NERVOUS IMPULSE

The Conduction of the Nervous Impulse

By Prof. A. L. Hodgkin. (The Sherrington Lectures, VII.) Pp. 108. (Liverpool: The University Press, 1964.) n.p.

THE nervous impulse, how it is generated at a local site and how it is conducted along the nerve fibre, was the subject of the Sherrington Lectures given in 1961 by Prof. A. L. Hodgkin. These lectures, completed with an account of more recent work, have now been published in the form of a book. Hodgkin, working in the Physiology Laboratory at the University of Cambridge, is Foulerton Research Professor of the Royal Society and received the Nobel Prize in 1963.

Signals conducted along nerve fibres are transmitted as electrical events, as a rule as all-or-nothing signals with a sharp threshold, and an amplitude which is independent of the stimulus. The most essential structure for the nerve impulse is the cell membrane, which surrounds the axis cylinder of the nerve fibre. This membrane can be seen in the electron microscope, but not in the light microscope, and is about 10^{-6} cm thick. The membrane has permeability properties which are very intriguing because it is clear that these permeability properties are the basis for the nerve impulse. The electrolyte in the axis cylinder contains a high concentration of potassium and a low concentration of sodium, while the external solution contains much sodium and little potassium. The resting nerve membrane is only little permeable to ions, but the permeability to potassium is relatively large compared to that of other ions. The movement of potassium through the membrane results, therefore, in a current of such a direction that it tends to charge the membrane towards the electrochemical equilibrium potential for potassium, and, due to this current, a steady potential difference across the membrane is maintained during rest. The axis cylinder is some 60–90 mV negative relative to the external solution. The permeability to other ions, and the consequent ionic currents, of course also affects the membrane potential at rest, but to a smaller extent.

An analysis of the ionic currents associated with step changes of the membrane potential indicates that the membrane permeability depends on membrane potential and time. When the axis cylinder potential is changed in positive direction from its resting value, then the membrane undergoes an initial transient increase in sodium permeability. The sodium permeability increase is followed by an increase in potassium permeability. Both these permeability changes are continuous smooth functions of membrane potential and of time, but are independent of sodium and potassium concentrations. The result of complicated and tedious experimental and analytical work is a fairly complete formal description of these permeability changes. A number of empirical constants in a number of equations thus describe the specific ionic currents in the nerve membrane as associated with step changes in membrane potential. The same set of constants and basic equations might be expected to apply also for the condition that the membrane undergoes a more complicated change. Further, the equations might predict the action potential of the nerve. Numerical solutions indicate indeed that the equations very satisfactorily predict many of the known excitability properties of the nerve fibre.

The impulse mechanism in the nerve, and very likely also in muscle, is thus explained quantitatively on the basis of these specific permeability changes in the very thin surface membrane. The ionic currents are passive and are consequences of the permeability changes and the electro-chemical driving forces. Immediate energy requirement for impulse generation is thus available in the form of a concentration battery, that is, the unequal distribution of ions on both sides of the membrane.

The specific ionic membrane currents have also been measured with the aid of radioactive isotopes and the findings support the ionic theory for impulse conduction. While these measurements resolve the ionic fluxes in many ways more clearly than the electrical techniques, the isotope measurements lack a high time resolution. Isotope measurements are also directed towards another goal, to investigate the ionic fluxes against the electrochemical gradient. These fluxes require energy, and are ultimately necessary in order to maintain the concentration gradients across the membrane and thereby maintain the energy used during the impulse generation. Energy-rich phosphate compounds are evidently used by the nerve for the active ionic transports against the electrochemical gradients.

In the preface of *The Conduction of the Nervous Impulse*, Prof. Hodgkin writes: "... I talked mainly about experiments on which my colleagues and I had been engaged in the immediate past". When reading the book I found this true. The preface does not tell, however, that Hodgkin and his colleagues have radically changed our concepts of how the impulse is generated in the nerve. Neither is it mentioned that a very complete picture of the "Cambridge Nerve" is given.

The book is written in a straightforward style. Rather drastic simplifications of the basic material have been made, but made in a masterly fashion so that the distortions caused are negligible.

I like the book; without doubt it is the best summary of the nervous impulse I have read. I can warmly recommend it to students within the field, to students in neighbouring fields and to all those who want to obtain an understanding of how the nervous impulse is generated and conducted.

B. Frankenhaeuser

BIOLOGY OF THE ANTARCTIC SEAS

Biology of the Antarctic Seas

Edited by Milton O. Lee. (Antarctic Research Series, Vol. 1.) Pp. ix+186. (Washington, D.C.: American Geophysical Union of the National Academy of Sciences—National Research Council, 1964.) 10 dollars.

FIVE of the eight papers presented here are devoted to experimental biology, with contributions from J. S. Bunt, E. Z. El-Sayed, E. F. Mandelli and Y. Sugimura on primary organic production, D. E. Wohlschlag on the respiratory metabolism and ecology of some Antarctic fishes, and M. A. McWhinnie on temperature responses and tissue respiration in Antarctic crustacea, with special emphasis on the whale food, or krill, *Euphausia superba* Dana. Such investigations are fundamental to our understanding of the biology of the Antarctic seas, and it is hoped that many more of them will shortly follow from the laboratories that now girdle the continent. This does not, of course, imply that future research can now be left entirely to the experimenter ashore. For there is still much to do in the field, especially on the little-known floor of the wide circum-polar abyssal sea.

M. A. McWhinnie's examinations on live krill in U.S.N.S. *Eltanin* will interest all who have studied these animals from preserved specimens. He finds them, for example, most active and showing the lowest apparent mortality at temperatures of 0° C and below. The greatest activity, in fact, was seen at −1·5° C, the lowest temperature observed. This is perhaps to be expected, because a very large part of this vast circum-polar population, it has recently been shown[1], does exist for the greater part of the year at surface temperatures falling within this range.

At South Georgia, it is true, *E. superba* has been recorded commonly enough up to about 3·9° C, but this occurs only in summer, February—the warmest month. In warmer water it occurs only as a rare straggler. Once or twice it has been recorded just north of the Antarctic convergence, but its occurrence there is anomalous and can be attributed to vagaries in the Antarctic surface current in which it spends so much of its existence. In the *Eltanin* experiments, 4° C proved lethal within 24 h. Euphausians as a whole are notoriously difficult to keep alive, and Dr. McWhinnie is to be congratulated on maintaining some dozens of his specimens for nine days, and five for thirteen days.

I was particularly interested in Robert Menzies's account of the latest techniques for deep-sea trawling used in U.S.N.S. *Eltanin*. These methods, notably in so far as they permit a fairly accurate determination of duration of contact with the bottom, and a useful estimation of the length of warp required, will doubtless be used with advantage on deep south polar bottoms, if they are not in fact already in use. But there is no mention of the Antarctic in this paper, the work described having been done in the tropics between Panama and Valparaiso. This is a great pity, as Antarctica has its own special problems, not the least being the cumbering of the sea-bed with erratic stones and boulders dropped by ice breaking away from the continental land. The deep terrigenous ooze so often encountered in these high latitudes presents a further problem, requiring, as I have shown elsewhere[2], its own technique. Moreover, it has yet to be shown that Menzies's contention, that in the deep sea contact with foul bottom is responsible for only trifling loss of gear, will hold good round the south polar continent. Rocky ground, and especially erratic boulders, can in fact play havoc with trawls and dredges there, especially on the rather deep continental platform. I have seen a large trawl, shot in 1,000 fathoms, torn almost to ribbons, evidently through contact with stony ground. He comments, too, on the exceptionally poor yield of the 'smaller dominant members of the deep sea' by the massive 'Herring Otter Trawl' (HOT) used by the Danes in *Galathea*. Surely the answer to this is to have fine nets attached to the back of the trawl, which is the technique that was so successfully used in the Antarctic by the late Stanley Kemp and his associates more than thirty years ago.

In "Antarctic Foraminiferal Zonation", O. L. Bandy and R. J. Echols have given an interesting zoogeographical investigation. In high southern latitudes animals so small are prone to 'faunistic displacement', floating icebergs, formerly aground, often being responsible for carrying massive accumulations of bottom material to be deposited eventually in the most unexpected places. The recognition and sorting out of the distributional and bathymetric anomalies that spring from these phenomena are competently handled in this important paper.

Concluding this volume is an exhaustive catalogue and bibliography of the benthic marine algae compiled from a very scattered literature by George A. Papenfuss. It has 240 references going back to 1822.

J. W. S. MARR

[1] Marr, J. W. S., *Discovery Reports*, **32**, 33 (1962).
[2] Marr, J. W. S., *Phil. Trans.* B, **246**, 327 (1963).

BIRD SONG

A Study of Bird Song

By Edward A. Armstrong. Pp. xv+335+16 plates. (London: Oxford University Press, 1963.) 45*s*. net.

IT is well known that bird song is much more than a sweet expression of domestic emotion, that it conveys many things, including aggression, courtship, territory ownership and other bits of information important to birds in their social life. Hence, the investigation of the vocalization of birds is being actively pursued by workers in behaviour, communication and systematics, who frequently become recording enthusiasts. In *A Study of Bird Song*, Armstrong has brought together most of the information accumulated by these workers, and presented it neatly in a condensed and systematic fashion.

The body of the book is made up of chapters on song structure, its development, the variety of forms and functions of song, and determinants such as light, climate, and the associated breeding cycle. There is an introductory chapter on bird utterances as language, and a concluding one on song as art and play. An appendix surveys acoustic communication in the animal kingdom, and eleven pages of addenda take account of work appearing in two and a half years of delay in publication. There is a thorough bibliography and finally an excellent index with four divisions.

In the treatment of such a broad-ranging subject, no unifying theme is found, and probably none is possible. The first chapter, on bird utterances, directs attention to the wide range of avian vocal signals and their rich content of information. A useful review is included of the sound signal repertoires found in the dozen or so species which have been examined in this respect.

Several chapters on the structure and components of song and on its development summarize much of the information available on this subject at the time of writing. Regarding structure, the emphasis placed on measures of pitch, loudness and quality of bird sounds gives undue importance to these aspects, neglecting the complex internal structure of the song as a whole, which is revealed in more recent audiospectrographic investigations. In this section there is an illuminating review and discussion of terminology in bird song. A typical suggestion for better usage is the substitution of 'song version' for 'song type' or 'song pattern'. Armstrong rejects,

however, the progress which has been made in the use of the important term 'subsong' by referring it to all forms of quiet song. Thorpe's restrictive definition based on acoustic characteristics which is discarded is, in fact, a model of clarity which ought to be imitated in the definition of other vague terms, such as that of song itself.

The strength of this book, its wide-ranging comprehensiveness, is also the source of a weakness, which is a tendency to overlook differences in the quality of evidence. The importance of results obtained with modern instruments and critical experiments is not sufficiently emphasized. It is disappointing to find the experimental results obtained by Thorpe on song in the chaffinch placed on the same footing as the observations of bird fanciers. The latter have known long ago that experience plays a part in the development of song, but such information is usually anecdotal in character and mixed with unreliable notions which must be tested.

In the later chapters there are many interesting pages on such topics as mimicry, dialects, duetting, and the variety of functions performed by song. There are numerous useful suggestions and critical comments on the interpretation of the facts, and indications of directions for fruitful future research. Anyone who has seriously asked if singing birds possess an æsthetic sense will enjoy the final chapter on bird song as art and play. Perhaps the disagreement reflected in the discussion of C. Hartshorne's ideas on the effect of the monotony threshold will stimulate research on this interesting hypothesis. The book will join earlier works by this author as an important contribution to the investigation of bird song, serving as a comprehensive guide to the work carried out and a valuable aid to the formation of general principles in this subject.

J. A. Mulligan

NATURAL PLANT GROWTH REGULATORS

Régulateurs Naturels de la Croissance Végétale

(Colloques Internationaux du Centre National de la Recherche Scientifique, No. 123, Gif-sur-Yvette, 15–20 Juillet, 1963.) Pp. 748. (Paris: Edition du Centre National de la Recherche Scientifique, 1964.) n.p.

Régulateurs Naturels de la Croissance Végétale reports the proceedings of the fifth in the series of international conferences on plant growth regulators, which was held at Gif-sur-Yvette in July 1963, under the auspices of the Centre National de la Recherche Scientifique. Most of the text is, however, in English. These colloquia provide an opportunity of surveying the rapidly developing subject of plant growth substances. This subject is now very wide, covering a large range of synthetic substances including weedkillers, so the restriction in this colloquium to natural growth regulators not only kept the subject within bounds, but also re-emphasized the importance of understanding the occurrence and action of naturally occurring substances which is essential to an understanding of the action of synthetic substances.

The organizers are to be congratulated on their choice of topics, so that the roles of plant growth substances from cell division to senescence were discussed in a comprehensive manner.

Twelve papers are concerned with the regulation of cell division. The introductory papers describe the complex molecular mechanisms of cellular regulation which are necessary for cell division and the processes which anticipate mitosis. Interest in cell division factors in plants is at present centred on the environment of the embryo, and several papers describe the isolation and characterization of substances from developing seeds and fruits. Coconut milk contains cell division factors, and it is not surprising, therefore, that much of the effect in this field has been directed to the isolation and characterization of substances which induce cell division in plant tissue cultures. The results are, at the moment, somewhat contradictory, and several substances, belonging to widely different chemical groups, have been identified which accelerate cell division; however, the isolation of three different phytokinins in coconut milk is probably significant. Progress is hampered by lack of a comprehensive range of bioassay methods for cell division factors. The interesting fact is recorded that the bleeding sap of *Vitis* also contains phytokinin-like substances which are probably synthesized in the root. Phytokinins may have effects other than on cell division, and their regulating effects on attraction and retention of nutrients have important implications in the transport of such substances to 'sinks', especially immature seeds and fruits. The largest section (sixteen papers) is concerned with the regulation of cell enlargement. Much of the effort is at present concerned with the form in which indoles occur in the plant and their transformation during growth. There is evidence that auxin interacts with macromolecular constituents of plants, for example, RNA. Six of this group of papers discuss the role of gibberellins, including their biosynthesis.

We know less of the occurrence and nature of natural growth inhibitors than of growth-promoting substances. One difficulty is to know whether a substance which acts as a growth inhibitor *in vitro* has the same role *in vivo*. This problem is well illustrated in five papers concerned with synergists and natural inhibitors. Some naturally occurring phenolic substances inhibit the growth of plant tissues at high concentration, but at lower concentrations growth may be stimulated. This suggested that such substances exert their effect through the indoleacetic acid oxidase system. In a discussion on dormancy it is pointed out that 'rest' stages of plant organs may be due either to the presence of growth-inhibiting substances or absence of growth-promoting substances. The first hypothesis is supported by a correlation between seasonal changes in bud dormancy and corresponding variations in the level of endogenous inhibitors.

Success in isolating the true growth-promoting substances from plants depends on the methods used. This problem is discussed in five papers using techniques of gas, paper and thin-layer chromatography and spectrophotofluorimetry.

Our knowledge of how growth substances regulate vegetative growth is still meagre. Widely ranging aspects of this topic are presented in seven papers and include the relative contributions of cell division and cell expansion to growth, the relation between contents of auxin and gibberellins to leaf growth, the substances governing root initiation and root growth. The polar transport of auxin and its modifications by unilateral influences have important implications in explaining geotropism and phototropism. The demonstration of the presence of minute crystalline bodies in apical cells of the *Avena* coleoptile which may act as photoreceptors in phototropism is a significant advance. The chapters on the regulation of flowering are mainly concerned with identification of substances which cause bolting or induce flower differentiation. No one can yet claim to have characterized such a substance, but it is encouraging that active crystalline extracts have been prepared. Finally, the four papers on senescence indicate the progress made towards an understanding of abscission processes and the role of phytokinins in regulation of leaf senescence.

This volume summarizes our knowledge of plant growth substances up to 1963 but, with rapid developments in this field, the information will soon be out of date. It is perhaps unfortunate, therefore, that it was considered necessary to have such a luxurious production.

E. C. Humphries

British Defence Policy and Nuclear War

By Emmaniel J. de Kadt. Pp. 148. (London: Frank Cass and Co., Ltd., 1964.) 21*s*. net.

THE question whether Britain should or should not continue to centre her defence policy on an independent nuclear force is now under re-examination by a new Government, and the responsibility may well force a more searching examination of the question than it has received from some of its opponents when out of office. Mr. de Kadt's book should assist in the independent and critical appraisal of a problem, inherently difficult and not to be further obscured by prejudice or preconceived ideas. His chapters outlining the nuclear strategies of the Western world and the development of the British nuclear deterrent are perhaps a little sketchy, but they display the inconsistencies and defects of successive White Papers on defence. The real value of *British Defence Policy and Nuclear War* lies in the five chapters which examine pragmatically the limitations or failure of deterrence, the function and limitations of civil defence and the situation which is likely to be encountered in Britain as a result of the use of nuclear weapons under conditions which are the most conceivable if, in fact, such weapons are used. The issues raised are inescapable, if often neglected or ignored, and Mr. de Kadt's treatment is sound, though perhaps open to criticism on detail. He does, perhaps, rather less than justice to the missile-firing submarine—on this Capt. S. W. Roskill's final chapter in *The Strategy of Sea Power* offers a firmer guide—but Capt. Roskill is just as emphatic as Mr. de Kadt that it is imperative for Britain to do everything possible to minimize the chances of nuclear attack ever happening. Even more interesting is its insistence that with the examination of the potential consequences of war must come the investigation of apparent barriers to peace. It is not for economic reasons alone that the appointment of a Minister of State to deal with disarmament is a logical part of foreign policy as well as of defence. It is a practical recognition of the fork in the road emphasized by Mr. G. Piel, and a definite step towards the policy of national security which Mr. de Kadt seeks to promote.

The Fabulous Isotopes

By Robin McKown. Pp. 189. (London: Macmillan and Co., Ltd., 1964.) 21*s*. net.

THIS book, intended for the general reader or as background reading for the senior school pupil, is an American attempt to popularize, in the best sense of the word, a field in atomic physics. It is simply and engagingly written and is generally accurate. The sections on the uses of isotopes seem to be better than the theoretical introduction concerning their nature. The illustrations are clear and helpful. The English reader will have to master American spellings which his English teacher might not accept. It is doubtful whether "about the size of a cantaloupe" will assist him in visualizing the dimensions of a miniature atomic power plant, to the working of which, incidentally, no clue is given.

Like so many other popular works, this book reveals the tremendous difficulties which beset an author of this kind of literature. To achieve the accuracy of statement which is the *sine qua non* of scientific writing is difficult enough. The present work, though largely concerned with neutrons, fails to mention the name of Chadwick. A simple explanation of the cloud chamber is given, but, although the Geiger counter is constantly mentioned, no explanation of its mode of action, or who Geiger was, is forthcoming. The old myth of Henri Becquerel accidentally leaving a piece of uranium ore on top of a photographic film is perpetuated and, incidentally, the date of his experiment is given wrongly. Here a real chance of a simple explanation of a planned experiment is lost.

The truth of the matter is that within a few weeks of the publication of Röntgen's X-ray paper, Becquerel, having read it, intelligently experimented to try to find a complementary or reciprocal phenomenon. The result of his experiments did not verify his hypothesis but quickly led him to the discovery of radioactivity.

The date of the discovery of artificial radioactivity is given as 1934 in the text and two years earlier in the summary. The positron appears in the text with the Curie-Joliots and in the summary with C. D. Anderson. These small points could be cleared up in a second edition.

Finally, the book raises some pedagogical questions in my mind, of which the following are specimens: Is there any virtue in printing a decimal fraction with twenty-seven noughts, to give an idea of the mass of an electron? The equation $e = mc^2$ is given, and the velocity of light appears as 186,000 miles per second. What units will the enquiring secondary-school pupil give to e? If text-books and popular books on science are to achieve optimum results their authors will have to consider painstakingly the nature of concept formation in relation to the intelligence and maturation levels of their readers.

W. L. SUMNER

Tumours of the Kidney and Ureter

Edited by Sir Eric Riches. (Neoplastic Disease at Various Sites, Vol. 5.) Pp. xv+416. (Edinburgh and London: E. and S. Livingstone, Ltd., 1964.) 90*s*. net.

COMPARED with other forms of neoplastic disease, tumours of the kidney evoke particular interest not only on account of their varied nature and behaviour, but because of recent developments in their earlier diagnosis and treatment. In bringing out this important monograph, Sir Eric Riches is to be congratulated on enlisting the support of a number of colleagues who have been carefully chosen to supplement his own unique experience. Thus, in addition to the statistical data which provide a regular feature of the series, authoritative sections are contributed by Dr. Cuthbert Dukes on the causation of renal neoplasms, by Dr. A. C. Thackray on their pathology and by Mr. D. Innes-Williams on tumours of the kidney in infancy and childhood. Among other contributions those by Sir Brian Windeyer on radiotherapy and by Dr. H. J. G. Bloom on hormone treatment are of particular practical importance.

The main message of the book, however, is conveyed by the description and analysis of renal tumours in adult life—by Sir Eric Riches, whose experience in this field is probably unparalleled and in which his numerous contributions have achieved international renown. This is supported by a wealth of illustrations in which the macroscopic features of the tumour are superimposed on the X-ray films so as to emphasize the importance of radiological interpretation. This theme is elaborated further by Dr. C. G. Whiteside and Dr. John L. Emmett, who refer respectively to the role of pyelography and arteriography, and nephrotomography in X-ray diagnosis. Other subjects of present-day interest which are discussed include the value of cytological diagnosis, the significance of polycythæmia and the association of renal tumours with hypertension. Perhaps the most important practical feature, however, is the section devoted to prognosis in which the results of an earlier series presented to the British Association of Urological Surgeons are expanded. Here the outlook is correlated with the pathological grading and extension of the disease at the time of surgical treatment in such a manner as to provide a valuable indication of what to expect.

In summary, the book, which is excellently produced, provides a comprehensive yet discriminative account of existing knowledge regarding the detection and management of renal neoplasm which will surely prove of value to all engaged in the investigation of this form of disease.

J. D. FERGUSSON

35-Gc/s, 70-Gc/s AND 94-Gc/s CYTHEREAN RADIATION

By Prof. C. W. TOLBERT and Prof. A. W. STRAITON
Electrical Engineering Research Laboratory, Austin, Texas

INTEREST in Cytherean radiating characteristics, defined by measurements at many different wavelengths on many different occasions, has been heightened by the prospect of physical exploration of Venus by the National Aeronautics and Space Administration, and many and varied models have been proposed to explain the level of radiation[1–5]. To resolve some of the degrees of modelling freedom allowed by the observed spectrum an extension of measured values of brightness temperatures into the domain of the millimetre wave-lengths is required. The millimetre wave-length observations must be conducted with the resolution of both line and continuum spectra in order to obtain information on the physical nature of the source that is available through the radiation and absorption of gaseous line spectra.

The measurements to be described in this article were conducted at frequencies of 35 Gc/s, 70 Gc/s and 94 Gc/s with a 16-ft. diameter antenna[6]. The 35-Gc/s measurements were made to associate the relative brightness temperature of the three frequencies to the brightness temperature of a frequency at which a number of measurements have been reported. While the three frequencies are too widely spaced to yield significant line spectra and even the bandwidth of the radiometer at each frequency is excessively wide for low-pressure gas line resolution, the absorption minima of the Earth's atmosphere at 35 Gc/s and 94 Gc/s make these frequencies attractive for Earth-based observations. By including 70 Gc/s, a frequency at which components are conveniently available and a frequency as far removed from the other two as the oxygen absorption in the Earth's atmosphere will allow normal Earth-based observations to be, a crudely defined spectrum was hoped for.

Conventionally designed Dicke-type a.c. radiometers with 30-c/s mechanical modulators were used in conjunction with the 16-ft. mm wave-length antenna system for the measurements. The root mean square uncertainties of the radiometers were 1° C, 5° C and 7° C for integrating periods of 1 sec at the frequencies of 35 Gc/s, 70 Gc/s and 94 Gc/s, respectively. The radio frequency components and the 10-Mc/s bandwidth intermediate frequency pre-amplifiers were incorporated at each frequency with the antenna feed horns and supported singularly by the antenna spars at the prime focusing of the 16-ft. parabola. The feed horns accepted 10 dB periphery tapered energy from the parabola with resulting antenna gains of 62·5 dB, 68·5 dB and 70·9 dB, at the frequencies of 35 Gc/s, 70 Gc/s and 94 Gc/s, respectively. The remainder of the radiometers, the intermediate frequency amplifier, the narrow bandwidth amplifier-synchronous detector and the assorted power supplies, were commonly used at three frequencies.

Analogue recordings of the millimetre telescope output were made during right ascension scans of the antenna beam across Venus. At each frequency the polar axis drive rate was adjusted to scan in the period of 10 radiometer time constants an angle relative to Venus equal to the antenna half-power beamwidths. Scans were repeated over an angle of ± 3 antenna beamwidths as frequently as possible when Venus was within ± 2 h of the meridian, and markers were applied to the data sample recordings when optical telescopes collimated with the millimetre telescope beam pointed at Venus.

The optical telescopes were collimated with the antenna beam by pointing the millimetre telescope for equal signals at the north–south point of the Moon and aligning

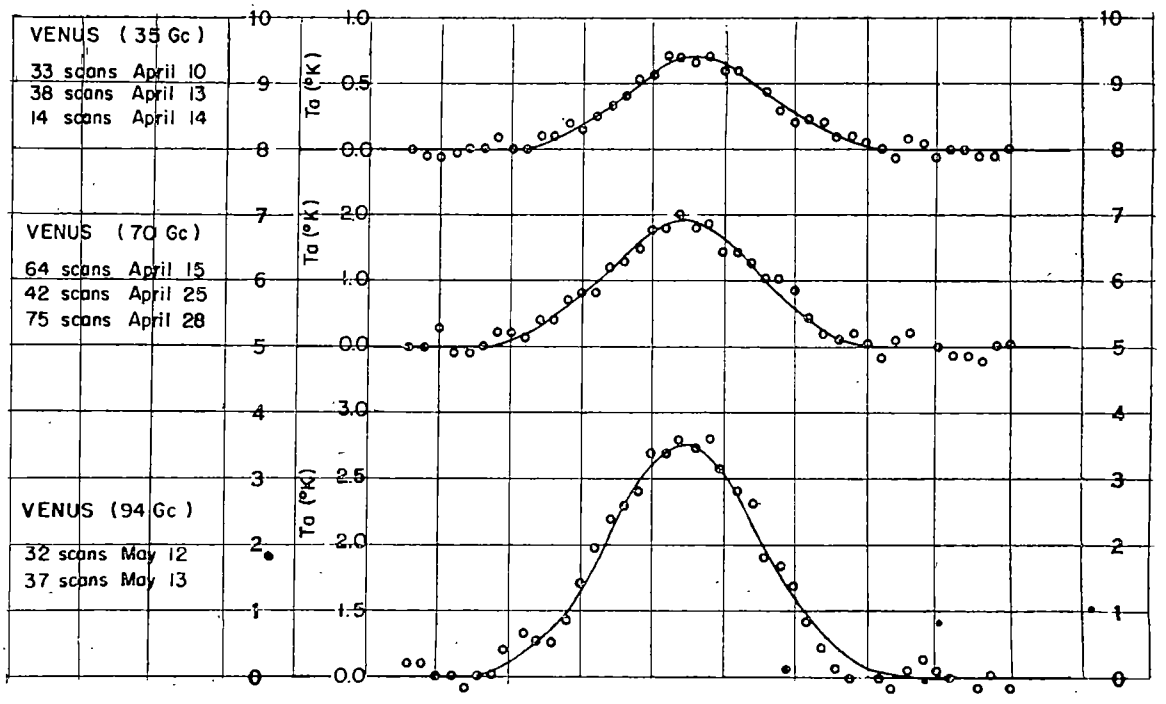


Fig. 1. Examples of antenna temperatures obtained from averages of data samples near eastern elongation

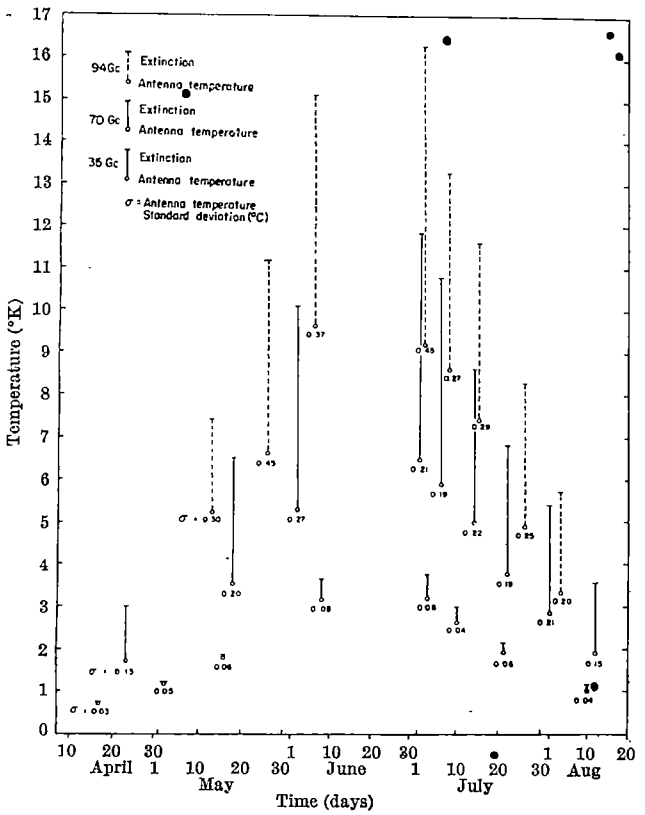


Fig. 2. The 35-, 70- and 94-Gc/s antenna temperatures and atmospheric extinction corrections

the cross-hair radicals of the telescopes with the limbs of the Moon. Collimation was performed at declination angles as near as possible to that of Venus to minimize optical-millimetre wave-length refraction differences and mechanical antenna beam-optical beam differences.

A single value of the atmospheric attenuation for an average value of altitude angle was used to evaluate the millimetre wave-length extinction each day. The attenuation values were derived from radiosonde data of the total precipitable water in the atmosphere. Wave-guide terminations immersed in boiling and tap water temperatures, electrically heated black bodies and gas noise tubes were used to calibrate the radiometer. The average full-scale pen and paper antenna temperature recordings were 6° C, 30° C and 40° C at the frequencies of 35 Gc/s, 70 Gc/s and 94 Gc/s, respectively.

Observations were attempted each day between April 10 and August 22, with the exception of those days omitted when Venus appeared within ± 1 h of the Sun setting. Data were excluded when clouds prevented optical confirmation of the antenna pointing, when during the approximately 3–6 min of a data sample scan, the variability of the emission and absorption of the atmosphere drastically disturbed the low-frequency components of the signal level or when the positions of the antenna side lobes relative to the Sun drastically disturbed the low-frequency components of the signal-level. Examples of the results obtained by averaging at 1/12 antenna beam-width intervals data samples from single days or groups of days are shown in Fig. 1. The solid curves are radiometer time constant transformed antenna pattern responses to point sources best-fitted to the measured values.

The brightness temperatures of Venus were evaluated from the antenna temperatures with the following expressions:

$$T_s = T_a \left[\frac{41{,}253}{(Ag)(K\Omega_s)(\Omega_s)^2}\right] e^{0.23\Gamma}$$

Where T_s is the source brightness temperature (°K), T_a, the antenna temperature (°K), Ag, the antenna gain (maximum relative to isotropic source), $K\Omega_s$, the antenna gain correction for source size, Ω_s, the angle subtending source (deg.) and Γ, the attenuation through the Earth's atmosphere (decibels), such that:

$\Gamma = 0{\cdot}10 + 0{\cdot}11\ (\rho_\omega)\ \sec\varphi$ (at 35 Gc/s)
$\Gamma = 1{\cdot}70 + 0{\cdot}16\ (\rho_\omega)\ \sec\varphi$ (at 70 Gc/s)
$\Gamma = 0{\cdot}65 + 0{\cdot}33\ (\rho_\omega)\ \sec\varphi$ (at 94 Gc/s)

ρ_ω is precipitable atmospheric water vapour (g/cm²), φ is zenith distance (degrees).

The uncertainties of the brightness temperatures are represented by the following expressions:

$$\Delta T_s = T_s \cdot A_p \pm \sqrt{(Ag^2) + (T_a)^2 + (T_c)^2 + (e^{0.23\Gamma})^2}$$

Where ΔT_s is the source temperature uncertainty, A_p, the antenna pointing uncertainty (5, 3 and 0 per cent at 94, 70 and 35 Gc/s, respectively), Ag, the antenna gain uncertainty (12, 8 and 5 per cent at 94, 70 and 35 Gc/s, respectively), T_a, the antenna temperature uncertainty (variable with number of data samples averaged), T_c, the temperature calibration uncertainty (5 per cent at 94, 70 and 35 Gc/s), and $e^{0.23\Gamma}$, the absorption uncertainty (5 per cent at 94, 70 and 35 Gc/s).

Graphs of the 35-, 70- and 94-Gc/s antenna temperatures as a function of time, graphically displaying the magnitude of the correction for atmospheric extinction, are shown in Fig. 2. The 35-, 70- and 94-Gc/s brightness temperatures of Venus as functions of time, Cytherean phase (included angle between the Earth, Venus and the Sun) and fractional Cytherean disk illuminated are shown in Fig. 3. The nominal brightness temperature uncertainties are +14 per cent −14 per cent at 35 Gc/s, +17 per cent −11 per cent at 70 Gc/s and +19 per cent −9 per cent at 94 Gc/s.

Temperatures of approximately 250° K and 350° K are prescribed for rapidly and synchronously rotating bodies, respectively, with an albedo of 0·7 at the position of Venus relative to the Sun. The spectral brightness temperature of Venus as defined by some of the measured values is

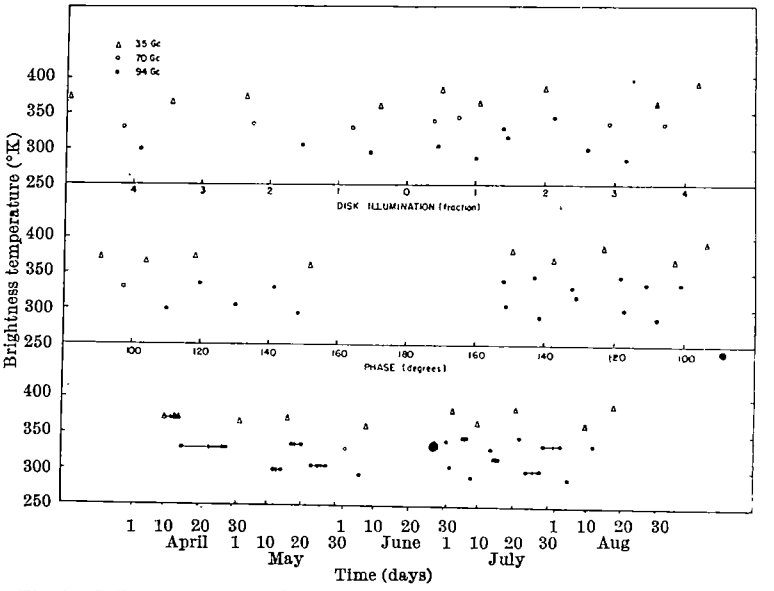


Fig. 3. Cytherean 35-, 70- and 94-Gc/s brightness temperatures as functions of time, phase and disk illumination

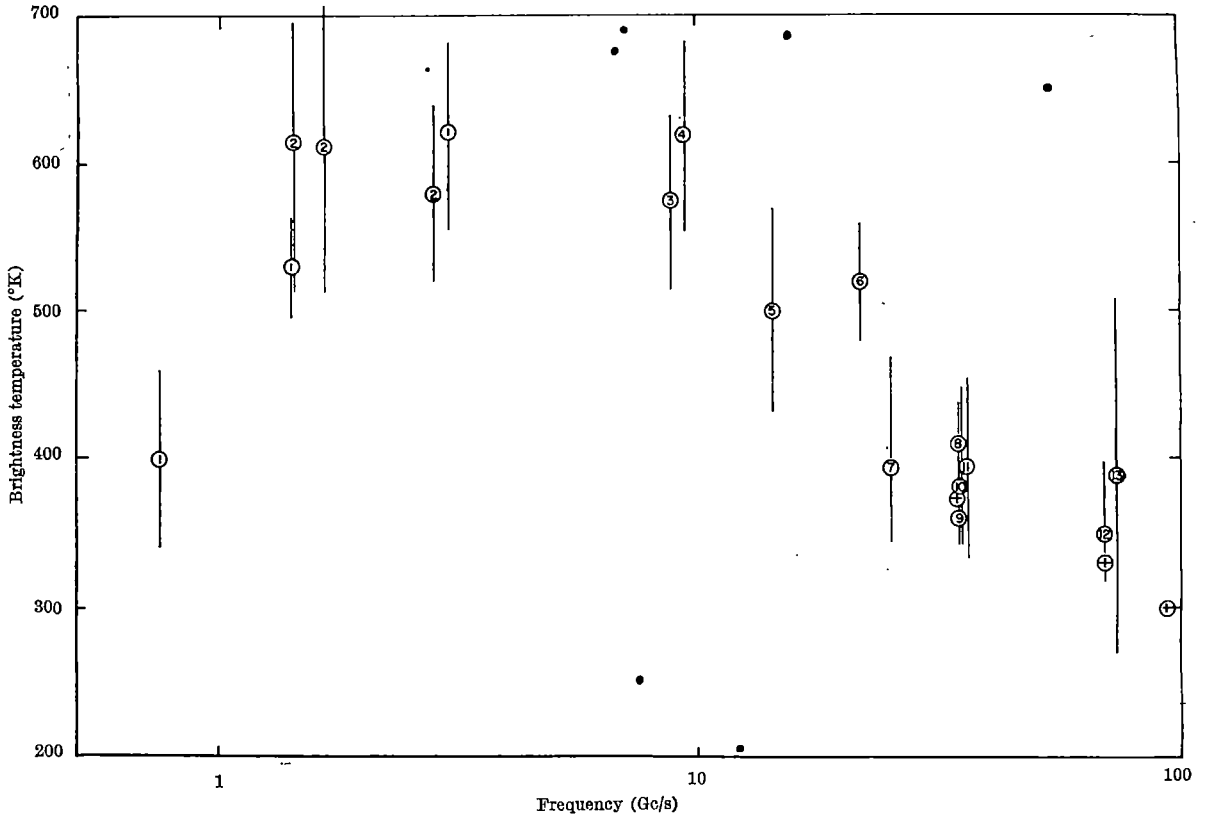


Fig. 4. Data representative of observations defining the spectral brightness temperature of Venus. 1, Drake, 1964; 2, Clark and Spencer, 1964; 3, Alsop, Giordmaine, Mayer and Townes, 1958; 4, Mayer, McCullough and Sloanaker, 1963; 5, McCullough and Boland, 1964; 6, Gibson and Corbett, 1963; 7, Staelin, Barrett and Kusse, 1964; 8, Gibson, 1963; 9, Copeland and Tyler, 1964; 10, Lynn, Meeks and Sohigian, 1964; 11, Thornton, 1964; 12, Grant, Corbett and Gibson, 1963; 13, Kislyakov, Kuz'min and Salomanovich, 1962; +, Tolbert and Straiton, this paper

shown in Fig. 4. The 35-, 70- and 94-Gc/s values are average temperatures measured between April 10 and August 22, 1964.

Planetary emission (T_e) can be represented by the expression

$$T_e = \sum_{i=1}^{n} \varepsilon T_i \, (1 - e^{-\alpha_i \Delta z_i \sec\theta}) \, e^{-\sum_{i=1}^{i-1} \alpha_{i-1} \Delta z_{i-1} \sec\theta}$$

T_e is emission temperature, T_i and α_i is temperature and attenuation of the element of depth Δz inward through the planetary mantle (atmosphere) and core (surface), ε is emissivity of planetary element, θ is angle of the observation relative to the emitting element zenith.

A summation of T_e for all values of θ over the planetary disk yields the brightness temperature. The zenith emission from an isothermal mantle and an isothermal core is:

$$T_e = T_m(1 - e^{-\tau}) + T_c e^{-\tau}$$

T_m is mantle temperature, T_c is core temperature, τ is $\int \alpha dz$.

The Cytherean spectrum can be described either by radiation from a hot core and a cold mantle increasingly opaque from 10 Gc/s to 100 Gc/s (thermal model)[1,3,4,7] or by radiation from a hot mantle decreasingly opaque from 10 Gc/s to 100 Gc/s and a cold core (non-thermal model)[2,5,8].

To obtain a hot-surface temperature, 'greenhouse' heating by a mantle of low optical wave-length opacity and high infra-red wave-length opacity (caused by a small density of a strongly absorbent gas, such as H_2O, or a large density of a weakly absorbent gas, such as carbon dioxide) or friction heating at the surface by wind-driven dust aerosols (agitated by solar radiation absorbed at high altitudes by dust clouds) have been proposed.

To obtain a hot-mantle temperature, free–free radiation from a high-density ionosphere (caused by solar ionization of the mantle in the absence of magnetic shielding) or radiation from charging and discharging of the large aerosol particles (resulting from the charge accumulation of precipitating particles) has been proposed.

Representative examples of the 10–100 Gc/s spectrum of each model are displayed with the measured brightness temperature values in Fig. 5. Within the uncertainty limits of the absolute brightness temperatures all models describe the observed spectrum.

Brightness temperatures at 3 Gc/s and 9·53 Gc/s as functions of Cytherean phase (i) are described by the following expressions[9,10]

(3 Gc/s) $\quad 622° \text{ K} + 41 \cos(i - 21)° \text{ K}$

(9·53 Gc/s) $\quad 621° \text{ K} + 73 \cos(i - 11{\cdot}7)° \text{ K}$

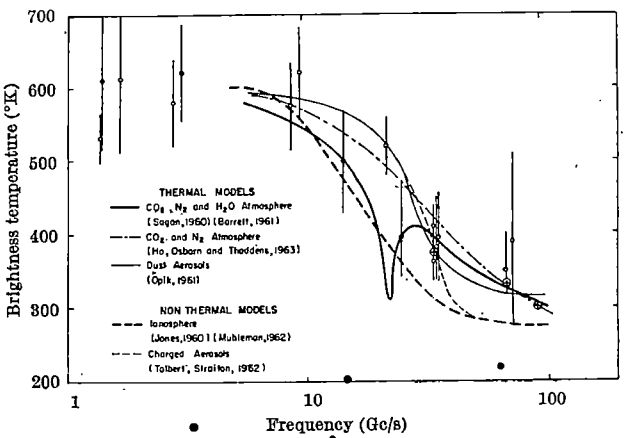


Fig. 5. Examples of spectra of proposed models describing the Cytherean radiating mechanism

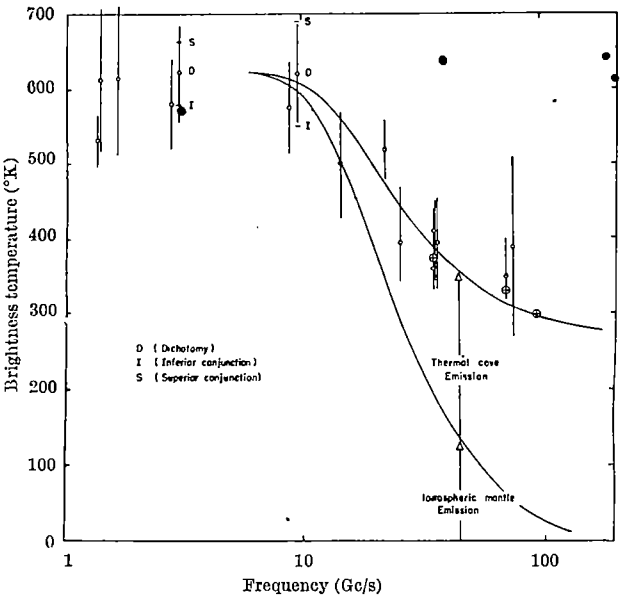


Fig. 6. Calculated ionospheric Cytherean spectrum best describing observations

Expressions of the Cytherean phase dependencies of the 35-, 70- and 94-Gc/s brightness temperature are unobtainable from the data due to the apparently small dependency factors relative to the uncertainties and sparseness of brightness values at each of the frequencies. The rank difference correlation coefficients of the Fraunhofer Institut sunspot indices, adjusted for the position of Venus relative to the Earth-based observations, with the millimetre brightness temperatures at 0·80 at 35 Gc/s, 0·75 at 70 Gc/s and 0·35 at 94 Gc/s. The quality of the correlation of the millimetre wave-length brightness temperature perturbations with the solar indices enhances the possibility that the Cytherean radiation is, in part, ionospheric.

Scaled millimetre wave-length phase-brightness temperatures from an average of the 3 Gc/s and 9·53 Gc/s phase-temperature characteristics based on an ionospheric model having a hot mantle and cold core of 620° K and 280° K, respectively (electron temperature $\sim 620°$ K, electron density $\sim 10^9$ cm^{-3} and integrated squared electron density through ionosphere is 10^{26} cm^{-5}), are as follows:

(35 Gc/s)	$375° \text{ K} + 17 \cos(i-x)° \text{ K}$
(70 Gc/s)	$330° \text{ K} + 9 \cos(i-x)° \text{ K}$
(94 Gc/s)	$300° \text{ K} + 4 \cos(i-x)° \text{ K}$

If any phase characteristic of the millimetre data of Fig. 3 is to be recognized there is an apparent phase advance of the millimetre wave-length minimum brightness temperature relative to that of the centimetre wave-lengths to a time preceding inferior conjunction. Such an interpretation implies, for an ionospheric model, that the predominating core radiation at the millimetre wave-lengths originates from a slightly retrograde rotating planet. The spectral contributions from the mantle and the core of the ionospheric model are shown in Fig. 6.

There are acknowledged objections to the ionospheric model based on the intensity and character of the radar returns from Venus[8]. It is also to be recognized that each model in itself probably oversimplifies a complex environment and that a combination of the elements of the models is required to describe the radiation in detail: certainly the inclusion of water vapour would improve the 'spectral fit' around 20 Gc/s and provide the characteristic of increasing the rate of extinction between 35 Gc/s and 180 Gc/s as exhibited by the median 35-, 70- and 94-Gc/s values.

This work and the continuation of the investigations of millimetre wave-length radiation from bodies of the solar system are supported by a grant *NsG*–432 from the National Aeronautics and Space Administration.

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MAGNETIC, GRAVITY AND DEPTH SURVEYS IN THE MEDITERRANEAN AND RED SEA

By T. D. ALLAN
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ALTHOUGH a relatively small and land-locked basin, the Mediterranean has many oceanic features. About three-quarters of the area is deeper than 1,000 fathoms, with extensive areas deeper than 1,500 fathoms and isolated depths of more than 2,500 fathoms. The western and eastern basins are geophysically different.

In both western basins there are extensive areas of abyssal plain, whereas the floor of the eastern basin, in spite of the sediment from the Nile, is characterized by an unusual type of small-scale relief. To the north of the eastern basin the Cretan island arc bounds the irregular topography of the Aegean Sea. Geophysical investi-

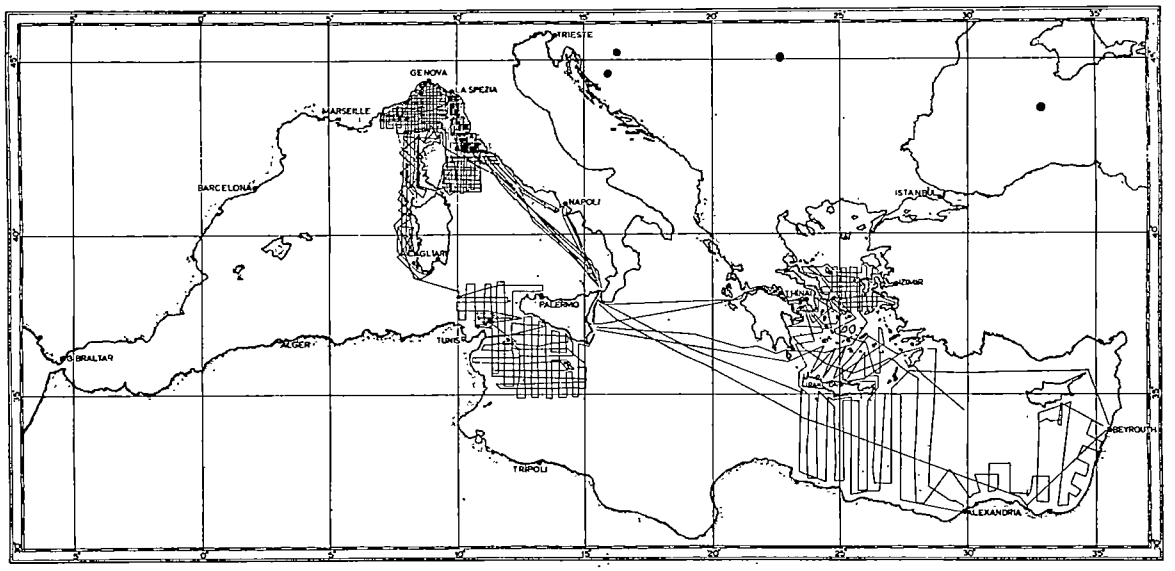


Fig. 1. Tracks of S.S. *Aragonese* in the Mediterranean Sea during the period July 1961–March 1963

gations of the differences between the eastern and western basins may provide clues as to their origin.

The Red Sea is another area where systematic observations may be of more than local interest. Previous observations have shown the similarity between the rift valley of the southern Red Sea and that of the mid-Atlantic Ridge.

The Saclant ASW Research Centre was commissioned in 1959 and its Oceanography Group formed the following year. Submarine geophysical observations in both the Mediterranean and the Red Sea have formed part of the group's programme. A summary of the geophysical work is given in this article.

Facilities

S.S. *Aragonese*, a freighter 293 ft. long, displacing 3,000 tons, was chartered for conversion into a marine research vessel. Radar, gyrocompass, precision depth recorder and other essential equipment were fitted initially. A Loran-*C* receiver and an electromagnetic log were fitted later.

The number of scientific personnel directly involved with the geophysical programme was small and the group frequently co-operated with other research establishments.

An agreement was reached with the Osservatorio Geofisico Sperimentale, Trieste, on a joint programme of gravity, magnetic and bathymetric survey work. The gravity meter, a Graf-Askania, was owned by the Osservatorio and operated by its staff throughout the cruises.

The gravity values were usually tabulated at 15-min intervals. The accuracy of the free-air gravity value depends not only on the performance of the meter and stabilized platform but also on the accuracy of the Eötvös correction which is calculated from the ship's speed and course. In this respect the Mediterranean has presented fewer problems than those in the ocean generally. In the earlier cruises radar signals on numerous islands provided good control: for most of the work the probable error in position was $\pm 0{\cdot}25$ mile. For the later surveys, in areas far from land, Loran-*C* positions proved reliable to better than $\pm 0{\cdot}5$ mile.

The magnetometer was the nuclear spin model described by Hill[1] and manufactured by Bruce Peebles, Ltd. It had a sensitivity of $\pm 0{\cdot}5$ γ. Readings were taken every 30 sec and recorded on punched paper for later reduction on the Centre's computer. The greatest inaccuracy in the measurement of the absolute value of the field lay in the uncertain knowledge of its diurnal variation.

Depth was recorded on a precision depth recorder which could be read to ± 1 fathom.

During the period July 1961–March 1963 seven cruises were made. These were devoted almost entirely to magnetic, gravity and depth surveys of selected areas, but brief trials of other techniques, such as coring and seismic reflexion profiling, were also made.

About 40,000 miles of track have been covered: the distribution in the Mediterranean and Red Sea is shown in Figs. 1 and 2.

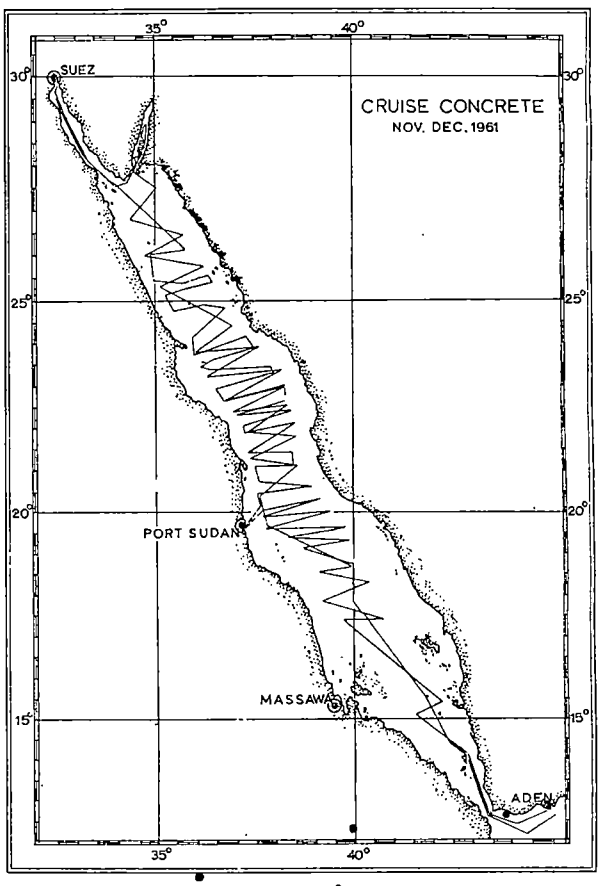


Fig. 2. Tracks of S.S. *Aragonese* in the Red Sea during November–December 1961

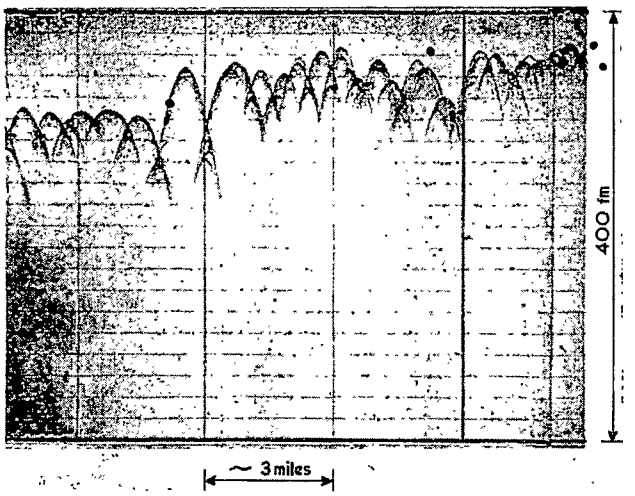


Fig. 3. Profile across the unusual floor of the eastern Mediterranean Sea

Western Mediterranean

The first cruise was used to compare the performance of different types of gravity meter. The Trieste Graf-Askania and a LaCoste-Romberg instrument provided by the U.S. Office of Naval Research were installed in the ship. Observations were made in three areas between La Spezia and Sicily where earlier bottom gravity readings[2] provided a precise reference. Consequently, not only relative but absolute comparisons of the surface-ship meters were possible. In the area near La Spezia precise navigational control was achieved by using theodolites on nearshore hills.

The gravity meters agreed within 10 m.gal in calm conditions, but modifications to the LaCoste-Romberg were necessary to allow for the 'Browne' corrections in rougher weather. An account of the comparisons has been published[3].

Other cruises in the western basin have examined the continental slopes which border the Balearic and Tyrrhenian abyssal plains. These slopes are cut by submarine canyons resembling those found in other parts of the continental margin but which have been interpreted as former sub-aerial valleys.

Between Sicily and Malta the survey did not appear to change the existing conception of the geology of the area. There were large magnetic anomalies around volcanic islands such as Pantelleria, and the negative Bouguer gravity anomaly known to exist in western Sicily was shown to change to a positive anomaly in the centre of the Strait.

Eastern Mediterranean

In the eastern Mediterranean the islands of Crete, Scarpanto and Rhodes appear to form an island arc structure with contrasting geophysical properties in the areas to the north and south of the arc. These areas have been extensively surveyed during three of our cruises. Dr. K. O. Emery took part in one of these, during which a relief map of the sea-floor was prepared from a study of the type of bottom displayed on the echo-sounder.

Much of the sea-floor in the eastern basin is characterized by the type of small-scale relief shown in Fig. 3. The lack of deposition appears to be caused by a series of trenches which border the area and act as a trap for sediment spreading from the Nile.

Perhaps the most interesting aspect of the eastern basin, in spite of the lack of sedimented area, is the dullness of its magnetic field. Thus, apart from the latitudinal variation, there is no significant change of magnetic field between North Africa and Crete. This can be seen from Fig. 4, which shows a typical north south profile of magnetic field, gravity and depth across the island arc. To the north there are several isolated magnetic anomalies, probably associated with vulcanism.

The gravity field is broadly similar to that associated with the island arcs of the major oceans. Generally negative in the southern part of the eastern basin, there is a free air anomaly of about −100 m.gal in a band associated with the deep trenches just to the south of the arc. To the north the gravity field is generally positive, reaching +140 m.gal.

The Red Sea

A survey of the Red Sea and Gulf of Aqaba was made between October and December 1961. A second Graf-Askania gravity meter, supplied and operated by scientists of the German Hydrographic Institute and the Bundesanstalt für Bodenforschung, was embarked for this cruise and provided a good opportunity of comparing the performance of two meters of the same type. Over most of the cruise the agreement was excellent.

Preliminary surveys in the Red Sea[4,5] had shown the existence of a magnetic anomaly associated with a steep-sided medial valley about 30 miles wide. This anomaly was studied in much more detail during the 1961 cruise. A total of fifty-four transverse crossings were made. Records from a typical crossing (20° N.) are shown in Fig. 5.

A preliminary analysis of the results shows that the median valley, which is more fully developed in the south,

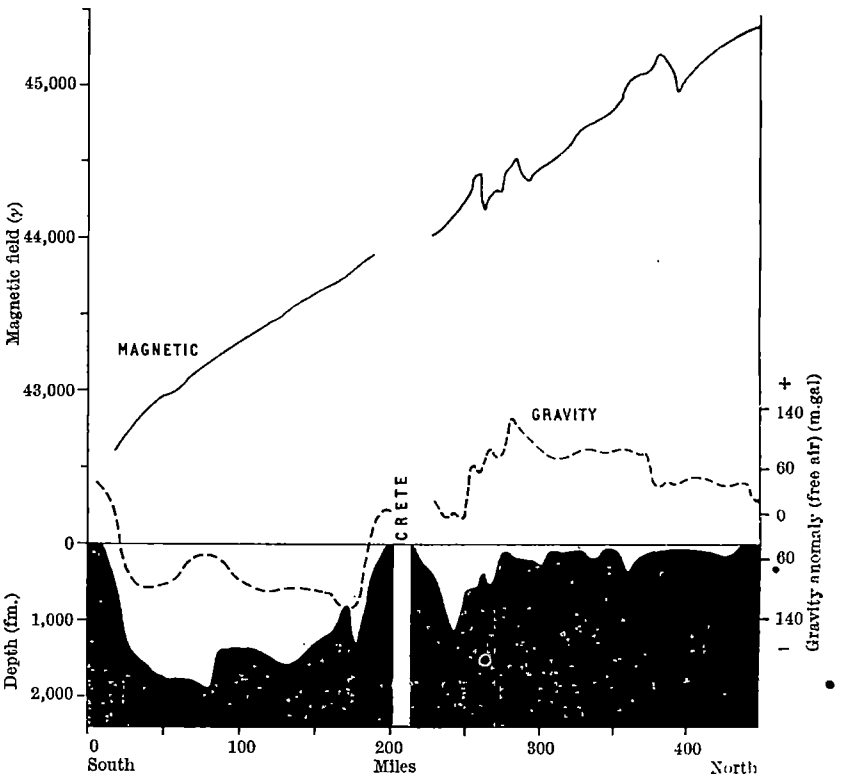


Fig. 4. Profile along meridian 26° E. of gravity (free-air) anomaly, total magnetic field and depth. The southern limit is the African coast and the northern limit lies at latitude 39° N. in the central Aegean

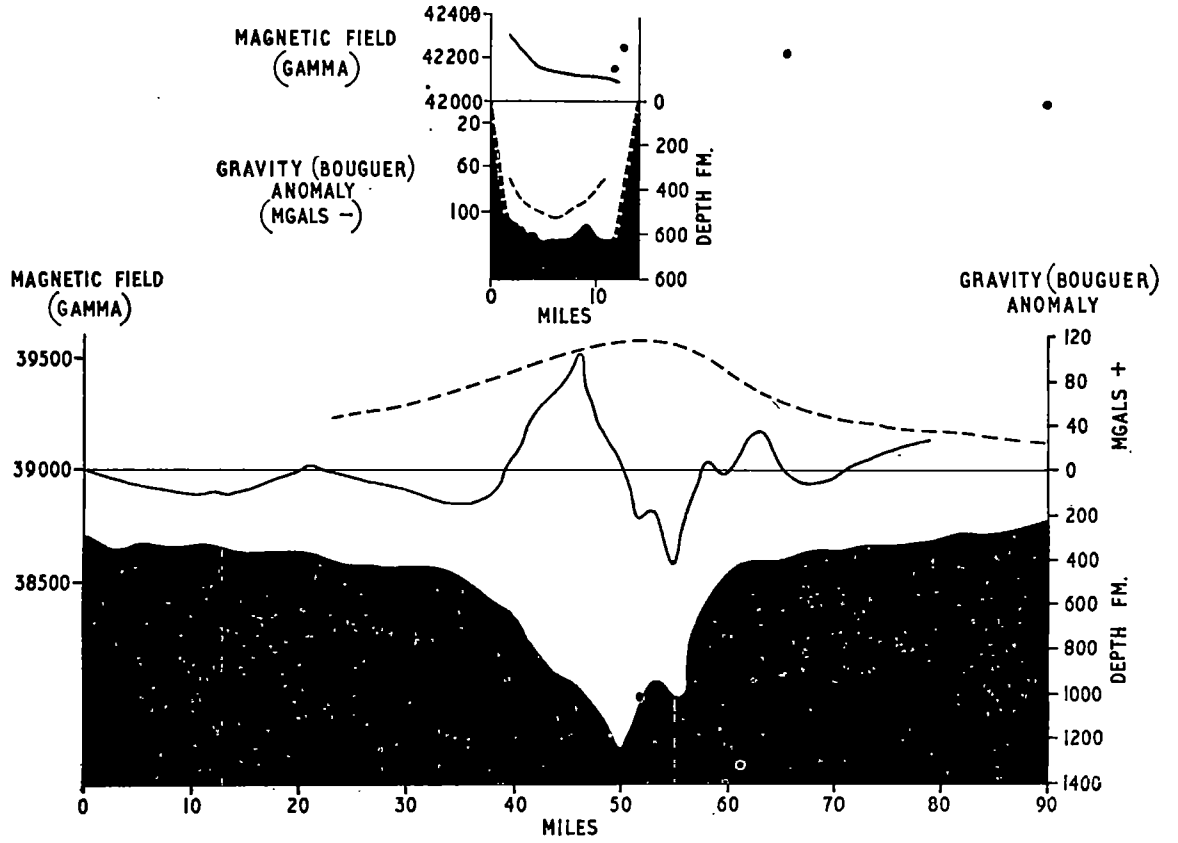


Fig. 5. Gravity and magnetic profiles across the Gulf of Aqaba (top), and across the Red Sea at 20° N. (bottom). The profiles are drawn to the same scale

is underlain by a considerable thickness of dense, magnetic rock. The lateral extent of this material appears to be confined to the width of the valley.

At the northern end of the Red Sea the Gulf of Suez is shallow and dull both in its magnetic and gravity structure. The Gulf of Aqaba, however, provides an interesting contrast. The Aqaba rift is everywhere less than 15 miles wide and about 600 fathoms deep. It is flanked on both sides by steep mountains. There is a strongly negative gravity anomaly, reaching −200 m.gal (free air) and −100 m.gal (Bouguer), while the magnetic field is featureless. The structure of the Gulf of Aqaba resembles that of the Gulf of Corinth; both may be thickly sedimented grabens.

The contrasting geophysical properties of the Gulf of Aqaba and the Red Sea, shown in Fig. 5, must be taken into account in any attempted interpretation of the origins of the Rift system.

We thank Dr. E. T. Booth and Dr. J. M. Ide for their advice during their service as scientific director of the Saclant ASW Research Centre; and our colleagues, ashore and afloat, and the captain, officers and crew of S.S. *Aragonese* for their assistance.

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SYNTHESIS OF PYROPHOSPHATE UNDER PRIMITIVE EARTH CONDITIONS

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It is reasonable to assume that some of the polymerization and dehydration reactions of simple organic compounds on the primitive Earth were carried out by high-energy phosphates. Experiments based on this assumption have been performed by Schramm *et al.*[1], who reported that ethyl metaphosphate can effect a number of polymerizations including the synthesis of polynucleotides from nucleotides. These polymerizations are carried out in anhydrous dimethylformamide as solvent, and they apparently require closely controlled conditions since they are difficult to repeat in detail[2–4]. Ponnamperuma, Sagan and Mariner[5] have synthesized adenosine triphosphate from ADP, AMP or adenosine in dilute aqueous solutions using ethyl metaphosphate and ultra-violet light. The role of the ultra-violet light is not clear, since the ethyl metaphosphate is already a high-energy phosphate.

Ethyl metaphosphate is prepared from phosphorus pentoxide, diethyl ether and chloroform. It is most unlikely that such a polymer could have occurred on the primitive Earth, at least if synthesized in this way. The most unlikely aspect is not so much the diethyl ether or chloroform, but rather the P_2O_5. Phosphorus pentoxide does not occur naturally on the Earth, and if it were

formed by some thermal process it could not be made available for synthetic reactions before it was hydrated by traces of moisture. The occurrence of polyphosphoric acid, which has been used in a 'thermal model of prebiological chemistry'[6-8], is also improbable for similar reasons.

It would be more plausible to assume the presence of meta- or poly-phosphates. These compounds are well known[9] and easily prepared by heating alkali metal or calcium dihydrogen phosphates above ~300°. However, metaphosphate minerals are not known to occur in Nature. This is not surprising since $Ca(H_2PO_4)_2$, which is the starting material for a thermal dehydration to calcium metaphosphate, is itself not a known mineral[9]. Pyrophosphate could be formed by the thermal dehydration of brushite ($CaHPO_4 \cdot 2H_2O$) or monetite ($CaHPO_4$), which are relatively rare minerals, but this apparently does not occur since not a single pyrophosphate mineral is known[10-12].

Although pyro- or meta-phosphate minerals may eventually be discovered, it is clear that they do not occur on an extensive scale. Greater volcanic activity on the primitive Earth would not change this conclusion. The phosphate in igneous and metamorphic rocks, which would be the most likely places to find thermally produced metaphosphates, occurs principally as hydroxyl apatite ($Ca_{10}(PO_4)_6(OH)_2$) with varying degrees of substitution of the hydroxide by fluoride and chloride. The principal phosphorus mineral in sedimentary rocks is also apatite.

The reducing conditions on the primitive Earth would not result in reduced phosphorus compounds to any great extent, since all the lower oxidation states of phosphorus are thermodynamically unstable in an aqueous environment[13]. Schreibersite (Fe_3P) occurs in the iron phase of meteorites; but the phosphorus would be oxidized to phosphate by water.

We have therefore looked for a low-temperature synthesis of pyrophosphate using various compounds with high free energies of hydrolysis that might have occurred on the primitive Earth, and we have found that pyrophosphate can be synthesized by the reaction of cyanate with hydroxyl apatite. Recently, Steinman, Lemmon and Calvin[14] have used cyanamide and its dimer to synthesize glucose-6-phosphate, pyrophosphate and AMP. These dehydration reactions were carried out in dilute solution at a *p*H of 2.

The apatite was prepared by adding 0·04 M sodium dihydrogen phosphate to 0·025 M calcium chloride, both buffered at *p*H 8 with *tris* [*tris*(hydroxymethyl) aminomethane]. The apatite formed was centrifuged and washed several times with water. This apatite was finely divided, and its X-ray powder pattern showed good crystallinity. The apatite was suspended in 0·05 M *tris* buffer (0·09 M in phosphate if dissolved), and the *p*H was adjusted with hydrochloric acid or sodium hydroxide. The Ca^{+2} concentration in solution was brought to 0·01 M with calcium chloride, and the cyanate was added as KNCO. The mixtures were shaken in a water bath at 35° for 20 days to complete the reaction. The apatite and calcium pyrophosphate formed were then dissolved with ethylenediamine tetraacetic acid, and this mixture was chromatographed on 'Dowex 1' (ref. 15). The pyrophosphate in the fractions was hydrolysed, and the phosphate was determined by the Fiske–SubbaRow procedure[16]. The identification of product was confirmed by paper chromatography[17], and by comparing the infrared spectrum with an authentic sample of calcium pyrophosphate.

The yield of pyrophosphate as a function of *p*H with 0·001 M KNCO is shown in Fig. 1. The yields are expressed as moles of pyrophosphate synthesized per mole of cyanate added, and the maximum is 27 per cent at *p*H 6·5. In addition to the pyrophosphate, a small amount of triphosphate (about 5 per cent of the pyrophosphate) was obtained. The yields of pyrophosphate

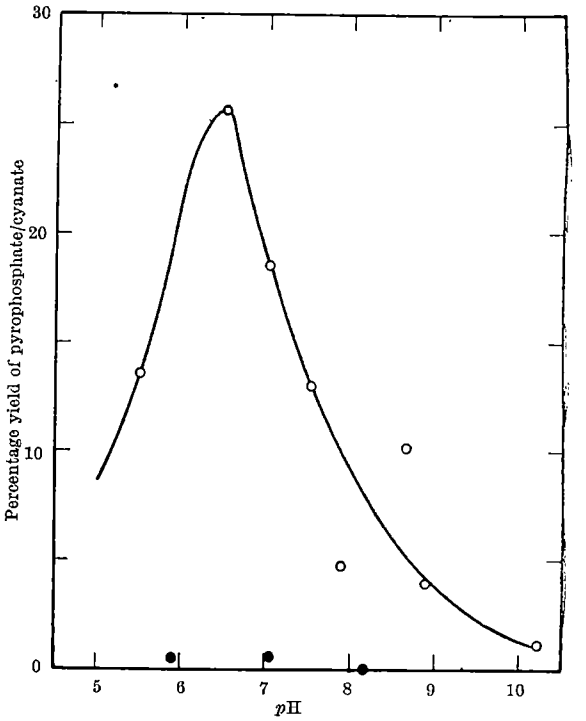


Fig. 1. The yield of pyrophosphate as a function of *p*H. Apatite + 0·001 M KNCO, 0·05 M *tris* buffer (0·1 M borate added for *p*H > 8) (○); 0·64 M sodium phosphate buffer + 0·1 M KNCO (●). Samples shaken at 35° for 20 days

from concentrated phosphate buffers and cyanate are very low (Fig. 1).

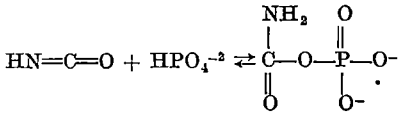
Although the synthesis of pyrophosphate is reproducible, there is considerable variation in the yields with the apatite reaction as would be expected with a surface reaction. One preparation of apatite gave lower yields and did not show the *p*H maximum.

The yields with apatite are not affected appreciably by the concentration of Ca^{+2} or by adding Mg^{+2}. Pyrophosphate is also synthesized with apatite prepared in the absence of *tris* and buffered with phosphate. The effect of fluoride, trace elements and various organic compounds on this reaction is being investigated.

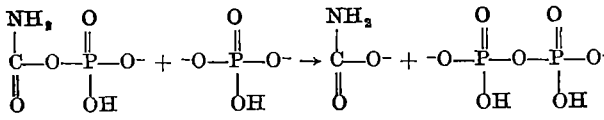
The free energy of hydrolysis of cyanate to NH_4^+ and HCO_3^- is about -12 kcal at a *p*H of 8. There are a number of other compounds such as hydrogen cyanide, thiocyanate, cyanamide, and cyanogen which also have a sufficient free energy of hydrolysis to synthesize a pyrophosphate bond and which could have occurred on the primitive Earth. Under the conditions used, hydrogen cyanide, thiocyanate and cyanamide did not react with the apatite to give pyrophosphate. Cyanogen gave significant yields of pyrophosphate with apatite and with soluble phosphate, the yields being greater with the apatite. However, this reaction gives erratic results and is being investigated further.

Cyanate is a reasonable compound to have occurred in the oceans of the primitive Earth. Urea has been synthesized from methane, ammonia and water by electric discharges[18] and by high-energy electrons[19]. The urea in these experiments was probably synthesized from ammonia and cyanate by the Wöhler synthesis. This reaction is reversible[20], $NH_4^+ + NCO^- \rightleftarrows CO(NH_2)_2$, $K_{25°} = 3 \times 10^4$. Although most of the cyanate produced by various sources of energy would react with the NH_4^+ in the oceans, the urea produced thereby would on decomposition yield a low steady-state concentration of cyanate to react with the apatite.

This synthesis could reasonably occur by the reaction of cyanate with phosphate on the surface of the apatite to form carbamyl phosphate:



Carbamyl phosphate ordinarily decomposes back to cyanate and orthophosphate. The equilibrium constant in solution is 3·1 (ref. 21). The reaction of carbamyl phosphate with another phosphate at the O–P bond rather than at the C–O bond would give pyrophosphate by the displacement of carbamate,



The carbamate would then decompose to carbon dioxide and ammonia. These equations refer to reactions taking place on the surface, probably involving chelation by calcium, and the states of ionization have been written arbitrarily.

Jones and Lipmann[21] have proposed that carbamyl phosphate may have been an important source of high-energy phosphate bonds on the primitive Earth. The data here presented support this hypothesis, provided the reaction occurs on the surface of the most abundant phosphate mineral.

It has been pointed out by Gulick[22] and emphasized by Miller and Urey[13] that the synthesis of organic phosphates on the primitive Earth presents difficulties because of the very low solubility of apatite in the ocean (3×10^{-6} M phosphate). The results reported here suggest that a solubilization mechanism for calcium phosphates may not have been needed. The phosphate could have been made available for primitive syntheses by reactions taking place on the surface of phosphate minerals, the concentration mechanism for this relatively non-abundant element being a simple precipitation.

It would appear that pyrophosphate minerals, which are not found on the Earth at the present time, may have occurred in significant amounts on the primitive Earth during the period when the organic compounds leading to the origin of life were being synthesized. This pyrophosphate may have played an important part in polymerization and dehydration reactions of these organic compounds.

This work was supported by U.S. National Science Foundation grant *G*–22000. We thank Prof. G. Arrhenius for obtaining the X-ray powder patterns.

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SCIENTIFIC SOCIETIES IN INDIA

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RECENT investigations of the sociology of science have suggested a major shift in the organization of science and scientists. The general suggestion is that although science previously was a closed system[1], now it is not so. Science is now a part of society and, as such, is subjected to the same pressures as any other social process[2]. This change, it is contended, has also effected a change in its values. In the period when science was a closed system, as pointed out by Merton[3], its values were centred around the professional recognition of scientists. In this system societies had a major part to play, their transactions published the papers, and these, along with other non-published reports, were discussed in the formal and informal meetings of the societies. This criticism, or appreciation, of the work of fellow scientists built up the reputation of scientists and led to recognition. This process, as Storer mentions[4], suggests that the values of science are rooted in the requirements for continued, adequate allocation of professional recognition rather than in the utilitarian relationship of the value to the aim of science.

With the rapid development of science, and the fact that it has become a large-scale activity, scientific criticism is losing much of its edge[5] and hence professional recognition does not really enjoy the same status as before. In addition, money, prestige and power have come to enjoy greater status than professional recognition[2]. The informal atmosphere has also been replaced by more business-like methods. Greater specialization and the larger number of scientists have also broken the scientific community as of old. What is the impact of these changes on the role of scientific societies? Have societies continued to grow as of old, or have they also undergone changes to meet the new requirements and trends? These questions have great relevance to the growth and development of science.

A number of roles are ascribed to scientific societies, that is, bringing together scientists specialized in the same area of knowledge, affording them an opportunity for intercommunication of ideas within a discipline or between various disciplines through conferences, symposia and publication of journals, serving as a platform for general discussion by scientists of problems of science, and acting as a forum for crystallization of ideas regarding the relation of science to society. They are also supposed to popularize science and confer distinctions on members as a recognition of contributions to science.

No investigation has so far been carried out as to whether these are pertinent roles of scientific societies and how far the various national and international societies are fulfilling these and other related roles. Is there a need to change the role of scientific societies, and in what direction? What are the activities they should undertake and how should they organize themselves? Could there be

various levels of organization according to the needs of specialization and possible social role?

In the context of the growth of science, these questions are very pertinent and, in our opinion, a time has come when we should ask these questions and carry out specific investigations to find the answers.

To fill this lacuna, a survey was conducted by us. The aims of the survey were limited to the collection of data and examining the historical development and activities of the societies and their financial condition. A detailed questionnaire was sent to 150 societies and 115 responded. A directory of the societies was also compiled. The societies were classified as physical sciences, biological sciences, medical sciences, engineering and technology and general societies.

Historical Development

India, before the beginning of the twentieth century, had only four scientific societies, namely, Asiatic Society (1784), Royal Agricultural Horticulture Society of India (1860), Indian Engineering Association (1895), and Bombay Textile and Engineering Association (1900). By the end of the First World War, the total number increased to 16, of which one was physical, three were medical, two biological, six engineering and technological and four general science societies. It is interesting to note that, even though engineering and technology were not very much developed during this period, India had the maximum number of engineering and technological societies, most of which were of a professional nature rather than academic.

Prominent societies founded during 1900–20 were the Indian Association for the Cultivation of Science (1909), Indian Mathematical Society (1907), Indian Science Congress Association (1914), Indian Botanical Society (1920), and Institution of Engineers (1920).

They provided a forum for specialists to discuss academic problems in their fields and also provided scope for scientists of different disciplines to meet to discuss and gain knowledge of the developments occurring in other fields or in border-line disciplines. In fact, in the age of non-specialization in specific areas such general science societies had a special part to play.

The total number of societies increased to 38 by the end of 1940. Between the two World Wars, more specialized societies were founded. During this period, India had four physical, seven medical, five biological, 10 engineering and technological and four general science societies. Most of the non-professional societies, up to this period, used to promote active research work besides providing forums for scientists to meet and discuss; the shift from this activity now becomes noticeable.

Prominent societies which were formed during this period were: Institution of Chemists (1928–29), Indian Academy of Sciences (1934), National Institute of Sciences of India (1935), Indian Physical Society (1939), etc.

During the decade 1941–50, the number almost doubled, the number of societies increasing to 71. The greatest relative increase in societies occurred in the physical sciences group, which increased from four in 1940 to 11 in 1950. The number of medical societies more than doubled, from seven in 1940 to 15 in 1950. Biological science societies increased to 15; engineering and technological societies increased to 10 by the end of 1950.

Independence brought a spurt in the growth of science societies in India. The next decade also saw an equal increase in number. Among those who responded to our questionnaire we find that 33 societies were founded during 1951–60. At the end of 1960, we find that we had 17 physical, 23 medical, 26 biological, 26 engineering and 12 general societies. It is interesting to note that with the increase in specialization in the country the proportion of general societies decreased. Another interesting point is that most of the societies which were established during the 1951–60 decade, in contrast to those established earlier, do not conduct or support scientific research, but only provide facilities for specialists to meet, discuss and cross-fertilize ideas and publish journals or look after the professional interest of members.

Since 1961, the tempo of the growth in number has been slower. During 1961–63, only six societies have been established. Perhaps there may be a few more to which our attention has not yet been directed.

Concentration and Branches of Societies

There is a heavy concentration of scientific societies in the big cities (Calcutta, New Delhi, Bombay, Bangalore and Madras). As many as 118 out of 150 societies are located in these places. Most of the professional societies (in the field of medicine and engineering and technology) have large numbers of branches, but that is not true for other societies. Out of 18 physical science societies, 14 have no branches, and four have 1–5 branches; out of 28 biological science societies, 22 have no branches, three have 1–5 branches, one has 6–10 branches and two have more than 10 branches; out of 15 general science societies, 12 have no branches and three have 1–5 branches. The medical, engineering and technological societies, which have a pronounced professional character, have a large number of branches; for example, out of 26 medical science societies, 12 have no branches, six have 1–5 branches, four have 6–10 branches and four have more than 10 branches. Among the 28 engineering and technological societies, nine have no branches, 11 have 1–5 branches, three have 6–10 branches and five have more than 10 branches.

Membership of Societies

It is significant that the membership in 1962 of professional societies was higher than that of the academic societies. Out of 18 physical science societies, seven had membership below 100, eight had 100–499 and three had more than 500 members. None of the societies had more than 1,000 members.

Out of 25 medical societies whose membership figures are available, four had less than 100 members, 14 between 100 and 499, four between 500 and 999, and three had between 1,000 and 4,000.

Biological science societies usually have a larger membership than physical science societies. Out of 27 (whose data were available), six had less than 100, 16 between 100 and 499, three between 500 and 999, and two between 1,000 and 5,000 members.

It is observed that the membership of engineering and technological societies is heavy, mainly because of their professional nature. The Institution of Engineers is the only society which has more than 5,000 members; in fact, it had more than 42,000 members in 1962. Out of 28 societies in this group, four have fewer than 100 members, 10 between 100 and 500, seven between 500 and 1,000, six between 1,000 and 5,000, and only one more than 5,000.

Membership of general societies is usually limited to 100–500 members. Out of 16 such societies whose membership figures were available for the year 1962, 13 have membership between 100 and 500, two between 500 and 1,000, and one (Indian Science Congress Association) had a total membership of 4,683.

Mode of Becoming Members of Societies

There is not much group difference here. The common mode is by election and payment of subscription. Out of 115 who have reported, in 48 societies, membership is through paying a fee, in 52 through election and payment of fee, in four by election only; in eight engineering, two physical and one medical science societies, one can become a member by passing examinations conducted by the society.

Only 16 out of 115 societies conduct examinations, and, of these, 10 are engineering and technological societies,

two physical, one medical, two biological societies and one general science society.

Activities of the Societies

The societies which conduct examinations also provide library facilities. About 50 per cent of the societies confer some kind of honour, by way of award of a gold medal, cash prize or membership or fellowship, to their members.

About 88 per cent of the societies publish one or more journals; in all, out of 115 societies which replied, 101 publish 123 journals. The details are as follows:

Out of 18 physical science societies, 15 publish journals and they publish 19 journals in all; out of 26 medical societies, 21 publish 24 journals; out of 28 biological societies, 26 publish journals and they bring out 32 journals; out of 28 engineering and technological societies, 26 publish 33 journals; and out of 15 in the 'general' group, 13 publish 15 journals.

It is interesting to note that 49 out of 115 societies had not conducted any symposium, seminar or conference during the past 10 years. Out of 66 who arranged any of the aforementioned meets, scarcely 20 per cent did so as an annual feature; many of them do meet a number of times in a year but only to conduct the business of the society. This may suggest that societies are playing a limited part in the promotion of science, and that only through publishing journals. They give little opportunity to members to discuss specific research topics or academic problems.

Financial Support

Our investigation reveals that, in all, only 41 societies get direct or indirect support from the Central Government, 35 societies get little financial support from State Governments, 21 societies get support from industry and 16 from endowment funds. It is interesting to note that those who receive support from Central or State Governments also get support from other sources. Of those who receive support from the Central Government, many of them get support from State Governments also. The support which is available mostly goes to a selected few societies, while the rest depend solely on their own resources. Seventy-five per cent of the funds which come from Central or State Governments are distributed to a few of the societies, with the result that funds for most of the others are not enough even to run their journals, let alone arranging seminars or symposia at an all-India level, which usually demands heavy expenditure.

This investigation reveals the following features:

(1) Growth of scientific societies has kept pace with that of science and technology.

(2) General and professional societies (that is, engineering and medical) were founded earlier and were followed by more specialized societies. Recent trends have been towards the establishment of more sophisticated societies in the newly developing fields of science and technology like Operations Research Society of India, Institute of Information Scientists (of India).

(3) Societies are mostly concentrated in the big cities of Calcutta, Bombay, Madras, Delhi and Bangalore. This is in keeping with the concentration of scientific research institutes in large cities. It may be observed here that most of the earlier academic type of scientific societies were founded in the Calcutta region. In recent years, however, new societies are being formed at a faster rate in the Delhi and Bombay regions.

(4) Earlier societies of the academic type were mostly institutional in nature, that is, as a part of their activity they not only provided a forum for scientists to meet, arrange symposia and publish journals, but also they encouraged scientific research or carried out research as a part of their activity. The last-named activity is not a feature of the more recent societies.

(5) Professional societies in the field of engineering, technology and medicine have usually a larger number of branches and members. Specialized societies in more academic fields have usually smaller membership. The increase in membership of the more academic type of societies is not in keeping with the rise in number of research workers in those fields.

(6) Most of the societies do not receive any support from the Government, either Central or State. Those who receive from one source, receive from others also. Whatever support is available from the Government, most of it goes to a few. Many of the societies are publishing journals from their own resources, in view of their limited finance, and the standard and regularity in publishing the journals suffer. It is of interest to note that most of the support from Government goes to the more academic type of societies rather than to professional engineering and medical ones.

(7) The most curious feature is the lack of activity of societies by way of organizing academic activities, namely, symposia, seminars and specialized discussions. This may be due to the lack of financial resources available to them, as against the established institutions*.

Two major conclusions could be drawn from this work: one for India and the other more generalized. For India, the conclusions are the need for critical examination of the activities of the societies and the support they are receiving from the Government. There is an urgent need for the developing of a proper policy for support of scientific societies, particularly for those which cover the newer fields of science and technology and those which deal with the social problems of science or with science as a whole.

It is suggested that the Government should not only finance the academic societies to arrange the seminars and symposia but should also make sufficient provision in the budget of the research institutes to enable active research scientists to attend seminars or symposia, organized by the societies. Finally, there is also a need for associating the scientific societies in forming decisions. This is vital for the healthy growth of science, and its democratization in India where most of the decisions are taken by a discrete group. This would not only add to the prestige of the societies but would also enable them to become alive and take an active part in the promotion of their field of specialization and science as a whole.

The more generalized conclusions are with regard to the role of specialized societies in giving direction to research in their field of interest. At the moment the quantum of information and the nature of symposia are such that at no place could a critical appreciation of the trends of research in a particular field be made. This requires immediate attention. Secondly, with the growth of greater degrees of specialization in science, there is a need for more generalized discussions, where more than one discipline could be integrated and where problems of policy, planning and other social aspects of science could be discussed. To have a specialized society for such discussion would not serve the purpose. What is needed is the participation of scientists from different fields of specialization to take a synoptic view of science and contribute to such generalized problems, which means a greater emphasis on general societies.

Finally, there is a need to re-examine some of the traditional activities, the impact they are creating and the efficiency with which they are fulfilling their objectives.

In the light of such an analysis and the trends in science, the need for re-orienting the activities of societies might emerge.

* It appears from the historical records that the work of earlier societies had greater impact on India of their day, though the standard of work and publication appears higher to-day.

[1] Merton, R., *Amer. Sociol. Rev.*, **22**, 635 (1957).
[2] Storer, Norman W., *Science*, **142**, 464 (1963).
[3] *Op. cit.*
[4] Storer, Norman W., *Amer. Behavioural Scientist*, **4**, 27 (1962).
[5] Hubbert, M. K., *Science*, **139**, 884 (1963).

OBITUARIES

Sir James McNeill, K.C.V.O., C.B.E., F.R.S.

JAMES MCFADYEN MCNEILL, who died on July 24, was born in 1892, at Clydebank, where, in 1870, the establishment of a shipyard and engine works had led to the foundation of a burgh. His schooling was first local, and then in Glasgow at Allan Glen's, a school notable for the opportunity offered to aspirants toward engineering. His father was a foreman in the shipyard of John Brown and Co., and in 1908 James McNeill began an apprenticeship there, and an association which, with only a war-time interruption, was to be continuous. In those days, a technical education was a luxury which had to follow a normal apprenticeship, and it was not until 1912 that James McNeill, with a good knowledge of shipbuilding as a background, matriculated at the University of Glasgow. In 1915 he graduated as B.Sc., the leading student of naval architecture in his group, winner of prizes in engineering and in mathematics as well as in his own specialized subject, and, withal, quiet and unassuming; an indication of another interest was attendance at the class of political economy. Graduation was soon followed by enlistment, with the Royal Artillery as the choice. In the German offensive of early 1918 the conduct of Captain James McNeill as a battery commander gained for him a mention in despatches, a Military Cross, and promotion to field rank.

Back to the shipyard after the War, James McNeill was placed in charge of the design and technical section associated with the ship drawing office. It was fairly soon apparent that in the years of war there had been marked development of latent qualities—acceptance of responsibility and exercise of leadership. The development was recognized when, in 1928, following an unexpected vacancy, the young man was appointed naval architect to the Company, a position carrying responsibility for the preparation of all shipyard plans, from the design stage onwards to completion, and leading in a few years to a seat on the local board of directors. The first major work carried out by James McNeill was the design and completion of the *Empress of Britain*, the largest ship built in Great Britain since the completion of the *Aquitania* in 1914; there had been keen competition for the contract, and there were stringent conditions to be fulfilled. In the launching of this large ship a feature new in Britain was the use of ciné cameras, and the painting of 'register' marks on the hull, in order that a complete and reliable record could be obtained of the travel of the ship down the launching ways and, especially, of the 'plunge' as the fore end of the launching cradle dropped off the end of the ways; the placing of some of the cameras involved some of the technique of the skilled gunner.

The record of the launching of the *Empress of Britain* was valuable in itself. There was, however, special intent behind it. Consideration was already being given to the problem of building and launching a ship about 1,000 ft. long, and the cost of the dredging necessary to permit the launching into a restricted river was a factor to be taken into account. From the records of the launch of the *Empress of Britain* it was found that the dredging need not be so extensive as had been feared. The successful launching of the *Queen Mary* in 1934 was, in a way, a development from the launching of the *Empress of Britain*. There was also, however, much fresh and patient investigation of the whole range of problems involved in what is at once a crude and a highly complicated operation. A comprehensive record of the investigations and of the treatment of the problems was presented to the Institution of Naval Architects in 1935 in a paper by Mr. McNeill which is a masterpiece.

The patient and perceptive thoroughness which Mr. McNeill had displayed in his first major work for John Brown and Co. was carried on throughout his career in the shipyard. The range of his activity was wide, and although the *Queen Mary* and *Queen Elizabeth* are his masterpieces, he left his mark on lesser-known craft of all types. In 1948 he became managing director of the establishment to which he had come as an apprentice forty years before: and then in 1953 he became deputy chairman of the Company, a position he held until retirement in 1962. A catalogue of honours includes first the honorary degree of LL.D. from the University of Glasgow in 1939, election as a Fellow of the Royal Society in 1948, Royal appointment as C.B.E. in 1950, and as K.C.V.O. in 1954. Appreciation of colleagues in the shipbuilding and engineering industry was shown by selection for high office in the ancillary organizations of the industry, and in the professional institutions.

In Sir James McNeill there was much more than technical capacity. There was staunch integrity, allied to wisdom and kindliness, qualities which won the respect of all who were concerned with ships produced at Clydebank, whether as owners of ships or as officers of Government. With a streak of the puritan in him, he could come down hard on any tendency toward vulgarity in the conversation across the shipyard lunch-table. He could express strong dislike of income tax 'fiddling', and also of bullying of juniors—to him, bullying was the resort of the man who was not up to his job. A. M. ROBB

Prof. Louis Fage

THE well-known French zoologist, Louis Fage, member of the Académie des Sciences, Commandeur de la Légion d'Honneur, died in Dijon on May 28, in his eighty-first year. Formerly professor at the Muséum National d'Histoire Naturelle, Paris, and at the Institut Océanographique, he was outstanding as investigator, lecturer and administrator. His publications include the volumes on Amphipoda (with Chevreux, 1925) and Cumacea (1951) in the *Faune de France*, and the sections on Merostomata and Pycnogonida in Grassé's *Traité de Zoologie* (1949).

He was born on September 30, 1883, in Limoges (Haut-Vienne), France, son of René Fage, barrister and corresponding member of the Académie des Inscriptions et Belles-Lettres. He was educated at the Collège St. Martial, Limoges, and entered the University of Paris in 1900, still undecided as to his course of study. It so happened that Gaston Bonnier and Edmond Perrier were old family friends; on their advice he chose the natural sciences and obtained his doctorate in 1906. Forty years later Goodrich referred to his thesis on the segmental organs of polychæte worms as the most important of the general works on nephridia and allied subjects which had appeared since 1895.

For the next fourteen years he served as naturalist to the Service Scientifique des Pêches Maritimes, attached to the Laboratoire Arago, Banyuls, of which Racovitza was the resident deputy director. His work on Mediterranean fishes attracted the attention of the oceanographer, Johannes Schmidt, and led to a life-long association with Danish scientists. He found the local methods of catching pelagic shoaling fishes rudimentary in the extreme and suggested fishing with lights at night. He also used this method extensively for the collection of plankton; this work, started in the Mediterranean,

was continued over a period of years at Concarneau with Legendre and greatly extended our knowledge of the biology of many littoral invertebrates. During this period he also became interested in Arachnida and in speleology. He joined Racovitza and Jeannel, founders of the association known as Biospeologica, in their explorations and described the cavernicolous spiders. This had an interesting sequel; in 1947 Fage put the case for a subterranean research laboratory to the director of the Centre National de la Recherche Scientifique (C.N.R.S.) so successfully that soon afterwards the laboratory at Moulis (Ariège) was opened.

The year 1920 marked a turning point in Fage's scientific career. At the invitation of Charles Gravier he joined the staff of the Muséum National d'Histoire Naturelle, Paris, as deputy director of the zoological laboratory, in charge of various invertebrate phyla. He became director of the laboratory in 1938, a post he held conjointly with his professorships until his retirement in 1953. To commemorate his scientific jubilee he was presented just after his seventieth birthday with a gold medal specially designed by Robert Cochet.

Fage also played a prominent part in scientific activities outside the Museum and outside France. For example, during 1924–27 he was secretary of the Société de Biogéographie, of which he was a founder member. He was president of the Société zoologique (1929), the Société entomologique (1935) and the Académie des Sciences (1955). He was secretary-general of the Permanent Committee of the International Congresses of Zoology (1948–53) and represented the Institut Océanographique on the Conseil International pour l'Exploration de la Mer, the Commission pour l'Exploration de la Mediterranée and the Commission internationale d'Océanographie. A member of the C.N.R.S., he was chairman of the Bathyscaphe-Calypso Committee and of the Moulis Laboratory Committee. For many years he was editor or co-editor of various periodicals such as the *Archives de Zoologie expérimentale et générale* and the *Annales de l'Institut Océanographique*; he was a co-editor of *Deep Sea Research* from its inception in 1953.

Over a period of almost sixty years Fage published some 250 scientific papers, monographs and books dealing with many quite unrelated groups of animals from Protozoa to fishes. Although much of his museum work was of necessity taxonomic, he was pre-eminently a field naturalist interested in the living animals, their habits, habitats and life-histories, their geographical distribution in relation to environmental factors, their evolution. An authority on Arachnida, he described the habits of spiders that lead an amphibious life among tropical corals or the encrusting calcareous algal growths (the 'trottoir') of Mediterranean shores, that live normally in the urns of pitcher plants or as commensals in the nests of ants and termites. He studied the development, metamorphosis, growth, spawning and migrations of many coastal fishes, and applied biometric methods to the elucidation of the different races of sardine and anchovy. He found that many bottom-living invertebrates, especially annelids, cumaceans, amphipods, mysids and sea-spiders, have pelagic phases which are subject to nycthemeral, lunar and seasonal rhythms, and that many supposedly rare sedentary species may be captured in thousands at certain times. His *Dana Reports* on the primitive mysidacean order Lophogastrida (1941) and (1942) and on the pelagic amphipod family Oxycephalidae (1960) deserve special mention. The vast amount of material (for example, *Gnathophausia* 875, *Ceratolepis* 174 and oxycephalids 35,000 specimens), available for the first time, enabled him to add much to our knowledge of the systematics, chorology, sexuality and parasites of the species represented. In the field of oceanography he investigated the influence of the organic content of marine sediments on the distribution and density of the abyssal benthic fauna; also the variation in the amount of oxygen dissolved in the sea-water immediately above the sediments. For an account of Fage as speleologist the reader is referred to Prof. Vandel's obituary note in *Annales de Spéléologie* (19 (2), 245; 1964).

Fage was a foreign member of many scientific societies, including the Zoological and Linnean Societies of London. Of the many honours which were conferred on him, he must, one imagines, have valued highly the gold medal of the Johannes Schmidt Foundation for the Exploration of the Sea (1958). All this notwithstanding, he was without ostentation, modest, sincere and kind, at his charming best when entertaining a few friends in his own home. His wife, *née* Marie Marguerite Gigaud-Lafont, predeceased him by about three years; they had one son and one daughter.

Isabella Gordon

NEWS and VIEWS

The Royal Society : S. G. Brown Medal and Award

In January 1964 the Council of the Royal Society announced the establishment of a fund, following a bequest under the will of the late S. G. Brown, the income from which would be used to offer annually an S. G. Brown Award and Medal for an outstanding contribution, based on work carried out during the preceding five years, to the promotion and development of mechanical inventions (*Nature*, 201, 556; 1964). Recommendations for the award were to be made to the Council by the Councils of the Institutions of Civil, Mechanical and Electrical Engineers in rotation. It has now been announced that the first award has been made to Mr. F. M. Adler a civil engineer, for his work in the promotion and development of the 'Jackblock' technique, a system for the construction of tall buildings providing factory conditions during erection. The roof is constructed first, at ground level, and then raised by jacks for the top-most floor to be constructed beneath it, and so on. During the process each floor travels vertically through a 'factory' in which all the services, fittings and finishes are installed. A 17-storey block of flats has recently been completed at Coventry using this technique.

Biochemistry in University College, Dublin : Prof. E. J. Conway, F.R.S.

Prof. E. J. Conway retired from the chair of biochemistry and pharmacology in University College, Dublin, on October 29. After a brilliant undergraduate career in that University, he graduated in science in 1916 and in medicine in 1921. There followed a vigorous research programme on renal physiology in the Physiology Department under Prof. J. M. O'Connor on the basis of which he was awarded the D.Sc. degree in 1927. As a Rockefeller Fellow he worked for two years with Embden at Frankfurt. On returning to Dublin he was appointed to the chair of biochemistry and pharmacology in 1932. During the thirty-two years that he occupied the chair, Prof. Conway published two books and more than 150 research papers, monographs and review articles. In addition to his earlier work on renal physiology, he is well known internationally for his contributions to the mechanisms of electrolyte exchanges between cells and their environment, the evolutionary origins of such environments, the mechanism of gastric acid secretion and the technique of microdiffusion analysis. His outstanding place in world science can best be assessed in terms of the honours which

have been bestowed on him. Chronologically, the most important of these include: fellowship of the Royal Society (1947); fellowship of the Royal Society of Arts (1952); membership of the Académie Septentrionale, Paris (1961); membership of the Pontifical Academy of Sciences (1961); honorary fellowship of the Royal Institute of Chemistry (1963); fellowship of the Institute of Biology (1964). In 1960 he was appointed chairman of the Institute for Advanced Studies in Dublin. In 1960 the Irish Government made a special grant to institute a Unit of Cell Metabolism under his directorship as part of the Medical Research Council of Ireland. Now, as he leaves the chair to which he has brought such honour, it is to be hoped that in these two capacities his active research programmes will continue, and his advice and encouragement will continue to be available to the younger generations of Irish biochemists.

Prof. M. G. Harrington

Dr. M. G. Harrington, lecturer in biochemistry in University College, Dublin, has been appointed to the chair of biochemistry in succession to Prof. Conway. A graduate in science of University College, Cork, Dr. Harrington was in 1952 awarded the travelling studentship in biochemistry of the National University of Ireland during the tenure of which he carried out, in association with Prof. E. F. Gale at Cambridge, investigations of the action of antibiotics on Gram-negative organisms. In 1954 he was appointed to the Department of Biochemistry and Pharmacology in Dublin, becoming lecturer in biochemistry in 1958. From 1954 until 1963 he worked in collaboration with Prof. Conway on various aspects of electrolyte physiology; in particular, investigations of the uptake of sodium by frog sartorii, and the application of electron microscopy to such investigations. During 1963–64 he was visiting associate professor of physiology at Tufts University School of Medicine, Boston, where he carried out investigations on the fate of chromium-labelled proteins injected intravenously in mice.

Microbiology in the University of Birmingham : Prof. H. Smith

Dr. H. Smith, at present head of chemical pathology at the Microbiological Research Establishment, Porton, has been appointed to the chair of microbiology in the University of Birmingham, as from January 1, 1965, in succession to Prof. D. G. Catcheside, who has been appointed to the chair of genetics within the John Curtin School of Medical Research, the Australian National University, Canberra (*Nature*, 199, 536; 1963). Dr. Smith graduated in the University of London in 1942 and then spent three years with Boots Pure Drug Co. After a brief period as an assistant lecturer in the Department of Chemistry at University College, Nottingham, he moved in 1947 to the Microbiological Research Establishment, Porton. During the past seventeen years he has been concerned with various aspects of microbiology, but his most outstanding work has been concerned with the chemistry of microbial virulence. Dr. Smith is a prominent member of the Society for General Microbiology, and has been a member of its Council since 1960.

Deputy Director, Water Research Association: Dr. E. Potter

Dr. E. Potter has been appointed to the newly established post of deputy director of the Water Research Association. Dr. Potter, who is forty-one, is a graduate of the University of London, having taken the special chemistry degree by part-time study in 1944. In 1940 he joined the Metal Box Co., where he was engaged on packaging problems, and in 1947 entered the Imperial College of Science and Technology. There he carried out research on electrochemical kinetics, gaining his Ph.D. in 1950. Following this, he returned to applied science and, after a year at the Paint Research Station, Teddington, he joined the then British Electricity Authority to set up a research unit for the investigation of corrosion and water chemistry in steam power plant. During the next decade his team achieved fundamental advances in this field, applying them to the practice of large-scale steam generation. His procedure for the analysis of oxygen dissolved in water, for example, is sensitive to one part in five thousand million, and by means of it the oxidation of boiler steel in high-temperature water could be clarified in practical detail for the first time. He received the Beilby Memorial Award in 1957 for these and similar developments. In 1963 Dr. Potter lectured in Australia and New Zealand, and has made many other technical visits, notably to the United States and the U.S.S.R.

Science in Parliament : Aircraft Industry

In a statement to the House of Commons on December 9, the Minister of Aviation, Mr. R. Jenkins, announced that Lord Plowden had accepted the Government's invitation to serve as chairman of a committee to consider the future place and organization of the aircraft industry in relation to the general economy of Britain. The committee would take into account the demands of national defence, export prospects, the comparable industries of other countries and the relation of the industry with Government activities in aviation, and would make recommendations on any steps and measures necessary. The members of the committee would include Mr. Aubrey Jones, Mr. A. Albu, Mr. D. H. Barran, Mr. F. Hayday, Admiral of the Fleet Sir Caspar John, Mr. C. McMahon and Sir William Penney, with Mr. N. Craig as secretary. In reply to questions Mr. Jenkins said that the committee would review the whole field of the aero-space industry, and its deliberations would not postpone decisions on projects already under review. It did not replace the Transport Aircraft Requirements Committee. A similar statement was made in the House of Lords by Lord Shackleton, who added, in reply to a question, that as full an account of the committee's report would be published as matters of national security and commercial confidence allowed.

Manpower Research Unit

In reply to a question in the House of Commons on December 7, the Minister of Labour, Mr. R. J. Gunter, said that the future programme of the Manpower Research Unit would include full consideration of the effects of automation on manpower: he would himself keep under review the need for further work on the effects of human relations in industry. The Unit was exploring the possibilities of manpower forecasts on the basis of the estimated trends in output and productivity, including the effects of automation. Moreover, it was planning to follow its current studies of changes in the occupational structure of the metal industry and the effect of computers on office employment with new studies of the changing structure of particular industries, and on the introduction of particular new techniques.

Solway Barrage

In reply to a question in the House of Commons on December 9, the Minister of State for Scotland, Mr. G. Willis, said that a preliminary examination had already been made of the Solway Barrage proposals. The new step was for the new planning machinery to examine the project, along with similar proposals for Morecambe Bay, in the context of the very substantial and competing claims on the nation's capital investment resources over the coming year. The Scottish Development Group had already had discussions with local authorities on both sides of the Border and also with the North West Study Group on this matter, as well as discussions with Dr. R. L. Drew and experts from the University of Strathclyde.

These had established the fact that the feasibility of impounding fresh water in the way proposed required a further technical survey, and this very considerable undertaking would be considered in due course.

Poisonous Substances used in Agriculture

In reply to a question in the House of Lords on December 2, the Minister of State for Education and Science, Lord Bowden, said that the Government had accepted the recommendations of the Cook Committee on Poisonous Substances used in Agriculture and Food Storage. As assurances had been received of the co-operation of all interests concerned, it had been decided to give effect to the recommendations through the present voluntary scheme. The Cook Committee was now examining the present voluntary safety arrangements, and the need for legislation would be considered in the light of its advice. The Earl of Haddington, Lord Douglas of Barlock and Lord Saltoun all protested that voluntary action was unlikely to be effective and referred to the serious side effects on the breeding of birds, particularly birds of prey. However, Lord Bowden, in view of the agricultural value of these pesticides, merely emphasized that the Cook Committee and the manufacturers were anxious to produce insecticides which had the desired effects with as few side effects as possible. This was the subject of earnest discussion and research. In a written answer in the House of Commons on the same day, the Minister of Agriculture, Fisheries and Food, Mr. F. Peart, stated that investigations by his Department supported the view of the Cook Committee in the review of the persistent organo-chlorine pesticides, published in March 1964, that the restrictions placed on the use of aldrin and dieldrin in cereal seed dressings were serving their purpose and had greatly reduced the number of deaths of seed-eating birds through these chemicals.

Long-term Role of Nuclear Energy in Western Europe

The European Nuclear Energy Agency of the Organization for Economic Co-operation and Development is to carry out a special study of the long-term role of nuclear energy in Western Europe. The study will include evaluations of reserves of uranium and thorium available to European member countries in various cost ranges, of long-term energy demands, and of the likely contribution from nuclear energy towards meeting these demands under the technical and economic conditions which may be expected to develop. The decision to make this study was taken by the Agency's Steering Committee at a recent meeting in Paris, under the chairmanship of Prof. Urs Hochstrasser, Swiss Federal Delegate for Atomic Energy Questions, and results from a preliminary proposal of Prof. Lew Kowarski, the Agency's scientific adviser, which was first discussed by the Steering Committee last July and has since been elaborated by a group of experts from Austria, France, the German Federal Republic, Sweden and the United Kingdom under the chairmanship of Prof. Kowarski. The study is expected to be completed by the summer of 1966.

Occupational Health Service in Dundee

Four years ago the Nuffield Foundation decided to spend a certain sum on experimental group occupational health services to well-established older industries spread over a city or town and mixed with residential and other property. The professor of public health and social medicine in St. Andrews University and the district factory inspector aroused interest and enthusiasm for a group service among prominent business men in the city of Dundee. Dundee's claim as a suitable area was put forward by them and was accepted by the Nuffield Foundation in 1962. This was the beginning of the Dundee and District Occupational Health Service, the progress of which is described by the nursing superintendent, Miss Elizabeth J. Tyren, in *Occupational Health*, 16, July–August 1964.

There are several reasons why Dundee was considered a suitable area for this experiment. It is a medium-sized city of about 190,000 people, contained in an area about five miles by three miles, and is the third-largest city in Scotland. The city stands on the north shore of the Firth of Tay, linked with Fife to the south by a fine rail bridge and the new road bridge.

Dundee has variety in industry, although there are still many people employed in the jute trade. The other industries include linoleum manufacture, light engineering, carpet manufacture, confectionery, and flax spinning and weaving, and the city is a potentially prosperous one. These industries are scattered throughout the area among residential and other property, although an industrial estate is gradually taking shape to the north-west of the city. The member firms of Dundee and District Occupational Health Service are mostly of medium and small size and are, therefore, probably representative of the factories of Great Britain as a whole. The outlying parts of the city are gradually being filled with new houses as the centre of Dundee is replanned and rebuilt.

Another reason for the choice of Dundee was the fact that it is a university city containing the Queen's College of the University of St. Andrews. It was planned that, should the city be accepted for the group experiment, the student health service for Queen's College would become a member of the occupational health service, and this was an added attraction, as no other group health service has a university among its members.

The aims of the service were formulated early and are gradually being fulfilled in the various factories. They are: to promote and maintain the health and safety of people at work; to assist in the provision at work of immediate treatment for sick and injured people; to advise on the rehabilitation and placement in suitable work of those temporarily or permanently disabled by illness or injury; to advise on the provision of safe and healthy conditions by informed scientific assessment of the working environment; to educate all concerned in the attainment and preservation of good health; to carry out or promote research into the causes of occupational diseases and injury, and into the means of their prevention.

Publications of the Medical Research Council

Under the title *Current Medical Research*, the Medical Research Council, following its usual practice, has reprinted articles which appeared in the Report of the Council for the year 1962–63 (Pp. iii+51+3 plates. London: H.M. Stationery Office, 1964. 4*s*. 6*d*. net). An important feature this year is the statement by the Council on the responsibility of investigators who conduct experiments on human subjects. The other articles deal with: the theoretical basis of organ transplantation; damage to human chromosomes due to ionizing radiations; clinical genetics; recent work on the control of ovarian function; the drug-resistances of micro-organisms; the chemotherapy of cancer; protein turnover in the central nervous system; and epidemiological research in psychiatry.

Housing Problems

Public Health Papers, published by the World Health Organization, provide a medium for the publication of reports usually prepared as contributions by the Organization to a particular health question, which have been considered to be of interest to a wider circle of readers than that for which the papers were originally written. *Public Health Paper No. 25* is entitled "Housing Programmes—the Role of Public Health Agencies" (By A. Andrzejewski and seven other authors. Pp. 197. Geneva: World Health Organization; London: H.M. Stationery Office, 1964. 8 Sw. francs; 13*s*. 4*d*.; 2.75 dollars). It discusses the responsibility of public health administrations in the field of housing; the basic principles of housing hygiene in

the U.S.S.R.; the planning of housing programmes; the participation of the community in housing and environmental hygiene in Ceylon; and economic and financial problems associated with housing.

Procter and Gamble, Ltd.

PROCTER AND GAMBLE, LTD., with world-wide ramifications, but operating in Britain at Gosforth, Newcastle upon Tyne, manufactures a large variety of products, of which perhaps the most familiar in domestic circles are many well-known brands of detergents popular with the housewife and others. Other products include edible materials for the bakery trade, industrial soaps, textile brands, air entraining agents and mortar plasticizers, refined and hardened edible oils, soap for emulsifying raw materials in synthetic rubber manufacture, glycerine, fat additives for animal feeds, etc. In the *Financial Statement* for the year ended June 30, 1964, apart from the commendably summarized profit and loss and balance sheet figures, a considerable amount of space is devoted to the aims and activities of this organization, not only in meeting present-day retail demands, but also in being 'one step ahead' by both technical and economic research, to discount the future (Pp. 16. Newcastle upon Tyne: Procter and Gamble, Ltd., 1964). The stress is on 'innovation and change'. "Product Innovation results from the effort to provide better goods; Operational Change from the search for better methods. In the competitive fields in which our company operates, we have to seek both those things continuously. Over the years it is not just the health of the company but its survival that depends on our ability to serve the consumer with better products more efficiently produced." There is a great deal of sound common sense, quite apart from business acumen, disclosed in this booklet; it is a pleasure to read an annual *Statement* which breaks away from many of the cut-and-dried documents conventionally issued to shareholders and others concerned. In this case, that pleasure is enhanced by the inclusion of some beautiful illustrations of Newcastle and Northumberland scenes in colour, the work of David Knight. This modest and artistic approach to the public, devoid of all the usual photographs of factories, plant and people, is in itself quite sufficient advertisement, a model which could well be emulated by some of the larger industrial groups in Britain.

Personnel Management

THE control and welfare of workers in industry, whether in the open air or under cover, in agriculture, civil service, factory, office or shop, have in recent years become the province of people specially trained and selected to take care of the humanities of mass employment such as is experienced to-day in Britain. The qualifications are essentially 'A-levels' at least in practical knowledge of the work concerned, whatever and wherever it may be, and, perhaps at even higher rating, the psychological effects of administration and environment on both men and women. The issues affect everyone, the 'small man', the business executive, and the boards of major industrial and nationalized concerns. It may not be generally recognized that to co-ordinate, organize and publicize much of this important relationship between employers and employees, there exists the Institute of Personnel Management (80 Fetter Lane, London, E.C.4), whose quarterly publication, *Personnel Management*, always reflects the public interest in this subject. Quite a number of people have not fully appreciated the impetus of recent legislation on the new legal status of the average employee, quite apart from mandatory improvements in the conditions under which he or she works. An article by J. E. Genders entitled "The Contracts of Employment Act: A Challenge" (*Personnel Management*, 46, No. 369, September 1964) directs attention to the implications of at least this phase of the problem of industrial humanities. Great changes in the deployment of manpower are inevitable in the near future, under the pressure of advances in automation, which none can stop. As Mr. Genders rightly says: ". . . the achievement of the . . . Act may lie not nearly so much in what it itself lays down, but in the incentive it offers for proper attention to be focused to-day on security of employment in order to provide the right conditions for mobility of employment to-morrow".

Sulphur Dioxide as an Air Pollutant

ONE of the most widespread and dangerous constituents of atmospheric pollution, especially in and around heavily industrialized areas, is sulphur dioxide, derived principally from the combustion of various fuels, for example, coal and the heavier mineral oils. Its well-known corrosive properties are injurious not only to human beings but also to animal health and to vegetation alike. At its conference at Harrogate in October 1964, the National Society for Clean Air presented a timely report on this subject arising from research carried out by the Technical Committee of the Society (*Sulphur Dioxide as an Atmospheric Pollutant*. Pp. 32. London: National Society for Clean Air, 1964. 2*s*.). From the outset it is frankly admitted that there is no single solution to this sulphur problem and therefore the purpose of the report is to ". . . break the problem down into its component parts, both in respect of the different sources of pollution and the possible lines of attack on each". Sulphur is present in certain fuels in both mineral and organic forms, and, since the continued use of sulphur-bearing fuels is to-day unavoidable, there is little hope of removing it before combustion. Thus the problem resolves into ways and means of efficiently dispersing the products of combustion where sulphur dioxide is potentially active, and, when it is practicable, using non-sulphur-bearing fuels and sources of heat and power that are virtually sulphur-free, such as nuclear and hydro-power, gas, and the lighter mineral oils. "For larger installations, such as power stations, the policy of discharge of flue gases from chimneys at high velocity, to secure effective dispersion, is regarded as preferable to the wet washing processes that have been tried. . . . Chimneys should be designed to be of a height that will give satisfactory dispersion of flue gases, and these needs should not be subordinated to architectural and landscaping considerations. . . . If the latter are of paramount importance . . . the building should be equipped with heat or power installations that do not require chimneys". The report also considers the importance of low-level sulphur dioxide emission from domestic chimneys, and here the picture is a somewhat brighter one, especially in smoke control areas, where modern preventive measures are aimed at eliminating smoke (hence winter fogs), incidentally reducing substantially the amount of sulphur dioxide polluting the air, or getting rid of this hazard altogether. It has been estimated that by using town gas, paraffin or light domestic oil, as, for example, in oil-fired domestic boilers, there is a 99 per cent reduction in sulphur dioxide pollution of air; with solid fuel room-heaters, up to 60 or 70 per cent reduction; with electricity low-level emission is entirely replaced by high-level emissions. In the final section of the report, "Conclusions", it is stated: ". . . the problem of sulphur dioxide in the atmosphere is one that calls for a variety of contributory solutions . . . there is no prospect in the immediate future of entirely eliminating this gas, but . . . through the means that have been indicated, much can be done to reduce its concentration in the air we breathe".

British Species of *Lepidodendron*

THE publication of *Fossil Plants of the Carboniferous Rocks of Great Britain* (Second Section), part 3, is welcome, for it has been long delayed (Memoirs of the Geological Survey of Great Britain. Palæontology, Vol. 4, part 3. Pp. 217–354. Pls. 59–81. London: H.M.S.O., 1964. 140*s*.

net). Kidston first prepared it long ago as a complete review of the British species of *Lepidodendron* and certain other genera, nearly all the specimens on which it is based being in his own collection. It was unfinished when he died and Dr. Crookall then undertook its revision and re-wrote it, but this again was not quite finished when he retired in 1952; it is now completed with the help of four other authors. The volume describes the bark types of all the British species of *Lepidodendron* and certain other genera: it deals with this aspect only. The treatment is full and careful, as one expects in a survey memoir, the synonymies are critical and often very long, and the photographs are beautiful, nearly all being at natural size or at low magnifications. The treatment is conservative; the criteria used to distinguish species are well-tried and long-accepted ones and very few new species are made. It is rather striking, however, to find how many of the species are rare and little known in Britain, though perhaps commoner elsewhere. After looking at the figures one should be able to identify a *Lepidodendron* reliably, and this should be useful. Most of the commoner kinds do indeed have rather long time-ranges, but these ranges extend over a fraction only of the Carboniferous, so that when a number of determinations are taken together it should be possible to correlate a horizon fairly well.

Imperial College of Science and Technology

The *Prospectus 1965–66* of the Imperial College of Science and Technology contains the customary information on senior academic staff, and on courses offered at the Royal College of Science, the Royal School of Mines, and the City and Guilds College, scholarships and postgraduate awards, etc. (Pp. viii+74+5 photographs. London: Imperial College of Science and Technology, 1964). Fuller information, including a short history of the College and a list of student awards, is contained in the *Calendar 1964–65*. A booklet has also been issued giving information on shorter postgraduate courses to be held at the College in the autumn term of session 1965–66, with advance information on those to be held in the spring and summer terms where known (Pp. 16. London: Imperial College of Science and Technology, 1964). Information is given separately on part-time and short full-time courses.

University College, London

The *Calendar 1964–65* of University College, London, includes a short history of the College and the usual particulars of officers, governors, committees and academic staff, together with prospectuses for the faculties of arts and science, law, engineering, medical sciences, the Bartlett School of Architecture; the Department of Town Planning, the Slade School of Fine Arts, and the School of Librarianship and Archives. Particulars of scholarships, exhibitions, etc., and lists of Honorary Fellows and Fellows, are also included (Pp. lxxxvii+470. London: University College, 1964).

Chelsea College of Science and Technology

The *Prospectus 1965–66* of the Chelsea College of Science and Technology contains particulars of the governing body, academic staff, and the courses leading to the new degrees of B.Sc. and B.Pharm. (Pp. 40+4 plates. London: Chelsea College of Science and Technology, 1964). The draft charter of incorporation of the new university will have been printed by the time the courses begin, in October, 1965. The policy of the College is to move to a site outside metropolitan London, to allow for expansion to some 3,000 students within a few years, and the title of the university will depend on this siting.

The Night Sky in January

New moon occurs on Jan. 2d 21h 07m U.T. and full moon occurs on Jan. 17d 13h 38m. The following conjunctions with the Moon occur: Jan. 6d 22h, Saturn 4° N.; Jan. 12d 17h, Jupiter 2° N.; Jan. 21d 15m, Mars 2° S. Mercury is 1°·2 N. of Venus on Jan. 7d 7h. Conditions are unfavourable for the observation of Mercury and Venus, both rising 2 h before the Sun at the beginning of the month and less than 1 h before the Sun at the end of the month. Mars rises at 22h 30m, 21h 50m and 20h 50m on Jan. 1, 15 and 30, respectively; its stellar magnitude is +0·2; its distance from the Earth is 89 million miles on Jan. 15 and is in Virgo. Jupiter sets at 3h 40m, 2h 40m and 1h 50 m at the beginning, middle and end of the month, respectively; its stellar magnitude is −2·2; its distance from the Earth on Jan. 15 is 425 million miles and is in Aries. Saturn sets at 20h 30m, 19h 40m and 18h 50m at the beginning, middle and end of the month, respectively; its stellar magnitude is +1·1; its distance from the Earth on Jan. 15 is 977 million miles and is in Aquarius (where it will be all year). Occultations by the Moon of stars brighter than magnitude 6 are as follows, observations being made at Greenwich: Jan. 11d 19h 56·1 m, 64 Cet (*D*); Jan. 11d 21h 06·4m, ξ′ Cet (*D*); Jan. 13d 19h 32·0m, 163B. Tau (*D*); Jan. 14d 19h 48·4m, ι Tau (*D*); Jan. 15d 03h 25·2m, 109 Tau (*D*); Jan. 15d 18h 04·4m, 1 Gem (*D*); Jan. 19d 23h 48·3m, 46 Leo (*R*)—(*D* refers to disappearance and *R* to reappearance). The Quadrantid meteors are active in the period Jan. 3–5. Conditions for observation are favourable but maximum occurs during daylight (Jan. 3d 12h). The Radiant is at 232° R.A., +50° Dec.

Announcements

Prof. Hans K. A. S. von Euler, one of the greatest pioneers of modern biochemistry, in particular enzymology, died peacefully in Stockholm on November 6. Prof. Euler celebrated his ninetieth birthday on February 15, 1963 (*Nature*, **197**, 436; 1963). In spite of his great age he remained active until the end.

Sir Edmund Hirst, immediate past-president of the Royal Society of Edinburgh and Forbes professor of organic chemistry in the University of Edinburgh, has been awarded the Gunning Victoria Jubilee Prize for 1960–64 of the Royal Society of Edinburgh, in recognition of his distinguished contributions to the chemistry of the carbohydrates.

A seminar on "Fatty Acids" will be held at the Regional Research Laboratory, Hyderabad, during the first week of February. Further information can be obtained from the Director, Regional Research Laboratory, Hyderabad 9.

A conference on "Electronics Design" will be held at the Institution of Electrical Engineers, London, during February 8–9. Further information can be obtained from the Secretary, Institution of Electrical Engineers, Savoy Place, London, W.C.2.

The second regional conference on "Electron Microscopy in Far East and Oceania", arranged under the auspices of the International Federation of Societies for Electron Microscopy, will be held in Calcutta during February 2–6. Further information can be obtained from the Electron Microscope Society of India, Saha Institute of Nuclear Physics, 92 Acharya Prafulla Chandra Road, Calcutta 9.

A symposium on "Catalysis", organized by the Bradford Chemical Society and the Department of Chemical Technology of the Bradford Institute of Technology in conjunction with the Yorkshire Council for Further Education, will be held at the Bradford Institute of Technology on February 6. Further information can be obtained from Mr. W. A. Straw, Department of Chemical Technology, Bradford Institute of Technology, Bradford 7.

PRIVATE FOUNDATIONS IN THE UNITED STATES

INFORMATION on research and other activities of 272 private philanthropic foundations gleaned from a survey conducted by the U.S. National Science Foundation in 1960 is summarized in a report issued by the Foundation *.

Research funds accounted for 20 per cent of the total expenditure of 437 million dollars for all activities of these foundations, and of the remainder 72 million dollars represented funds for medical research or research related to health and education, of which 47 million dollars was for research and 25 million dollars for education. Eighty-nine million dollars of the total research expenditure came from 177 of these foundations and of this 85 per cent was for current operating expenses and 14 per cent for new capital expenditure. Seventy-six million dollars went to research carried out by the foundations' personnel in the United States and abroad, and almost 15 million dollars was spent on intramural research. All but 12·5 million dollars of the extramural expenditure was spent within the United States, and of the 62 million dollars so spent 35 million went to colleges and universities, 11 million dollars to hospitals and 8 million dollars to independent non-profit research institutes.

More than half the 76 million dollars spent on research by the 177 private foundations went on basic research projects, the total in 1960 reaching 42 million dollars, of which 8 million dollars represented funds for basic research by individuals and 34 million dollars for projects conducted in outside organizations. Of the total, 22 millions were spent on the life sciences, 10 millions on the social sciences, 6 millions on the physical sciences, and 2 million dollars on psychology. Three-quarters of basic research funds for the life and physical sciences were used extramurally. The financial support of applied research by private foundations totalled 34 million dollars, and four-fifths of this was allocated to the social and life sciences.

In January 1961, 59 of the 272 foundations replying reported employment of 758 scientists and engineers, and of this number 650 were working full-time including 441 scientists and engineers engaged on research.

The four foundations with the largest research programmes accounted for 52 per cent of the total research expenditure. Out of the 177 private foundations twelve spent more than 1 million dollars on research in 1963. These accounted for 63 million dollars of the total. Intramural research amounted to almost 10 million dollars and extramural research to 53 million dollars, or 71 per cent of the total funds spent by all the foundations surveyed.

Research grants going to educational institutions from the twelve foundations spending more than 1 million dollars amounted to 24 million dollars, or more than two-thirds of the total of research funds from this source, while 28 million dollars (three-quarters of the total) went to hospitals, and 4 million dollars to independent non-profit research institutions.

* National Science Foundation, Washington. *NSF* 64–14: *Research and other Activities of Private Foundations, 1960.* (*Surveys of Science Research Series.*) Pp. xi+33. (Washington, D.C.: Government Printing Office, 1964.) 35 cents.

WOOL SCOURING

THE large international attendance at a colloquium on "Wool Scouring", held at the Commonwealth Scientific and Industrial Research Organization Division of Textile Industry, Geelong, Australia, on October 26, was indicative of the growing scientific interest in this traditional process. Participants included representatives from industry, universities and technical colleges, research organizations, the Australian Wool Board, and the International Wool Secretariat.

In his opening paper on "The Importance of Scouring", Dr. M. Lipson, chief of the Division, gave a broad survey of the requirements of good scouring. From a consideration of the contaminants present on the fleece, he went on to discuss the various methods of removing them, and some of the effects of these methods on the subsequent behaviour of the wool in processing. He deprecated the still-common attitude of merchants who demand a conventional appearance rather than a good processing performance from the wool they buy, but forecast that this would disappear as the industry took more account of scientific advances in processing.

Mr. W. W. Mansfield's talk on "The Fundamentals of the Detergent Process" covered the behaviour of surface-active materials in solution, adsorption at interfaces, the removal of solid and liquid contaminants from surfaces, and the behaviour of charged particles in an emulsion. He emphasized the importance of a kinetic approach to detergency problems, particularly in industrial problems where conditions are far removed from the ideal conditions of classical surface chemistry.

Recent research on scouring at C.S.I.R.O. was described by Dr. G. F. Wood. The first part of his talk covered the development of the detergent-jet scouring process, and presented the results of investigations into the effects of various factors on scouring efficiency. In the second part, he described anomalies in the behaviour of certain non-ionic detergent solutions when used for scouring wool by the jet method. Differences in the ease of removal of oxidized and unoxidized wool wax from the fibre did not explain the anomalies, and it was suggested that they might be due to surface phenomena at the air/liquid interface of the jets.

In the final paper of the morning session, Prof. M. Chaikin made a statement on the present-day position of the "Compression Jet Scouring" process, which has developed from work carried out at the University of New South Wales.

Mr. P. H. A. Strasser outlined the history of synthetic detergents in relation to the textile industry and described the manufacture of non-ionic detergents. He discussed the changes that are taking place in the detergent industry, and showed how the demand for biodegradability was affecting the raw materials used for both anionic and non-ionic detergents.

There followed a critical appraisal by Mr. L. E. Way of the present situation in the scouring industry. He discussed quality requirements and cost limitations in relation to possible improvements resulting from chemical and mechanical modifications, and pointed out that quality control was hampered in some cases by lack of suitable measuring and control instruments.

The final paper, from Dr. C. A. Anderson, reviewed the present position in recovery of wool grease and effluent disposal. Dr. Anderson described the structure of wool scour liquors, and showed how various factors affected the centrifugal recovery of wool grease. He presented estimates of the capital and operating costs of the available effluent treatment processes, and concluded that solvent

scouring (which does not produce a noxious effluent) is the most attractive to mills where this scouring method is acceptable.

The sessions were admirably chaired by Dr. E. G. Carter and Dr. G. Laxer of the International Wool Secretariat, Mr. W. A. May of the Australian Wool Scourers' Association and Dr. F. G. Lennox of the C.S.I.R.O. Division of Protein Chemistry. The papers were followed by lively discussion, which contributed considerably to the success of the colloquium.

The lectures will be published in full in the next few issues of the *Textile Journal of Australia*.

C. A. Anderson
G. F. Wood

HIGH-TEMPERATURE DYEING

A HALF-DAY conference on "Continuous High-temperature Dyeing" was held at the Shirley Institute, Didsbury, Manchester, on November 18. The conference consisted of two lectures given by staff from the Institute, each followed by an interval for discussion. In a joint paper, entitled "The Principles and Scope of Continuous High-temperature Dyeing", Mrs. B. J. Bonwitt and Mr. M. G. Corless dealt with the technical requirements of the process and the range of fibres and dyestuffs to which the technique is applicable. The differences between American and European practice were described. Whereas in America processing speeds are usually of the order of at least 100 yd./min, in Europe it is uncommon for speeds to exceed 30 yd./min. The difference in approach can be ascribed to the difference between the structures of the two industries. Rather surprisingly it appears that machinery designed to run at lower speeds has to be somewhat more refined than that designed for high-speed operation.

In the second paper, entitled "Modern Continuous Dyeing Machinery", Mr. L. J. Gibson reviewed the machinery at present available from Continental machinery makers and then went on to discuss economic factors. 'Thermosol' and pressure steam ranges are not economical for short runs. In general 4,000–5,000 yd. must be regarded as the minimum length at one shade; runs in the United States are commonly of the order of 20,000 yd., and 10,000 yd. is regarded as a minimum.

'Thermosol' machinery is being used primarily for the dyeing of polyester/cotton blends; padding systems are not yet sufficiently well developed to deal with the majority of continuous filament synthetic fabrics. Pressure steam ranges are only just coming into use and are primarily used for continuous filament viscose rayon fabrics. However, they show considerable promise for the dyeing of woven nylon fabrics. It seems certain that a major reorganization of the merchanting system will have to take place before continuous high-temperature dyeing can be exploited to the full, despite its many technical attractions.

The questions which followed both lectures led to discussions on continuous preparation, the desirability or otherwise of heat-setting before padding, the merits of infra-red pre-drying and several other points. A measure of the interest in the subject of the conference lies in the fact that 160 people accepted invitations to attend (the conference had to be repeated on November 19, it being impossible to accommodate all on one day). In addition to members of the Shirley Institute, representatives were present from the major Continental machinery and dyestuff manufacturers.

BIOENERGETICS

A SYMPOSIUM on "Bioenergetics" was held at the University of Western Ontario, London, Canada, during October 15–16 under the auspices of the Biochemistry Division of the Chemical Institute of Canada. The papers dealt, in three successive half-day sessions, with energy transformations in the plant, microbial, and animal kingdoms. Interest in the symposium was evident from the attendance: 225 scientists were present from places as far away as Halifax, Edmonton and California.

One of the highlights of the meeting was the after-dinner speech by Dr. R. Y. Stanier (University of California) on comparative aspects of the cytological structures involved in energy transformations. Most aerobic bacteria possess no recognizable intracellular membrane components comparable with mitochondria. While it is possible to isolate fragments (presumably derived from outer cytoplasmic membranes) which contain oxidative enzymes, it is probably correct to view the whole bacterial cell as the smallest respiratory unit. The forms of photosynthetic structures present great variety in procaryotic cells. The chromatophores of the purple bacteria resemble the internal lamellæ seen in chloroplasts of higher forms. The lamellæ may appear as intrusions of the cytoplasmic membrane, as vesicular elements in the cytoplasm, or as transitions between the two forms. These primitive structural analogues of the chloroplast may be interpreted as early experiments on the path to evolution of the discrete, membrane-contained organelles of present-day higher organisms.

Mechanisms and intermediates of the phosphorylation reactions associated with photosynthesis were explored by Dr. D. I. Arnon (University of California) and Dr. A. T. Jagendorf (Johns Hopkins University). Central to these and subsequent discussions on photosynthesis was the role of ferredoxin, a low-molecular-weight protein containing stoichiometric proportions of non-hæm iron and sulphide, and possessing a redox potential at physiological *p*H in the neighbourhood of that for the hydrogen electrode. One of the more interesting functions of this strongly reducing electron carrier is its ability to promote net synthesis of pyruvic acid from acetyl coenzyme A and CO_2, a reaction of especial significance to *Chromatium* where the primary products of photosynthesis are amino acids. The photoreduction of ferredoxin by activated chlorophyll appears to be a primary reaction in photosynthesis leading to generation of high-energy phosphate compounds, the production of reduced pyridine nucleotides for CO_2-fixation, and the evolution of molecular oxygen. By alternating light and dark periods of exposure of isolated chloroplasts it is possible to demonstrate formation in the light of a high-energy compound with a short half-life which is capable of later transferring its energy equivalents to ATP in the dark. The nature of the intermediate is not established as yet, but its formation is associated with structural changes in the chloroplast and the uptake of hydrogen ions. Dr. Jagendorf provided some illuminating speculations concerning the relationship of these events to the known contractile changes of mitochondria and the anisotropic membrane ATPase theory of Mitchell.

The primary event of photosynthesis, the quantum-trapping, charge-separating mechanism which leads ultimately to the splitting of water into molecular oxygen and reducing equivalents of hydrogen, was described by Dr. N. E. Good (Michigan State University) and Dr. B. Kok (Research Institute for Advanced Studies, Baltimore). The process appears to involve an aggregate of

chlorophyll molecules, which acts as a light-gathering structure analogous to a lens, transferring and focusing the energy from a single photon to a photochemically active electron sink. Kinetic studies, based on combinations of selective inhibitors of oxygen evolution with the photochemically active site, have shown that the aggregate unit possesses approximately 2,500 chlorophyll molecules for each active oxygen-producing site. Losses in the energy transfers are compensated by an extension of half-life of the activated pigment molecules; an additional factor extending the stability of the excited pigments involves reaction with an associated electron carrier (probably cytochrome *f*) in a charge transfer complex. The entire process of photosynthesis may be viewed as an extension of the life-time of energy storage forms, from the activated singlet state of chlorophyll (half-life about 10^{-8} sec) to reduced pyridine nucleotide (half-life about 1 sec).

The problem of energy-production in certain autotrophic bacteria was broached by Dr. W. W. Umbreit (Rutgers University) and Dr. H. Lees (University of Manitoba). The inability of obligate autotrophs such as *Thiobacillus thio-oxidans* to grow with carbon sources other than carbon dioxide is unexplained since the organism possesses a full complement of enzymes for glucose metabolism, and converts ^{14}C-glucose into cellular protein. *Thiobacillus* can be adapted to survive in the absence of carbon dioxide in a glucose medium under certain conditions but does not remain viable. The reduction of pyridine nucleotide for fixation of carbon dioxide by *Nitrobacter* does not appear to proceed by a direct pathway, since the redox potential for the oxidation of nitrite to nitrate is too high to permit a direct coupling of the two reactions. The initial electron transfer is to cytochrome *c*; reduction of pyridine nucleotide occurs by an energy-requiring reversed electron flow from cytochrome *c*, a reaction demonstrated previously in mammalian mitochondria and also reported for *Escherichia coli*.

Studies of oxidative phosphorylation in *Mycobacterium phlei* were described by Dr. A. F. Brodie (University of Southern California). The oxidation of malic acid by cytochrome-containing particles from this organism proceeds via two different enzyme reactions. The first is the typical pyridine nucleotide-linked dehydrogenase; the second path proceeds by a flavin-mediated reduction of a naphthoquinone. Depletion of particulate naphthoquinone by irradiation with long-wave ultra-violet light causes inactivation of oxidation and phosphorylation with a variety of substrates. Restoration of activity on addition of synthetic and natural quinones revealed two sites of interaction with the electron transport system for pyridine nucleotide-linked substrates, and an additional site for succinate. The session on bacterial energetics was fittingly concluded by Dr. R. M. Hochster (Department of Agriculture, Ottawa) with a discussion of some unsolved problems in this area. Major difficulties in assessing the efficiency of oxidative phosphorylation in microbial systems arise from the presence of high phosphatase activity and the existence of by-passes in the oxidative reactions.

The dynamics of carbohydrate metabolism in mammalian cells was discussed by Dr. G. R. Williams (University of Toronto) and Dr. E. Shrago (University of Wisconsin). by continuous monitoring of $^{14}CO_2$ released from carboxyl-labelled substrates by respiring liver mitochondria *in vitro*, it was possible to calculate the rate constants for utilization of intermediates of the tricarboxylic acid cycle. The rate constant for fumaric acid was considerably greater than that for succinic acid, suggesting that succinic dehydrogenase is a limiting component of the cycle. On the *in vivo* level, studies of enzymes in animals rendered diabetic by alloxan treatment revealed marked alterations in pathways of carbohydrate metabolism. The most dramatic change was the marked increase in activity of the soluble enzyme, phosphoenolpyruvic acid carboxykinase, in liver; since the increase was reversed by injection of insulin it would appear that this enzyme plays a major part in the endocrine regulation of gluconeogenesis.

Dissection of the mitochondrial oxidative phosphorylation process into its partial reactions was presented by Dr. C. L. Wadkins (Johns Hopkins University). A protein of low molecular weight which catalyses an exchange reaction between adenosine diphosphate and adenosine triphosphate may be extracted from liver mitochondria; simultaneously, the phosphorylation coupled to cytochrome *c* oxidation is abolished, and may be restored by addition of the purified exchange enzyme to the depleted mitochondria. The exchange enzyme appears to correspond in its action and properties to the coupling factor III described by Green *et al.* Kinetic studies indicate that ferro-cytochrome *c* combines at two sites on the exchange enzyme; interaction at one site is blocked by dinitrophenol, while azide or oligomycin *A* prevents combinations at both sites.

Dr. W. Chefurka (Agricultural Research Institute, London) presented comparative data on the lability of phosphorylation reactions in mammalian mitochondria and insect sarcosomes. The rapid ageing of insect mitochondria is related to the rapid release of free fatty acids from mitochondrial lipids. The most sensitive indicator of the ageing process is the adenosine triphosphate cleavage occurring in the presence of dinitrophenol. The disappearance of the latter enzyme on ageing may be reproduced by *in vitro* addition of fatty acid.

The symposium was well organized by a local committee under the chairmanship of Dr. K. P. Strickland. Fortunately, sufficient time was allowed for free discussion of the stimulating data and ideas presented in the formal papers. All present at the symposium were able to participate in several interesting functions concerned with nutrition, digestion, and the products of fermentation. Plans are under way to publish the papers in the *Canadian Journal of Biochemistry*.

W. C. McMURRAY

OCEANOGRAPHY IN BRAZIL

THE recent symposium on the oceanography of the western South Atlantic organized by the Brazilian Academy of Sciences (Rio de Janeiro, September 14–18) was an important step in the development of the investigation of the sea in South America. For many years there has been an active interest in oceanography in Brazil. Initially this manifested itself in the establishment of the Instituto Oceanografico in the University of São Paulo and the acquisition of field stations, one at Ubatuba situated close to an inviting range of sub-tropical marine environments, and the other at Cananéia amidst an extensive mangrove swamp with its exciting scientific problems. These facilities encouraged an energetic interest in marine biology.

The Brazilian contribution to the symposium strongly reflected this initial interest but also showed how widely the field of work has since developed. Other laboratories have entered the field, and the papers presented included investigations of the Brazil current, of water movements nearer to the coast, the drift of littoral sediments, the production of organic matter, the biology of the plankton and benthos, and geophysical investigations of the sea-bed.

Delegates from Argentina and other South American countries contributed accounts of similar investigations extending southwards to the waters of the Antarctic, and it was clear that an important aspect of a meeting such as this lay in bringing together for the first time

scientists who often have to work in comparative isolation at widely separate places. A number of invited papers from abroad on the oceanography of adjacent regions or on more general problems of oceanography formed a background for the symposium.

The amount of resources devoted to marine science in Brazil is still not great, but as a visitor one could not help being deeply impressed by what has been achieved on slender resources and particularly by the great enthusiasm displayed by the younger scientists. In all fields the awareness of the need for aiming at quality rather than quantity of work stood out very clearly. There is a growing appreciation of the importance of oceanography to the country. The need to learn more about available fisheries and to deal with the problems of defence of a very long coastline has stimulated interest in the Government and in particular in the Navy. Indeed, the Hydrographic Office has with the help of Unesco already almost completed the reconstruction of a research vessel, *Almirante Saldanha*, which will rank among the largest in the world. She will have an enormous potential for making much needed observations in the Brazil current, but it is to be hoped that such splendid facilities for making observations will not submerge the promising laboratory work which is also gaining impetus. Descriptive oceanography in all disciplines must be paralleled with adequate theoretical and experimental work before we can expect to reach a real understanding of what happens in the sea. Brazil has the potential to undertake these investigations, and it is hoped that adequate support will be found for them.

Ronald I. Currie

APPLICATIONS OF MICROFILM READERS IN PETROGRAPHY

MICROFILM recording of original documents is established practice, so also is the use of microfilm readers in their enlargement. In the more specialized field of petrographic investigations of igneous, metamorphic and sedimentary rocks, the use of microfilm readers in direct analysis of these materials may not be so generally known. In an article by Radco Kühnel, of the Institute for Mineralogy, Petrography and Geochemistry, Mining Academy, Ostrava, U.S.S.R. (English translation), entitled "Microfilm Reader in Petrography" (*Jena Review*, No. 3, 1964; VEB Carl Zeiss Jena, Jena (Saale)), the potentialities of this adaptation of a modern technique are both lucidly explained and convincingly illustrated.

Taking the ordinary thin section of a rock of conventional thickness about 30μ, as normally examined under a petrological microscope, and assuming that the average size of the slide fits comfortably within a 35-mm microfilm frame (which according to European custom it should do), then the simple process of placing the thin section under the microfilm reader results in a magnified natural image of the section, coloured if appropriate minerals are present, giving not only a clue to identification of at least some of the more easily recognized constituents, but equally to the microstructure and texture of both rock and minerals scrutinized. One initial advantage of this approach to a petrographic investigation is that the whole rock-slice, within the reader frame, can be seen as one field and in complete continuity, avoiding any interruption in analytical assessment of mineral composition and microstructure sometimes occasioned by constantly changing horizontal and vertical orientations of these features in the normal course of microscopical examination. There is of course nothing new in projection of part or, if the original image is small enough, the whole of a thin section of rock or particles by a petrological microscope and suitable optical bench and illumination, on to an appropriate wall-screen, especially for class-instructional or other demonstration purposes; but the microfilm reader, properly adapted, simplifies this operation.

Among advantages claimed by the author for microfilm reader technique, he mentions the following: ". . . measurements may be carried out on mm-graph paper, etc. . . . for determining the absolute size of the individual grains . . . the grain-bonding figure (average number of adjoining grains)". "As regards the texture, it is a simple matter to ascertain the regularity of the spatial distribution as well as the orientation of the individual minerals. . . . In certain rocks (especially those rich in melanocratic constituents) it is possible to carry out modal determinations by means of a self-produced lattice. . . . Especially in sediment petrography it has given invaluable service in the investigation of the fine stratification, in post-sedimentary deformations, rhythmic precipitations, of gradation symptoms and other lithological phenomena." It is also possible to use this apparatus to explore thin sections of rocks in polarized light and with crossed nicols on the available projection area of the instrument, and with a more powerful light-source if it is desired to project the image on to a wall-screen for instructional purposes. Another application of the microfilm reader is in photographic documentation of rock micro-structure and micro-texture. In this connexion the author briefly describes his work on 'negative micro-exposures', that is, on direct exposure of the section to photographic paper; he also indicates the possibility of producing direct micro-colour photographs of rocks and minerals by means of coloured reversal paper.

VEB Carl Zeiss Jena (C. Z. Scientific Instruments, Ltd., London, W.1) manufacture what they call a 'Dokumator Film Reader' which appears to be ideal for the purposes described here. In this apparatus the image appears on a 300 mm × 390 mm screen of high reflective power. An attachment for slides permits viewing of glass slides up to 50 mm × 50 mm in area, but it is to be noted that some special arrangements would have to be made if conventional British 75 mm × 25 mm glass slides are involved; alternatively, the smaller foreign-type mounts might have to be used.

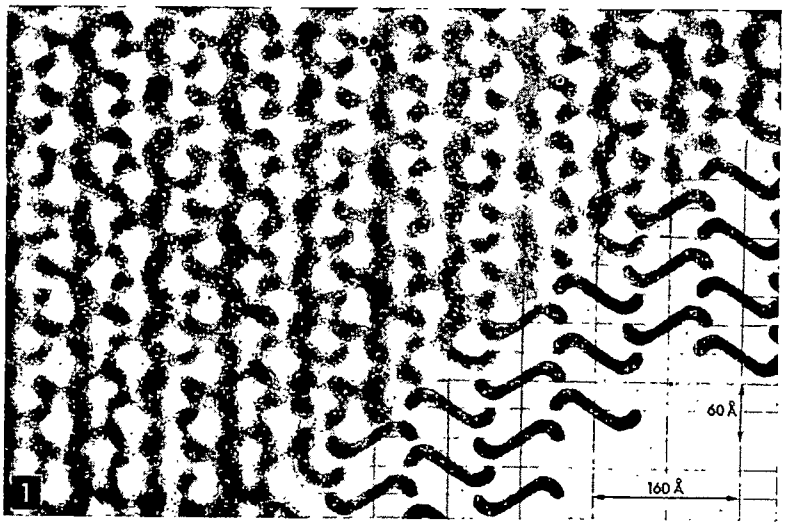
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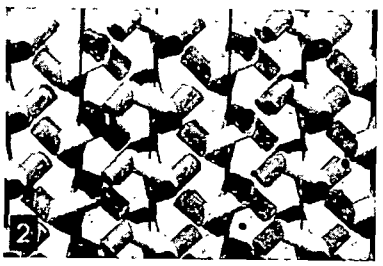
SUB-UNITS OF THE CATALASE MOLECULE SEEN BY ELECTRON MICROSCOPY

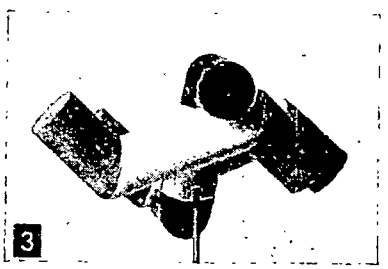
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CLEAR pictures of protein molecules are difficult to obtain with the electron microscope, although in size the molecules should be easily within its resolving power. At present, negative staining is the most generally successful way of showing detail down to the molecular level. This is done by drying some dense but unreactive substance with the specimen; the relatively electron-transparent objects are outlined by the far more opaque 'negative stain'. When applied to protein molecules, this technique works less well with some proteins than with







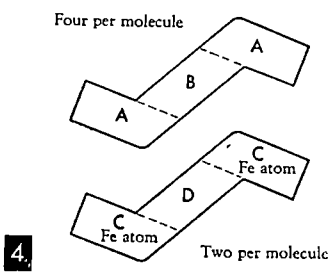


Fig. 1. Part of an electron microscope picture of a catalase crystal at a magnification of 1,250,000 times. The sub-units seen in the plane of the picture are indicated at the lower right. Fig. 2. A model of the suggested arrangement of the crystal showing how two perpendicular sets of similar sub-units can give a pattern resembling that of Fig. 1. Fig. 3. A model of the proposed half-molecule of catalase containing three sub-units each of 42,000 molecular weight. The sub-unit with the darker ends, though similar to the other two, is not identical with them. Fig. 4. A diagram showing one way of reconciling the electron microscope appearance with the chemical data. The whole molecule might be built from 18 polypeptide chains of four kinds *A*, *B*, *C*, and *D*. Each chain contains 120 amino-acid residues and has a molecular weight of 14,000. The *C* chains are the only ones to contain a hæm group. In the half molecule (Fig. 3), two sub-units containing chains *A* and *B* are linked by one (shown with darker ends) containing *C* and *D*. Thus the whole molecule of six sub-units has 8 *A*-chains, 4 *B*-chains, 4 *C*-chains, and 2 *D*-chains. One iron atom in each *C*-chain (or alternatively one in each *B*-chain) then gives 4 iron atoms per molecule

others. The difference is not related to molecular size, and the explanation, though very likely concerned with penetration of the stain into the molecules, is not clear. However, in some cases the pictures seemed good enough to suggest the general shape of the molecules[1]. The conclusion was that the electron microscope did not support the cigar-shape which had been the widely accepted picture derived mainly from ultracentrifuge data. As a first approximation, non-fibrous proteins appear to have spherical molecules; axial ratios greater than two were not seen.

The chances of obtaining detailed electron microscope pictures of protein molecules are very much improved when it is possible to examine the proteins in crystalline form. The advantage of crystals as specimens was first pointed out by McLachlan[2], who suggested a scheme that might allow the electron microscope to 'see atoms'. The regularly arranged molecules in the crystal would not be very clear on the original electron micrograph. But the suggestion was made that it should be possible when enlarging the negative to superimpose tens or hundreds of different molecules. This is done by making multiple exposures in the enlarger with the image shifted between each exposure by exactly the repeat distance. Such a synthesized picture should show much more detail than was clear in the original, and indeed a successful application of this sort of idea to tobacco mosaic virus has recently been described[3]. An additional advantage of crystalline material is that there is less denaturation of the molecules during preparation of the specimen. The only difficulty is to obtain thin enough crystals.

Catalase is an enzyme which forms thin micro-crystals very suitable for electron microscopy. Crystalline bovine-liver catalase (C. F. Boehringer und Soehne, Mannheim, Germany) was therefore examined after treatment with a high-contrast negative stain (sodium silicotungstate or uranyl acetate). A Philips *EM* 200 electron microscope was used which had been adjusted to give a resolution measured on Fresnel fringes of at least 5 Å.

The molecular weight of catalase is 250,000, and in agreement with this, as already reported, the microscope shows the free molecules around the crystals as roughly spherical, or perhaps cubical, objects 70–80 Å in diameter. But molecular detail could not be clearly distinguished from background grain. The crystals themselves showed a remarkable variety of repeating patterns which were

seen to change when the specimen was tilted in the microscope. From the contrast of the unstained crystals, it was estimated that many of them were 200–500 Å thick; they would therefore contain several layers of molecules. The variation in pattern with orientation is thus understandable, though it adds to the complication of interpreting the pictures. However, it did become clear after an examination of many high-resolution electron micrographs that it would be difficult to maintain the initial idea[1] that the crystal was a simple array of the 70-Å molecules. Lucy and Glauert[4] have therefore suggested that the crystal is composed of an array of sub-units of the molecule and have devised a model on this basis which gives a much better explanation of the pictures.

More than twenty-five years ago Sumner and Gralén[5] showed that catalase could be split into smaller molecules in acid or alkali. Recent work with the ultra-centrifuge[6–10] and chemical investigations[11,12] now clearly show that the molecule of 250,000 molecular weight is composed of sub-units. How many, however, is a matter of some dispute. An approximate treatment of the ultra-centrifuge data suggested that the molecule could be split into four identical sub-units[9]; this conclusion was based on the assumption that the sub-units had nearly the same shape as the molecule. Other workers, after much fuller investigations, have come to the conclusion that there must be six similar sub-units[8,10]. The only difficulty in accepting this is that catalase contains four atoms of iron per molecule. Thus, if it is built of identical sub-units, one would strongly suspect *a priori* that there should be two or four but not six of them. The chemical estimations of the number of sub-units have changed the other way. Initially the workers favoured six sub-units[11], and after further work[12] found it "not unreasonable to incline towards four". What does seem to be established[10] is that at *p*H 3 catalase molecules can split into identical half-molecules. These are enzymatically unreactive but can be recombined, and up to half the enzyme activity is then restored.

In the light of this evidence the electron micrographs of the crystals of catalase were reconsidered and an attempt was made to interpret them assuming that the molecule contains either four sub-units of molecular weight 62,500 or six of molecular weight 42,000. The possibility was also considered that the crystal, like that of hæmoglobin, might be an array of the half-molecules. In examining the pictures it was found, as McLachlan had predicted, that superimposition in the enlarger of ten or twenty images from the same negative shifted by the repeat distance considerably improved the clarity of the relevant detail. Unfortunately imperfections in the crystals occur when they are dried and limit the number of repeats that can be usefully superimposed. Detail below about 15 Å is still not seen.

In what is taken as the key pattern, a sub-unit can be made out shaped something like an integral sign (Fig. 1). The width of the unit is between 20 and 25 Å and, straightened out, its length would be 120 Å. The density of catalase is 1·3 g cm^{-3} (or more strictly the partial specific volume is 0·75 (ref. 5)), and with the assumption of an approximately circular cross-section the molecular weight of the unit comes out as between 30,000 and 45,000; it is almost inconceivable that it could be as high as 60,000. The sub-unit is thus almost certainly one-sixth of the molecule and will have a molecular weight of 42,000. Fig. 1 shows the units in neighbouring rows alternately packed and the repeat distance is 160 Å (twice the distance between the rows). The repeat distance up the rows is 60 Å but between the units are clear elliptical spots about 40 Å by 20 Å. If, as the centrifuge evidence suggests, all the sub-units are similar, these spots have to be interpreted as end-on views of sub-units. The complete pattern is then explained by assuming that the crystal is built by half-molecules consisting of two parallel sub-units linked by one at right angles. A model of the crystal structure suggested is shown in Fig. 2 and the appearance of the half molecules in Fig. 3.

Although it is not clear how the two halves of the molecule fit together, there are several ways that will give an approximately spherical shape. In view of the highly elongated sub-units (the axial ratio of which is about 6), the fact that the molecule does not sediment in the centrifuge as a sphere but as an ellipsoid of axial ratio 5 (ref. 10) is understandable. Some high-resolution electron microscope pictures of glutamate dehydrogenase have been published by Horne and Greville[13], and again the ultra-structure appears to be elongated. These workers have also published[14] some preliminary pictures of catalase crystals in various orientations and suggested that they contain 'structure units'.

Recent chemical evidence[12] about catalase has already been mentioned as favouring four sub-units, but it can also fit the picture of catalase just described. In fact, since unfortunately *N*-terminal amino-acids could not be detected, a clear answer about the number of sub-units or polypeptide chains was not obtained and light on this point only comes indirectly. In outline the chemical work consisted of isolating and largely characterizing the peptides in tryptic hydrolysates of bovine-liver catalase. The catalase molecule itself (molecular weight, 250,000) has about 2,150 amino-acid residues. If the molecule did not contain any identical sub-units, the peptides obtained by trypsin digestion of one molecule would almost certainly all be different. Then, on isolating each peptide and characterizing the amino-acids in each, a total near to 2,150 should be found. If the molecule contains identical sub-units, each peptide will be repeated by up to as many times as there are sub-units in the molecule. In fact the hydrolysate contained at least 2·5 times the amount of some peptides which would be expected from a molecule of 250,000 molecular weight. This seems to establish beyond question the presence of at least three identical sub-units in catalase. On the other hand, if there were five or more identical sub-units, there should not be more than 430 (that is 2,150/5) residues found in the peptides. Since at least 460 were found, four sub-units were favoured.

The sub-units seen in the electron microscope pictures, however, are perhaps not all identical since they pack in the crystal in two different ways. Two-thirds appear to be of one kind and one-third of another. Moreover, the sub-units look symmetrical about their centres and consist of a straight portion of lower contrast than the two similar ends. The electron microscope appearance and the chemical data can be most simply accounted for by supposing that catalase is built of 18 polypeptide chains of four different kinds, each of about 14,000 molecular weight (120 residues). This would make each chain similar in size to the single chain of ribonuclease. The hypothetical structure is illustrated diagrammatically in Fig. 4. It allows there to be six similar sub-units, four of one kind and two of another, with only 4 atoms of iron per molecule and 480 residues in the tryptic digest.

I thank Mr. N. G. Wrigley for the photographic techniques and model-building involved in this work.

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GOLGI CELL INHIBITION IN THE CEREBELLAR CORTEX

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THE Golgi cells or large stellate cells of the granular layer were first described by Camillo Golgi in 1886 (ref. 1). These cells are among the largest neurones in the cerebellar cortex, being second in size only to the Purkinje cells. As illustrated in Fig. 1*A*, the bodies of the Golgi cells are located in the granular layer (*G*) of the cerebellar cortex, immediately below the Purkinje cell bodies, their dendrites ramifying extensively in the molecular layer (*M*) and their axons branching profusely to form a thick plexus, which is restricted to the granular layer and which enters into contact there with a special synaptic structure called the glomerulus[2]. This glomerulus is formed by the mono-synaptic contact between the terminal sacs of the most numerous cerebellar afferent fibres, the mossy fibres, and the dense surround made by the terminals of granule cell dendrites[2-4]. Although quantitative data are not available, it can be said that the axon of a single Golgi cell may enter into contact with thousands of these glomeruli. Its commonest site of termination appears to be on the dendrites of the granule cells. There are three synaptic inputs to the Golgi cells, the main one being the parallel fibre system which contacts the dendritic spines of these cells in the molecular layer[4]. These parallel fibres are formed by the terminal bifurcations of the granule cell axons at all depths in the molecular layer[2,5-7]. Thus an afferent volley in the mossy fibres will activate granule cells and these, in turn, through the parallel fibres, the Golgi cells. The other two types of synapse are located on the bodies, and are the axon collaterals of Purkinje cells[3] and the collaterals of the climbing fibres[8,9], and are presumed to be of secondary significance. The function of the Golgi cells on the mossy fibre-granule cell synapse has been studied by microelectrode recordings at various depths in the cerebellar cortex (laminar field analysis) and also by intra- and extracellular unit recordings.

The experimental procedure was similar to the one reported in previous publications[10,11]. Cats were anæsthetized with 'Nembutal' and artificially ventilated after being paralysed with 'Flaxedil'; a bilateral pneumothorax was performed in order to minimize the movements of the rib cage. Microelectrodes filled with 3 M potassium chloride or 2 M potassium citrate with an average resistance of 10 MΩ were used for intra-cellular recording. Laminar field analysis and extra-cellular unit recordings were carried out with microelectrodes filled with 4 M sodium chloride solution. The part studied was the vermal region of the cerebellum in the folia immediately anterior (culmen) or immediately posterior (lobus simplex) to the fissura prima. The cortex was covered with an agar gel which served to suppress the pulsation movements and to insulate the cortex from environmental influences, such as cooling and evaporation. Stimulation of the surface of the cerebellum (local and transfolial) and of the underlying white matter (juxta-fastigial) was done through concentric bipolar electrodes. Similar electrodes were used to stimulate the inferior olive and the external cuneate nucleus. The superficial and deep radial nerves were stimulated with platinum electrodes under paraffin oil.

When a microelectrode is lowered into the granular layer, and a large population of granule cells is simultaneously excited, a typical negative field potential is recorded. This negative field, which represents for the most part summated spikes of these cells, can be evoked by any form of stimulation capable of activating mossy fibres. For example, in Fig. 1*B* stimulation of an adjacent folium has been used to excite granule cells by a type of axon reflex along the mossy fibres, which branch profusely in the granular layer and extend in a U-shaped fashion from one folium to the next[2].

In the upper trace of Fig. 1*B* a progressive increase of such a transfolial (*T.F.*) stimulation evoked a corresponding increase in the granule cell spike potentials. In the lower trace it can be seen that a conditioning stimulation of the parallel fibres (*LOC*) removed this spike component and left only a slow negative potential wave, which is presumably the excitatory post-synaptic potential (EPSP) evoked by the mossy fibre synapses on the granule cells. In Fig. 1*C* a larger granule cell spike potential was produced by a graded juxta-fastigial (*J.F.*) stimulation. Lower traces show that the conditioning parallel fibre stimulation also depressed this granule cell spike, though it was less effective against the more powerful synaptic action produced by the *J.F.* stimulation.

In both Fig. 1*B* and *C* it will be noted that there is a pre-synaptic spike, which is small in Fig. 1*B* and larger in Fig. 1*C*. In Fig. 1*B* this spike would be produced mainly by impulses in mossy fibres, but in Fig. 1*C* the impulses in Purkinje axons and climbing fibres would make a large contribution. This inhibition of the granule cell spike potentials reached its maximum at about 20 msec from the preceding stimulus to the parallel fibres, and passed off at intervals beyond 100 msec.

The inhibition of granule cells can also be shown when action potentials from single granule cells are recorded with a microelectrode in the granular layer. In Fig. 1*D* a single volley from the superficial radial nerve evoked a repetitive discharge in a granule cell. This discharge was inhibited by a preceding stimulation of the parallel fibres (*L*). The inhibition of the repetitive spikes is complete up to 50 msec and is still present at 120 msec. This inhibitory action is also demonstrated in Fig. 1*E*, where the spontaneous discharge of several granule cells was completely inhibited for as long as 110 msec by applying a stimulus to the parallel fibres (*L*) or the underlying white matter (*J.F.*), whereas a stimulus to the inferior olive (*I.O.*) produced a shorter-lasting inhibition.

These results demonstrate the existence of a powerful inhibitory action exerted by the parallel fibres on their cells of origin, the granule cells. Anatomically, the only neural elements capable of such negative feed-back at cortical level are the Golgi cells, since they represent the only system linking the molecular layer with the granule cells, and the only synaptic input to the granule cells apart from the mossy fibres. Parallel fibre stimulation by the surface electrode (*LOC*) would excite Golgi cells through their dendrites in the molecular layer (Fig. 1*A*) and so produce inhibition of the granule cells. The inhibition effected by white matter stimulation (*J.F.*) is similarly explained, for this stimulus would directly excite mossy fibres, which, in turn, would activate granule cells and the corresponding parallel fibres.

This inhibitory depression contrasts with the climbing fibre activation, where a similar parallel fibre stimulation does not diminish the climbing fibre response evoked by inferior olive stimulation[11]. The difference is to be expected, since the Golgi cell action is exerted on the mossy fibre–granule cell synapse, whereas the climbing fibre, being in mono-synaptic contact with the Purkinje cell, has no synaptic relay at which inhibitory action can be exerted[2,9].

Another example of Golgi cell inhibition is shown in Fig. 2. An intra-cellular recording from a Purkinje cell

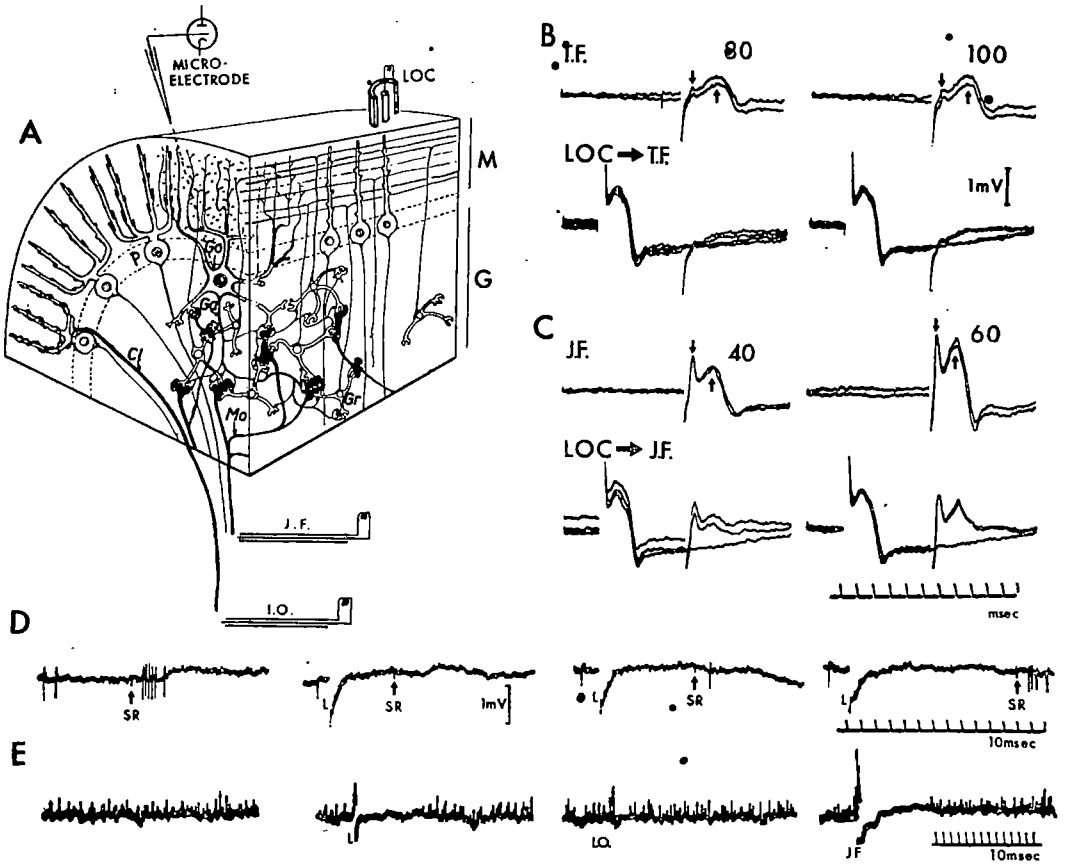


Fig. 1. *A*, Diagram of the cerebellar cortex (modified from Szentágothai, 1964 (ref. 4)) and experimental arrangementas, described in the text: *G*, granular layer; *M*, molecular layer; *Cl*, climbing fibre; *Ga*, Golgi cell axon; *Go*, Golgi cell soma; *Gr*, granule cell: *Mo*, mossy fibre; *P*, Purkinje cell soma. *B* and *C*, Potential fields recorded at 550μ depth: downward arrow, presynaptic spike; upward arrow, granular spikes. *D* and *E*, Extracellular granule cell spikes at 500μ depth

shows in Fig. 2*A* the synaptic activity evoked by a single stimulus applied to mossy fibres through the *T.F.* electrode. By means of the mossy fibre to granule pathway an EPSP is produced by the direct action of the parallel fibres on the Purkinje cells. This potential is followed by an inhibitory post-synaptic potential (IPSP) due to the inhibitory action of basket cells on axon and soma of the Purkinje cell[10,12], and by the wider inhibitory action of the stellate cells on the dendritic tree of the Purkinje cell[13]. As shown in Fig. 2*B*, both the EPSP and the IPSP of Fig. 2*A* are greatly decreased by a preceding stimulus (*L*) to the parallel fibres. On the other hand, if the testing volley is not applied to the mossy fibres, but is applied directly to the parallel fibres one synapse later (Fig. 2*C*), a preceding stimulus to the parallel fibres does not depress the synaptic potentials of the Purkinje cell (*D*). Evidently the inhibition in *B* must be occurring earlier in the pathway than the parallel fibre–Purkinje cell synapses, and hence must be effected at the mossy fibre–granule cell synapse. It should be pointed out that the large apparent increase in the EPSP evoked by the testing stimulus in *D* relative to *C* is attributable to the occlusion of its IPSP by the large IPSP produced by the conditioning stimulus.

The results presented in Figs. 1 and 2 demonstrate that there is a very effective inhibitory action on the mossy fibre–granule cell synapses, and that the excitatory action of the parallel fibres *per se* on the Purkinje cells is unimpaired by this inhibition. It is concluded that the Golgi cells have a strong inhibitory action on the granule cells and thus serve to regulate the output of the cerebellum, acting as a negative feed-back system on the dominant afferent system to the cerebellar cortex, the mossy fibres.

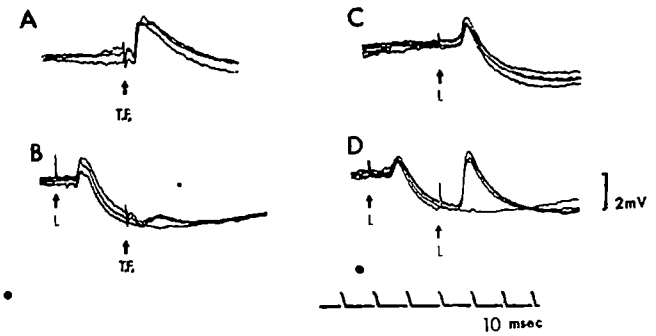


Fig. 2. Intra-cellular recording from a Purkinje cell. *A*, Excitatory and inhibitory synaptic potential evoked by mossy fibre (*T.F.*) stimulation. *B*, Depression of these synaptic potentials by a preceding parallel fibre (*L*) stimulation. *C*, Excitatory and inhibitory synaptic potentials evoked by parallel fibre stimulation. *D*, No depression by a preceding parallel fibre stimulation

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RELATIVE EFFICIENCY OF HIGH-LET RADIATION (BRAGG-PEAK LITHIUM IONS) ON NORMAL RABBIT SKIN, USING INTEGRAL DOSE AS A BASIS FOR COMPARISON

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THE biological effectiveness of the Bragg-peak region of a high-LET (linear energy transfer) particle relative to that of a low-LET heavy particle was examined by comparing the reactions produced in the skin of rabbit ears which had been irradiated with a beam of either lithium-7 ions (LET = 122·0 keV/μ) or hydrogen-2 ions, deuterons (LET = 17·9 keV/μ). Both beams (from the *Hilac*, Lawrence Radiation Laboratory, Berkeley, California[1]) were monitored with a parallel plate ionization chamber, previously described by Brustad *et al.*[2], with degrading foils placed after the ionization chamber to adjust the range of the particles so that they would stop within the skin and short of the cartilage of the ear. (Histological investigations showed that in animals of this size the inner skin surface averages 300μ in thickness.) The experiment was planned so that equal volume doses at the Bragg peak would be delivered within the epidermis and dermis with each of the two ions. Integral dose was used as the basis for comparison of the two qualities of radiation used in this experiment, and in this situation it is perhaps imprecise to speak of 'relative biological effectiveness' in the usual sense (see ref. 3 for further discussion of this point). Therefore, to avoid any possible confusion in terminology we shall use relative efficiency rather than relative biological effectiveness in discussing the results of these experiments.

Five groups of ten rabbits each were used (all 7·5 week-old males). Each animal had the inner surface of one ear exposed to radiation by 6·3 MeV Bragg-peak deuterons (low-LET) and of the other ear to 35 MeV Bragg-peak lithium-7 ions (high-LET). Table 1 gives the ranges and LET's of these particles at entry and within the skin of the ear. The average LET values were obtained by dividing the incident particle energy by the range. The volume dose in the skin was doubled from one group to the next, the Group V animals thus receiving 16 times the dose of Group I animals. The doses received by Group I animals are shown in Table 2. (It was noted that because of a technical error during irradiation the integral dose was slightly higher for the deuteron-irradiated ears in each group.) The deposition of energy is not uniform throughout the range of the particles: the estimated ratios of maximum dose to surface dose for lithium-7 and deuteron ion beams are 3·0 and 1·6 respectively.

In order to avoid any bias or systematic error in the investigation, involving a subjective as well as an objective evaluation of radiation response, an elaborate system for randomizing the animals in their numbering sequence and their cage assignments throughout the experiment as well as a method for weighting the assigned response scores were worked out, and these have already been described[3]. Briefly, the acute responses were graded subjectively on a 1 to 4+ scale, the observer taking into account the usual objective criteria of radiation reactions such as erythema, œdema, desquamation, keratosis, tanning and thinning. Later, 6·5 weeks after irradiation when the peak reactions for the acute response were passed, a second system was adopted for evaluating the chronic reactions. These were scored on a 1 to 6+ basis, and the criteria were various degrees of pigmentation, atrophy and telangiectasia, as well as scarring and necrosis which were considered to be accentuations of the responses.

Table 1. PHYSICAL CHARACTERISTICS OF TREATMENT BEAMS

	Ion	
	^{7}Li	^{2}H
Residual energy at entry (MeV)	35·0	6·3
Residual range at entry (μ)	287·0	352·0
LET (keV/μ)		
At entry	64·8	11·2
Within skin	122·0	17·9

'Entry' denotes the superficial skin surface. The thin skin of the rabbit ear has been assumed to be of water density for calculation purposes. To obtain the range and LET in the density-independent units mg/cm^2 and MeV/g cm^{-2} respectively, the values for the ranges given above must be divided by 10, and those for the LET must be multiplied by the same factor.

Table 2. DOSES RECEIVED BY GROUP I ANIMALS

Ion	Incident energy flux (MeV/cm^2)	Average dose (rad)	Integral dose (g rad)
^{7}Li	$1·90 \times 10^9$	1,060	86·5
^{2}H	$2·17 \times 10^9$	988	98·8

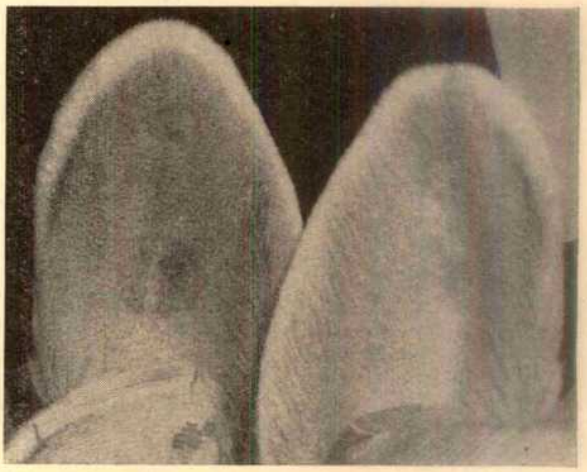


Fig. 1. Ears of Group IV rabbit three weeks after irradiation with heavy ions: right ear, lithium-7 (LET = 122·0 keV/μ); left ear, hydrogen-2 (LET = 17·9 keV/μ). Following comparable volume doses to each ear, note the pronounced reaction in the ear receiving the higher-LET radiation whereas there is only a faint response in the ear receiving the lower-LET radiation. (The unirradiated central strip on the right ear results from an unavoidable longitudinal folding of the ear during treatment)

The analysis of the acute reactions for the lithium-7 and the deuteron-irradiated ears of the five groups of animals revealed the following four patterns of response.

(1) A prompt response which increased steadily, reaching a maximum by the third to fourth week after treatment. This was observed in both ears of the Group V (highest dose) animals, although the response in the deuteron-irradiated ears consistently lagged behind that in the lithium-7 irradiated ears.

(2) A prompt response which waned and then recurred rapidly after the second week, reaching a maximum by the third week or later. This was observed in the lithium-7 irradiated ears of Groups III and IV animals.

(3) No initial reaction, but a subsequent response during the second week at the time when reactions observed in the ears of Group V animals had passed their peak and were in a subsiding phase. This was observed in

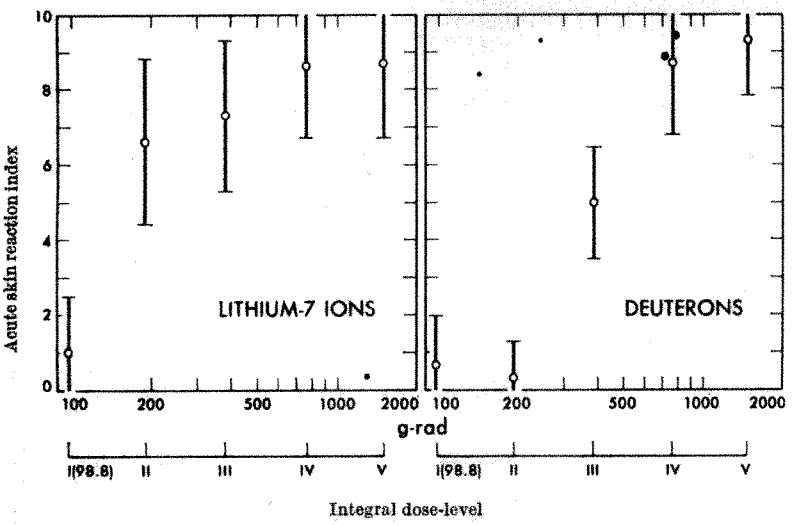


Fig. 2. Dose-response curves for lithium-7 ions and hydrogen-2 ions (deuterons). A skin reaction index of 10 represents a maximum response in all animals. Differences in reactions at dose levels II ($P < 0.001$) and III ($P < 0.02$) lead to curves of different configuration in their low-dose portions, with consequent rapid changes in relative effectiveness

deuteron-irradiated ears of Groups III and IV, and in lithium-7-irradiated ears of Group II.

(4) Minimal reactions, if any at all, which appeared 3–4 weeks after treatment. This was observed in both ears of Group I (lowest dose) animals and in deuteron-irradiated ears of Group II animals.

The acute reactions faded progressively up to the sixth week, and from then until the end of the experiment at 6 months there was little change.

Thus, these investigations of the acute reactions in rabbit ears showed that at equal times after therapy the responses were more severe in lithium-7-irradiated ears for each group (Fig. 1). The overall patterns of response before maximum reactions developed were consistent; the average reactions in lithium-7-irradiated ears at a given integral dose were equal to, or slightly greater than, those observed in deuteron-irradiated ears receiving twice that integral dose, but were not so pronounced as those in deuteron-irradiated ears receiving about four times that integral dose (that is, an efficiency of between 2 and 4). Finally, as shown in Fig. 2, the dose-response curves for the maximum acute skin reaction indices obtained (disregarding time) with each of the ion beams differed distinctly in the low-dose regions; the curve for deuterons is clearly sigmoid whereas that for lithium-7 ions had a low-threshold or no-threshold configuration. This results in a rapid change in relative efficiency as dosage is increased, the variation being from about 5 : 1 to 1 : 1 as the dosage is doubled.

The chronic reactions were also analysed. In the rabbit ears receiving single high doses of lithium-7 ions there were pronounced, but not disproportionately severe, late post-radiation changes, as compared with those irradiated in the same way with deuterons. There was no suggestion in any animal, at any time during the 6-month period of observation, of a progressive or abrupt deterioration of the chronic response towards complete necrosis or catastrophic damage.

This work was carried out under the auspices of the U.S. Atomic Energy Commission.

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RATE OF LABELLING OF MITOCHONDRIAL PHOSPHOHISTIDINE BY RADIOACTIVE INORGANIC PHOSPHATE

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In 1961, Boyer *et al.*[1] made the important observation that when $^{32}P_i$ is added to mitochondria pre-incubated with or without substrate in the absence of added P_i or ADP, the ^{32}P is rapidly incorporated into a protein-bound phosphorus compound, later identified as protein-bound phosphohistidine[2]. Boyer[2,3] concluded that these experiments give convincing evidence that protein-bound phosphohistidine participates as an intermediate of oxidative phosphorylation. In simple terms, the course of the phosphate uptake was envisaged as:

$$P_i \longrightarrow \text{phosphohistidine} \longrightarrow \text{ATP}$$

Clearly, this theory required experimental evidence that the rate of formation of phosphohistidine from P_i is sufficient to account for the rate of synthesis of the ATP. In their first paper, Suelter *et al.*[1] showed that the maximum labelling of the mitochondrial protein-bound phosphohistidine was reached before the maximum labelling of the mitochondrial ATP, which satisfies one of the criteria for a precursor-product relationship. However, the amount of label finally appearing in the ATP was about 200 times that appearing in the phosphohistidine, and already at the time of taking the first sample (5 sec), when labelling in the phosphohistidine had reached its maximum value, 70 times more label was found in the ATP than in the phosphohistidine. Although the results were not inconsistent with Boyer's conclusion, it was clear that a more extensive investigation of the kinetics of the initial incorporation of $^{32}P_i$ into phosphohistidine and into ATP was required.

In a previous publication (Fig. 2 of ref. 4), we reported the results of such an investigation, carried out under the same conditions as in the experiments of Suelter *et al.*[1], with the exception that, in order to extend the time-scale, the mitochondria were incubated at 0°. The labelling of the phosphohistidine, measured by the procedure of Peter and Boyer[3], followed zero-order kinetics for the first 30 sec, without a measurable initial lag, and the incorporation of $^{32}P_i$ into ATP, after an initial lag lasting for about 30 sec, followed zero-order kinetics for the following 90 sec. The initial (that is, maximal) rate of labelling of phosphohistidine in the zero-order phase

was only 1·5 per cent of that of the rate of labelling of the ATP in its zero-order (also maximal) phase. Oligomycin had no effect on the initial rate of labelling of the phosphohistidine, whereas it inhibited the labelling of the ATP by 95–99 per cent. We concluded that the relatively slow rate of labelling of the phosphohistidine would seem to preclude the latter as intermediate on the main pathway of oxidative phosphorylation, and it was suggested that the phosphohistidine might be an intermediate in a slow oligomycin-insensitive pathway for the synthesis of ATP.

Pressman[5] reported evidence of a different nature which also cast doubt on the role of phosphohistidine as an intermediate of respiratory-chain phosphorylation. He found that addition of dinitrophenol, Ca^{2+} or valinomycin caused a rise in the amount of phosphohistidine and a decline in the amount of ATP.

Afterwards, Boyer[6] showed that succinyl-coenzyme *A* synthetase (*EC* 6.2.1.4) is responsible for the incorporation of $^{32}P_i$ into protein-bound phosphohistidine by extracts of liver mitochondria. A phosphorylated form of this enzyme had previously been isolated from *Escherichia coli* by Upper[7], and Boyer[6] showed that the phosphate in this enzyme is bound to a histidine residue. We have confirmed these findings using the enzyme isolated from heart muscle (Table 1).

Table 1. INCORPORATION OF ^{32}P FROM $^{32}P_i$ INTO PROTEIN-BOUND P IN SUCCINYL-COENZYME *A* SYNTHETASE

Exp.	Reaction time (sec)	Enzyme preparation (mg protein)	Addition	c.p.m. in protein-bound P
1	120	1·2	None	222
	120	2·4	None	501
	120	4·9	None	1,100
2	3	2·5	None	66
	6	2·5	None	145
	9	2·5	None	216
	13	2·5	None	335
	40	2·5	None	462
3	15	2·5	None	566
	15	2·5	Arsenite (5 mM)	566
	15	2·5	Arsenate (5 mM)	331

The succinyl-coenzyme *A* synthetase was isolated from pig heart. The reaction mixture contained 5 mM $MgCl_2$, 1 mM EDTA, 6 mM KCl, 200 mM sucrose, 100 mM *tris*-HCl buffer (*p*H 7·4), 0·09–0·12 mM succinyl-coenzyme *A*. The reaction was started by addition of $0·7 \times 10^6 - 1·3 \times 10^6$ counts/min $^{32}P_i$ (0·05–0·10 mM). Reaction volume, 1·3 ml. Reaction temperature, 0°. The amount of protein-bound P was determined by the method of Bieber *et al.*[8].

In our previous publication[4], we suggested that the slow oligomycin-insensitive pathway for the synthesis of ATP, involving phosphohistidine, might be derived from the same high-energy intermediates of oxidative phosphorylation ($A \sim C$) as in the oligomycin-sensitive pathway. However, using a highly sensitive method, we have been unable to find any measurable net oxidative phosphorylation linked with the oxidation of β-hydroxybutyrate, succinate or tetramethyl-*p*-phenylenediamine, in the presence of oligomycin. With glutamate as substrate, there was a small oligomycin-insensitive phosphorylation, which was to be expected since the substrate-linked phosphorylation step of α-ketoglutarate oxidation is not inhibited by oligomycin[9]. The addition of glutamate, but not of other substrates, was found to increase the rate of incorporation of $^{32}P_i$ into mitochondrial protein-bound phosphohistidine (measured by the new procedure described by Bieber *et al.*[8]), under the conditions of our earlier experiments.

Arsenate, in a concentration of 5 mM, strongly inhibited the incorporation into both protein-bound P and ATP, the ratio of the incorporations, after 40 sec at 0°, remaining the same (Table 2). Arsenite, on the other hand, had a much larger effect on the rate of incorporation into protein-bound P than into ATP, the ratio dropping from about 5 to about 1.

The sensitivity to arsenite and insensitivity to oligomycin suggest that the incorporation of $^{32}P_i$ into mitochondrial protein-bound P is associated with the substrate-linked phosphorylation step of α-ketoglutarate oxidation. We should now wish to modify the summary scheme shown in our previous paper[4] in the sense that the $A \sim C$ compound involved in the labelling of phosphohistidine is not the same compound as that involved in the labelling of ATP by the oligomycin-sensitive pathway. The synthesis of phosphohistidine involves succinyl-coenzyme *A*, the oligomycin-sensitive pathway the high-energy intermediates of oxidative phosphorylation. The P_i-ATP exchange is represented by a rapid oligomycin-sensitive reaction and a slower oligomycin-insensitive reaction. Phosphohistidine is involved in the latter. In the presence of oligomycin there is little discrepancy between the rate of labelling of ATP and of phosphohistidine[4]. Clearly all the data can be satisfactorily explained on the basis that phosphohistidine is an intermediate of the reaction catalysed by succinyl-coenzyme synthetase. Although protein-bound phosphohistidine, discovered by Boyer, is clearly a compound of great interest as an intermediate in certain enzyme-catalysed phosphorylation reactions, there is now no experimental evidence in support of the view that it is an intermediate of respiratory-chain phosphorylation.

Table 2. EFFECT OF ARSENITE AND ARSENATE ON THE INCORPORATION OF $^{32}P_i$ INTO PROTEIN-BOUND P AND INTO ATP

Addition	C.p.m. in Protein P	ATP	Ratio (%)
None	2,670	56,400	4·7
Arsenate (5 mM)	210	4,200	5·0
Arsenite (5 mM)	250	28,500	0·9

Procedure as in Fig. 2, ref. 4. 40 sec at 0°.

During the symposia before, during and after the International Congress of Biochemistry in New York, July 27–August 1, 1964, all participants, including Dr. Boyer, appeared to be in agreement with this conclusion. On the other hand, Dr. Boyer reiterated the objections to our kinetic argument[4] which he and his colleagues published shortly before the Congress[8]. Since our argument is applicable not only to the now-abandoned theory that phosphohistidine is an intermediate of respiratory-chain phosphorylation, but to any other compound which is to be brought forward as a possible phosphate-containing intermediate of respiratory-chain phosphorylation, we should like to reiterate our views and to discuss Boyer's objections to them.

The point under discussion is essentially the validity of kinetic criteria for distinguishing between possible reaction pathways in mitochondria. The use of ^{32}P to follow the pathway of phosphate in the synthesis of ATP is fundamentally similar to the use of spectroscopic methods to follow the pathway of electron transfer. When the passage of electrons from NADH to oxygen, catalysed by the Keilin and Hartree heart-muscle preparation, is inhibited by cyanide or by removal of oxygen, the uptake of electrons by cytochrome *b* is much slower than the rate of oxidation of NADH in the uninhibited system; this finding induced us to remove cytochrome *b* from the main respiratory chain for the oxidation of NADH in this preparation[10]. Chance[11], using a similar criterion, came to the same conclusion with respect to the oxidation of succinate by this preparation. Similarly, Redfearn[12] has questioned whether the reduction by succinate of ubiquinone in respiratory-chain preparations, which proceeds at 55–59 per cent of the rate of the overall reaction, is sufficiently rapid to permit ubiquinone to be placed on the main succinate oxidase chain. It should be pointed out, however, that in this case the discrepancy between the two rates is much less than in the experiments cited relating to cytochrome *b*. Moreover, Green[13] has pointed out that the ubiquinone, which is present in much larger concentrations than other components of the chain, may be kinetically inhomogeneous and that only about one-half of the total amount present may transfer electrons along the chain. In this case, then, there is good reason

to keep open the possibility that ubiquinone is on the main pathway, particularly since there is other evidence in favour of this view.

In our experiments[4], the maximum rate of labelling of phosphohistidine, both in the presence and the absence of oligomycin, was only 1·5 per cent of the maximal rate of labelling of ATP in the absence of oligomycin. Applying the same criteria as above, we conclude that phosphohistidine is not an intermediate in the formation of ATP.

Boyer and colleagues[1–3], on the other hand, by plotting, as a function of time, the percentage of maximum incorporation in phosphohistidine and ATP, obtained curves in which the former reached its maximum before the latter, and concluded that this is a sufficient criterion for a precursor-product relationship. In our view, it can be very misleading to plot the data in this way when the amount of product (ATP) is two orders of magnitude greater than the amount of the proposed precursor (phosphohistidine). To return to the comparison of the respiratory chain, incorrect conclusions on the relative rates of reduction of ubiquinone and cytochrome *b* would be drawn if one only measured the rate of changes of the degree of the reduction of these two compounds, without taking account of the much larger amount of ubiquinone present.

In their recent paper, Boyer *et al.*[8] claim to have demonstrated an initial lag in the incorporation of $^{32}P_i$ into phosphohistidine (3 sec at 0°, 5–15 msec at 25°). They conclude that other reactions or processes precede the phosphohistidine labelling, so that our results[4] "only suffice for estimation of a minimal value for the rate of phosphohistidine turnover. The actual rate could far exceed this minimal value. Thus nothing in the experimental findings rules out an exchange sufficiently rapid so that the specific activity of the phosphohistidine is close to the same as that of the P_i at the phosphorylation site(s) when the label is first detected in phosphohistidine. Under such circumstances, if the pools of phosphohistidine and ATP are catalytically homogenous, the participation of phosphohistidine as an intermediate would only demand that its rate of approach to maximal labelling equal or exceeds that of ATP. Such is observed to be the case. With demonstration of a readily anticipated lag in the labelling of phosphohistidine, the use of data on labelling rates in mitochondria to estimate turnover rates has no more validity than the attempted measurement of turnover rates of phosphorus compounds in tissues by measurement of rates of incorporation of intravenously administered $^{32}P_i$".

The analogy with experiments on the uptake of intravenously administered $^{32}P_i$ appears quite inappropriate. The difficulty with such experiments is that the rate-limiting step is often the uptake of $^{32}P_i$ by the tissues, so that the rate of incorporation of $^{32}P_i$ into intracellular phosphate compounds underestimates the phosphorylative capabilities of the cells. In the experiments with mitochondria, the end-product (ATP) is rapidly labelled.

Even if the small time-lag in the synthesis of phosphohistidine claimed by Bieber *et al.* exists (it is not clearly visible to us in their published results), this does not affect our conclusion drawn from the relative rates of uptake of $^{32}P_i$ into phosphohistidine and ATP during the zero-order portions of the curves. In fact, if the formula of Calvin and Duffield[14], which is often used by biochemists to calculate the rates of exchange from incorporation data, is applied to our data, the same discrepancy between the two rates is found, although the calculated rates of both incorporations vary with time, because the formula of Calvin and Duffield is based on first-order kinetics.

Experimental support for our view that the measured rates of incorporation have quantitative significance is given by the effects of arsenate and arsenite on these rates, shown in Table 2.

For these reasons, we wish to reaffirm our view that the criterion that a given compound is an intermediate in oxidative phosphorylation should be that the initial rate of its labelling from $^{32}P_i$ should not be less than the maximal rate of labelling of the product. As a model to test this postulate, we have chosen succinyl-coenzyme *A* synthetase. Since, in the complete system (succinyl-coenzyme *A*, P_i, GDP and GTP), the steady-state level of protein-bound phosphohistidine is too low to allow reliable measurements of the rate of incorporation of $^{32}P_i$ into the phosphohistidine, this was measured in the absence of GDP or GTP. This corresponds to the situation where a possible intermediate of respiratory-chain phosphorylation is tested in the presence of oligomycin. In Fig. 1, the rate of incorporation of $^{32}P_i$ into phosphohistidine, in the absence of GDP or GTP, is compared with the rate of incorporation into GTP when GDP and GTP were added. It may be seen that the initial rate of incorporation into the phosphohistidine is about the same as that into the GTP in the overall system. This is consistent with the suggestion[6,7] that protein-bound phosphohistidine is an intermediate in this reaction.

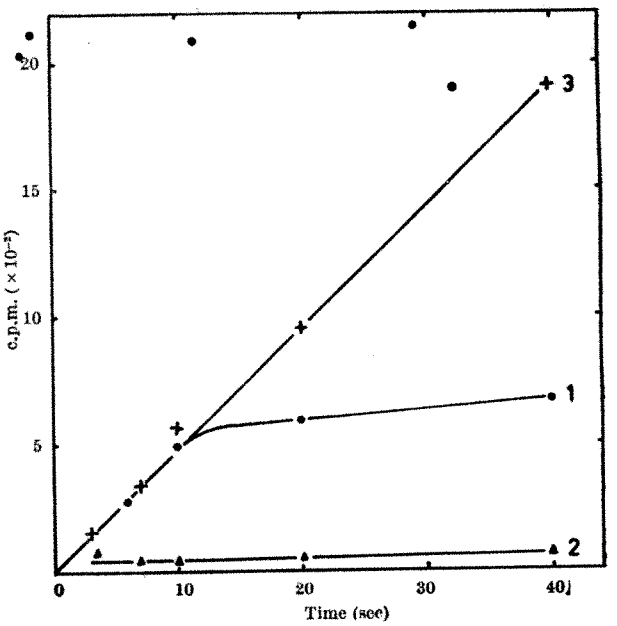


Fig. 1. Incorporation of $^{32}P_i$ into protein-bound phosphohistidine of succinyl-coenzyme *A* synthetase under different circumstances. The reaction mixture contained 5 mM $MgCl_2$, 1 mM EDTA (*p*H 7·4), 6 mM KCl, 100 mM *tris*-HCl buffer (*p*H 7·4), 70 μM potassium phosphate (*p*H 7·4), 200 mM sucrose, 0·17 mM succinyl-coenzyme *A* and 5 mg of a crude preparation of succinyl-coenzyme *A* synthetase[8] in a final volume of 1·3 ml. In curves 2 and 3, 0·08 mM GDP and 0·8 mM GTP were also present. After 15 min at 0° the reaction was started by addition of 0·1 μmole $^{32}P_i$ ($1·5 \times 10^6$ c.p.m.) and different samples stopped at different time intervals by the addition of 8·0 ml. of a solution 0·3 M in trichloroacetic acid and 3 mM in potassium phosphate and by 0·2 ml. 40 per cent trichloroacetic acid, respectively. Curves 1 and 2 give the incorporation into protein-bound phosphohistidine, measured by the method of Bieber *et al.*[5]. Curve 3 gives the incorporation into GTP measured as the activity in the protein-free supernatant after removal of $^{32}P_i$ as phosphomolybdate into benzene-isobutanol

There remain for discussion other criticisms brought forward by Bieber *et al.*[8].

(1) They suggest that the limited labelling of mitochondrial ATP which we observed at 0° might not occur by the main reactions of oxidative phosphorylation. Since, however, the labelling which we study at 0° is almost completely sensitive to oligomycin, we consider that it is related to oxidative phosphorylation. Furthermore, the high-energy intermediates of oxidative phosphorylation are readily utilizable at 0° for the reversal of the respiratory chain[15] and for ion uptake[16]. In any event, the kinetic data of Bieber *et al.*[8] obtained at 25° are in essential agreement with our own.

(2) Bieber *et al.*[8] point out that the lack of inhibitory effect of oligomycin on phosphohistidine is not conclusive, since the mode of action of oligomycin is not known.

For example, it might prevent the transfer of a phosphorylated intermediate from a hydrophobic locus, where it is formed, to a locus allowing reaction with ADP. We agree that lack of inhibition by oligomycin of the incorporation of $^{32}P_i$ into phosphohistidine is not conclusive evidence, of itself, against the participation of phosphohistidine as an intermediate in ATP synthesis, and, contrary to what Bieber *et al.* write, we did not use this argument, since the possible explanation given by them had been pointed out to us by Dr. J. B. Chappell. The only assumption which we have made is that oligomycin prevents phosphorylation of ADP, a fact which is well established and which we demonstrated under our experimental conditions.

(3) Bieber *et al.*[8] suggest further that more than one form of bound phosphohistidine may be present, with an oligomycin-insensitive form as the principal component observed under the experimental conditions used. If a second, oligomycin-sensitive phosphohistidine is present, it has not yet been revealed experimentally, since the amount of phosphohistidine labelled at isotopic equilibrium is not decreased by addition of oligomycin. Although the possibility that a small amount of phosphohistidine, undetectable so far, might be an intermediate in respiratory-chain phosphorylation, cannot be excluded, this postulate is at present pure speculation, without any experimental basis. The total amount of protein-bound phosphohistidine in rat-liver mitochondria is only about 0·05 μmole/g mitochondrial protein[1], rather less than that of the individual cytochromes.

The fact that phosphate strongly inhibits arsenate-stimulated mitochondrial respiration in the absence of ADP is rather good evidence that a relatively stable ~P compound is an intermediate in oxidative phosphorylation[17]. The method introduced by Boyer is the most direct way of searching for this compound. It is our view that it is necessary to show that the rate of labelling of any compound proposed as a ~P intermediate should be of the same order as the rate of labelling of the ATP and that its labelling should show the susceptibility to inhibitors expected from studies of oxidative phosphorylation with intact mitochondria.

We thank Mr. R. Stoeckart for his collaboration and assistance in many of the new experiments reported. We also thank Dr. J. M. Tager for his advice.

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ELUCIDATION OF THE BIOSYNTHETIC PATHWAY OF THYROIDAL 3:5:3′ TRIIODOTHYRONINE BY MEANS OF ISOTOPE POSITION EXPERIMENTS

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So far only two theoretically probable routes for triiodothyronine biosynthesis have been proposed. Gross and Pitt-Rivers[1] suggested that it might arise by removal of one iodine atom from thyroxine. Roche, Michel, Michel and Lissitzky[2], being unable to demonstrate deiodination of thyroxine by thyroid slices, concluded that the biosynthesis probably involved coupling between one molecule each of mono- and di-iodotyrosine. The evidence of Pitt-Rivers[3] supporting the latter hypothesis must now be regarded as questionable because the triiodothyronine isolated for specific radioactivity measurements by that author is known to have been contaminated by another component[4]. This leaves the problem of triiodothyronine biosynthesis undecided at the present time.

The work of Plaskett[5,6] showed that in theory the biosynthetic pathway of triiodothyronine could be determined by studying the way in which iodine-131 is distributed between the rings of triiodothyronine in the thyroid gland during the early intervals after administering [^{131}I]-iodide. This possibility exists because if triiodothyronine is derived from thyroxine, then both the iodine-containing rings of triiodothyronine would be derived from the same precursor, namely diiodotyrosine, which is the sole precursor of thyroxine[7,8]. In this case, therefore, one must expect that even at the shortest times after administration of [^{131}I]-iodide the two rings of triiodothyronine will be labelled at equal specific activity with ^{131}I.

Alternatively, if triiodothyronine arises by the coupling of one molecule each of mono- and di-iodotyrosine, then the two rings have different precursors. In a situation in which the precursors have different specific radioactivities the iodine on the two rings of triiodothyronine will become unequally labelled with ^{131}I.

This theoretically predicted situation is illustrated in Fig. 1.

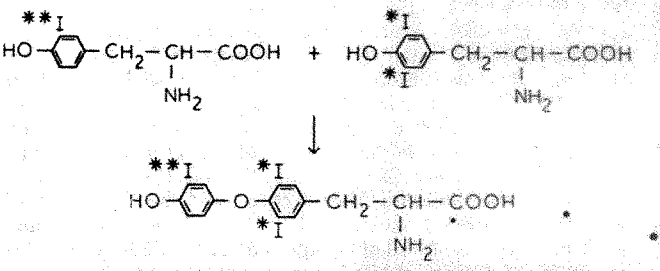


Fig. 1. Suggested scheme for triiodothyronine biosynthesis

We have been working for some time with the view of finding out which of these pathways applies *in vivo* in the rat thyroid gland. Some results have already been published and include: (1) confirmation that thyroxine, as predicted by theory, is always equally labelled in the two rings after administration of [^{131}I]-iodide[6]; (2) develop-

Table 1. Incorporation of [^{131}I] into Monoiodotyrosine and Diiodotyrosine in the Rat Thyroid Gland at Different Times following Administration of ^{131}I-iodide

Time after administration of [^{131}I]-iodide	No. of determinations	Total thyroid gland radioactivity (%) Monoiodotyrosine	Diiodotyrosine	*R* value, i-e [^{131}I]-monoiodotyrosine [^{131}I]-diiodotyrosine
30 min	8	34·7 ± 0·4*	43·4 ± 0·3	0·80
1 h	6	32·9 ± 0·3	42·7 ± 0·3	0·77
24 h	11	22·4 ± 0·3	45·4 ± 0·4	0·50

* *S.E.M.*

Table 2. Distribution of ^{131}I between the Rings of 3 : 5 : 3′ Triiodothyronine (*T*3) isolated from the Thyroid Glands of Rats at Different Times following Administration of [^{131}I]-iodide. Random labelling would correspond to 66·7 per cent of ^{131}I on the 3 : 5 Positions. The Value predicted on the Basis of the Hypothesis that *T*3 arises from Thyroxine would be 66·7 at all Times examined

Time after administration of [^{131}I]-iodide	*R* value	No. of degradations performed	% of ^{131}I on the 3 : 5 positions: Value found	Value predicted for newly formed *T*3 on the basis of the *R* value and the hypothesis that *T*3 arises from monoiodotyrosine and diiodotyrosine
30 min	0·80	12	56·5 ± 0·6	55·6
1 h	0·77	6	55·7 ± 1·2	56·5
24 h	0·50	15	65·4 ± 1·0	66·7

ment of a chromatographic technique for isolation of triiodothyronine from the thyroid gland at a high level of radiochemical purity[4,5]; (3) development of a degradation procedure for triiodothyronine which permits investigation of the distribution of ^{131}I between the rings[5].

Unlike many other workers[3,9,10] our experimental conditions were such that we were unable at first to produce a situation in which the specific radioactivities of monoiodotyrosine and diiodotyrosine were different[11]. This difficulty has been overcome recently by feeding the animals with commercial rat pellets instead of the powdered low-iodine diet formerly used. It is now known that in rats fed the pellet diet, the specific radioactivity of iodine in monoiodotyrosine is considerably greater than that in diiodotyrosine at 30 min after the dose of [^{131}I]-iodide. The specific radioactivities of the two compounds become increasingly similar at later times and are the same at 24 h[12]. This effect is shown in Table 1, which gives the results of analysis of rat thyroid tissue at 30 min, 1 h, and 24 h after administration: the diminution with time in the value of the ratio *R*, of ^{131}I-monoiodotyrosine to [^{131}I]-diiodotyrosine, shows that the monoiodotyrosine was initially the more heavily labelled. The value of *R* does not undergo any significant change at times later than 24 h, the value measured at 38 h being 0·49. Fig. 2 illustrates the change in the value *R* with time in an experiment with a single rat which received ^{125}I 24 h before death followed by ^{131}I 30 min before death. On death the thyroid gland was removed, homogenized and hydrolysed enzymatically; three single-dimensional chromatograms, each measuring 38 cm from origin to solvent front, were run with the hydrolysate. After development the chromatograms were cut into thirty-eight 1-cm segments and each segment was assayed for both ^{125}I and ^{131}I. Fig. 2 shows the mean results of these assays. It can be seen that iodide was very much more heavily labelled with ^{131}I than with ^{125}I. Monoiodotyrosine was also more heavily labelled with ^{131}I than with ^{125}I. The most important fact emerging in relation to the present work is that the labelling situation for the two iodotyrosines is opposite. Monoiodotyrosine contained 18·6 per cent of the total ^{131}I and 17·2 per cent of the total ^{125}I. Diiodotyrosine contained 36·2 per cent of the ^{131}I and 45·4 per cent of the ^{125}I. Monoiodotyrosine is thus a rapidly labelled component and diiodotyrosine is a relatively slowly labelled component of thyroid tissue.

From this double isotope experiment and from the evidence of measurements of *R* in rats given ^{131}I (Table 1) we are now able to suggest, in common with workers in other laboratories[3,10], that the rats used go through a period following injection of ^{131}I-iodide during which the two iodotyrosines in the thyroid gland have different specific radioactivities.

This finding has made the determination of the pathway of triiodothyronine biosynthesis possible. It is clear that if the biosynthesis occurs by coupling between one molecule each of mono- and di-iodotyrosine, then it is likely that at 30 min or 1 h after a dose of ^{131}I-iodide the two rings of triiodothyronine will be found to be labelled unequally with ^{131}I.

In the present work samples of triiodothyronine have been isolated from rat thyroid glands at 30 min, 1 h, and 24 h after administration of [^{131}I]-iodide and degraded by the diazo-coupling technique[5] to determine the distribution of label between the rings. The isolation procedure consisted of two-dimensional chromatography in the solvent systems butan-1-ol/dioxan/aq. 2 N-ammonia (4 : 1 : 5) (first) and butan-1-ol/aq. 9 N-ammonia (1 : 1) (second). Location of the triiodothyronine spot was determined by autoradiography and by reference to a spot of carrier triiodothyronine detected during brief exposure under an ultra-violet lamp. The result shows conclusively that at 30 min and 1 h after the dose the β-ring iodine atom of triiodothyronine (position 3′) is labelled more heavily than the iodine on the α-ring

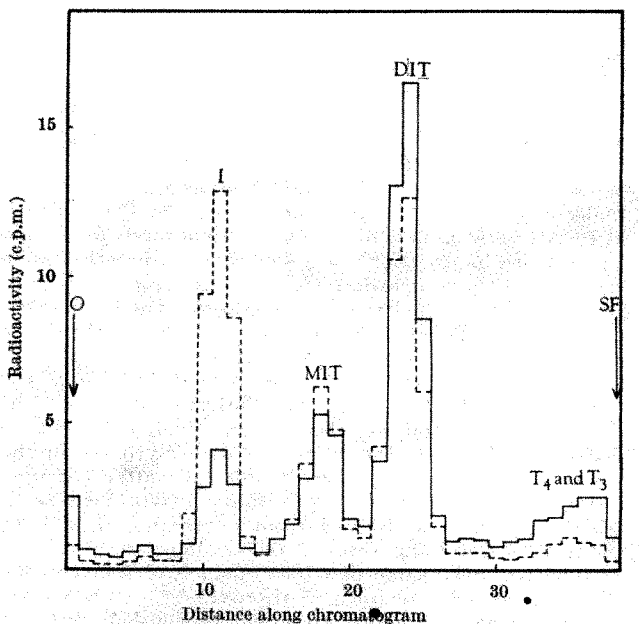


Fig. 2. Chromatogram scan of a rat which received ^{125}I 24 h before death followed by ^{131}I 30 min before death. ·-·-·-·, ^{125}I; ———, ^{131}I; *O*, origin; I, iodide; MIT, monoiodotyrosine; DIT, diiodotyrosine; T_3, triiodotyrosine; T_4, thyroxine; SF, solvent front

(positions 3 and 5) (Table 2). This is consistent with origin of the β-ring from monoiodotyrosine and origin of the α-ring from diiodotyrosine. Furthermore, it appears to be incompatible with an origin of triiodothyronine from thyroxine. It is predicted by theory and has been shown experimentally[6] that thyroxine in the rat thyroid is labelled equally on the two rings at all time-intervals studied following administration of [^{131}I]-iodide. Therefore, if thyroidal thyroxine is homogeneous with respect to turn-over the triiodothyronine isolated in the present work cannot have arisen from thyroxine.

The question arises as to whether this constitutes an unequivocal demonstration that triiodothyronine is formed by the coupling of mono- and di-iodotyrosine. At the present time we can see only two possible objections.

(1) Several workers including ourselves have recently obtained evidence suggesting that thyroglobulin may be heterogeneous with respect to turn-over[11,13,14]. While this has not yet been shown definitely to apply to the thyroxine-containing fraction, the possibility that it may do so should be taken into account. The likelihood that this would affect the conclusions of the present work must be considered.

In theory the precursor of triiodothyronine may be a fraction of the total thyroxine in a separate metabolic pool in which preferential labelling of the β-ring occurs. However, the mean distribution of ^{131}I between the rings of thyroxine is known always to be such that the two rings are equally labelled; therefore, the existence of a separate pool of this type would necessitate the existence of a second separate pool of thyroxine in which the degree of preferential labelling was at all times exactly equal but opposite to that in the first. This would seem to require a degree of coincidence of unlikely events that makes it extremely improbable.

(2) The evidence presented here in favour of the coupling of mono- and di-iodotyrosine in triiodothyronine formation is, first, that the isotopic data are compatible with this mechanism, and, secondly, there are no alternative hypotheses. The second of these pieces of evidence is negative in character and cannot exclude as yet unconceived hypotheses which would give rise to the experimentally-determined labelling pattern.

In spite of these two theoretical objections it is our opinion that the evidence of the present paper establishes, beyond reasonable doubt, the pathway of triiodothyronine biosynthesis as the coupling between one molecule each of mono- and di-iodotyrosine.

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ISOLATION OF A HIGHLY PURIFIED ENCEPHALITOGENIC PROTEIN FROM BOVINE CORD

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EXPERIMENTAL allergic encephalomyelitis (EAE) has been produced by both proteolipid and water-soluble, basic, protein fractions of central nervous system tissue. Encephalitogenic activity of proteolipids in rabbits was demonstrated by Waksman *et al.*[1], whereas basic protein but not proteolipid was shown to be encephalitogenic in guinea-pigs by Kies and Alvord[2]. Kibler and Barnes[3] later demonstrated EAE activity of a basic protein fraction in rabbits. This article describes the isolation of a highly purified, low-molecular-weight, encephalitogenic protein from a water-soluble, basic, protein fraction of bovine cord.

Fresh, whole bovine spinal cord was freed of its meninges and stripped clean of its grey matter. 140 g were homogenized in 300 ml. cold (−20° C) acetone for three 1-min periods at high speed in a Servall 'Omni-mixer' (Ivan Sorvall, Inc.). An additional 900 ml. of cold acetone were added and extraction was continued with constant mixing by a magnetic stirrer for 10 min at −12° C. The residue was taken to near dryness on a Buchner funnel and extracted in 700 ml. of 1 : 1 acetone, ethyl ether for 5 min at −12° C. The latter extraction was repeated three additional times and after the last filtration the residue was dried by gentle melding with a spatula on large pieces of blotting paper. Approximately 30 g of a coarse, beige-coloured powder were obtained. This powder was extracted for 24 h in 400 ml. of 0·1 M sodium citrate, *p*H 4·3 at 4° C with constant stirring. The suspension was spun at 12,800*g* in a high-speed centrifuge (Model *AX*, Lourdes Instrument Corp.) in the cold room, but complete clearing of the supernatant was not achieved. The supernatant was dialysed against distilled water at 4° C for 24 h in 1-in. dialysis tubing (Visking Corp.) with 2 changes of the dialysate. The supernatant now was easily cleared by spinning at 500*g* in a refrigerated centrifuge (model *PR-2*, International Equipment Co.) at 4° C. Lyophilization yielded an average of 500 mg of a fine white powder.

100 mg of this water-soluble protein fraction (WSPF) were chromatographed on a 3-g carboxymethylcellulose ((CMC) 0·9 ± 0·1 m.equiv./g, Mann Research Lab.) column in an airless system and at a constant flow rate. The materials and techniques employed in this method were the same as those previously described for the chromatography of bovine pancreatic ribonuclease[4] with the exception that no condenser jackets were used with the columns and the procedure was carried out at room temperature. A linear gradient was provided by adding 400 ml. of 0·005 M borate buffer, 0·05 M NaCl to the mixing flask and 400 ml. of 0·005 M borate buffer, 0·4 M NaCl to the second flask. Both solutions were adjusted to *p*H 8·6. Fractions between 2·0 and 2·4 ml. were collected every 17 min and frozen before reading at 280 mμ in a Beckman *DU* spectrophotometer.

The chromatographic pattern of the WSPF is shown in Fig. 1. Two major, symmetrical peaks were observed; peak 1, tubes 10–16, and peak 2, tubes 50–56. Peak 1, after dialysis and lyophilization, yielded 15 mg and consisted of 4 bands on disk polyacrylamide gel electrophoresis, *p*H 4·4 (ref. 5), and was inactive in rabbits in a dose of 200 γ/animal. Since overnight dialysis of peak 2 resulted in a major loss of the protein, the peak was first

lyophilized and then desalted on a *G–25*, coarse, beaded 'Sephadex' (Pharmacia, Uppsala, Sweden) column, 2·1 cm in diameter and 54 cm high. The 'Sephadex' was equilibrated with 0·01 N HCl and this same solution was used to elute the protein by simple gravity flow. 4 ml. fractions were collected every 30 sec and read at 280 mμ in the Beckman *DU* spectrophotometer. The protein came through as a single, symmetrical peak at the end of the void volume, that is, tubes 14–17. Lyophilization yielded 7–10 mg of a fine, white powder. This material proved to be highly purified by electrophoretic and ultracentrifuge analysis.

Fig. 2 shows the pattern of 50γ each of the WSPF and the CMC 2nd peak on disk polyacrylamide-gel electrophoresis, *p*H 4·4. The WSPF pattern consists of 4 minor and 5 major bands while the CMC 2nd peak shows two faint minor bands and a single major band corresponding in position to one of the major bands of the WSPF. On disk polyacrylamide-gel electrophoresis, *p*H 2·3 (*Chemical Formulation for Disc Electrophoresis*, Canalco), there was considerably less resolution of the WSPF, but the CMC 2nd peak pattern was similar to the pattern at *p*H 4·4 with the exception that the two minor bands were more distinct. The ultracentrifuge pattern of the CMC 2nd peak is shown in Fig. 3. A 1·0 per cent solution of the protein in phosphate buffer, *p*H 6·9, was placed in a valve-type, synthetic boundary cell and centrifuged at 59,800 r.p.m. at 18° C. The frame on the left was taken at the time full speed was first reached and the frame on the right 48 min later. The sedimentation constant calculated from the distance the peak of the ascending limb moved in 48 min was $0{\cdot}42 \times 10^{-13}$, which suggests that the molecular weight of the 2nd peak protein may be less than 10,000. The slight asymmetry and thickening of the descending limb in the frame taken at 48 min indicate contamination with one or more heavier molecular species.

The encephalitogenic activity of this material was tested in male albino rabbits, 12–14 weeks of age. The materials and methods used in these investigations were similar to those reported in previous experiments with a water-soluble, basic protein fraction of rabbit cord[3].

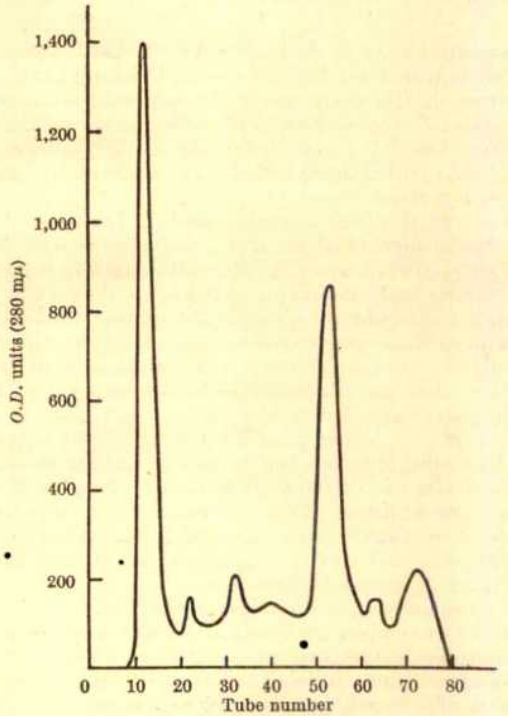


Fig. 1. Column chromatography of the WSPF on CMC. A linear gradient consisted of 400 ml. of 0·005 M borate buffer, 0·05 M NaCl in the mixing flask and 400 ml. of 0·005 M borate buffer, 0·4 M NaCl in the second flask. Both solutions at *p*H 8·6

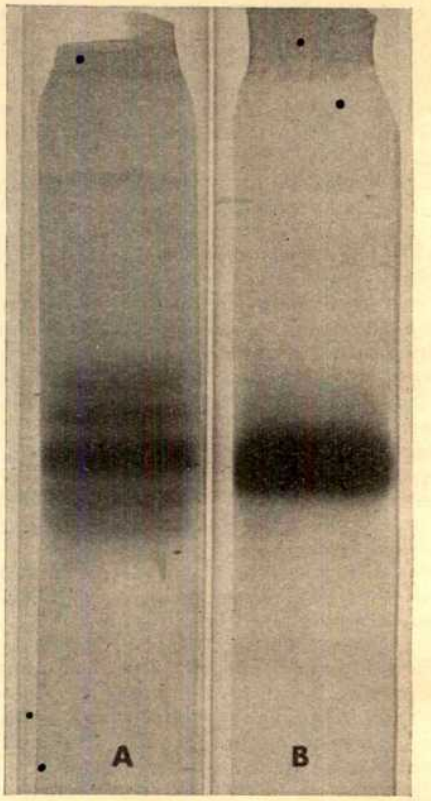


Fig. 2. Disk polyacrylamide gel (0·5 cm × 6·5 cm) electrophoretic patterns of the WSPF (*A*) and CMC 2nd peak (*B*) at *p*H 4·4

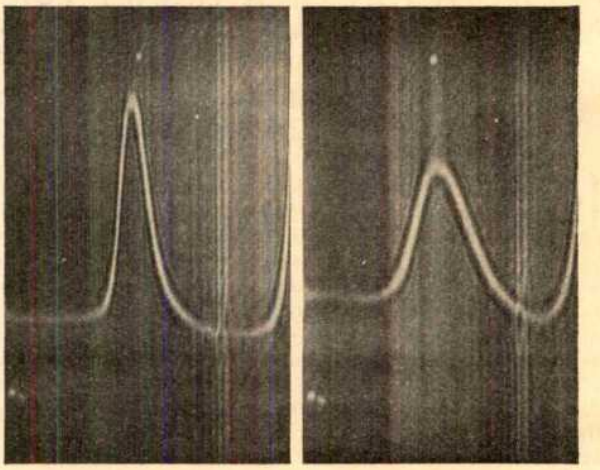


Fig. 3. Ultracentrifuge pattern of the CMC 2nd peak. On the left is the pattern at the start and on the right the pattern after 48 min of centrifugation at 59,800 r.p.m. at 18° C

A 1 : 1 ratio of oil (Difco incomplete adjuvant) to water and a 3 : 1 ratio of *Mycobacterium butyricum* to protein were used. Each rabbit received 0·25 ml. of the emulsion intradermally, equally divided between the 4 foot-pads.

Table 1 shows the results obtained with the CMC 2nd peaks from five different WSPF preparations. Preliminary experiments suggested that the CMC 2nd peak protein reached maximal activity around 200γ. In these investigations 200γ produced experimental allergic encephalomyelitis (EAE) in 100 per cent of 31 rabbits with an average day of onset of 14·5 days after challenge. Two of the rabbits had mild disease, that is, either had incomplete hind-leg paralysis or developed complete paralysis after more than a 4-day interval following onset. Sections of the spinal cord from two randomly selected rabbits

Table 1. ENCEPHALITOGENIC ACTIVITY OF THE CMC 2ND PEAK PROTEIN COMPARED TO THE WSPF AND TO WHOLE BOVINE CORD

Antigen		Dose	Incidence of EAE	Day of onset Range	Day of onset Mean	No. with mild disease
CMC 2nd peak WSPF prep.	72	200 γ	5/5	13–20	15·4	0/5
	80	200 γ	5/5	14–17	15·0	1/5
	81	200 γ	5/5	13–16	14·4	0/5
	83	200 γ	5/5	13–19	14·8	0/5
	85	200 γ	6/6	13–14	13·5	1/6
			26/26		14·5	2/26
WSPF prep.	81	500 γ	6/12	13–21	16·0	3/6
Whole cord		250 mg	24/25	11–17	12·7	2/24

showed the typical histological features of EAE. For comparison the activity of a representative WSPF preparation and whole bovine cord is also shown in Table 1. The injection of the WSPF was handled in the same manner as the CMC peaks. With the whole cord injections a 1 : 1 ratio of oil to cord was used and each rabbit received a total of 0·5 ml. of the emulsion containing 5·0 mg *Mycobacterium butyricum*. 500γ of the WSPF caused disease in 6 of 12 rabbits, with mild disease in 3, and an average interval to onset after challenge of 16·0 days. Comparable results for 250 mg whole cord were a disease incidence of 24/25 with mild disease in 2 and a 12·7 average day of onset.

Although the CMC 2nd peak protein was considerably more encephalitogenic than the WSPF, it did not quite display the full activity of whole cord. This is apparent from the average day of onset, an important quantitative index of encephalitogenic activity[6]. The difference between 12·7 and 14·5 days is highly significant, and similar differences have been a consistent feature of experiments prior to and subsequent to the present ones. As already indicated, 200γ of the CMC 2nd peak protein seems to represent a maximally active dose. Doses up to 500γ do not enhance the day of onset. This could mean that the purified protein is an incomplete antigen or only one of several antigens. There is also the possibility that the lipid content of whole cord enhances the adjuvant effect of the water-in-oil emulsion. In any event, the high degree of encephalitogenic activity and chemical purity of the CMC 2nd peak protein makes it a very desirable antigen for future investigations in EAE.

The small molecular size of the CMC 2nd peak protein suggests that it may be similar to the dialysable peptide reported to produce EAE in guinea-pigs by Robertson *et al.*[7]. A highly purified, dialysable preparation of bovine cord with encephalitogenic activity in guinea-pigs has also been reported by Nakao and Einstein[8].

This work was supported by a research grant from the U.S. National Multiple Sclerosis Society.

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β-LACTOGLOBULINS

By K. BELL and DR. H. A. McKENZIE

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MILK is the natural food of the new-born mammal and it must be well balanced to serve as the sole nourishment. The needs of the young vary considerably from species to species, and it is not surprising to find marked variation in the composition of milks of various species. Furthermore, the composition of the milk of a given species varies with the stage of lactation. Under natural conditions milk passes directly from mother to offspring. In the case of man successful lactation is not necessary for survival: substitute milks are readily available. However, numerous problems arise in their use. It is not surprising, therefore, that most attention has been given to the properties and composition of the most common substitute, cow milk.

It is the purpose of the present article to summarize studies which we have carried out over the past seven years on the β-lactoglobulins of milk of a number of different species. The unusual properties of these proteins make them of interest from the physico-chemical as well as the biological point of view. Our findings are considered in relation to those of other workers.

Bovine β-Lactoglobulins

The main whey proteins of mature bovine milk are: β-lactoglobulin (about 0·3 g/100 ml.), α-lactalbumin (about 0·1 g/100 ml.), serum albumin (about 0·04 g/100 ml.) and immune globulin (about 0·08 g/100 ml.)[1]. The predominant protein, β-lactoglobulin, was first crystallized by Palmer[2] in 1934. Sørensen and Palmer[3] showed that a 3-fold variation in the amount of solid phase did not appreciably change its solubility. These observations led to the conclusion that β-lactoglobulin was a pure protein. Thus it became a favourite protein for study by biochemists and chemists. McMeekin and Warner[4], using the X-ray data of Crowfoot, calculated an anhydrous molecular weight of 35,800 in agreement with values obtained by other methods.

The first real indication of the possible heterogeneity of β-lactoglobulin prepared from pooled milk came in 1946 from the heterogeneity studies of Li[5]. In 1955 Ogston and Tilley found differences in samples prepared from milks of individual cows. The main cause of these differences was the subject of an important discovery by Aschaffenburg and Drewry[6]. They found that β-lactoglobulin from pooled cow milk is a mixture of two genetically different proteins (*A*, *B*) and showed that some cows produce only β-lactoglobulin-*A*, others only β-lactoglobulin-*B* and the rest a mixture of the two proteins in approximately equal amounts.

Aschaffenburg and Drewry distinguished the two proteins by the difference in their mobilities during electrophoresis of the 'lactalbumin' fraction on filter paper at *p*H 8·6 (the β-lactoglobulin of higher mobility being designated *A*). Since then considerable development of methods of zone electrophoresis of higher resolution has occurred. One of us (K. B.) initiated an investigation of bovine milk using the method of starch-gel electrophoresis, to determine more precisely what genetic variants of β-lactoglobulin were present. This work has led *inter alia* to the detection of an additional variant[7] which has been designated β-lactoglobulin-*C*. The details of the method developed for typing of whey proteins will be given in detail elsewhere. It suffices to point out here that the method enables typing to be

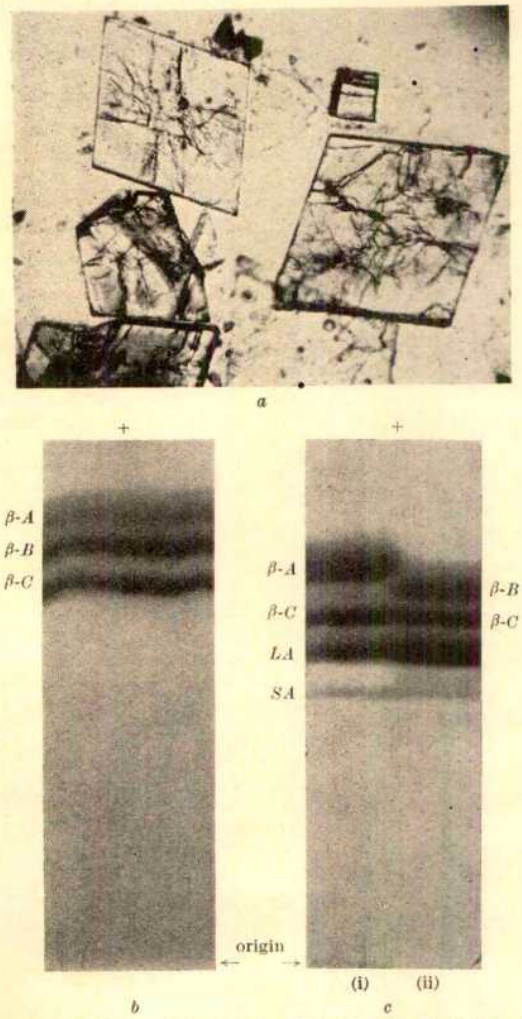


Fig. 1. (*a*) The first crystals of bovine β-lactoglobulin-*C* isolated in 1962 (× *c.* 60). The crystals are very fragile. (*b*) Starch-gel electrophoresis patterns of a solution of the three crystalline homozygous bovine β-lactoglobulins (*A*, *B*, *C*) at *p*H 8·5 (0·028 M H_3BO_3–0·012 M NaOH buffer), 7·5 V cm^{-1}, 5 h. (*c*) Patterns of 'lactalbumin' solutions from milks containing (i) β-lactoglobulin-*AC*, (ii) β-lactoglobulin-*BC*, under similar conditions to (*b*)

carried out on skim milk without prior separation of the casein group of proteins; it is more rapid than the filter paper method of Aschaffenburg and Drewry and gives superior resolution.

Occurrence and genetic implications. One of us (K. B.) has examined milks of 928 'pure bred' cows of five breeds (Jersey, Guernsey, Australian Illawarra Shorthorn, Friesian, Ayrshire) and has found that either β-lactoglobulin *A*, *B* or *C* occurs, singly or together with either of the other two proteins in the milks of individual cows in approximately equal concentrations. (In a preliminary communication it was thought that *A*, *B* and *C* could occur together but this result was artefactual and the explanation of it will be given elsewhere.)

Genetic investigations showed that the genes controlling the β-lactoglobulin synthesis were autosomal alleles.

In contrast to our work, Moustgaard *et al.*[8], using a different method of starch-gel electrophoresis, failed to detect type *C* in the milks of 158 Danish Jersey cows. It was considered of interest to determine if β-lactoglobulin-*C* has originated from a mutation in Australian Jersey cattle. In 1963 we provided a sample of β-lactoglobulin-*C* to Dr. R. Aschaffenburg, for use as a reference in looking for the possible occurrence of β-lactoglobulin-*C* in British cattle. In a random sample of 80 milks from British Jersey cows he found only two milks containing *C*: these were heterozygous (*BC* and *AC*). Thus β-lactoglobulin-*C* has not originated from an Australian mutant. Recently, Aschaffenburg[9] has demonstrated a similar polymorphism for bovine β-casein, three genetic variants (*A*, *B*, *C*) occurring in major British breeds. The third variant (*C*) was found only in the milk of Guernsey cows. Aschaffenburg suggested that if the β-casein allele is of ancient origin, it could be used in determining differences in the origins of Jersey and Guernsey cattle. If the β-lactoglobulin-*C* allele is restricted to Jerseys, as the present results indicate, and is of ancient origin, it could be used also in determining differences in the origins of the Channel Island breeds.

Isolation. In 1962 an attempt was made by one of us (H. McK.) to isolate β-lactoglobulin-*C* from the milk of a homozygous Jersey cow, using a method essentially similar to that developed by Aschaffenburg and Drewry for types *A* and *B*. It was found difficult to crystallize type *C*, but finally a successful procedure for crystallization was developed. The first crystals obtained in August 1962 are shown in Fig. 1*a*. During October 1962 we obtained crystals[10] from the milk of another homozygous cow using method 1 of two methods for isolation of β-lactoglobulins developed by Armstrong and McKenzie[11]. Since then β-*C* has been prepared some dozen times using both methods 1 and 2. Its properties[10] will now be compared with those of β-*A* and β-*B*.

Electrophoresis. On starch-gel electrophoresis at *p*H 8·6 β-*A* is the fastest-moving type, followed by β-*B* and then β-*C* (Fig. 1*b* and *c*). Since their particle sizes are similar under these conditions the differences arise from differences in mobility rather than a molecular sieving effect. It was expected, therefore, that a similar difference would be found in electrophoresis in agar (ion agar and agarose), acrylamide and on filter paper. The expected separation has been found with these methods. However, the concentration at which the protein is applied is of considerable importance.

Molecular size. Ogston and Tombs showed by sedimentation studies that β-lactoglobulin-*A* associated at *p*H 4·7 and low temperature[12]. Timasheff and Townend[13] confirmed this association between *p*H 3·5 and 5·2 by light-scattering measurements and showed that it was rapid, reversible and highly specific, giving a 'tetramer' of particle weight 4 × 35,500. They found that these samples of β-lactoglobulin-*A* contained 10 per cent of a non-associating component. On the other hand, while McKenzie and Smith found a similar association of *A* to form 'tetramers' from sedimentation investigations (velocity and Archibald), they did not find any evidence of a non-associating component in their samples[14–16]. In 1963 we supplied a sample of our β-lactoglobulin-*C* and information on its occurrence to Dr. R. Townend at his request for him to check if his samples of *A* and *B* lactoglobulin were contaminated with *C*. Townend *et al.* concluded that their *A* and *B* samples were free of *C*. We understand they have since isolated *C* from an American herd and confirmed some of our results.

Timasheff and Townend[17] observed that below *p*H 3·5 both *A* and *B* β-lactoglobulins dissociated into particles of molecular weight 17,750. Other evidence would seem to indicate that the units from a given type are identical single chains, but that the type *A* chain is different from that of type *B* (ref. 18). Hybridization experiments show that reassociation can only occur specifically and no β-*A* *B* hybrids are formed[18].

Tanford and Nozaki have shown from *p*H titration curves that β-*A* has two more free carboxyl groups than *B* per 35,000 particle weight[19]. Timasheff and Townend[17] showed that maximal association of β-*A* occurred at *p*H 4·7, which is in the *p*H range of side-chain carboxyl ionization. These findings suggest the possibility of the extra aspartic acid residues known to be in β-*A*, being involved in the association. Recently, Armstrong and one of us (H. McK.)[20] have blocked the carboxyl groups in

β-*A* using a method developed by Armstrong for blocking carboxyl groups in proteins. This blocking prevents the association occurring. This work implicates strongly the role of the side-chain carboxyls of the extra aspartic acid residues in β-A being involved in its tetramerization. The significance of this work will be discussed in detail elsewhere.

We have found that in its association–dissociation β-*C* resembles β-*B* and not β-*A*.

Amino-acid composition. Dr. D. C. Shaw has collaborated with us in examining the chemical differences between the three types[21]. Amino-acid analyses of the HCl hydrolysate show that, for a monomer molecular weight of 17,800, β-*A* has the following residue differences compared with β-*B*: +1 aspartic acid residue −1 glycine, −1 alanine and +1 valine. (These differences for *A* and *B* are similar to those reported by Piez *et al.*[22] and Gordon *et al.*[23]). The amino-acid composition of type *C* is similar to type *B*, having no difference in aspartic acid, glycine, alanine and valine, but it is different from *B* in showing a difference of +1 histidine, and −1 glutamic acid residues. In the protein the glutamic acid residue may occur as glutamine. Two-dimensional electrophoresis of the tryptic hydrolysate from performic acid oxidized β-*C* confirmed the existence of three histidine-containing peptides whereas β-*B* has only two.

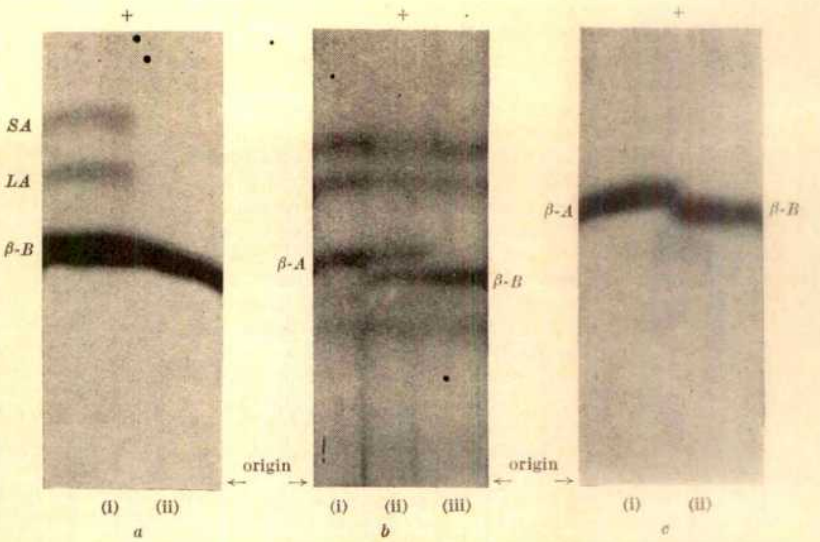


Fig. 2. (*a*) Starch-gel electrophoresis patterns of (i) sheep 'lactalbumin' solution containing β-lactoglobulin-*B* and (ii) of the isolated sheep β-lactoglobulin-*B*, at *p*H 7·6 (*I* 0·2, phosphate buffer), 7·5 V cm^{-1}, for 5 h. (*b*) Patterns of sheep skim milks containing (i) β-lactoglobulin-*A*, (ii) β-lactoglobulin-*AB* and (iii) β-lactoglobulin-*B*, at *p*H 7·6, 6·5 V cm^{-1} for 5 h. (*c*) Patterns of the isolated sheep β-lactoglobulins (i) β-*A*, (ii) β-*B*, similar conditions to (*a*)

Optical rotatory properties. The β-lactoglobulins exhibit unusual optical rotatory properties. Many proteins show co-variance of the rotatory parameters characteristic for a simple mixture of helical and disordered regions[24]. Because of differences in amino-acid composition and the effect of solvent conditions on the denatured form, some lack of correspondence between the optical rotation at a given wave-length ($[m']_\lambda$) and its change on denaturation ($\Delta[m']_\lambda^{H-D}$) with other rotatory dispersions parameters (λ_c, b_0 and a_0) may be expected[24]. However, the discrepancies in the case of the β-lactoglobulins are so unusual as to warrant further investigation. McKenzie *et al.*[25] have found values of $[m']_\lambda$ and $\Delta[m']^{H-D}$ signifying high helical content for the three bovine lactoglobulins. The change in a_0 found on denaturation indicates about a 70 per cent helical content. The native value of λ_c (245 mμ) only decreases by a small amount during urea denaturation and b_0 remains fairly constant at values indicative of a small helical content. Other workers have obtained similar results for β-lactoglobulin −*A B*. Since the β-lactoglobulins have a compact folded structure other chain arrangements must be considered to explain these unusual rotatory properties. Tanford *et al.*[27] explain the low b_0 as indicating a negligible helical content. However, Urnes and Doty[24] have made the alternative suggestion that the β-lactoglobulins have a mixed helical (60 per cent) and β-chain content. In this connexion it is of interest that the X-ray crystal investigations of Green *et al.*[28] indicate that the same proportion of the chain may be helically folded as in hæmoglobin.

McKenzie and Smith[14–16] have found that the absolute magnitude of the optical rotatory power of the cow β-lactoglobulins at *p*H 6·9 shows a slight increase with increasing temperature. At *p*H 2, where splitting into single chains is complete, there is also slight increase with temperature. At *p*H 9·1 there is an immediate increase in absolute magnitude of optical rotatory power compared with *p*H 6·9 at each temperature, but there is only slight increase of this value with increasing temperature. Although at *p*H 9·1 the lactoglobulins are denatured there must still be considerable constraints imposed (cf. the rule of Kauzmann and Eyring).

The other interesting optical rotatory properties are exhibited only by bovine β-lactoglobulin-*A*. At *p*H 4·7 the behaviour of β-*B* and β-*C* is similar to that at *p*H 6·9. On the other hand, in the temperature region where β-*A* associates to form tetramers (4 × 35,000) at *p*H 4·7 there is a considerable decrease in absolute magnitude of optical rotatory power with decreasing temperature. The optical rotatory dispersion parameters, thermodynamic data and the significance of these results will be discussed further elsewhere.

Sheep β-Lactoglobulins

We found that the boric acid–sodium hydroxide buffer of *p*H 8·5 used in the bovine lactoglobulin work and *tris* discontinuous buffers were unsatisfactory for demonstrating polymorphism of the sheep β-lactoglobulins by starch-gel electrophoresis[7]. However, a phosphate buffer of *p*H 7·6 and *I* 0·2 was found to be suitable. Examination of the Armstrong–McKenzie 'lactalbumin' fraction of milks of individual ewes showed that there are three main proteins in this fraction. These proteins have been identified as serum albumin, α-lactalbumin and β-lactoglobulin using as criteria of identification chemical, physical and immunological properties. This identification is in conflict with that of Darcel *et al.*[29] from filter-paper electrophoretic patterns. It can be seen from Fig. 2*a* that in the starch gel at *p*H 7·6 the order of mobility of these sheep proteins is serum albumin > lactalbumin > β-lactoglobulin. Serum albumin is not apparent in the electrophoretic pattern on paper of the concentrated 'lactalbumin' solution at *p*H 8·6, but the mobility of the β-lactoglobulin is greater than that of the α-lactalbumin. One-dimensional starch-gel electrophoresis at *p*H 7·6 of skim milks of individual ewes (73 Merino, 3 Romney Marsh, 35 Suffolk) showed the presence of two forms of β-lactoglobulin (Fig. 2*b*) of which either one or both (equal amounts) were present in all milks examined. These results suggested that the genes controlling production of the two types were alleles. A random sample of 62 Merino ewes from one flock were typed and the two-allele hypothesis tested. There was good agreement between the observed and expected numbers.

The faster-moving sheep β-lactoglobulin in starch gel at *p*H 7·6 has been designated β-*A* and the slower β-*B*. Both lactoglobulins have been isolated by the Armstrong–McKenzie procedure (Fig. 2*c*), but so far it has only been

possible to crystallize type *A*. The crystals resemble those of bovine β-*B* and β-*C*. It has been found by sedimentation investigations that sheep β-lactoglobulin-*A* does not associate at *p*H 4·7 and low temperature. Its sedimentation behaviour resembles that of bovine β-*B* and β-*C* and not bovine β-*A*. It is of interest that its aspartic acid residue content is similar to those of bovine β-*B* and β-*C*, affording further circumstantial evidence for the implication of the excess aspartic acid residues in bovine β-*A* being involved in the association at *p*H 4·7.

In collaboration with Dr. D. C. Shaw[21] we have found a general similarity between the amino-acid composition of the sheep and cow β-lactoglobulins, but there are differences which are sufficiently complex to preclude a simple comparison between species. The differences of sheep β-*A* compared with cow β-*B* (per 17,800 monomer) include: −1 lysine, −1 serine, −2 glutamic acid, +1 glycine, +1 valine, −3 leucine. The peptide maps of the two species have a gross similarity, but there are obvious differences.

Comparison of the amino-acid analyses of sheep β-*A* with sheep β-*B* indicates that *A* has −1 histidine and +1 tyrosine. Investigations in progress on the tryptic hydrolysates from performic acid oxidized material have shown that there is not a simple replacement of histidine for tyrosine. Two-dimensional electrophoresis (*p*H 1·9 and 4·7) resolved the extra histidine containing tryptic peptide in β-*B*, while the pattern from β-*A* had two other peptides. The amino-acid composition of one of these peptides from β-*A* is the same as the histidine peptide (containing 26 residues) from β-*B* except that it contains an extra glutamic acid or glutamine residue instead of histidine. The other peptide from β-*B* is not evident in the system used and so other means of separation are being explored.

Goat β-Lactoglobulins

The skim milks of 30 Saanen goats have been examined by starch-gel electrophoresis in the phosphate buffer (*p*H 7·6, *I* 0·2). Three bands were found in all the milks. It was shown by starch-gel electrophoresis at *p*H 7·6 and 8·5 of the 'lactalbumin' fraction, and of isolated β-lactoglobulin, that the slowest-moving band towards the anode under both conditions was β-lactoglobulin. No polymorphism of β-lactoglobulin was detected. This limited study does not exclude such polymorphism in goat milk, but it is of interest that Sen and Chaudhuri[30] were unable to detect any polymorphism by filter-paper electrophoresis investigations of 75 milks of individual goats from three Indian breeds.

Human β-Lactoglobulins

It has generally been considered that human milk contains β-lactoglobulin. Lunsford and Deutsch[31] demonstrated that serum albumin was present in human whey. Johansson[32] separated the whey proteins into three fractions: I, containing 55 per cent of the whey protein and similar in sedimentation behaviour to bovine α-lactalbumin (1·75 *S*); II, identical in electrophoretic and immunological behaviour to serum albumin; III, mainly a salmon-coloured protein (containing iron).

One of us (K. B.), in collaboration with Prof. O. E. Budtz-Olsen, has examined milks from 152 women (122 British, 6 Australian Aborigines, remainder various origins) by starch-gel electrophoresis at *p*H 8·5. The 'lactalbumin' fraction showed three bands, which were also exhibited in the skim milk patterns. The fastest-moving protein was present in the greatest amount and probably corresponds to the 1·7 *S* protein of Johansson. The second protein migrated at the same rate as serum albumin. The slowest protein had a very low mobility and possibly corresponds to fraction III of Johansson. On adjustment of the *p*H of the 'lactalbumin' fraction to 2, all three proteins were precipitated. Thus none of them corresponds in behaviour to the β-lactoglobulins of other species examined by us. No polymorphism was detected in any of the proteins. Further work on these proteins of human milk is in progress (K. B. and H. McK.).

Immunological Properties

Larson and Twarog[33] found that bovine β-lactoglobulins *A* and *B* were weak antigens compared with serum proteins and that they were immunologically identical. In the present work antisera against cow β-lactoglobulin-*AB* and β-lactoglobulin-*C* were produced in rabbits. The β-*AB* antiserum gave a single precipitin line with all three proteins with the Ouchterlony double-diffusion technique. On immunoelectrophoresis a single precipitin arc was obtained with β-*A*, *B* and with β-*C* against the β-*AB* antiserum. Similar results were obtained when the proteins were mixed two at a time (1 : 1) and subjected to immunoelectrophoresis. The experiments were repeated using β-*C* antiserum. All the results indicated that bovine β-*A*, β-*B* and β-*C* are immunologically identical.

Using similar methods it was found that sheep β-*A* and β-*B* are immunologically identical. Furthermore, the sheep β-lactoglobulins reacted strongly with complete cross-reaction with antiserum to the bovine proteins. Thus, either the antigenic portions of each of the bovine and sheep β-lactoglobulins are identical and the difference amino-acid residues are not involved in them, or the rabbit is incapable of detecting the differences between the two molecules and producing specific antiserum.

At the beginning of this article it was pointed out that the failure of many human mothers to breast-feed their babies has resulted in a number of problems arising in the use of milk substitutes. Many investigations have indicated that respiratory, dermal, gastrointestinal, anaphylactic and other reactions may result from allergy to cow milk[34]. Ratner *et al.*[35] considered β-lactoglobulin to be the dominant allergen in cow milk. Recent clinical and immunological studies cast some doubt on the validity of their conclusions[36]. Some workers have claimed allergic reactions in infants to human milk and/or to the milks of one or more of several ruminant species, β-lactoglobulin being implicated. The immunological identity of the ruminant β-lactoglobulins investigated in the present work and the failure to find similar β-lactoglobulin in human milk lead us to question the finding that β-lactoglobulin is the dominant allergen.

We thank Prof. O. E. Budtz-Olsen and A. G. Ogston for their interest and encouragement; the Australian Cattle and Beef Research Committee for a studentship (K. B.), the C.S.I.R.O. and the Australian Dairy Board for encouragement and financial assistance; and the Rural Veterinary Centre of the University of Sydney; the Herd Recording Section of the Queensland Department of Primary Industries and the Comptroller General of Queensland Prisons for access to their herds.

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MECHANISM OF β-KETOADIPATE FORMATION BY BACTERIA

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THE oxidative attack on many benzenoid substrates by bacteria leads to formation of either catechol or protocatechuate (3,4-dihydroxybenzoate) as the last intermediate possessing an aromatic structure (Fig. 1). These *o*-diphenols are substrates for oxygenative ring cleavage. Some bacteria attack them by oxygenation of the two adjacent carbon atoms which bear hydroxyl groups, to yield *cis, cis*-muconate and β-carboxy-*cis, cis*-muconate from catechol and protocatechuate, respectively. Both compounds are eventually converted to β-ketoadipate, which is in turn decomposed via acetyl-coenzyme *A* and succinate. This metabolic pathway is not confined to bacteria, since the mould *Neurospora crassa* can also convert protocatechuate to β-ketoadipate. Among bacteria, there are organisms that de-aromatize catechol and protocatechuic acid by a different oxygenative mechanism: only one atom of oxygen is added to a hydroxylated carbon atom, and a second is added to a neighbouring, non-hydroxylated carbon. The products of this mode of ring cleavage are α-hydroxysemialdehydes of the muconic acids, which are metabolized through pathways distinct from the β-ketoadipate pathway (Fig. 1)[1,2].

The general nature of these metabolic sequences has been known for some years, but the detailed biochemistry of the steps subsequent to ring cleavage has remained obscure. The purpose of this article is to describe the reactions which lead to the formation of β-ketoadipate from both catechol and protocatechuate, and to discuss their comparative biochemistry.

Formation of a muconic acid as the product of bacterial attack on catechol was first demonstrated by Hayaishi and Hashimoto[3]. The definitive characterization of this acid as the *cis, cis* isomer followed the elucidation of the chemistry of muconic acids and their lactones by Elvidge, Linstead *et al.*[4,5]. Evans and Smith[6] showed that only *cis, cis*-muconate can be converted to β-ketoadipate by extracts of benzoate-induced bacteria; and, in collaboration with Linstead and Elvidge[7], they showed that such extracts can also form β-ketoadipate from muconolactone (γ-carboxymethyl-Δ-α-butenolide, Fig. 2, structure III). The intermediacy of this lactone was confirmed by Sistrom and Stanier[8], who separated the enzymes of *Pseudomonas fluorescens* which convert *cis, cis*-muconate to β-ketoadipate into two fractions. One fraction catalysed the formation of (+)-muconolactone from *cis, cis*-muconate; the other converted (+)-muconolactone to β-ketoadipate. Evans *et al.*[7] suggested that another intermediate, γ-carboxymethyl-Δ-β-butenolide (Fig. 2, structure IV), might lie between (+)-muconolactone and β-ketoadipate, but without direct evidence for its occurrence.

The cleavage product formed from protocatechuate by *Pseudomonas fluorescens* was identified as β-carboxy-*cis, cis*-muconate by MacDonald, Stanier and Ingraham[9]. These workers found that only the *cis, cis* isomer of the tricarboxylic acid was converted to β-ketoadipate. We shall refer to the biologically active isomer as β-carboxymuconate. MacDonald *et al.*[9] chemically synthesized a carboxymuconolactone from β-carboxymuconate and found that this lactone was not attacked by extracts of protocatechuate-induced *Pseudomonas fluorescens*. Gross, Gafford and Tatum[10] established the structure of the

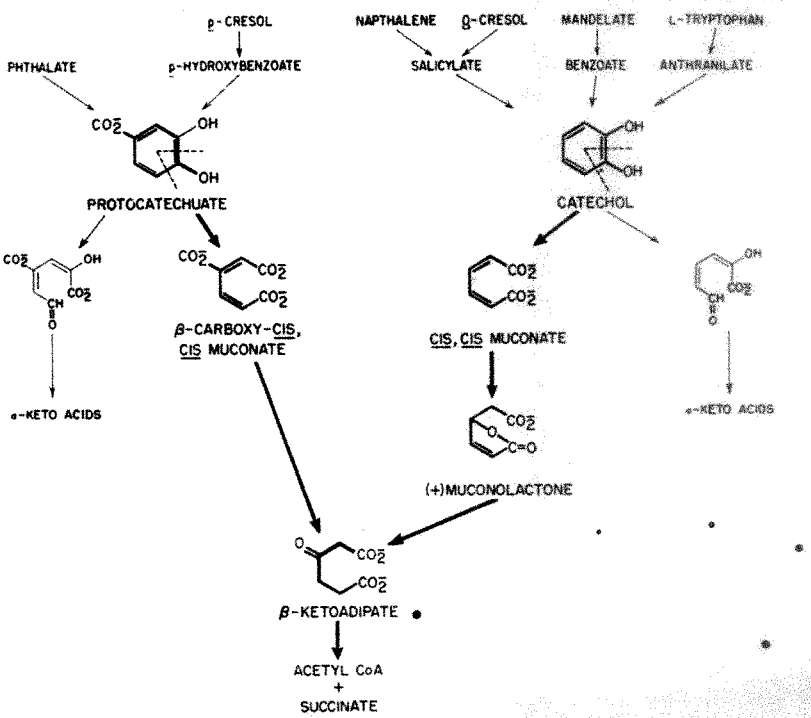


Fig. 1. A condensed metabolic map to show key positions occupied by catechol and protocatechuate in the bacterial degradation of aromatic compounds, and the divergent pathways for the breakdown of these diphenols

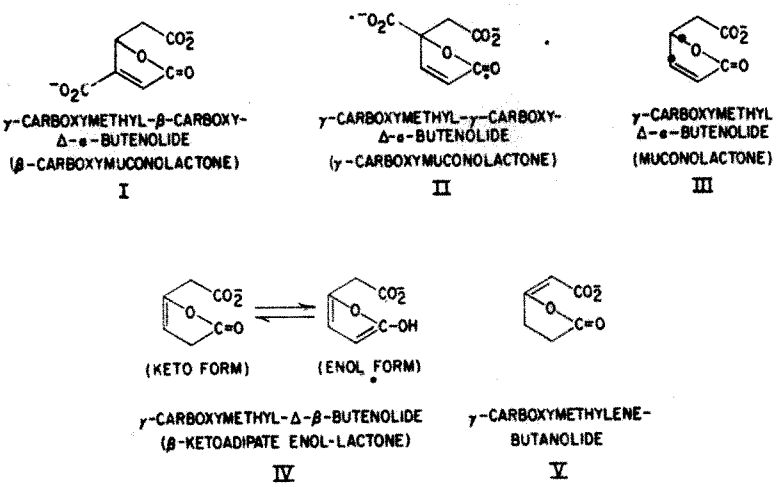


Fig. 2. Lactones related to the muconic acids. The names in brackets are the common names used in the text

chemically synthesized lactone as β-carboxymuconolactone (γ-carboxymethyl, β-carboxy-Δ-α-butenolide, Fig. 2, structure I) and showed that one of the optical isomers of this compound was an intermediate in the degradation of β-carboxymuconate by *Neurospora*. In contrast, Cain, Ribbons and Evans[11] found that β-carboxymuconolactone was not attacked by extracts of other protocatechuate-decomposing bacteria: *Nocardia erythropolis*, *Moraxella* sp. (*Vibrio* 01) (ref. 12) and a phthalate-utilizing pseudomonad.

Further evidence that the bacterial and fungal pathways diverge after ring cleavage was provided by the tracer experiments of Gross *et al.*[10], who analysed the labelling pattern in β-ketoadipate formed enzymatically from protocatechuate which had been specifically labelled with carbon-14 in the ring. The distribution of radioactivity in the β-ketoadipate formed by *Neurospora* extract was consistent with its formation via β-carboxymuconolactone, whereas the distribution in β-ketoadipate formed by an extract of *Pseudomonas fluorescens* was not, and seemed to indicate that the reaction proceeded through a symmetrical intermediate.

In reviewing this work, Elsden and Peel[13] suggested that the requirement for symmetry could be satisfied by 2,6-dioxa-3,7-diketo-bi*cyclo* (3,3,0) octane, the dilactone of *cis*, *cis*-muconate. However, both the dilactone and another possible symmetrical intermediate, β,γ-dihydroxyadipate, were afterwards excluded as intermediates by Cain, Ribbons and Evans[11], leaving the isotopic data of Gross *et al.*[10] without any satisfactory chemical explanation.

Far from clarifying the problem, subsequent work on the bacterial metabolism of protocatechuate introduced further complications. Cain and Cartwright[14] reported that an unidentified lactonic intermediate was produced in the conversion of β-carboxymuconate to β-ketoadipate by *Nocardia erythropolis*. Later observations by Cain[15] pointed to (+)-muconolactone (Fig. 2, structure III) as an intermediate in this conversion by *Moraxella* sp. He found that a heated extract of cells grown on *p*-hydroxybenzoate produced (+)-muconolactone from β-carboxymuconate, and that the unheated extract could convert this lactone to β-ketoadipate. Cain *et al.*[11] reported that extracts of *Nocardia erythropolis* and of a phthalate-utilizing pseudomonad could likewise convert (+)-muconolactone to β-ketoadipate, but confirmed the earlier observation of MacDonald *et al.*[9] that extracts from *p*-hydroxybenzoate-induced cells of *Pseudomonas fluorescens* cannot do so. These results implied that in addition to the distinctive *Neurospora* pathway, there are two distinct bacterial pathways for the conversion of β-carboxymuconate to β-ketoadipate: one characteristic of *Nocardia* and *Moraxella*, which passes through (+)-muconolactone, and one, characteristic of *Pseudomonas fluorescens*, which does not.

Our work was undertaken primarily to clarify the mechanism of β-ketoadipate formation from β-carboxymuconate by *Pseudomonas fluorescens*, which had been left completely obscure as a result of earlier investigations. During the course of a screening programme designed to obtain mutants of *P. fluorescens* strain *A*.3.12 with genetic blocks in the metabolism of benzoate, a considerable number of mutants which had lost simultaneously the ability to grow on benzoate and on *p*-hydroxybenzoate were isolated. The enzymes of the catechol pathway could be induced in these mutants by growth on asparagine in the presence of benzoate. Some of these mutants were clearly blocked below the level of β-ketoadipate; but extracts prepared from benzoate-induced cells of three of these mutants were unable to catalyse the formation of β-ketoadipate from precursors on the catechol pathway, indicating that the blocked step-reaction lay between (+)-muconolactone and β-ketoadipate (Fig. 1).

We shall refer to these particular strains as ELH⁻ mutants. Since (+)-muconolactone was known not to be an intermediate in the protocatechuate pathway in *P. fluorescens*, the inability of these mutants to grow on *p*-hydroxybenzoate indicated that the catechol and protocatechuate pathways must converge in an unknown intermediate immediately prior to β-ketoadipate, and that the affected step-reaction was the conversion of this common intermediate to β-ketoadipate. This inference was confirmed by the finding that an extract of wild-type cells grown on *p*-hydroxybenzoate, which cannot itself convert (+)-muconolactone to β-ketoadipate, can nevertheless confer this ability on extracts of benzoate-induced ELH⁻ mutant cells. By the use of (+)-muconolactone as a substrate in the presence of an excess of mutant extract, it became possible to measure in extracts of *p*-hydroxybenzoate-grown wild-type cells the activity of the enzyme responsible for the terminal step in the formation of β-ketoadipate. A typical experiment is illustrated in Fig. 3.

In principle, extracts of benzoate-induced ELH⁻ mutant cells should be able to accumulate the unknown

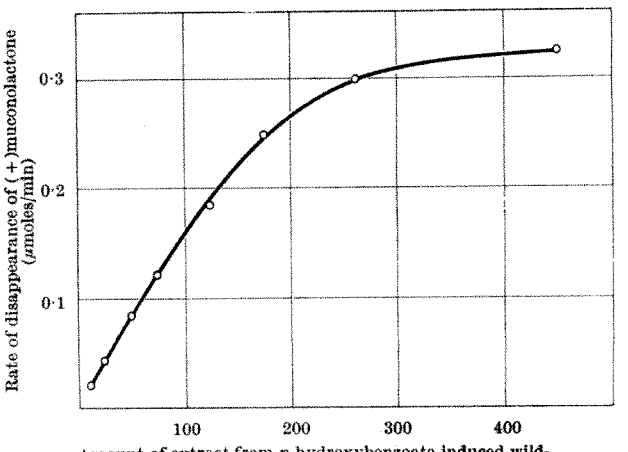


Fig. 3. Use of benzoate-induced ELH⁻ mutant for measurement of the activity of the enzyme which forms β-ketoadipate. Varying amounts of crude extract from *p*-hydroxybenzoate-induced wild-type cells were added to a series of cuvettes containing, in 3 ml., 100 μmoles *tris* (hydroxymethyl) aminomethane buffer, pH 8·0, 3 μmoles (+)-muconolactone and 250 γ-protein of extract from benzoate-induced cells of the ELH⁻ mutant. The reaction mixture was incubated at 25° C. Disappearance of the (+)-muconolactone was followed at 230 mμ

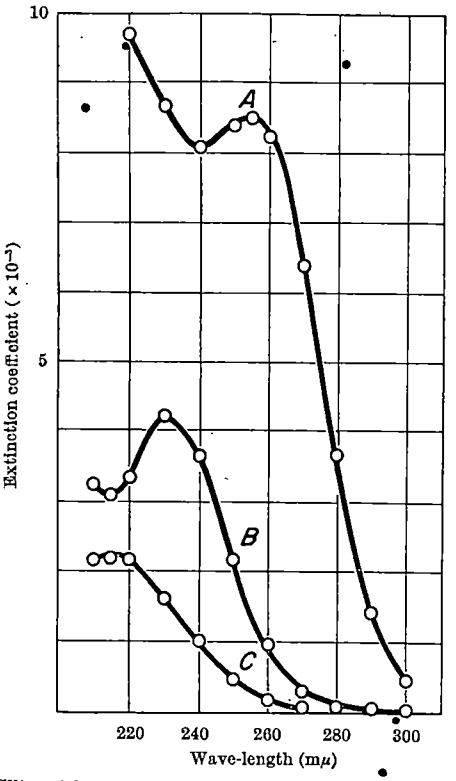


Fig. 4. Ultra-violet absorption spectra of intermediates between protocatechuate and β-ketoadipate, measured in aqueous solution at *p*H 8·0. Curve *A*, β-carboxymuconate; curve *B*, γ-carboxymuconolactone; curve *C*, β-ketoadipate enol-lactone

intermediate from precursors on the catechol pathway. However, proliminary examination showed that the principal compound which accumulates when such extracts are furnished with catechol or *cis, cis*-muconate is (+)-muconolactone, suggesting that the equilibrium between (+)-muconolactone and the unknown intermediate is strongly in favour of (+)-muconolactone. Accordingly, further attempts to prepare the intermediate enzymatically from precursors on the catechol pathway were abandoned in favour of an attempt to prepare it from precursors on the protocatechuate pathway. Since a decarboxylation occurs during the conversion of β-carboxymuconate to β-ketoadipate, it seemed reasonable to hope that the intermediate might arise in the protocatechuate pathway by an essentially irreversible reaction.

An extract was prepared from *p*-hydroxybenzoate-grown cells of the wild type, and the individual enzymes of the protocatechuate pathway were separated from one another and extensively purified by a combination of ammonium sulphate fractionation, heat treatment and column chromatography on DEAE cellulose and 'Sephadex *G*-100'. The problem was greatly facilitated by the existence of the indirect assay, described here, for the last enzyme of the sequence. The activities of the individual enzymes catalysing the successive steps between β-carboxymuconate and β-ketoadipate could each be measured spectrophotometrically, since β-ketoadipate is relatively transparent in the ultra-violet region, whereas β-carboxymuconate and the two intervening intermediates all show strong and characteristic ultra-violet absorption (Fig. 4). Purified enzymes were used for preparing these intermediates so that subsequent enzymes could be assayed.

The enzyme which attacks β-carboxymuconate is a lactonizing enzyme. One equivalent of acid per mole of substrate disappears on addition of the purified enzyme, and one equivalent of acid reappears on subsequent addition of crude extract, which converts the reaction product to β-ketoadipate. The product is a highly unstable carboxymuconolactone, probably identical with the lactone which Cain and Cartwright[14] reported to be produced by *Nocardia* extracts. It decarboxylates spontaneously to yield (+)-muconolactone (Fig. 5). We have not been able to isolate this intermediate; but the spectral properties, the lability of one carboxyl group, and the decomposition to (+)-muconolactone are compatible with only one structure, γ-carboxymuconolactone (γ-carboxymethyl-γ-carboxy-Δ-α-butenolide, Fig. 2, structure II).

The next enzyme in the sequence brings about the decarboxylation of this unstable lactone. As shown in

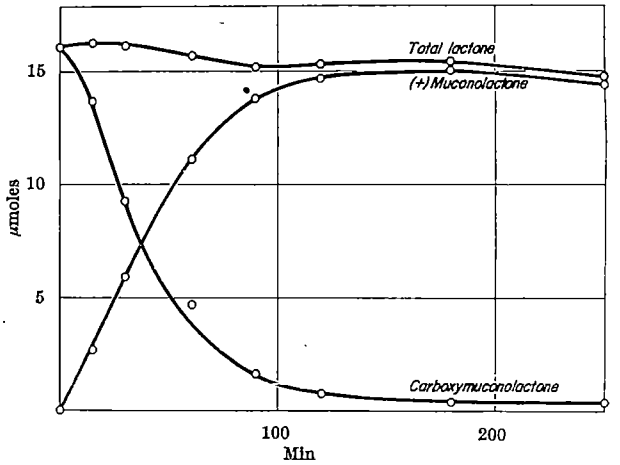


Fig. 5. Spontaneous decarboxylation of γ-carboxymuconolactone to (+)-muconolactone. The γ-carboxymuconolactone was generated enzymatically from β-carboxymuconate. Flasks contained, in 3 ml., 16·2 μmoles β-carboxymuconate, 200 μmoles sodium potassium phosphate and 20 μmoles citrate buffer at *p*H 6·0, and enough purified lactonizing enzyme to convert the β-carboxymuconate completely to γ-carboxymuconolactone in ten minutes. The reaction mixture was incubated at 30° C. Disappearance of γ-carboxymuconolactone and appearance of (+)-muconolactone were followed spectrally in aliquots of the reaction mixture

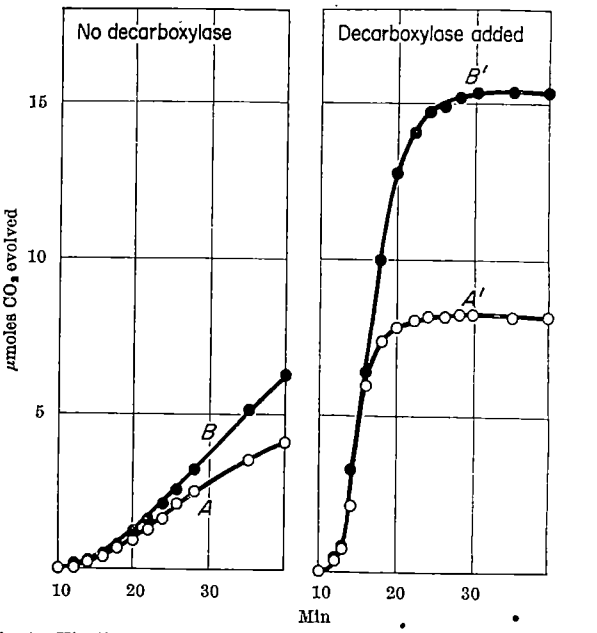


Fig. 6. Kinetics of the spontaneous and enzyme-catalysed decarboxylations of γ-carboxymuconolactone. The general conditions were the same as those for the experiment shown in Fig. 5. Flasks *A* and *A′* were furnished with 8·1 μmoles of β-carboxymuconate, flasks *B* and *B′* with 16·2 μmoles. All four flasks received at zero time an amount of purified lactonizing enzyme sufficient to lactonize the substrate at a rate of 2 μmoles/min. Flasks *A′* and *B′* received simultaneously an amount of purified decarboxylase sufficient to decompose γ-carboxymuconolactone at a rate of 5 μmoles/min. Accordingly, evolution of carbon dioxide in flasks *A* and *B* reflects the spontaneous decarboxylation of two different concentrations of γ-carboxymuconolactone, formed enzymatically from β-carboxymuconate; evolution of carbon dioxide in flasks *A′* and *B′* reflects the enzyme-catalysed decarboxylation of the same amounts of enzymatically-formed lactone

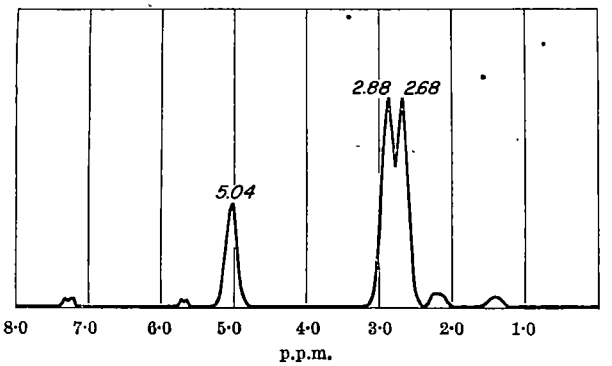


Fig. 7. Schematic magnetic resonance spectrum of β-ketoadipate enol-lactone in deuterated acetone. Values given for chemical shifts are in parts per million

Fig. 6, the addition of this enzyme to a reaction system containing β-carboxymuconate and the purified carboxymuconate lactonizing enzyme greatly increases the rate of production of carbon dioxide.

The product of the enzyme-catalysed decarboxylation of γ-carboxymuconolactone is also a lactone; a release of protons accompanies its conversion to β-ketoadipate. It is, however, clearly different from (+)-muconolactone, the product of spontaneous decarboxylation, since it can be converted to β-ketoadipate by crude extracts of *p*-hydroxybenzoate-induced wild-type cells. The new lactone was produced enzymatically from β-carboxymuconate and extracted from the acidified reaction mixture. The compound was crystallized (colourless needles, m.p. 72°–74° C) from a mixture of chloroform and *n*-hexane. Physical investigations indicated that this lactone was a structural isomer of muconolactone. The equivalent weight was 142, in agreement with the value for a monocarboxylic acid of the empirical formula $C_6H_6O_4$. Elemental analysis showed C, 50·02 per cent; H, 4·34 per cent; calculated values for $C_6H_6O_4$ are C, 50·71 per cent; H, 4·26 per cent. It was concluded that the new lactone was either γ-carboxymethyl-Δ-β-butenolide (Fig. 2, structure IV) or γ-carboxymethylenebutanolide (Fig. 2, structure V). Four lines of evidence indicate that the double bond is endocyclic, and hence that structure IV is the correct formulation. First, the spectrum of aqueous solutions of the lactone is identical at *p*H 8·0 and *p*H 2·0, which shows that the chromophore is independent of the carboxyl group. Secondly, its melting-point differs radically from the melting-point (176°–179° C) reported by Eisner, Elvidge and Linstead[16] for γ-carboxymethylenebutanolide. Thirdly, the nuclear magnetic resonance spectrum (Fig. 7) is consistent with structure IV, but not with structure V. The lactone dissolved in deuterated acetone exhibits in its proton nuclear magnetic resonance spectrum two protons at 2·68 p.p.m., two protons at 2·88 p.p.m. and one proton at 5·04 p.p.m. These chemical shifts correspond to two protons on the α-carbon, two protons on the Δ-carbon and one proton on the β-carbon, respectively, of γ-carboxymethyl-Δ-β-butenolide. Lastly, nuclear magnetic resonance investigations showed that the two protons on the α-carbon of the new lactone exchanged with deuterium ions when the compound was dissolved in deuterium oxide, indicating the occurrence of an equilibrium between the keto and enol forms as portrayed in Fig. 2, structure IV. The furan structure of the enol form no doubt explains the observed ultra-violet absorption of the compound in solution. We shall refer to this compound as β-ketoadipate enol-lactone.

When β-ketoadipate enol-lactone is added to a crude extract of benzoate-induced cells of the ELH⁻ mutant, it is almost completely converted to a mixture of (+)-muconolactone and *cis*, *cis*-muconate. This confirms the assumption, mentioned earlier, that the equilibrium between (+)-muconolactone and the subsequent intermediate in the catechol pathway is strongly in favour of (+)-muconolactone. At the same time, it demonstrates the position of β-ketoadipate enol-lactone as the first common intermediate in the catechol and protocatechuate pathways.

The enzyme responsible for the conversion of the enol-lactone to β-ketoadipate, enol-lactone hydrolase, has been extensively purified from cells of the wild type grown on *p*-hydroxybenzoate, but it occurs at comparable levels of activity in benzoate-grown cells. The purified enzyme, when added to crude extracts of benzoate-induced ELH⁻ mutant cells, permits a conversion of (+)-muconolactone to β-ketoadipate. Hence the genetic defect in these mutants, which prevents growth at the expense of both benzoate and *p*-hydroxybenzoate, is the inability to synthesize enol-lactone hydrolase.

The parallel pathways leading from catechol and protocatechuate to β-ketoadipate are shown in Fig. 8. In each sequence, there are three chemically homologous reactions which are, however, catalysed by two distinct series of enzymes, different both in substrate specificity and in inducibility. Convergence occurs with the formation of β-ketoadipate enol-lactone. Even though the formation of this lactone in the protocatechuate pathway involves

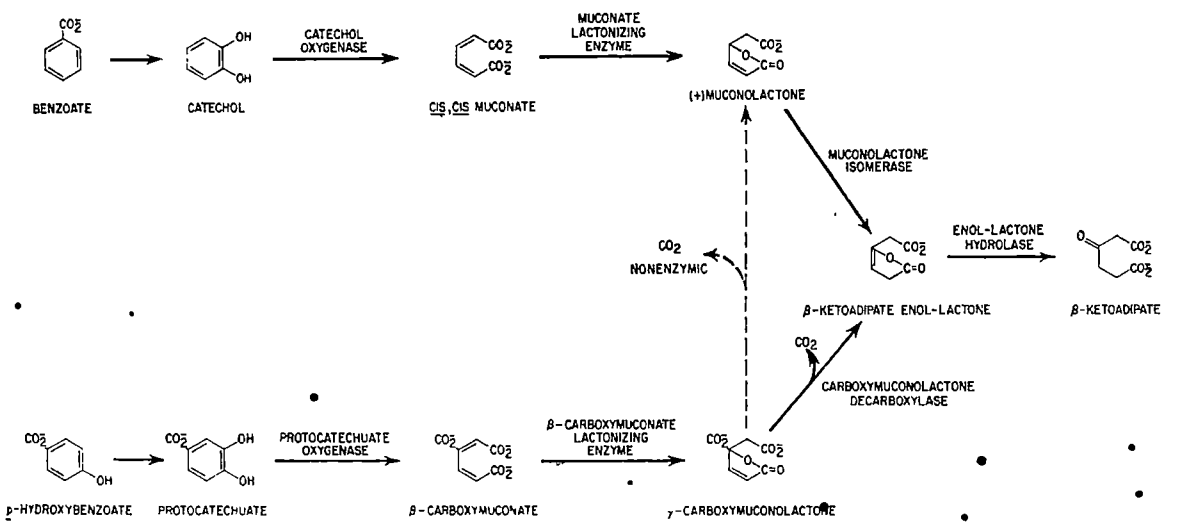


Fig. 8. The mechanism of β-ketoadipate formation by bacteria

a decarboxylation, the enzyme which catalyses this step is probably not a true decarboxylase; by analogy with the parallel step in the catechol pathway, its function is no doubt to catalyse the migration of the endocyclic double bond, decarboxylation being a necessary consequence of this structural change.

The pathway from protocatechuate to β-ketoadipate now established does not include a symmetrical intermediate, the existence of which was postulated by Gross, Gafford and Tatum[10] as a result of the finding that equilibration of a specific ring label initially present in protocatechuate occurs on its enzymatic conversion to β-ketoadipate by a crude extract of *P. fluorescens*. In fact, the only symmetrical intermediate in either the catechol or the protocatechuate pathway is *cis, cis*-muconate. Therefore, the only chemically plausible explanation of the findings of Gross *et al.*[10] is that the extract which they employed contained the enzymes necessary to catalyse a back-reaction which permitted an intermediate in the protocatechuate pathway (probably β-ketoadipate enol-lactone) to equilibrate with *cis, cis*-muconate.

The elucidation of the protocatechuate pathway in *P. fluorescens* has amply confirmed the surprising fact established by Gross *et al.*[10] that this bacterium and *Neurospora* convert carboxymuconate to β-ketoadipate by different biochemical mechanisms. We must now consider the evidence[14,15] which suggested the existence of an additional pathway for this conversion, passing through (+)-muconolactone, among bacteria. Since the evidence was obtained largely with *Moraxella* sp. ('*Vibrio* 01'), we have examined this organism, using the strain (NCIB 8250) used by previous workers[11,15]. Cain's allegation that heat-treated extracts of *p*-hydroxybenzoate-induced cells can form (+)-muconolactone from β-carboxymuconate is indeed correct; but this interpretation is not. Heating largely destroys carboxymuconolactone decarboxylase and completely destroys enol-lactone hydrolase. The γ-carboxymuconolactone which accumulates in the reaction mixture decomposes in part spontaneously to (+)-muconolactone, and in part enzymatically to β-ketoadipate enol-lactone. In the absence of enol-lactone hydrolase, the latter compound is back-converted enzymatically to (+)-muconolactone. The reported 'delactonization' of (+)-muconolactone to β-ketoadipate by unheated extracts of *p*-hydroxybenzoate-grown *Moraxella*[15] involves the action of two enzymes, muconolactone isomerase and enol-lactone hydrolase (Fig. 8). The former enzyme is, in *P. fluorescens*, specifically associated with the catechol pathway, whereas the latter is common to both the catechol and protocatechuate pathways. Hence the physiological significance of the 'delactonization' observed by Cain[15] depends on the level of specific activity of muconolactone isomerase in extracts of *Moraxella* grown on *p*-hydroxybenzoate. We have measured the level of this enzyme in extracts of *Moraxella* gathered during exponential growth at the expense of benzoate and of *p*-hydroxybenzoate. The specific activity of muconolactone isomerase in extracts of *p*-hydroxybenzoate-grown cells is clearly inadequate to account for the observed rate of conversion of β-carboxymuconate to β-ketoadipate, being forty times less than in benzoate-grown cells. Accordingly, none of the evidence which has been adduced to support the existence of a second pathway for this conversion in bacteria can be considered valid.

We thank Mr. George Hegeman and Miss Margaret Leroux, who isolated and generously furnished us with the ELH⁻ mutants. We also thank Prof. Rapoport and Mr. W. F. Benisek for their assistance in the determination and interpretation of nuclear magnetic resonance spectra.

The work was supported in part by a grant from the U.S. National Institutes of Health. One of us (L. N. O.) thanks the U.S. National Science Foundation for a pre-doctoral fellowship, during which this work was conducted.

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TERMINAL DISTRIBUTION IN THE SUPERIOR COLLICULUS OF FIBRES ORIGINATING IN THE VISUAL CORTEX

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TOPOGRAPHICAL localization in the terminal distribution of fibres from the retina to the visual cortex and to the superior colliculus has been shown in a number of animals. Suggested interactions of these two end-stations of the visual input have been described by many workers on the basis of behavioural experiments; but the detailed anatomical organization of the pathways interconnecting them and the relationship of these pathways with the retinotectal and retino-geniculo-cortical tracts have not been considered.

No satisfactory evidence exists to demonstrate a pattern of localization in the termination of fibres arising in the visual cortex and ending in the superior colliculus. Evidence for restricted degeneration in the latter following partial lesions of the visual area is presented by Gerebtzoff and Wauters[1] for the rabbit and guinea-pig and by Nauta and Bucher[2] for the rat. Unfortunately both made too few lesions to allow a system of localization to be deduced. A further investigation of the problem by Crosby and Henderson[3] suggests a localization pattern, but their conclusions depend on inconclusive evidence.

The work recorded here was undertaken on the albino rat using the Nauta and Gygax method[4] to examine the pattern of degeneration in the superior colliculus following aspiration of areas within the visual cortex. To reduce subjective bias in assessing localization, sections were mounted randomly and serialized with reference to a Nissl-stained series after analysis. The lesion area was

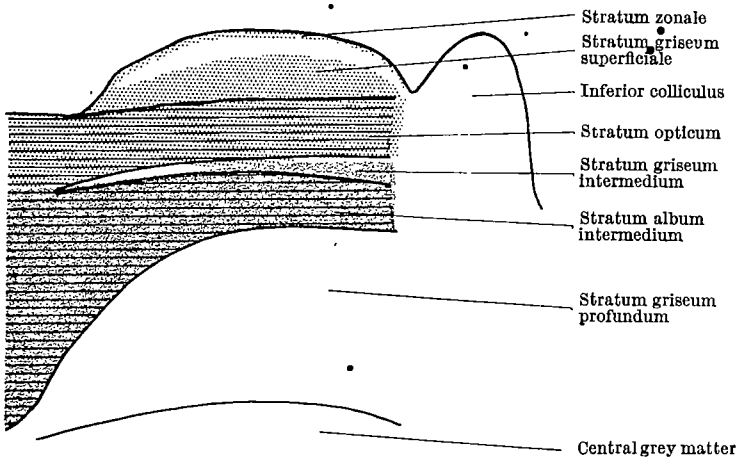


Fig. 1. Diagram of a parasagittal section through the superior colliculus of the rat. The lines indicate the afferent fibre laminæ, the dots show the area of degeneration following lesions of the visual cortex, and the stippling the degeneration after more rostral cortical lesions

mapped in relation to the posterior and medial borders of the cortex. In four brains with cortical lesions extending into the superficial part of layer IV or into layer III, no degeneration is seen in the thalamus, pretectum, or superior colliculus. As a result the area of the lesion was measured at the depth of layer IV when oblique sucker tracks were made or when lesions tapered from the surface inwards. Except for some of the large lesions which involve the superficial part of the underlying white matter, none extends deeper than layer VI.

The results summarized in Fig. 1 indicate that fibres from the visual cortex enter principally the stratum opticum of the colliculus and terminate in this lamina and the deeper part only of the stratum griseum superficiale. The density of degeneration is continuous from one lamina to the other. A few fibres run more deeply to terminate possibly in the most superficial part of the stratum griseum intermedium. However, since the boundary between this lamina and the stratum opticum is somewhat diffuse in the rat, it is not always clear within which layer this degeneration lies.

A small number of fibres run in the stratum zonale, a poorly developed layer in the rat, and evidence of termination can be seen here with the Nauta method. Degenerating fibres have never been seen passing from this layer to the subjacent stratum griseum superficiale. The paucity of fibres in the stratum zonale makes systematic localization impossible there. However, in the stratum opticum and the deeper part of the stratum griseum superficiale, there is good evidence of restricted degeneration following small lesions in the visual cortex, and the system of localization is the same in the two laminæ. The total area causing degeneration in these laminæ corresponds closely with the visual cortex defined by Lashley[5]. Ablation of the lateral part of this area causes preterminal degeneration in the rostral part of the superior colliculus, while damage to the medial part causes degeneration in the caudal part of the colliculus. Rostral lesions within the area cause medial degeneration and caudal lesions cause lateral degeneration. The area of degeneration relative to the total area of the stratum opticum is greater than is the area of the lesion relative to the total area of the visual cortex, even at the depth at which the lesion is most extensive. The effect of this apparent dispersion of fibres may be produced in part by spreading of the neighbouring cortex into the cavity of the lesion, so reducing its apparent extent. However, such expansion should be reflected in the packing density of the cells close to the lesion. This does not alter enough to account for the high ratio of degeneration area to lesion area.

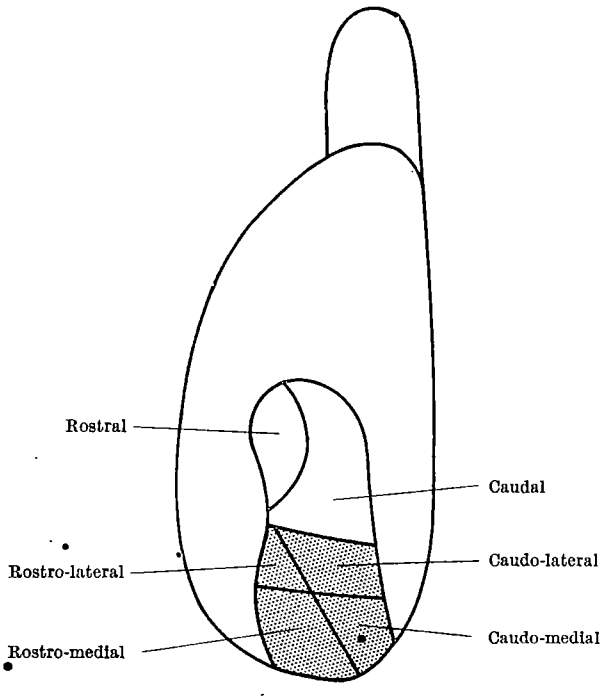


Fig. 2. Diagram of the left-hand cortex of the rat viewed from above showing the area that projects to the more superficial laminæ of the superior colliculus (stippled) and to the intermediate laminæ (clear area). The collicular receiving areas are indicated in relation to the areas of origin of corticotectal fibres

There is a relationship between this system of localization and that described by Lashley[6,7] for the retinotectal and retino-geniculo-cortical pathways of the rat. The area of cortex receiving fibres from the upper quadrant of the temporal retina of the contralateral eye projects to the area of the superior colliculus receiving retinotectal fibres from the same retinal quadrant. A similar situation is found with the other three retinal quadrants.

A further survey of degeneration produced in the superior colliculus has been made following lesions surrounding the cortical visual area to determine whether this area can be defined from neighbouring cortex in terms of its tectal projection. An area rostral to the visual area (Fig. 2) also projects to the colliculus. However, the fibres all enter by the stratum album intermedium and terminate in the posterior two-thirds of this lamina and of the stratum griseum intermedium. The preterminal distribution in the latter does not overlap that of fibres originating in the visual area. The passage and preterminal degeneration produced by rostral lesions is shown in Fig. 1. Localized degeneration is produced by small lesions; but there is considerable overlap of degeneration areas from lesions separated by several millimetres. There appears to be a pattern of localization as indicated in Fig. 2; but its significance is at present obscure. Lesions in the cingulate cortex, lying medial to the visual area, again produce no degeneration in the superficial laminæ, but some is found in the rostral part of the stratum album intermedium. Lesions in the auditory area, lying lateral to the visual cortex, show no evidence of degeneration in the superior colliculus.

From this work it appears that the superior colliculus of the rat may be divided into two sets of laminæ with regard to its afferent supply. One comprises the stratum opticum, griseum superficiale, and zonale, and possibly

part of the stratum griseum intermedium, and it receives fibres from the retina and visual cortex. The other comprises the rest of the stratum griseum intermedium and the stratum album intermedium, and receives fibres from cortical areas other than visual cortex, and from the spinal cord and other regions. How the individual inputs in each group are interrelated and what relation the two groups bear to one another are problems which are now under investigation.

I thank Prof. J. Z. Young for his advice. This work was carried out during the tenure of a University of London postgraduate scholarship.

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SUCCINATE METABOLISM IN *Salmonella typhimurium*

Utilization of Succinate as Carbon and Energy Source by *Salmonella typhimurium*

SEVERAL mutants of *Salmonella typhimurium* were found to have a specific growth requirement for relatively high concentrations of aspartic acid when tested in the Davis–Mingioli medium[1] containing glucose, sodium citrate, and mineral salts. In the absence of glucose, however, the mutants grew readily without aspartic acid using citrate, fumarate, succinate, malate, or acetate as the sole carbon source. The results were similar to those reported by Gilvarg and Davis[2], in which a mutant requiring glutamic acid grew on citrate in the absence of glucose, the metabolic lesion being in the condensing enzyme which forms citrate. The growth of some of our mutants on the four carbon acids was repressed by glucose; others were not affected. Since the requirement in the present mutant was satisfied by 4-carbon as well as 6-carbon compounds, the results suggest that this strain lacked the ability to fix carbon dioxide via the reaction mediated by phosphoenol pyruvic carboxykinase, a condition previously described in a similar mutant[3]. In contrast, the wild-type parent *S. typhimurium* grew only very slowly with succinate, fumarate, malate or acetate as the sole carbon source; the mutant had gained the ability to utilize these organic acids in addition to losing the ability to grow with glucose. This paper presents some preliminary results which may explain this metabolic difference.

The *S. typhimurium* (wild type) used was a typical strain virulent for mice; the mutant (*X*39) was one of several auxotrophs isolated from the wild type after treatment with ultra-violet light[4]. The mutant grew with succinate either in the presence or absence of glucose but not with glucose alone. The basal medium, *p*H 7, contained (in per cent as w/v) 0·27 per cent $(NH_4)_2SO_4$, 0·5 per cent K_2HPO_4, 0·01 per cent of a solution of trace minerals which consisted of 10 per cent $MgSO_4$, 1 per cent $MnSO_4$, 0·1 per cent $FeSO_4$ and 3 per cent HCl. The carbon source was added in the amount of 0·2 per cent, and for investigations using solid media 0·85 per cent 'Ion Agar No. 2' (Oxoid) was included. Inocula were grown in the basal medium containing glucose and L-aspartic acid as the carbon source. Broth cultures were incubated in 250-ml. flasks on a rotary shaker at 37° C. The optical density was measured at 425 mμ; respiration studies were made using the Warburg respirometer with air as the gas phase.

Both the mutant, *X*39, and the wild type respired succinate at approximately the same rate; neither strain respired citrate, although both could utilize it as a growth substrate. This was in keeping with the findings of other workers that bacterial cells are impermeable to citrate unless a specific permease has been induced. The inability of the wild type to grow rapidly on succinate as the sole carbon source could not be ascribed to the impermeability of the cells to succinate.

The ability of both strains to grow readily on citrate as the sole carbon source suggested that the tricarboxylic acid cycle was functional in both organisms. A number of supplementary compounds were added to the succinate minimal medium to determine if any would enhance growth. Of the compounds tested, acetate, pyruvate and glutamate stimulated the growth of the wild strain, acetate being most effective. Rapid growth occurred only on the combination; the organism grew slowly on acetate alone (Table 1).

Table 1. GROWTH RESPONSE OF THE WILD-TYPE *Salmonella typhimurium* TO SUCCINATE AND ACETATE

Addition to basal medium (%)		*O.D.** after 9 h
Sodium succinate	0·2	0·03
Sodium acetate	0·1	0·06
Sodium succinate	0·2	0·47
+sodium acetate	0·06	
Sodium succinate	0·2	0·80
+sodium acetate	0·1	

* Measured at 425 mμ.
Note: a comparable culture of the mutant *X*39 reached an *O.D.* of 0·80 in a similar period of time in the medium containing 0·2 per cent sodium succinate.

These results suggested that the wild-type *S. typhimurium*, when grown on succinate, lacked sufficient endogenously formed acetate to permit rapid growth. Pyruvate was considered a precursor to acetate. Since both strains oxidized succinate at about the same rate, and to about the same extent, it seems unlikely that acetate functioned solely as a precursor in the essential formation of citrate in the growing culture. The economy of acetate in the wild type may have been such that the rate of those biosynthetic pathways dependent on acetate became limiting.

Finally, on occasions, rapidly growing strains of the wild type (prototrophs) were obtained which behaved on succinate media as did mutant *X*39 except that they also grew on glucose as the sole carbon source. This observation confirmed our belief that gaining the ability to utilize succinate for growth was independent of the ability to utilize glucose. Studies are being continued in hopes of clarifying the metabolic distinction between these strains.

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Succinate Metabolism and Virulence in *Salmonella typhimurium*

Two kinds of strains of *Salmonella typhimurium* which can be distinguished by their behaviour on succinate as sole carbon source have been described in the preceding communication. One kind grows rapidly, yielding colonies

Table 1. Virulence of *Suc-E* and *Suc-L* metabolic types of *Salmonella typhimurium* administered intraperitoneally in albino mice

Dose	Dead/total (21 days)		Days to achieve 50% mortality	
	Suc-L	*Suc-E*	*Suc-L*	*Suc-E*
10^2	0/6	0/6	—	—
10^3	2/6	1/6	—	—
10^4	4/6	4/6	10	17
10^5	6/6	4/6	7	12
5×10^5	6/6	6/6	4	10
10^6	6/6	6/6	3·5	6
LD_{50}	4×10^3	8×10^3	—	—

* Calculated by the method of Reed and Muench[2].

visible at 24 h (designated *Suc-E*), and the other grows slowly, requiring 48 h for colonies to appear (designated *Suc-L*). This behaviour is also apparent in liquid medium in which *Suc-E* reaches maximal growth in 24 h while comparable inocula of *Suc-L* require 52 h to reach the same crop. Both were prototrophic strains derived from the same culture (strain wild 7, described by Herzberg[1]). Both strains grew well on glucose as sole carbon source, were antigenically smooth, were of equal endotoxicity, and both remained stable on sub-culture. The LD_{50} values, of the two strains, determined intraperitoneally in albino mice, were not significantly different and we would have assumed that the two strains did not differ in virulence. However, an examination of the time required for equivalent doses to kill showed that the TD_{50} values (days required for 50 per cent mortality) were consistently greater for the *Suc-E* strain (Table 1). Organisms were cultured from liver and spleen of animals infected with each strain and were tested for time required to yield visible colonies on succinate medium. The *Suc-L*-infected mice yielded only *Suc-L* organisms during a 12-day observation period. The bacterial population in animals infected with *Suc-E* changed from no detectable *Suc-L* in the inoculum administered to the animals to 40 per cent *Suc-L* by the 2nd day, 80 per cent by the 4th day and 100 per cent *Suc-L* by the 8th day of infection. Thus, the organisms which ultimately killed the mice were not the *Suc-E* strain inoculated, but rather *Suc-L* which had emerged from the *Suc-E* population *in vivo*. The delay in kill by the *Suc-E* infection was presumably due to the time required for the *Suc-L* population to grow out while the original *Suc-E* population diminished in numbers, and eventually was cleared by the animals. This pattern of a 'change-over' in population *in vivo* was consistently reproducible in a number of independent experiments. The phenomenon demonstrates the *in-vivo* selection of a metabolic type of *S. typhimurium* by animal passage, a procedure known to enhance virulence[3]. Whether this phenomenon is due to selection of *Suc-L* mutants present in the original population of *Suc-E* inoculated or to a 'mutagenic' or 'converting' mechanism present in the susceptible animals is at present under investigation. We can offer no hypothesis at this time for the correlation of virulence and succinate metabolism in this host-parasite interaction; we have found the correlation in a number of strains of *S. typhimurium* of independent origin. We believe that this is the first instance reported which links a metabolic phenomenon with virulence in this species.

This work was supported by grants *AI*–01663 from the National Institute of Allergy and Infectious Diseases and *5TI–GM*–869, National Institute of General Medical Sciences, U.S. Public Health Service.

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SOIL FERTILITY PROBLEM IN ARID AND SEMI-ARID LANDS

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THE population of the world, at the moment, is more than 2,700 millions. This figure will be doubled during the next fifty years if the present rate of growth continues. The majority of all human beings are very anxious to improve their living standard and want eagerly to reach the level of living which at present is enjoyed by only a privileged minority.

These two facts, namely the rapidly increasing pressure of population and the demand for high-quality foodstuffs, lead to the necessity of developing and making use of lands which until now have been overlooked. These regions were not taken into consideration so long as more favourable conditions could be found somewhere else. The vast soil resources of the sub-polar regions, of the humic tropical zones, and of the arid and semi-arid lands are potential agricultural *lebensraum*. The last group especially is of great importance, as more than one-third of the total continental globe suffers under these climatic conditions. It is worth knowing that only one-tenth of the continental surface is under agricultural cultivation.

Furthermore, the majority of the young developing nations, for which the quick development of all natural nutritional resources is decisive for their existence and future, are situated in the arid and semi-arid climatic belt. The problems connected with the agricultural use of these vast stretches of virgin lands are manifold and are of a very intricate nature.

The number of investigations of the agricultural prospects of soils in arid and semi-arid regions are growing steadily, but only a few are concerned with the practical use of these lands. Most of the investigations deal with hydrology in these zones and touch very briefly the importance of soils under the prevailing climatic conditions.

Measures for utilizing and improving arid and semi-arid soils, and for growing various crops, can be designed only after applying the general principles of soil and crop science to the specific conditions. This article deals only with the soil aspect.

Climate and Soil

Since the atmosphere is resting on the biosphere, air temperature, soil temperature, dew point, solar radiation, and moisture are important for the energy exchange in the biosphere. Sunlight, thermal radiation, evaporation, convection, and conduction all interact with the soil surface. They deliver a net energy to the soil or abstract energy from it. To understand the energy budget at the surface of the Earth the net radiation has to be known,

which is the difference between all downward streams of radiation and all upward streams of radiation, and is expressed in kg cal/cm²/year.

During a normal year, the atmosphere above arid and semi-arid lands takes up much more water evaporating from the soil surface and transpired by the plants than the amount of water which reaches the soil as precipitation. There is a negative balance between the yearly precipitation and the evapo-transpiration.

In the arid regions with low and sporadic rainfalls a thin soil-layer is formed which, by low leaching, is fairly rich in mineral plant nutrients. The lack of a continuous and closed vegetational cover prevents the formation of a thicker soil mantle. Organic residues, normally given off to the soil by the plant-root system, do not accumulate. Therefore, in the strict sense of the science of the soil, in most arid regions there are not soils, because the mineral component with the organic substances, together with the soil organisms do not form an inseparable entity.

On the contrary, in the semi-arid regions with their distinct summer or winter rainfall seasons, soil formation occurs. The more developed vegetational cover (typical steppe grasses) even leads to the meagre formation of humic compounds, giving these soils a slightly darker colour.

Apart from the deficiency in rainfall, the most characteristic factor of soil formation in arid and semi-arid regions is the dynamics of the weathering processes, the nature of which is primarily a physical one with slight chemical reactions. There is almost complete disintegration of the primary soil minerals, formation of secondary clay minerals of the kaolinite type, and accumulation of iron oxides and, sometimes, alumina. There is a very slight horizon differentiation, having a uniform distribution of the various fractions throughout the profile, sand (0·2–2·0 mm diameter per grain) being the most prominent part of it.

Unfortunately, the adsorption capacity of kaolinite for plant nutrients is rather low. Rapid leaching by torrential rainstorms in the semi-arid regions during the rainy season causes a severe deficiency in bases, making the soil complex especially poor in trace elements. The prevalence of free iron oxides in the mineral colloids of the soil produces a stable microstructure and makes for high porosity and water permeability.

These structural qualities of arid and semi-arid soils are very suitable for plant growth; but they should be considered when irrigation projects are planned.

Humic Compounds and Soil Fertility

The soil differs from its mineral parent material by its fertility as an indestructible and permanent property. In the formation of soil fertility, the organic substances reaching the soil as plant residues and animal remains, which are transformed by microbial activity into humic compounds, play the decisive part. These newly synthesized, dark brown, organic colloids have a beneficial effect on the physical, chemical and biological properties of the soil. They contribute prominently to the formation of a permanent and stable structure (crumby structure) of the soil which, on the other hand, has a favourable influence on the water-retention and heat turn-over of the soil. In addition to this is their importance for the nutrient supply of the higher plant. In the first instance, humic compounds possess a very high adsorption capacity which surpasses that of kaolinite by at least 50–80 times and is even considerably higher than that of the most active clay mineral, namely montmorillonite. Moreover, the main part of the total nitrogen in the soil occurs in the organic form (more than 85 per cent) of which the greatest part is aliphatically and heterocyclically incorporated into the humic compounds. For soil formation processes the chelating and complexing properties of the humic colloids are of primary importance.

Almost all the available phosphorus in arid and semi-arid soils is a product of biological accumulation, which prevents it from being fixed by the iron and alumina ions of the mineral colloids. There exists a mutual peptization effect between humic compounds and phosphorus complexes. This fact alone emphasizes once again the acuteness of the humus problem in arid and semi-arid soils, where phosphorus is the scarcest element of plant nutrition.

Very recently, the direct influence of the humic substances as highly active polymers with quinonoid character on the growth and the development of the higher plant has been established. During these processes in which humic compounds act as additional enzyme systems the osmotic value of the plant cells is increased, the transpiration considerably lowered, and therefore the wilting resistance strengthened. These results of modern humus research during the past two decades are of significant importance for the solution of soil fertility problems and practical agriculture in the regions which have still to be developed.

Humus Formation

The total reaction complex of the transformation of all plant residues and animal remains in the soil into humic compounds depends on the activity of all micro-organisms present in such a soil. They perform and exercise numerous biochemical functions, and the soil may be regarded as a tremendous biochemical laboratory.

The accumulation of humus in a given soil is not dependent only on the absolute amount of the total organic rests returned to it but depends to a certain extent also on the decomposability of these starting materials.

In one pathway of processes, the micro-organisms incorporate all organic material as energy sources into new biological processes, catabolizing them completely to carbon dioxide and water. This process normally is known as 'mineralization'. Under certain, more unfavourable environmental conditions, the plant cell-wall structures and animal tissues are broken down by micro-organisms only to specific building blocks. These low-molecular precursors of humic substances by condensation and polymerization yield high-molecular humic compounds, a process normally known as 'humification'. Mineralization and humification, therefore, are to a certain extent synonymous in their character, namely, energy-yielding pathways. Only in the second phase of humification, contrary to mineralization, does a synthesis of organic colloids take place which is mainly physico-chemical in its character.

The intensity of microbial action is in a great measure dependent on the hydro-thermic conditions which prevail in the upper crust of the Earth (0–15 cm as the most important horizon). Temperature increases up to 30° C stimulate the biochemical activity of the soil organisms, but this activity drops sharply after passing this optimum. Even numerous micro-organisms become active in the soil when the moisture content is rather low (*p*F values of about 4·0); they show full activity only when the soil reaches 40–60 per cent of its water-holding capacity (*p*F values of about 3·0). Simultaneous increase of both temperature and moisture above the optimal values (but also the decrease of both factors) delays the decomposition of the organic rests. As in any other biological activity, the decomposition rate is governed by the factor in minimum.

Generally, it can be said that the more favourable the environmental conditions (temperature, moisture, and aeration) the lower are the chances for the forming of humic compounds, since micro-organisms, as all biological entities, are inclined to break down energy sources indiscriminately and completely so long as starting material is available.

Apart from the importance of temperature and moisture conditions for the transformation of organic remains in the soil, the quality of the newly synthesized humic compounds, their particle weight and the number and kind of their exhibited active groups depend very much on these parameters. Consequently, the organic colloids in the soils of different climatic regions are very varied, not only quantitatively but also in their physicochemical character.

Breakdown of Humus

Humic compounds are not of a constant and permanent nature. They undergo continuous changes, multiplications as well as diminutions. Representing a dynamic system, they depend for their parent material, the organic rests, on a number of different factors. Fluctuations of the climate in the biosphere, soil biological activity, and activity of man during soil cultivation (seed-bed preparation) are the ruling parameters inseparably and intimately connected and influencing each other.

The humic colloids may be regarded as three-dimensionally cross-linked, predominantly aromatic systems. Apart from covalent bonds in their micro-building units, there are hydrogen bonds active in the cross-linking of 'monone' to 'polyone', and further to micelles. Such bridges exist between neighbouring phenolic and hydroxyl groups in the humic sphero-colloids. The presence of 'inner' and 'outer' hydrogen bonds leads to humic acid associations of coacervate structure, depending on their concentration in a medium and on the nature thereof. With decreasing particle concentration in humus soils a splitting-up of the micelles into 'polyone' occurs, accompanied by a decrease in viscosity.

Even though humic compounds by their aromatic nature are very resistant to microbial degradation they undergo fundamental changes with time. Pseudomonaceae, Myxobacteria, Proactinomycetes, Actinomycetes, *Penicillia*, and Basidiomycetes possess the necessary enzyme systems to use these complex substances as energy sources. Nitrogen-containing humic compounds especially are catabolized, the aliphatic and heterocyclic nitrogen being released by the breaking-up of the ring structures. But, in general, these organisms must be stimulated by certain environmental conditions before they attack the humus. The addition of small gifts of fresh plant residues to the soil is accomplished normally by a breakdown of the dark brown, colloidal substances. Moreover, during the numerous soil mechanization measurements for the preparation of the seedbed in order to cultivate the higher plants, a rapid loss of accumulated soil humus under a permanent vegetational cover (virgin or planted grassland) can be observed. Both treatments, incorporation of fresh plant rests and mechanical treatment of the soil, lead to a strong increase of biological activity in the soil. The microbial enzyme systems involved in the humus turn-over are polyphenoloxidases. During favourable oxygen tension and facilitated gas exchange, these oxidation enzymes become strongly activated. Every soil treatment, leading to the exposure of fresh soil surface, carries in itself the stimulation of biological activity and, therefore, is of acute danger to the maintenance of humus in the upper crust of the soil.

This is especially true for the light sandy soils in the arid and semi-arid regions which, by their high porosity (43·5 per cent of pore space), have already under normal circumstances a favourable gas exchange and therefore a limited humus content. These soils react especially sensitively on further exposure brought about by cultivation methods with modern machinery and implements.

But there arises another limiting factor for the maintenance and preservation of humus in soils of arid and semi-arid lands. During the summer or winter rainfall seasons these soils undergo a continuous drying and re-wetting, similar to the conditions of irrigated soils in such climatic belts. After strong, sporadic, and localized downpours the soils dry off very quickly, especially in the upper crust which contains the main part of the root system of the higher plant. During this process of drying, humic compounds are set free which previously were strongly adsorbed on soil minerals, especially on clay colloidal material These clay-humus complexes are very resistant to microbial attack. It seems that, during drying of the soil surface and its exposure to the solar radiation, photochemical processes are taking place. In these reactions water-soluble humic substances are re-formed from already highly polymerized humus and micro-organisms find them a suitable energy-source.

It must be remembered that the solar energy flux in most arid and semi-arid regions (North American desert zones, South American pampas, African dry lands, Asian continental steppes, and Australian scrublands) is fairly constant throughout the year. During daytime, very intense sunlight reaches the surface of the soil in these vast stretches of hostile land mainly undisturbed by clouds (about 9 h daily sunshine as annual long-term average). Also, the clearer skies above these lands cause much stronger nocturnal cooling at the soil surface, the clear and dry atmosphere allows almost full penetration of solar radiation, ranging from about 200 mμ to 23μ with energy peaks just beyond 1,000 mμ or 1μ. Twenty-nine per cent of the irradiated energy lies at wave-lengths greater than 1μ, and 50 per cent lies beyond the red of the visible spectrum.

By exposure to such radiation, especially in the range between 200 and 800 mμ, reactions of many types of synthesis and decomposition, polymerizations and dissociations, as well as oxidations and reductions, can be brought about. Hydrogen bonds and other short-range inter-molecular forces are most vulnerable to irradiation, and an unlimited number of degradation products may appear from large organic molecules during such reactions. In secondary processes, the building blocks of the complex molecules may again react with each other to form new compounds.

Consequently, in the very delicate process of humus dynamics, prominently occurring in the upper crust of soil, one cannot separate the biological world from the physico-chemical phenomena. The application of the newest results of 'phototropy' and 'photosensitized reactions' to soil fertility problems in arid and semi-arid regions—via humus research—is long overdue.

It is now essential that such basic investigations be started and properly integrated with hydrological investigations and planning in these climatic zones.

The agricultural usage of dry lands, predominant in the developing countries, is the greatest challenge to our knowledge in soil fertility. Only with great precaution and skill can complex cultivation methods be applied to such soils which by their peculiar nature are very sensitive to wind and water erosion. Sound knowledge of the two fundamentals of agriculture in arid and semi-arid zones—water and humus—is necessary if disappointing set-backs are to be avoided, causing not only large financial losses but in most cases forfeiting irretrievably the confidence of the farmers involved.

Nations with a long and successful agricultural tradition, planning the improvement of agricultural practices in order to make use of lands in arid and semi-arid zones, are well advised to start long-term research work on microclimatology, hydrology, and biochemistry of the soil. New methods of agricultural management, including the increased use of organo-mineral fertilizers (ammonized lignites) and of industrial waste-products (processed sulphite liquors) as prominent sources of high-quality humic compounds, will undoubtedly yield much new and surprising information and result in efficient agricultural usage of these critical but promising lands.

LETTERS TO THE EDITOR

GEOPHYSICS

Estimation of Half-lives from Meteoritic Data

MOST investigations of cosmic ray nuclear interaction effects in meteorites have been aimed at deducing information on the history of cosmic radiation or on the history of the irradiated solid material[1]. It is the purpose of this communication, in part, to use such investigations for the determination of an approximate half-life.

The case illustrated is that for ^{44}Ti, recently found to be 48·2 ± 0·9 years[2]. The results obtained from meteoritic data are within 25 per cent of this value, while results based on cross-section arguments have been in error by as much as a factor of 20. Experiments of a similar nature are planned to estimate the ^{32}Si half-life.

The first ^{44}Ti half-life estimate, ⩾23 years, was given by Sharp and Diamond[3]. This estimate was later revised upward to 1,000 years[4] and then to ~200 years[1]. Until very recently, however, no direct measurement had been made.

In principle it should be possible to measure the half-life of ^{44}Ti by determining its production rate in a meteorite with an appropriate terrestrial age, t, and comparing the observed ^{44}Ti activity, A, with the theoretical value, A_0. The decay constant, λ, can then be calculated by means of the well-known formula:

$$\frac{A}{A_0} = e^{-\lambda t} \qquad (1)$$

The production rate, however, is a function of the cosmic ray intensity- and energy-spectrum which, in turn, is dependent on the degree of shielding which the meteorite had while in space. For all meteorites studied thus far, the theoretical production rate is so uncertain that such a treatment is not feasible.

Another approach is possible, however. If the ratios of the concentrations of a number of cosmogenic nuclides can be obtained between two meteorites of known terrestrial ages, t_1 and t_2, a theoretical ^{44}Ti ratio, R_0, can be calculated which is already corrected for shielding effects. The observed ratio, R, can then be related to the decay constant, λ, by:

$$\frac{R}{R_0} = e^{-\lambda (t_2 - t_1)} \qquad (2)$$

As it happens, an appropriate pair of iron meteorites is Treysa (fell April 3, 1916) and Aroos (fell November 24, 1959). Table 1 lists those nuclides which have been measured in both meteorites. The stable nuclide ratios have been corrected for the different cosmic ray exposure ages (Aroos, 980 m.yr.; Treysa, 530 m.yr.) of the two meteorites[5]. The uncertainties in these corrections have been included.

Table 1. COSMOGENIC NUCLIDES IN THE AROOS AND TREYSA IRON METEORITES

All stable isotope ratios have been multiplied by a factor of 1·85 to correct for the different exposure ages of these meteorites (ref. 5)

Nuclide (half-life)	Units	Aroos (ref.)	Treysa (ref.)	Treysa/Aroos
^{21}Ne	10^{-8}scc/g	8·20 ±0·10 (5)	8·00 ±0·25 (5)	1·81±0·38
^{26}Al (7·4 ×10^{5}y)	dpm/kg	1·47 ±0·15 (5)	2·69 ±0·34 (5)	1·83±0·30
^{36}Cl (3·1 ×10^{5}y)	dpm/kg	14·0 ±1·4 (8)	22·0 ±2·0 (1)	1·57±0·21
^{38}Ar	10^{-8}scc/g	42·0 ±2·2 (5)	32·4 ±1·3 (5)	1·43±0·31
^{45}Ca	ppb	1·7 ±0·15 (7)	1·15 ±0·06 (7)	1·25±0·29
^{44}Ti (?)	dpm/kg	2·40 ±0·30 (5)	1·23±0·59 (5)	0·51±0·25
^{46}Ca	ppb	0·039±0·004 (7)	0·030±0·002 (7)	1·42±0·34
^{50}V	ppb	4·79 ±0·40 (7)	2·47 ±0·20 (7)	0·96±0·23

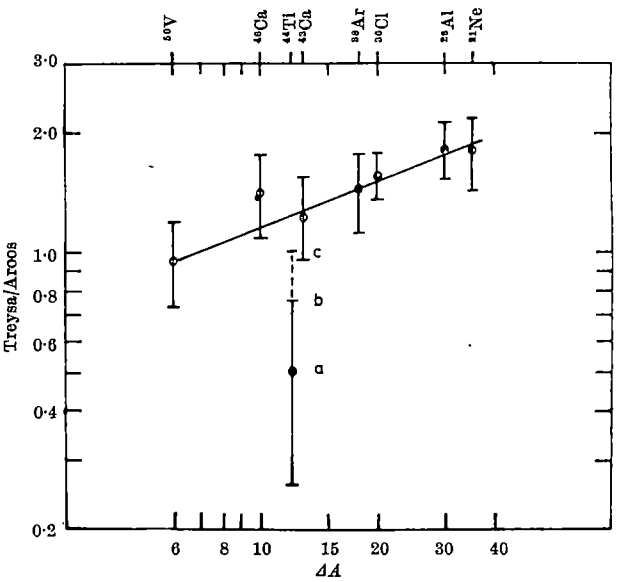


Fig. 1. Treysa/Aroos cosmogenic nuclide ratios versus ΔA, the difference in mass between ^{56}Fe and product nucleus. From these data the most probable value for the ^{44}Ti half-life is $36^{+\infty}_{-19}$ years. ○, stable nuclides; ●, radioactive nuclides

Fig. 1 shows the values of these ratios plotted versus ΔA, the difference in mass between the principal target nucleus (^{56}Fe) and the measured nuclide. Although many of these nuclides are non-cumulative, their position along a straight line indicates that the ratio of the spallation yields of the isobars may be assumed to be constant for the two meteorites. For a ΔA of 12, that is, ^{44}Ti, the production ratio between Treysa and Aroos should be 1·24. The observed value is considerably lower, 0·51 ± 0·25, and this 'depletion' may reasonably be ascribed to terrestrial decay.

The three points, *a*, *b* and *c*, which are indicated in Fig. 1 correspond to the most probable value, the 1σ limit (67 per cent confidence level), and the 2σ limit (96 per cent confidence level). The half-lives corresponding to these points are listed in Table 2. From these values it may be seen that the ^{44}Ti half-life almost certainly is less than 160 years, and its most probable value is about 36 years.

Table 2. COMPARISON BETWEEN THE ^{44}Ti HALF-LIFE CALCULATED FROM COSMOGENIC NUCLIDES AND THE MEASURED VALUE

Value	Half-life (years)
Most probable	36
67% confidence level	⩽65
96% confidence level	⩽160
Measured value (ref. 2)	48·2±0·9

Comparison with the actual ^{44}Ti half-life, 48·2 ± 0·9 years, indicates that the meteoritic technique seems potentially more accurate than cross-section arguments for such estimations.

A similar investigation for estimating the ^{32}Si half-life, now supposed to be about 500 years[6], requires a meteorite which fell before about 1650. Likely candidates are Elbogen (fell 1400) and Jalandhar (fell 1621).

In addition to allowing an estimate of the ^{44}Ti half-life to be made, several other facts can be deduced from Fig. 1. First, the meteorites, Treysa and Aroos, suffered either equal or no space erosion during at least their last few hundred thousand years of cosmic ray irradiation[7]. Secondly, it appears that the ratio of the two exposure ages (1·85) is

very nearly correct, although it may perhaps be very slightly too low. Thirdly, the value of k_2, 'the hardness value', is 2·12, which is in good agreement with the value of 2·13 obtained by Stauffer and Honda[7]. This, in turn, implies that the Treysa specimen analysed[5,7,8] came from rather less than 10 cm below the pre-atmospheric surface of the meteoroid (originally of the order of 50–100 cm in radius).

I thank Dr. Dieter Heymann for informing me of the progress of his investigation of the ^{44}Ti half-life. I thank the U.S. National Science Foundation for a post-doctoral fellowship at Bern and the Schweizerischen Nationalfonds.

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Magnetic Variations produced by Ocean Swell

As part of a programme to investigate geomagnetic micropulsations, the Pacific Naval Laboratory has constructed a floating buoy for recording these signals at sea. A non-magnetic (fibre-glass) case housing a rubidium vapour magnetometer is suspended in the ocean by a nylon line from a vertical spar buoy. The system is designed to de-couple the magnetometer case so far as possible from the motion of the sea surface. The magnetometer is connected by electric cable to a raft on the surface, whence the output signals are telemetered to recording equipment on the attending ship which is kept magnetically remote from the buoy.

In connexion with such measurements, it is necessary to take into account the varying magnetic field associated with the electric currents induced in the sea-water by its motion across the Earth's magnetic field. This problem has recently been receiving some attention. For example, formulæ giving the magnetic field induced by a sinusoidal gravity wave in a deep sea have been obtained by Crews and Futterman[1] for points above the sea, and by Warburton and Caminiti[2] for points within the sea. In both these papers, the method used was to integrate the contributions from each individual current element. Actually, however, the formulæ can be found more simply by solving the differential equations satisfied by the magnetic vector $\boldsymbol{H}$ within and above the sea, and applying the usual electromagnetic boundary conditions at the surface.

Let the sea occupy the half-space $z > 0$ in a rectangular co-ordinate system, and let the gravity wave progress in the x-direction with angular frequency ω, and amplitude a at the surface. Then, with g denoting the acceleration due to gravity and $m = \omega^2/g$, the wave is specified by the velocity potential:

$$\varphi = \frac{ag}{\omega} \exp(i\omega t - imx - mz)$$

and in electromagnetic units, $\boldsymbol{H}$ satisfies the differential equation:

$$\nabla^2 \boldsymbol{H} = 4\pi\sigma \{i\omega \boldsymbol{H} - (\boldsymbol{F}.\nabla)\nabla\varphi\}$$

where $\boldsymbol{F}$ is the (constant) magnetic vector of the Earth's field, and σ is the conductivity of the medium (4×10^{-11} E.M.U. in sea-water, 0 in air). The solution for $H_\parallel$, the component of $\boldsymbol{H}$ parallel to the Earth's field (which is the component measured by a rubidium vapour magnetometer) evaluated at $t = 0$, $x = 0$, is found to be:

$$H_\parallel = amF\left\{i(S+iC)^2(1-e^{\alpha z}) - \frac{\alpha^2(S^2+C^2)}{4\pi\sigma\omega}\right\} e^{-z(m+\alpha)}$$

where $\alpha = (m^2 + 4\pi i\sigma\omega)^{1/2} - m$

and $S = \sin I$, $C = \cos I \cos\theta$

I being the dip angle, and θ the declination (eastwards) of the direction of wave propagation from the magnetic meridian. This solution is exact; it reduces to the formula obtained previously[2] if second-order terms in $\sigma\omega/m^2$ are neglected in comparison with unity. Under most conditions this is a reasonable approximation.

In both the papers to which reference has been made here the discussion was confined to wind waves of short periods, the conclusion being that quite rough seas were required to produce significant magnetic fields. For example, a sea-state 5 (1·2–1·8 m amplitude) was claimed necessary to give a field of 0·1γ at an altitude of 100 ft. (ref. 1), and even for a 100-m wave a sea-state 6 (1·8–3·0 m amplitude) is required to produce the same field at a depth of 95 m (ref. 2). However, the foregoing formula shows that $|H_\parallel|$ increases not only with the surface amplitude a of the sea wave, but also with its period $2\pi/\omega$. The variation of $|H_\parallel|$ with period (wave-length) is depicted by the curve in Fig. 1 for a depth of 100 m and a constant 1-m surface amplitude of the wave. (Other values used in the calculation were $F = 0{\cdot}56\Gamma$, $I = 70°$, $\theta = 90°$.) The sharp rise in $|H_\parallel|$ with increasing period suggests that, even on a calm sea, the long-period swell may induce magnetic fields comparable with those produced by wind waves of much larger amplitude. This important fact was apparently overlooked by many previous authors. For example, it can be seen from Fig. 1 that a 15-sec swell of only 10-cm amplitude could generate a field of 0·1γ at a depth of 100 m.

Confirmation that the ocean swell does produce appreciable magnetic signals has been obtained by recent

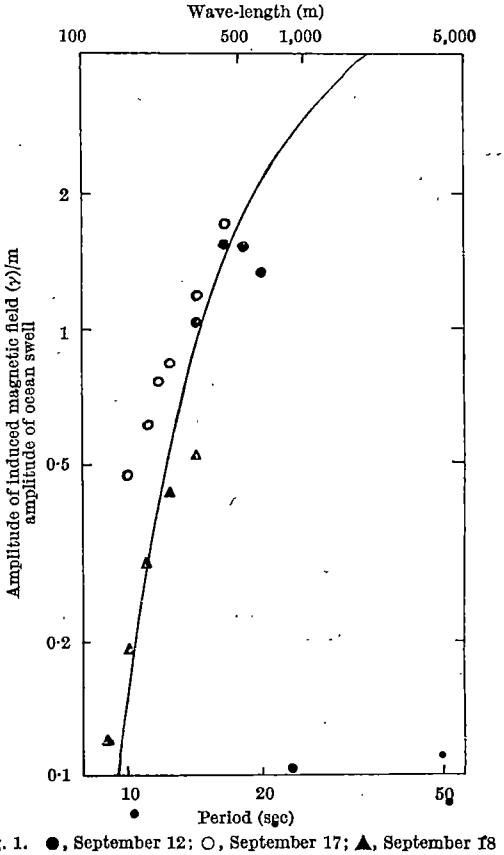


Fig. 1. ●, September 12; ○, September 17; ▲, September 18

experimental work in deep water off the west coast of Vancouver Island. Measurements were made under both calm and moderate sea conditions, but a long-period swell, measured by a vertical accelerometer fitted to the raft, was always present. On three different days the principal period of the swell (running approximately perpendicular to the magnetic meridian) was 16·7 sec, 11·8 sec and 11·1 sec, respectively, and in each case the magnetometer, suspended at a depth of 100 m, recorded a magnetic signal of approximately the same period. The power spectra and cross-correlation for the accelerometer and magnetometer signals were computed from about an hour's data for each of the three days. The coherence between the two signals was found to be very high indeed, and values of the magnetic signal per metre amplitude of the swell, which were computed for a few points in the neighbourhood of the dominant period on each of the days, were found to fit the theoretical curve reasonably well, as is shown in Fig. 1.

A further experiment was conducted to the east of Vancouver Island in Georgia Strait which is sheltered from ocean swell. A moderate sea was running at the time with short-period (3-sec) wind waves of about 0·6 m amplitude, but there was no swell. The magnetic field at a depth of 100 m was found, in this case, to be negligible (less than 0·03γ amplitude), which is also in agreement with theoretical calculations. Throughout these experiments a land-based rubidium vapour magnetometer, situated at Albert Head, on southern Vancouver Island, was used simultaneously to monitor the natural micropulsation background.

We wish to acknowledge the useful discussions with Drs. S. Z. Mack and J. E. Lokken during the planning of the experiment and the analysis of the results, and the assistance of Messrs. D. B. Edwards and J. G. Moore during the experiment.

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Strontium-90 and Cæsium-137 in Antarctic Snows

THE strontium-90 content of snow samples indicates that the main influx of fission products in Antarctica occurred in 1955. The movement of ^{137}Cs seems possible in Antarctic snow.

Samples of snow were dug from the New Zealand Antarctic Division snow mine at Scott Base and after melting sent to the Institute of Nuclear Sciences, Wellington, for analysis. The results for cæsium-137 and strontium-90 content are given in Table 1 in which the errors quoted are those which arise from counting statistics only. The sample labelled 1953, for example, represents snow taken from a layer which is considered to contain the 1952/53 summer snow and the 1953 winter snow.

Table 1. FISSION PRODUCTS IN ANTARCTIC SNOWS

Year of deposit	Activity at time of deposition (picocuries/100 l.) ^{137}Cs	^{90}Sr	^{90}Sr-deposition (microcuries/km²)
1939	49 ± 28	not detected	nil
1940	268 ± 27	not detected	nil
1953	301 ± 22	34 ± 2	66
1954	89 ± 22	9 ± 1	9
1955 (summer)	74 ± 22	19 ± 1	17
1955 (winter)	91 ± 20	302 ± 3	500
1956	127 ± 18	224 ± 3	210
1957	16 ± 15	129 ± 2	
1958	50 ± 14	179 ± 2	
1959	29 ± 12	135 ± 2	
1960	not detected	100 ± 2	
1961	47 ± 13	174 ± 2	

The samples labelled 1957–1961 were collected in November 1961, the snow being melted in a kettle, poured into plastic jerricans and returned to New Zealand, where it was filtered, and carriers were added and the activities analysed. The remaining samples were collected in November 1962, melted in a kettle with carriers added to the first kettleful of each 2-gallon sample, poured into a plastic jerrican and returned to New Zealand for analysis.

This work was done with the help of officers of the New Zealand Antarctic Division, principally A. J. Heine, who dug the snow samples, and assisted with the stratigraphy and dating. He noticed that the layer he ascribed to 1955 was much thicker than other yearly layers, 58 cm as against an average of 29 cm. Therefore he took from this layer two samples of equal thickness which I have designated as 'summer' 1954/55 and 'winter' 1955 snows. Vickers[1] also mentions that 1955 produced the largest snowfall of recent years in most of west Antarctica. Heine had to rely mainly on a study of snow crystals for dating, although Sharp and Epstein[2] and Gonfiantini *et al.*[3] have since recommended the additional measurement of isotope ratios such as $^{18}O/^{16}O$. Vickers[1], however, claims that deuterium determinations and oxygen isotope ratio measurements add little to the information gained from a study of the snow crystals.

The strontium-90 determinations show a major influx of ^{90}Sr in 'winter' 1955 following smaller depositions in 1953, 1954 and 'summer' 1954/55. The latter may be due to the November 1952 'Ivy' test. The 1955 influx, arriving at least 12 months after the March 1954 'Castle' tests, is presumably due to this series. The date of this influx agrees reasonably with the arrival time of February 1955 (± 2 months) found from the total β-activity measurements of Picciotto and Wilgain[4] on snows from a glaciological pit at King Baudouin Base. The ^{90}Sr determinations of Libby[5] and Drevinsky[6], which were discussed by Vickers[1], indicate increases at Admiral Byrd Bay, Atka Bay, Kainan Bay and McMurdo Sound in the spring/summer of 1954—somewhat earlier than shown by our results. Furthermore, Drevinsky's Kainan Bay figures appear lower than those at other stations and show a further major increase about spring 1955. It seems that further measurements coupled with accurate pit stratigraphy at different sites in the Antarctic are still desirable.

Our cæsium-137 results appear completely chaotic. The low figures for both strontium-90 and cæsium-137 in the 1957–61 samples may reflect the absence of carriers during melting and transport. The nearly constant $^{137}Cs/^{90}Sr$ ratio of about 1·8 almost invariably found in rain samples is not apparent. Furthermore, appreciable ^{137}Cs was found in the 1939 and 1940 samples—13 years before the first thermonuclear device was detonated in November 1952. The ^{137}Cs results suggest, therefore, either contamination, or migration of ^{137}Cs in Antarctic snows. These possibilities are being investigated.

The absolute values recorded in Table 1 must be regarded with caution. A complete profile is being re-sampled with greater precautions to melt the snow in the presence of carriers. The ^{90}Sr results for the 1957–1961 snows have been included in Table 1 because, although we suspect them to be low, they are still much higher than the pre-1955 values.

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GEOCHEMISTRY

Luminescence of Orthopyroxenes

In an examination of several stony meteorites Derham and Geake[1] reported on the luminescence of three enstatite achondrites under proton excitation. A later report[2] concludes that enstatite, the major mineral in the meteorites, is probably responsible for all the observed luminescence. Each meteorite yields a spectrum in which red emission with a peak at 6700 Å is accompanied by a weaker blue peak at 4000 Å. The red peak at 6700 Å in meteoritic enstatite has been attributed by Garlick[3] to $MgSiO_3$ containing manganese as an impurity. Sarver and Hummel[4] have shown that both photo- and cathodo-luminescence occur in the red spectral region (6310–6830 Å) of Mn-activated magnesium germanates and silicates. The position of peak emission of enstatite activated with Mn(II) is extrapolated from $MgGeO_3$–$MgSiO_3$ enstatite solid solutions to be 6730 Å. This is near the value found in synthetic clinoenstatite activated with Mn(II), namely, 6720 Å. By examining crushed grains of the Bustee meteorite, Derham, Geake and Walker[2] were able to separate grains which luminesced blue from a mixture in which most of the grains luminesced red. The luminescence spectrum of the 'blue' grains differs from that of the 'red' grains and from the luminescence spectrum of the meteorite in that the red peak at 6700 Å is weaker than the blue peak at 4000 Å. X-ray powder analyses of 'red' and 'blue' grains showed both to be orthoenstatite.

Recent investigations[5,6] have shown that orthoenstatite in enstatite achondrites is present in both ordered and disordered forms. We have made a preliminary examination of luminescence of orthopyroxenes and related minerals in an attempt: (1) to delimit the occurrence of this phenomenon in a number of diverse samples; (2) to show whether any correlation exists between luminescence and disorder in $MgSiO_3$.

Samples were excited by X-rays from a tungsten tube operated at 50 pkV and 35 m.amp. Luminescence was observed visually for overall colour and through appropriate filters to obtain a crude estimate of the red and blue components of the emission. X-ray single crystal and powder techniques were utilized to investigate disorder in the orthopyroxenes. The results of this survey are presented in Table 1.

Mg-silicates prepared in different laboratories from different starting materials yield similar luminescence. With the possible exception of the Stannern hypersthene, luminescence was observed only in orthopyroxenes containing little or no iron. None of the four terrestrial iron-bearing orthopyroxenes, all from plutonic environments, emits detectable luminescence. The presence of an appreciable amount of iron in the $MgSiO_3$ structure may inhibit the luminescence which is readily apparent in the virtually iron-free synthetic and meteoritic enstatites.

All the disordered orthopyroxenes examined exhibit luminescence, but there is no simple correlation between disorder and the intensity and colour of the light emitted. Differences in luminescence were found between ordered and disordered enstatites from the same meteorite (Khor Temiki and Pena Blanca Spring in Table 1). In both cases the luminescence of the disordered enstatite was weaker and the ratio of blue to red was higher. This suggests that some correlation does exist between the character of the luminescence and either disorder in the structure or some effect closely related to and perhaps cogenetic with disorder.

Note. In a communication which reached us just before this letter was submitted for publication, Dr. Geake informed us of his later work at Manchester on the Bustee meteorite. The X-ray powder patterns of 'red' and 'blue' grains from this meteorite[2] have been found by Prof. W. S. MacKenzie to match those of ordered and disordered orthoenstatite, respectively.

Table 1

Sample	Meteorite class or source	Structural state of orthopyroxene	Luminescence		
			Colour	Relative intensity of red and blue emission*	Intensity
Meteorites					
Aubres	Enstatite achondrite	Disordered	Red	Red > blue	Moderate
Bishopville	Enstatite achondrite	Ordered and disordered	Violet	Red ≈ blue	Moderate
Bustee	Enstatite achondrite	Disordered	Blue	No red	Moderate
Cumberland Falls	Enstatite achondrite	Disordered	Red	Red > blue	Moderate
Khor Temiki	Enstatite achondrite	Ordered	Red	Red > blue	Moderate
Khor Temiki	Enstatite achondrite	Disordered	Violet	Blue > red	Weak
Norton County	Enstatite achondrite	Ordered and disordered	Red	Red > blue	Moderate
Norton County	Enstatite achondrite	Disordered	Red	Red ≥ blue	Weak
Pena Blanca Spring	Enstatite achondrite	Ordered	Red	Red > blue	Moderate
Pena Blanca Spring	Enstatite achondrite	Disordered	Blue	Blue > red	Weak
Pesyanoe	Enstatite achondrite	Ordered and disordered	Red	Red > blue	Moderate
Manegaon	Hypersthene achondrite	Ordered	Nil		
Shalka	Hypersthene achondrite	Ordered	Nil		
Tatahouine	Hypersthene achondrite	Ordered	Nil		
Stannern	Pyroxene-plagioclase achondrite	Disordered	Blue	No red	Weak
Novo-Urei	Olivine-plagioclase achondrite		Nil		
St. Marks	Enstatite chondrite	Ordered	Nil		
Campo del Cielo	Enstatite from hexahedrite	Ordered	Nil		
Marjalahti	Olivine from pallasite		Nil		
Mount Egerton	Enstatite from stony iron	Disordered	Red and blue grains		Moderate
Terrestrial minerals					
Enstatite	Bamle, Norway	Ordered	Nil		
Enstatite	North Carolina	Ordered	Nil		
Bronzite	New Mexico	Ordered	Nil		
Hypersthene	Canada	Ordered	Nil		
Talc, white	New York		White	Red > blue	Moderate
Talc, green	Georgia		Nil		
Plagioclase	From anorthosite		White	Blue, no red	Weak
Synthetic samples					
Enstatite and amorphous material	Heated $MgSiO_3$ gel	Disordered	White	Blue > red	Moderate
Enstatite	From General Electric	Disordered	Violet	Blue > red	Strong
Protoenstatite	Heated $MgSiO_3$ gel		Blue	No red	Weak
Protoenstatite > clinoenstatite	Heated $MgSiO_3$ gel		White		Weak
Protoenstatite	Heated white talc		Red	Red > blue	Moderate
Forsterite	From General Electric		Violet	Blue > red	Strong
Forsterite	From Harbison–Walker		Violet	Blue > red	Moderate
Periclase	From Harbison–Walker		Red	Red > blue	Moderate
Spinel, $MgO.Al_2O_3$	From Harbison–Walker		Green		Strong

* When viewed through appropriate filters.

This work was supported in part by grant *NSG*–593 from the U.S. National Aeronautics and Space Administration.

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PHYSICS

Aggregation of Ice Crystals in Strong Electric Fields

A RECENT series of experiments[1] has demonstrated that the adhesion of ice crystals on collision is a sensitive function of temperature, humidity and crystal-type. However, the effect of electric fields on the adhesion was not examined. Electric fields may have an important effect on the aggregation of ice crystals to form snowflakes inside thunder-clouds where the crystal concentrations are high and fields of several thousand V/cm have been measured. It appeared possible that the attractive forces between the crystals, produced by polarization charging within the field and accentuated because of the small radius of curvature of the crystal edges, may produce increased aggregation which would be especially important under conditions close to the threshold, in the absence of a field, between adhesion and separation. The experiments described here were performed in order to test this hypothesis.

Ice crystals were formed at a predetermined temperature inside a cold room[2] by producing a water cloud and seeding it with pellets of solid carbon dioxide. While the crystals were growing inside the cold room, small water drops of known identical diameter were suspended from the tips of fine glass fibres located centrally within two identical ebonite tubes (Fig. 1), and were then frozen by exposing them to a stream of cold crystal-free air. The ebonite tubes were located on top of the cold room, and the cooling stream was produced by passing air through copper coils surrounded by solid carbon dioxide. Windows inserted in the walls of the ebonite tubes permitted visual observation or photography of the spheres by means of a telescope. A variable high-voltage source, connected between two electrodes in the form of copper rings straddling one of the ice spheres, allowed this sphere to

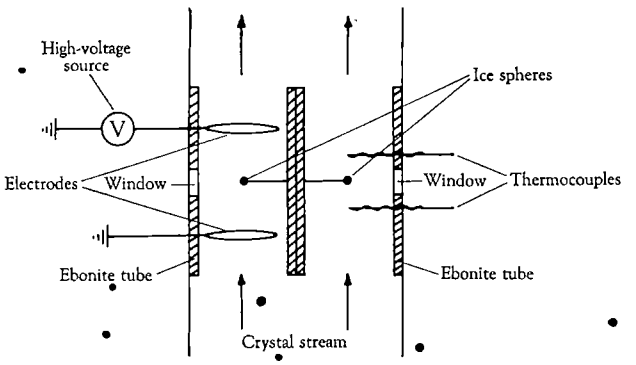


Fig. 1. Apparatus for measuring the aggregation of ice crystals in electric fields

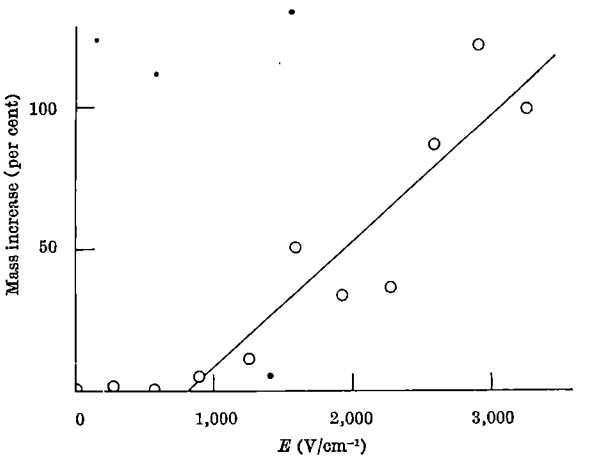


Fig. 2. The increase in mass of an ice sphere as a function of electric field strength E. Temperature of the crystal stream, − 9° C

be exposed to an electric field of up to about 4,000 V/cm^{-1} along the direction of the axis of the tubes. Subsidiary experiments showed that the field in the vicinity of the ice sphere could be calculated approximately by dividing the potential difference between the electrodes by their separation. In addition, the electrodes were found to have no appreciable effect on the crystal flow; dummy electrodes, not shown in Fig. 1, were situated inside the field-free tube in order to minimize differences between the air-flow inside the two tubes. The temperature at various points in the vicinity of the spheres was measured by means of thermocouples. The temperature of the air flowing past the spheres was continually adjusted in order to maintain that of the crystals inside the cold room. The rate of flow of air past the spheres could be controlled by varying the voltages applied to suction devices, and was measured by means of flow-meters.

When the ice crystals had been formed, the subsidiary cooling circuit was switched off and ice crystals were immediately drawn from the cold room to flow at a known speed past the two ice spheres, one of which was suspended in an electric field of pre-selected strength. The collection of ice crystals by the spheres was then observed through the telescope for the entire duration of the exposure of the ice spheres to the crystal streams. At the end of each experiment the crystal stream and electric field were switched off, and the aggregates of ice crystals and the spheres to which they adhered were melted by exposing them to a current of warm air of low velocity. The diameter of the two resultant water drops was measured using a micrometer eyepiece in the telescope and the mass increase of both drops was calculated from these measurements and a knowledge of the initial diameter of the drops.

Fig. 2 shows that although there was a large amount of scatter in the individual readings there was a definite increase in aggregation with increasing electric field. For field strengths below about 800 V/cm^{-1} there was no detectable difference between the masses collected by the two spheres but for higher fields the mass collected by the sphere situated inside the field was considerably greater than that collected by the sphere in the field-free tube. The visual observations confirmed these mass-charge measurements. If the electric field was below about 800 V/cm^{-1} the underside of the ice sphere collected a highly compact assemblage of ice crystals which was similar to that adhering to the ice sphere in the field-free tube. However, for higher fields, long chains of ice crystals protruded from the underside of the ice sphere (probably along the lines of force) and often attained a length of several hundred μ before folding inwards to adhere to other folded chains of crystals and thus creating a very open three-dimensional structure. Occasionally one of

the longer chains would be torn away from the ice sphere by aerodynamic forces; this probably accounts to a great extent for the large amount of scatter in the individual readings of increase in mass.

These experiments demonstrate that ice-crystal aggregation is appreciably influenced by the presence of strong electric fields. However, more comprehensive investigations are required in order to establish the influence of fields of thunderstorm magnitudes on ice-crystal aggregation under all the conditions of temperature, humidity and crystal-type which are of meteorological importance.

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Directional Property of Electrode Surfaces

It has been recognized for several years that when a conducting electrode is placed in an electrolyte in which there is an alternating potential gradient, it can assume a potential different from that expected[1]. In particular, it is generally found that the electrode potential is out of phase with the potential in the electrolyte by a variable amount. So far as I know, no explanation has been offered for the effect.

It is clear that it can occur only if the Helmholtz double layer at the electrode/electrolyte surface is non-uniform in the direction of the applied potential gradient, so that currents which pass from the electrolyte through the layer to the material of the electrode, and back into the electrolyte at a point of lower potential, are, electrically speaking, asymmetrical. Fig. 1 shows the section and plan of an irregular, non-uniform electrode, the surface of which is level with that of the insulating material in which it is embedded, the whole surface forming the boundary with an electrolyte in which there is a potential gradient E_z, unchanged by the introduction of the electrode. The electrode material is supposed to have a specific conductance high compared with that of the double-layer specific acceptance, so that the electrode potential is everywhere U_e, while the potential at the origin is arbitrarily taken as zero. The thickness of the double layer is small compared with the electrode dimensions, so that little error is introduced by assuming that current flow in the layer is everywhere normal to its surface.

With these assumptions, and noting that

$$\int_A j_y \mathrm{d}A = 0$$

where A is the total electrode area and j_y the current density normal to the electrode surface at any point, it follows that:

$$\int_{-a}^{a} w_z \sigma_z \, (U_e - E_z z) \, \mathrm{d}z = 0$$

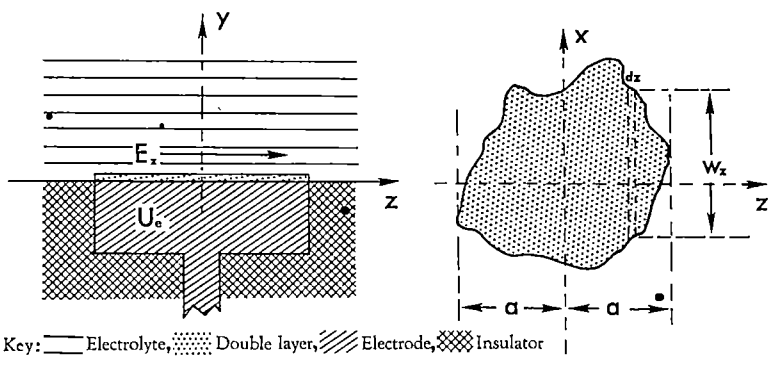


Fig. 1

where w_z is the length of a strip, dz, of electrode surface, and σ_z is the average admittance of the strip. Now both $w_z\sigma_z$ and U_e can be complex, and writing these $(p + iq)$ and $(U_p + iU_q)$ respectively $(i = \sqrt{-1})$, and solving the simultaneous equations which result from separation of the real and imaginary parts, it is found that:

$$\frac{U_q}{E_z} = \frac{\int_{-a}^{a} qz\mathrm{d}z \Big/ \int_{-a}^{a} q\mathrm{d}z - \int_{-a}^{a} pz\mathrm{d}z \Big/ \int_{-a}^{a} p\mathrm{d}z}{\int_{-a}^{a} p\mathrm{d}z \Big/ \int_{-a}^{a} q\mathrm{d}z + \int_{-a}^{a} q\mathrm{d}z \Big/ \int_{-a}^{a} p\mathrm{d}z}$$

$$\frac{U_p}{E_z} = \frac{\int_{-a}^{a} pz\mathrm{d}z \Big/ \int_{-a}^{a} q\mathrm{d}z + \int_{-a}^{a} qz\mathrm{d}z \Big/ \int_{-a}^{a} p\mathrm{d}z}{\int_{-a}^{a} p\mathrm{d}z \Big/ \int_{-a}^{a} q\mathrm{d}z + \int_{-a}^{a} q\mathrm{d}z \Big/ \int_{-a}^{a} p\mathrm{d}z}$$

Thus the quadrature component U_q is zero only when the centroid of the double-layer conductance coincides with the centroid of its parallel susceptance. Two obvious special cases are (*a*) p/q = constant, and (*b*) when both p and q are even functions of z. The results bear a close relationship also to the simple capacity bridge. Further, U_p is zero only in case (*b*).

The magnitudes of the components can quickly be assessed by considering the simple case of a rectangular electrode having its centre at the origin and its axes coincident with 0z, 0x. Let p be constant and equal to p_0, while q has values:

$$\begin{array}{rl} q_0 & -a < z < 0 \\ (1+\alpha)q_0 & 0 < z < a \end{array}$$

Then if $p_0 \approx q_0$ and α is small:

$$U_q \approx U_p \approx \alpha E_z a/8$$

and if $E_z = 2$ mV/cm, $a = 0{\cdot}025$ cm, $\alpha = 0{\cdot}1$, $U_q \approx U_p \approx 1$ μV.

The effect has been examined experimentally by rotating about their axes, relative to the potential gradient in the electrolyte, electrodes of 0·5 mm dia., and observing values of U_q and U_p at different angles. Typical plots of U_q using an electrode of bright gold are shown in Fig. 2. The effect is observed also with platinized electrodes, and a simple method has been discovered to make the surfaces reproducibly directional. The electrodes are platinized with their surfaces vertical, and the effect of gravity on the heavy platinum ions during electro-deposition is such as always to result in the double-layer asymmetry being along the vertical diameter. When platinization is carried out with the electrode surface horizontal, no asymmetry results. No surface variations of electrodes in good condition can be discerned under a low-power microscope (×40) whether or not asymmetry is found; on the other hand, any surface irregularity which can be discerned (for example, in damaged electrodes) can usually be related, from its angular position on the electrode surface, to the results of a rotational experiment.

The simple theory given here is inadequate when the current flow through the double layer is sufficient to produce a significant potential drop in the electrolyte. (This is true, for example, of platinized electrodes in 0·45 per cent sodium chloride solution.) In this case a quadrature potential gradient, which may be measured by means of an exploring probe, is produced in the electrolyte. If a pair of diametrically opposed electrodes are used, and the conducting medium between them be asymmetrical, an interelectrode quadrature potential is observed; and this would

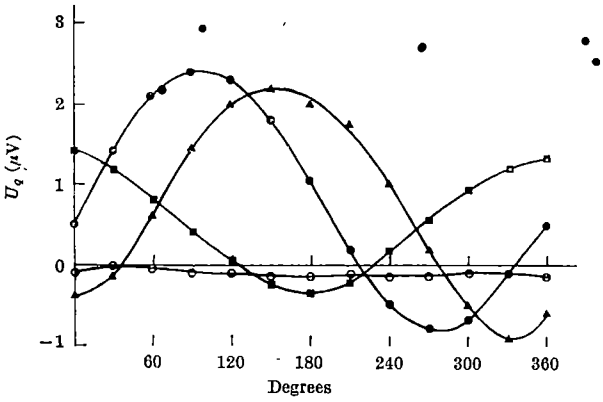


Fig. 2. ● Bright gold, 0·45 per cent NaCl; ▲ the same, after rubbing electrode surface along 0–180° diameter; ■ gold, platinized with 150°–330° diameter vertical; ○ gold, platinized with electrode surface horizontal

$E_z = 2{\cdot}2$ mV/cm, frequency 240 c/s.

be so even if the electrode surfaces were perfectly uniform. It appears to be this effect, and not that due to surface asymmetry of the electrodes, which has been observed by Hognestad[2].

The effect of surface asymmetry can be greatly reduced (as Schwan found) by fitting the electrode in a recess in the insulating wall, thus reducing the potential gradient at the electrode surface. A more detailed account of the experimental work, carried out in connexion with baseline errors in electromagnetic flowmeters for recording blood flow, will be given elsewhere.

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Laser Excitation of Powdered Solids

An understanding of the physical processes which occur when solids are subjected to intense surface heating is important to meteor and re-entry physics and to the physics of comets. These processes have been investigated spectroscopically in the laboratory by shock excitation[1,2], flash photolysis[3,4] and the impact flash[5]. As shown by Stoicheff[6], the high temperatures obtainable by using a focused ruby laser beam provide yet another means for the excitation of solids. It is the purpose of this note to report briefly on the spectroscopic investigations which have been made on laser excitation of molecular spectra from powdered solids during the past two years.

A commercial ruby laser with a nominal output of 2·5 joules and a pulse duration of 500 μsec has been used in this work. The beam is focused to a 0·5 mm diameter spot on the surface of the powder sample by an inexpensive microscope objective. The surface power density is thus about 3 MW cm^{-2}. Spectra of the 'flame' produced by the laser beam were recorded photographically in the wave-length range 2000 Å–7000 Å by Hilger *f*/4 Raman quartz and constant deviation glass spectrographs. The latter instrument was fitted with a 'Polaroid' camera back in place of its conventional film holder.

The powdered solid target materials used here were superior in performance to non-powdered solids because of their greater surface area, lower reflectivity and lower thermal diffusivity. Since most of the powders have also been shock excited[2], comparison between the two methods of excitation is possible.

The materials investigated and the gross features of their laser-excited spectra are displayed in Table 1. In all cases the experiment was performed in air at atmospheric pressure. As Debras-Guedon and Liodec[7] have

Table 1. Molecular Spectra produced by Laser Excitation of Powdered Solids in Air

Material	Observed molecular spectra*	Remarks
Al	AlO : Blue-Green ($A^2\Sigma^+ - X^2\Sigma^+$), UV($B^2\Pi - X^2\Sigma^+$)	Very strong
	AlH : ($A^1\Pi - X^1\Sigma$)	
Al_2O_3	AlO : Blue-Green ($A^2\Sigma^+ - X^2\Sigma^+$)	Moderate strength
B	BO : ($A^2\Pi - X^2\Sigma^+$)	Very strong
	BO_2 : Fluctuation bands	Very strong
BN	BO : ($A^2\Pi - X^2\Sigma^+$)	No BN bands
	BO_2 : Fluctuation bands	
$Ba(AlO_2)_2$	BaO : ($A^1\Sigma - X^1\Sigma$)	No AlO bands
C	C_2 : Swan ($A^3\Pi_i - X'^3\Pi_u$)	Very strong
	Deslandres-d'Azambuja ($c^1\Pi_g - b^1\Pi_u$)	Very strong
	CN : Violet ($B^2\Sigma^+ - X^2\Sigma^+$)	Very strong
CaS	"CaO" : Orange and Red systems at 5550 Å and 6230 Å	
CuO	Cu_2 : ($B^1\Pi_u - X^1\Sigma_g^+$)	
	CuO : Orange, Violet	
CuS	Cu_2 : ($B^1\Pi_u - X^1\Sigma_g^+$)	
	CuO : Orange, Violet	
Fe	FeO : A,B	
$H_3W_4O_8$	WO	
PbS	PbS : (A)	Very weak
	AbO : (A)? PbO: (B)	Identification uncertain
TiO_2	TiO : Red, ($A^3\Delta - X^3\Pi$), Blue-Green ($C^3\Pi - X^3\Pi$)	Strong bands, many atomic lines
V_2O_5	VO : ($^3\Delta - ^3\Delta$(?))	Many atomic lines
W	WO	

* Spectra cited in the same manner as by Pearse and Gaydon[9].

discussed laser-excitation of atomic spectra, the atomic features of our spectra are not listed in Table 1. All spectra were relatively free from overlapping continuum. No molecular spectra were observed when BeO, Bi, Cd, Mg, MgO, Pb, and S respectively were used as targets for the focused laser beam.

The strong preponderance of oxide band systems observed is consistent with the oxidizing atmosphere in which they were excited and supports the belief that much of the molecular spectrum arises from chemical reactions in the flame. This view is also supported by the work of Howe[8], who observed that the C_2 emission from laser-excited carbon begins promptly with onset of the laser pulse, while the accompanying CN emission is delayed by 15 μsec. It is interesting to note that the white powders, BN, TiO_2, CaS, give rise to laser-excited spectra while some black powders, Si, PbS, do not.

Intensity measurements are now being made on the molecular spectra to determine vibrational and rotational temperatures. Time-resolved investigations are also being made.

We thank Dr. B. Stoicheff for helpful discussions. The work is supported by grants from the Air Force Office of Scientific Research (*AF–AFOSR* 62–236*A*) and from the National Aeronautics and Space Administration (*NSG* 349).

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Annealing Behaviour of Recoil Cobalt-57 Atoms in Hexammine Nickel Chloride

Recoil chemistry of ^{57}Co (half-life: 270 days) produced by the (γ,p) reaction or by the decay processes (β^+ and *EC*) in hexammine nickel chloride has not been studied so far. In this work, studying behaviour of recoil ^{57}Co atoms in the nickel complex on thermal annealing, I have found remarkable difference between annealing reactions of ^{57}Co atoms produced by the $^{58}Ni(\gamma,p)^{57}Co$ reaction and those of ^{57}Co produced by the decay processes of ^{57}Ni.

Hexammine nickel chloride $Ni(NH_3)_6Cl_2$ was prepared by the orthodox method and recrystallized in concentrated ammonia[1]. $Ni(NH_3)_6Cl_2$ labelled with ^{57}Ni (half-life: 36 h) was prepared in the same way as aforementioned starting from metallic nickel powder irradiated by γ-rays of 20 MeV. γ-Rays were generated by an electron linear accelerator (maximum energy: 20 MeV, mean beam current: 30 μamp). The target material was irradiated while being cooled by dry ice. Radiation decomposition was minimized by cooling (< about 1 per cent). Chemical forms of ^{57}Co were analysed by a cation exchanger column ('Diaion *SK* 1', 100–200 mesh, 1 cm × 7 cm), after dissolving the irradiated complex in 0·1 N hydrochloric acid. The Co^{2+} fraction was further separated from Ni^{2+} or $Ni(NH_3)_6^{2+}$ using an anion exchange column ('Diaion *SA* 100', 100–200 mesh, 1 cm × 3 cm) under strongly acid condition (> 8 N HCl). By these procedures principal chemical forms of ^{57}Co could be separated satisfactorily[2]. Radioactivity of ^{57}Co samples was measured by a 20-channel γ-ray spectrometer of the Baird-Atomic Instrument Co.

The distribution of recoil species thus analysed is listed in Table 1. In the case of the (γ,p) reaction, Co^{2+} (or its precursor) changes into $Co(NH_3)_5Cl^{2+}$ and $Co(NH_3)_6^{3+}$ on thermal annealing at 87° C. Especially increase of the $Co(NH_3)_5Cl^{2+}$ fraction is the main event in this case. On the decay processes, however, the main event on thermal annealing is increase of $Co(NH_3)_6^{3+}$, which corresponds to the decrease of Co^{2+} (or its precursor). For the samples standing at room temperature, distribution of recoil products shows similar tendency to the annealed one.

These phenomena seem to be very important in interpreting annealing behaviour of recoil cobalt atoms because ^{57}Co recoil chemistry in nickel complexes eliminates possibilities of guided reactions to the parent species which are usually observed in ^{60}Co recoil atoms produced by the (n,γ) reaction. In this sense, annealing of ^{57}Co atoms is free from guiding to the parent species in the damaged zone in the crystal.

The reason why increase of $Co(NH_3)_5Cl^{2+}$ is the main event on annealing of ^{57}Co atoms produced by the (γ,p) reaction will be understood by considering a fairly large fragmentation zone around the ^{57}Co recoil atoms (maximum recoil energy: ~ 0·2 MeV). Annealing reactions here can be interpreted to be of synthetic type among the fragments such as Co^{2+}, Co^{3+}, $Co(NH_3)_x^{2,3+}$, NH_3, Cl^0, Cl^-, etc. Therefore, remarkable increase of $Co(NH_3)_5Cl^{2+}$ occurs by recombination of fragments.

In the case of ^{57}Co atoms produced by the decay processes fragmentation will be very small, and electronic change around ^{57}Co atoms will be important, because recoil energy of the atoms on the decay processes is small (maximum: 34 eV), and charge distribution will be created on the decay processes[3]. As in Wertheim's observation[4], a recoil cobalt atom will finally acquire +2 or +3 valence state in the crystal in very short time after the decay processes. At least some percentage of decayed ^{57}Co will remain in the chemical forms of $Co(NH_3)_6^{2+}$ or $Co(NH_3)_6^{3+}$, leaving electronic defects around the decayed atom. Annealing reaction in this region will be mainly of electronic type. For example, $Co(NH_3)_6^{2+}$ (unstable in an aqueous solution) will be converted into $Co(NH_3)_6^{3+}$ (stable in an aqueous solution) by giving up one electron to the neighbouring electronic defects. This possibility has already been discussed by Harbottle and myself[5,6]. In Table 1, increase of $Co(NH_3)_6^{3+}$ on annealing of decayed ^{57}Co atoms will most probably be interpreted by change of its electronic state. The difference between the main tendencies in annealing behaviour of ^{57}Co produced by the (γ,p) reaction and that of the decayed atom will be a good indication of two types of annealing reactions: one, a synthetic type, and the other, an electronic type.

I thank Dr. N. Shibata for his discussions, Mr. M. Hara and Mr. H. Nozaki for their assistance.

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Jets from Imploding Bubbles

Bowden has suggested that micro-Munro jets may play a part in the initiation of non-homogeneous explosive systems[1]. In addition, he and Brunton have shown that such jets can be formed by impacting a vessel containing a liquid with a hemispheric cavity located on its surface[2]. In an investigation of possible mechanisms for the initiation and propagation of low-velocity detonations in a cavitated liquid explosive, we have investigated the formation and subsequent behaviour of jets from bubbles in water and nitroglycerin–ethylene glycol dinitrate (NG–EGDN).

In these experiments, roughly hemispherical cavities were formed on the surface of the liquid under an overlay of vinylidene chloride film having a thickness of 0·0006 in. The cavities were then collapsed by a shock wave derived from a tetryl donor and attenuated by an aluminium plate 0·75 in. thick.

Selected views from a framing camera sequence showing water micro-jets are presented in Fig. 1. In this trial, a 1-in. diameter × 1-in. long tetryl donor was used; the depth of water, contained in a transparent tank with the attenuator serving as a base, was 1 in. In frame 2 of the sequence, a hemispherical shock wave can be seen approaching the four bubbles in the left side of the container. The

Table 1. Distribution of Recoil ^{57}Co Atoms in $Ni(NH_3)_6Cl_2$

Process	Irradiation	Storage	Annealing	Co^{2+}	$Co(NH_3)_5Cl^{2+}$	$Co(NH_3)_6^{3+}$
(γ,p)	−79° C 30 min	−79° C 15 min	No	82 ± 2%	4·4 ± 0·4%	14 ± 1%
		−79° C 15 min	87° C 30 min	54 ± 2	24 ± 1	22 ± 1
		−79° C 24 h*	No	78 ± 2	3·7 ± 0·4	18 ± 1
		−79° C 95 days	No	82 ± 2	3·3 ± 0·3	15 ± 1
β^+ and *EC*		−79° C 20 days	No	66 ± 2	1·6 ± 0·1	32 ± 1
		−79° C 21 days	87° C 60 min	52 ± 2	1·2 ± 0·1	45 ± 1
		Room temperature	No	44 ± 1	1·6 ± 0·1	51 ± 2

* Additional effects of the decay processes of ^{57}Ni (half-life: 36 h) produced by the (γ,n) reaction should be considered. However, the effects are small within 1 h after irradiation.

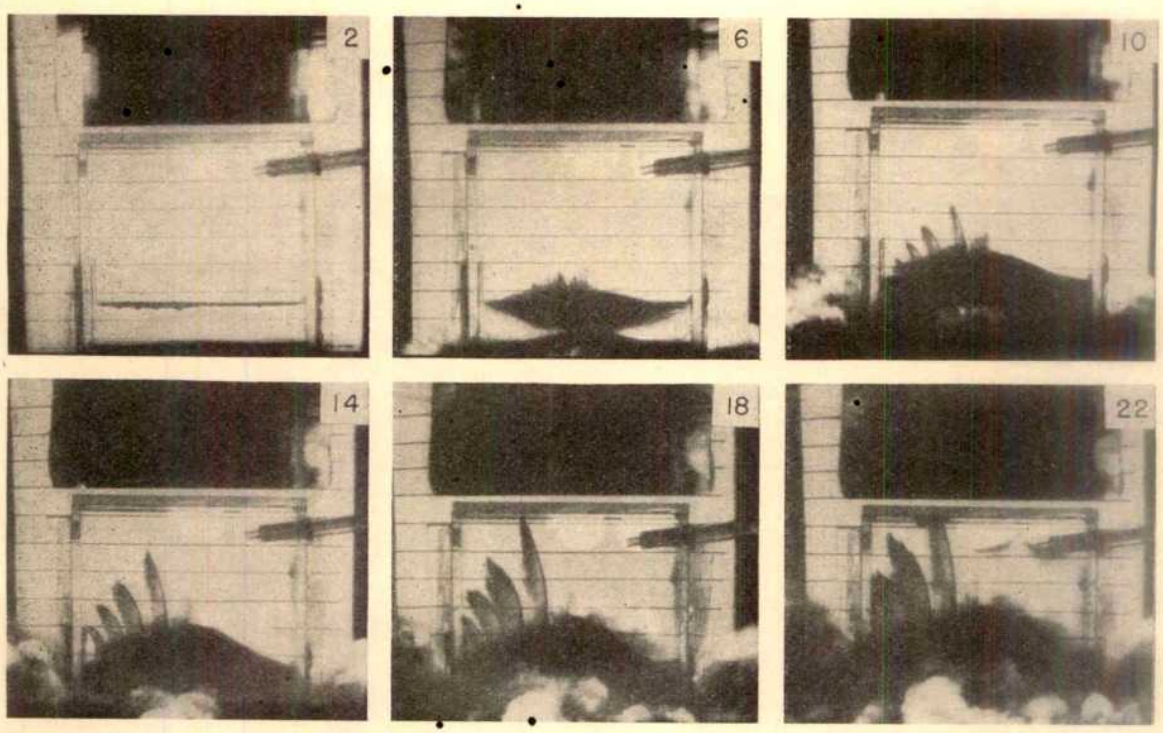


Fig. 1. Selected frames from a sequence showing micro-jets of water; the interframe time was 2 μsec

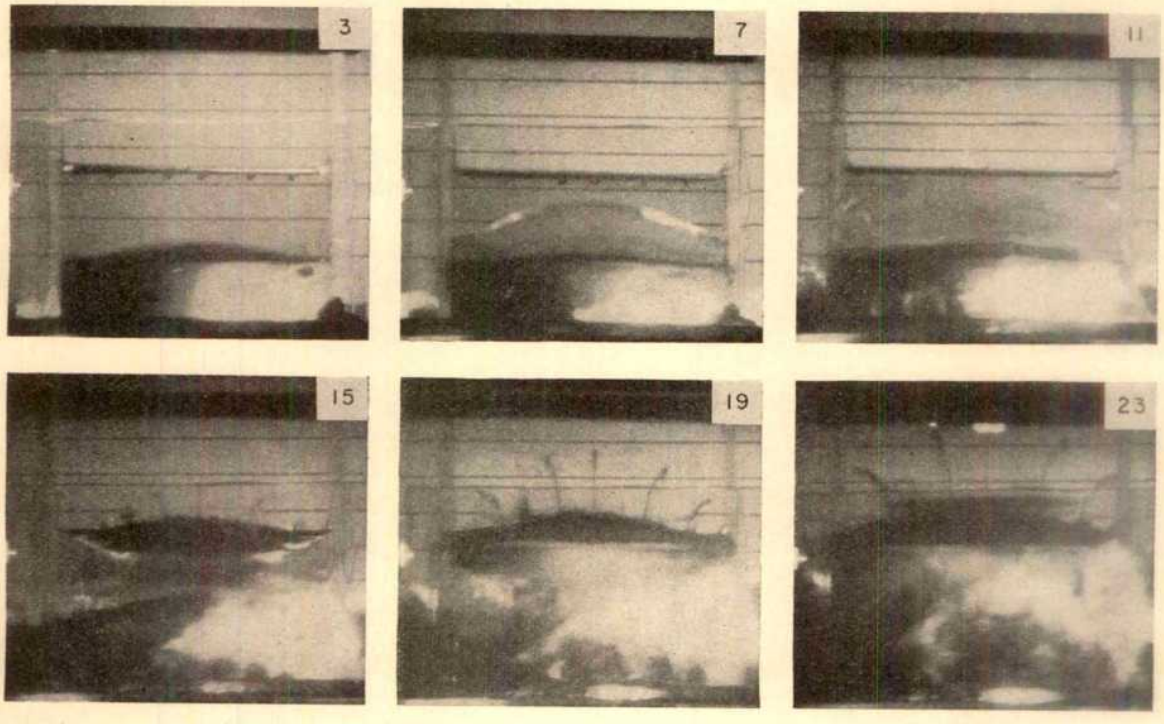


Fig. 2. Micro-jets of NG–EGDN impacting an aluminium barrier; the interframe time was 1 μsec

shock strength at this point, estimated from independent experiments, is approximately 10 kilobars. Frames 6, 10, 14 and 18 clearly show that each of the cavities produces a high-velocity jet. It will be noted that the velocity of the jets is strongly influenced by the divergence of the shock front. The velocity of the fastest jet, which can be seen striking a 0·25-in. aluminium barrier in frame 22, is estimated to be 1·9 mm/μsec. Subsequent examination of the barrier showed distinct pitting as a result of jet impact. It has been suggested[2] that the damage of solids by 'cavitation' is due in some part to this type of impact. Micro-jets of adequate velocity to produce 'cavitation' damage might arise from the interaction of two or more neighbouring cavities where the shocks generated by the collapse of one would serve to produce jetting in others.

Several frames from a sequence of a similar trial using NG–EGDN are shown in Fig. 2. In this case a $1\frac{5}{8}$-in. diameter × $1\frac{1}{2}$-in. long tetryl donor was used with a liquid fill of 0·5 in. The peak shock strength, inadequate for the initiation of a high-velocity detonation in NG–EGDN, is estimated to be approximately 38 kilobars when it enters the fluid; it has decayed to about 20 kilobars when it

encounters the cavities. Again, as in the case of the trial using water, each of the cavities produces a high-speed jet. Furthermore, while initially non-luminous and presumably non-reacting, each of the jets was observed to react explosively when it impinged on the aluminium barrier. Two such reactions can be seen in frame 23. The velocity at impact of the central jet is approximately 2·5 mm/μsec, which yields a peak impact pressure estimated to be in excess of 100 kilobars. Since for NG–EGDN this pressure is adequate for the initiation of a high-velocity detonation, the explosion in impact observed here can well result from shock heating.

These observations are believed to be especially pertinent to the initiation and propagation of a low-velocity detonation in liquid explosives. Earlier work conducted at the Bureau indicated that cavities played an important part in the initiation of liquid explosives[3]. More recent investigations have shown that cavities are generated by precursor waves propagated through container walls in the case of stable low-velocity detonations. These cavities serve as initiation centres, and while the exact initiation mechanism is not known micro-jetting appears to be a likely one. The results of this investigation into the nature of low-velocity detonations will be reported later.

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CRYSTALLOGRAPHY

Crystal Structure of Manganese *Cyclo*pentadienide

The crystallographic constants of manganese *cyclo*pentadienide[1] indicate that the crystal structure of this compound is different from those of other ferrocene-type molecules. Elucidation of its structure is imperative for an explanation of the strong metal–metal interactions found to exist in the crystals of manganese *cyclo*pentadienide. Magnetic measurements performed by Cotton, Wilkinson and Birmingham showed the crystals to be antiferromagnetic with a Néel temperature of 134° K (ref. 2).

A crystal of manganese *cyclo*pentadienide was obtained by vacuum sublimation and introduced in a Lindemann glass capillary in a nitrogen atmosphere. Its morphology was similar to that described by Weiss and Fisher[1], and the cell dimensions and systematic absences as determined by iron and molybdenum equi-inclination Weissenberg and precession photographs are essentially identical to those previously published: $a = 28{\cdot}03 \pm 0{\cdot}03$ Å, $b = 11{\cdot}74 \pm 0{\cdot}02$ Å, $c = 9{\cdot}96 \pm 0{\cdot}01$ Å, $\alpha = \beta = \gamma = 90°$, $Z = 16$; $hkl : h + k = 2n$, $0kl : k + 2l = 4n$, $h0l : h = 4n$, $hk0 : h = 2n$, and $k = 2n$ only. The space groups $Cm2a$ and $Cmma$ are consistent with the observed absences but do not allow for all of them. Moreover, it is impossible to find an acceptable arrangement of the 16 molecules in the unit cell consistent with both the symmetry and the additional absences.

The following observations indicate that the apparent orthorhombic symmetry results from the twinning of monoclinic crystals.

(1) High-order reflexions with both h and $k \neq 0$ on Fe $K\alpha$ $hk0$ and $hk1$ Weissenberg pictures are split, indicating that the observed pattern is a superposition of two almost orthogonal reciprocal lattices related by a mirror plane perpendicular to the common axis. The size of the splitting is consistent with a γ angle of 90° 17′.

(2) The three-dimensional Patterson map calculated from $hk0$–$hk8$ Mo $K\alpha$ Weissenberg and $0kl$ molybdenum $K\alpha$ precession camera data can only be interpreted as the superposition of two Patterson maps related by a mirror plane // (100) (or // (010)).

The relationship between the orthorhombic and the primitive monoclinic cell is shown in Fig. 1. The cell dimensions of the latter are: $a = 15{\cdot}20 \pm 0{\cdot}02$ Å, $b = 11{\cdot}74 \pm 0{\cdot}02$ Å, $c = 9{\cdot}96 \pm 0{\cdot}01$ Å and $\gamma = 114° 46' \pm 3'$. Though transformation of indices and co-ordinates from the large C centred cell to the monoclinic cell is straightforward we have found it convenient to refer indices and co-ordinates to the almost orthogonal C-centred cell.

The primitive monoclinic cell has the space group $P2_1/b$ ($Z = 8$) (c unique), which allows for the absences of $hk0$ with h and/or k odd and of $00l$ with l odd (indices refer to C-centred cell). The additional absences of the $0kl$ and $h0l$ reflexions require that the two molecules in the asymmetric unit are related as x, y, z and $\frac{1}{4} - x$, $y + \frac{1}{4}$, $z + \frac{1}{2}$. Manganese co-ordinates consistent with this condition could be derived from the Patterson map; they are:

$x_1 = 0{\cdot}033_5$	$y_1 = 0{\cdot}027$	$z_1 = 0{\cdot}25$
$x_2 = 0{\cdot}21_5$	$y_2 = 0{\cdot}277$	$z_2 = 0{\cdot}75$

These positions are shown in Fig. 1. Short Mn^{++}–Mn^{++} vectors are found in a plane roughly parallel to (100), the distance between two manganese ions related by the centre of symmetry at the origin being 5·36 Å, while the ions related by the b glide plane are 5·87 Å away from each other (almost equal to the 5·91 Å a axis translation in ferrocene[2]). In a direction perpendicular to the (100) plane all Mn^{++}–Mn^{++} distances are greater than 7·3 Å. This arrangement suggests that the anomalous magnetic properties of manganese *cyclo*pentadienide result from interionic interactions within the layers // (100) and it predicts a two-dimensional magnetic ordering in the anti-

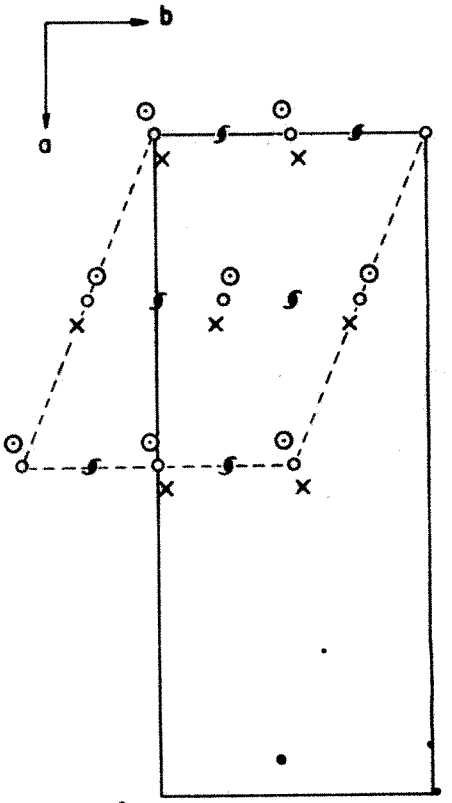


Fig. 1. Relationship between the two unit cells and the positions of the Mn^{++} ions. ×, Mn^{++} at or close to $z = 0{\cdot}25$; ○, Mn^{++} at or close to $z = 0{\cdot}75$

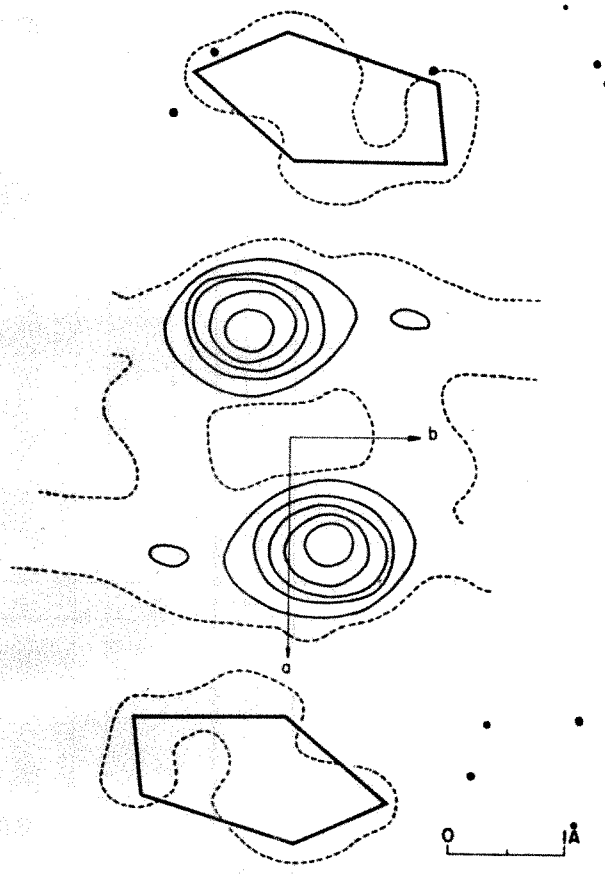


Fig. 2. *hk*0 Fourier map with Mn^{++} signs only. Contours at arbitrary intervals, zero contour broken. Maxima corresponding to ring atoms are seen on both sides of the large peaks representing Mn^{++} ions. One of these rings is drawn, the other overlaps the Mn^{++} peak

ferromagnetic state, similar to the ordering observed in Cu formate tetrahydrate[4,5].

As a result of the twinning an observed intensity I_{hkl} is a sum of the intensities of the hkl and $h\bar{k}l$ reflexions. Thus, for hkl reflexions one cannot obtain values of F_{hkl} by applying the usual correction values. However, it can be shown that for $h = 2n$, $k = 2n$, $|F_{hkl}| = |F_{h\bar{k}l}|$. Since the $hk0$ level contains only such reflexions it is possible to calculate the $hk0$ Fourier map in the usual way. This map, with signs based on Mn^{++} contributions only, is shown in Fig. 2.

Unresolved maxima appear which can be identified as the *cyclo*pentadienide rings. As anticipated, these rings are roughly parallel to the layers containing the short Mn^{++}–Mn^{++} vectors.

The details of the arrangement of the carbon atoms are as yet uncertain: a further investigation of the structure is in progress.

I thank Dr. D. R. Christman for preparing a sample of manganese *cyclo*pentadienide.

This work was carried out in part under the auspices of the U.S. Atomic Energy Commission.

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CHEMISTRY

Significance of the Solubility of Hydrogen Halides in Mercaptans and Thioethers

The low ideal (Raoult's law) solubility of hydrogen chloride has revealed the factors determining the real solubility of hydrogen chloride in a considerable number of compounds. From the data on the solubility expressed as moles of HCl per mole of an organic compound containing oxygen there appears to be a remarkable correlation between the solubility and the basic (or hydrogen bonding) function of the oxygen atom[1]. Extension of this work to observations on certain sulphonyl compounds[2] naturally led to the examination of mercaptans and thioethers. Solubility of hydrogen halides (HCl, HBr and HI) in these compounds concerns the basic or hydrogen bonding function of the sulphur atom.

Solubilities were determined by weighing the maximum amount of hydrogen halide absorbed at atmospheric pressure by about 2 g of compound in a U-tube at 0°. Although in previous investigations the solubilities were determined over ranges of temperature (see ref. 1 for refs. to particular papers), in this initial investigation of the sulphur compounds the temperature was maintained at 0°. In Table 1 are expressed the solubilities as moles of H*X* per mole of sulphur compound at 0°. The purity of each compound was checked by boiling point and gas-liquid chromatography, and after absorption of H*X* the compound was recovered by water treatment and so identified with the original compound.

Available results for the corresponding oxygen compounds are given in Table 1. For alcohols such as *n*-butanol the solubility of hydrogen chloride at 0° is more than 1 mole/mole, and although the solubilities in the corresponding mercaptans are much smaller they are materially larger than the corresponding ideal value, and point to a specific 'association' between sulphur and H*X*. To draw a line of demarcation between hydrogen bonding and basic function of a donor atom is a matter of definition, and we do not enter into this controversy here.

Table 1. Solubilities (moles of H*X* per mole of substance) at 0°

Substance	HCl	HBr	HI
Ideal	0·041	0·691	0·370
n-Decane	0·049	0·10	0·37
C_4H_4S*	0·034 —	0·14	‡
PhSH	0·093 (0·138)	0·18	0·39
Bu^nSH	0·125 (1·065)	0·35	0·67
Pr^iSH	0·225 (1·130)	0·29	0·45
Ph_2S	0·144 (0·117)	0·23	0·50
Bu^n_2S	0·640 (1·061)	2·53	2·89
Pr^i_2S	0·659 (1·157)	2·76	3·18
C_4H_8S†	0·673 (1·716)	1·0 §	1·0 §

The values in brackets are for the corresponding oxygen compound.
* Thiophene
† Tetrahydrothiophene
‡ Reacts further
§ White solid formed (see text).

Whenever the phenyl group is attached to oxygen, not only in phenol but also in phenyl esters, the basic function of oxygen is greatly reduced as compared with the corresponding *n*-butyl system. This effect is attributed to mesomerism involving an oxygen lone pair of electrons and the π-system of the ring[1]. A similar effect is clearly reflected in the thiophenol and diphenyl sulphide examples.

The dialkyl sulphides show lower solubilities for HCl than do the alcohols, but the solubilities are still much greater than ideal. Unfortunately data for HBr and HI are not available for the alcohols because of further quick enough reaction to give alkyl halides; but the sulphides show remarkably high solubilities, and it is a matter for further investigation to elucidate in precise terms the mode of 'association'.

Solubilities in thiophene are very low; but in tetrahydrothiophene the dialkyl sulphide value appears again for HCl. However, in the examples of HBr and HI a white crystalline solid was obtained at the 1 : 1 mole

ratio. By a suitable control in the passage of HBr, and by holding the temperature between 0° and about 20°, it appeared that if the actual formation of crystals was avoided, much more HBr was absorbed.

There have been diverse opinions on the hydrogen bonding function of the SH group with reference to donor molecules[3], and evidence for sulphur as the donor atom in hydrogen bonding is sparse[4]. In consideration of the increasing spectroscopic attention being given to hydrogen bonding and related basic function, the solubility data now presented give justification for further examination of sulphur compounds, particularly as the results might have a bearing on the function of sulphur in protein chemistry.

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Photochemistry and Radiation Chemistry of Fluorobenzene in Aqueous and Other Solutions

One of the problems in the radiation chemistry of solutions, and aqueous solutions in particular, is whether the chemical changes observed are due to ionization processes and free radical formation, or to excitation processes (which would resemble photochemical reactions). In a previous investigation[1] we discussed the action of ionizing radiations, compared with that of ultra-violet light on aqueous solutions of benzene. It was shown that in this case the action of ionizing radiations led to the formation of products different from that of ultra-violet light and, in particular, the *p*H-dependence of the two processes was entirely different. It was thus possible to differentiate between the two reaction mechanisms and to show that at least in dilute solutions excitation processes do not significantly contribute to the effects of ionizing radiations.

We have now investigated the related case of fluorobenzene. This was chosen since fluorine, as an aromatic substituent, has properties which in many aspects resemble those of hydrogen. In particular, the bond energy C—F on aromatic carbon is even stronger (115 ± 3 kcal) (ref. 2) than the aromatic C—H bond (102 kcal). The absorption spectrum of fluorobenzene in aqueous solution scarcely differs from that of benzene itself. The solubility of fluorobenzene in water is like that of benzene.

On the other hand, fluorine in aqueous solution, if displaced from the aromatic bond, would form the negative F^- ion accompanied by the gain of energy due to high electron affinity of the F-atom and the high solvation energy of the fluoride ion. If hydrogen is displaced at all, it should form the positive H^+ ion. Work on the radiation chemistry of organic fluorine compounds has generally indicated lack of reactivity[3]. It was now found when aqueous solutions of fluorobenzene were irradiated in the presence of oxygen, with ionizing radiations from a 200 kVp X-ray set, that F^- was formed in the solution with a *G*-value which was somewhat influenced by the *p*H varying from about 3·35 at *p*H 1·2 to about 1·6 at *p*H 8·4. This relatively high *G*-value of fluoride formation was accompanied by the formation of hydrogen peroxide showing a change with *p*H from 2·1 at *p*H 1·2–1·5 at *p*H 8·4. When irradiations were carried out in the absence of oxygen, the *G*-value of fluoride formation was

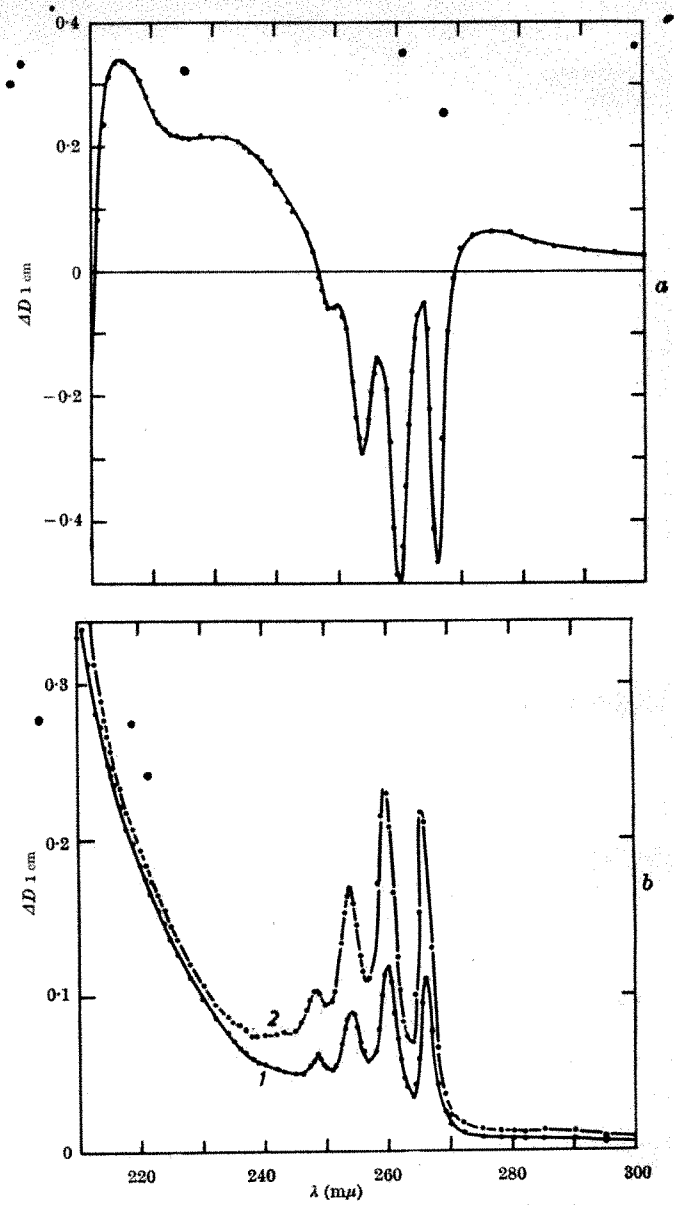


Fig. 1. Difference spectra obtained in 1 cm path-length optical cells of $9{\cdot}5 \times 10^{-4}$ M solution of fluorobenzene in ethanol. *a*, Difference between solution irradiated at 2537 Å *in vacuo* with $1{\cdot}2 \times 10^{-5}$ einstein/ml. and unirradiated solution; *b*, difference between irradiated solution after contact with air for (1) 1 h and (2) 19 h and the same solution before admission of air

4·55 at *p*H = 3·1 while that of hydrogen peroxide formation decreased to approximately 0·6. These results are entirely consistent with efficient displacement of fluoride from the aromatic ring both by OH radicals and by solvated electrons and H atoms. The high yields are to be noted in view of the low solubility (about 0·1 per cent or 0·01 molar) in aqueous solution.

By comparison on illuminating aqueous solutions of fluorobenzene in the presence or in the absence of oxygen with ultra-violet light of 2537 Å, it was found that the quantum yield of fluoride formation had the relatively low value of approximately 0·015, changing little with *p*H. It was found, however, that in these photolysed solutions another process proceeded with greater efficiency in oxygen-free solutions, leading to the disappearance of the absorption band of fluorobenzene in the 260 mμ region. This photochemical change had a quantum yield of more than 0·2 and was almost entirely reversible on admission of oxygen. No evidence for such effects was obtained using ionizing radiations.

In order to gain further information concerning the nature of this reaction, we investigated the process using solutions of fluorobenzene in ethanol or *n*-heptane as well. Here, too, a similar disappearance of the band due to fluorobenzene was observed, the change being largely reversible on admission of oxygen. The quantum yields are lower at comparable concentrations in ethanol and heptane than in water. We investigated the dependence of quantum yields on fluorobenzene concentration in the non-aqueous solvents and found results which, allowing for the number of quanta absorbed, indicated a first-order dependence on concentration. Therefore, the results would be consistent with a formation of a dimer. In view of previous results in the literature[4], it is not impossible that strongly polar fluorine might facilitate the formation of dimers containing, for example, *cyclo*butane rings. However, we have no definite evidence on this point and hope to elucidate this further.

We thank Prof. E. Lippert for his advice. One of us (L. M. R.) thanks the International Atomic Energy Agency, Vienna, for a fellowship.

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Production of Hydrogen in the Radiolysis of Methanol Vapour

DURING an investigation of the radiolysis of methanol vapour Baxendale and Sedgwick[1] quantitatively accounted for all product yields in terms of those initial ionization processes observed in the mass spectrometer[2], where the important reactions have been shown to be:

$$e^- + CH_3OH \rightarrow CH_3OH^+ + 2e^- \quad (1)$$

$$\rightarrow CH_2OH^+ + H + 2e^- \quad (2)$$

$$\rightarrow CHO^+ + H_2 + H + 2e^- \quad (3)$$

They further assumed that as a result of reactions such as:

$$CH_3OH^+ + CH_3OH \rightarrow CH_3OH_2^+ + \cdot CH_2OH \quad (4)$$

and:

$$CH_2OH^+ + CH_3OH \rightarrow CH_3OH_2^+ + CH_2O \quad (5)$$

the only important neutralization process is:

$$CH_3OH_2^+ + e^- \rightarrow CH_3OH + H \quad (6)$$

The foregoing ion-molecule reactions have been observed in the mass spectrometer by Lindholm[3]. The species e^-, H and $CH_3OH_2^+$ can therefore be considered the probable precursors of hydrogen. It was further suggested that the methanol molecule formed in reaction (6) is in an excited state and dissociates to molecular hydrogen and formaldehyde.

It has been found possible to identify H and e^- in the liquid phase radiolysis of both alcohols and water by the use of appropriate solutes. In the work recorded here we have attempted a similar approach in the gas phase, using nitrous oxide, methyl bromide and propylene as potentially efficient scavengers.

Irradiations were carried out with 260-kV electrons produced in single pulses of 0·2 μsec duration from a modified C.S.F. klystron unit coupled with a Field Emission Corporation pulser, model 233. The absorbed dose of 10^{17} eV per pulse was determined using nitrous oxide at 165 mm mercury pressure as dosimeter, assuming $G(N_2) = 9{\cdot}68$ (ref. 4). Air-free samples of methanol were irradiated at a pressure of 200 mm mercury and a temperature of 40°–42° in a 3-l. 'Pyrex' cell fitted with a 10μ thick stainless steel window. Methanol (Merck reagent grade) was purified as described previously[5]. Methyl bromide, nitrous oxide and propylene were purified by trap-to-trap distillation. The product gases were removed from the methanol after irradiation and the yields were determined by gas-solid chromatography.

The following yields were obtained for pure methanol:

$$G(H_2) = 10 \pm 2 \quad G(CH_4) = 0{\cdot}5 \pm 0{\cdot}1 \quad G(CO) = 1{\cdot}0 \pm 0{\cdot}2$$

These are the mean values for ten experiments investigated over the range 4×10^{17} eV/g to 5×10^{18} eV/g, the higher doses being obtained by accumulation of successive pulses. They are in good agreement with those of Baxendale and Sedgwick, apart from the methane yield which is appreciably higher.

The reactions:

$$N_2O + e^- \rightarrow N_2 + O^- \quad (7)$$

and:

$$CH_3Br + e^- \rightarrow \cdot CH_3 + Br^- \quad (8)$$

have been shown by electron impact investigations to occur effectively at or near zero electron energies[6–8]. In the methanol system these reactions will result in the production of nitrogen and methane respectively in the presence of the two additives, with a corresponding reduction in the hydrogen yield. However, the addition of up to 1 per cent v/v nitrous oxide was found to have no effect on $G(H_2)$, and the nitrogen yield, after correction by electron fraction for direct absorption by the solute, was found to be $G(N_2) < 0{\cdot}1$. In the presence of up to 3 per cent methyl bromide again no reduction in $G(H_2)$ was observed and $G(CH_4)$ remained constant. We therefore conclude that, at the highest additive concentrations used in this work, neutralization processes such as reaction (6) are not affected.

Alkyl halides are known to be efficient H atom scavengers in the liquid phase, and in the case of methyl bromide the reaction is:

$$CH_3Br + H \rightarrow \cdot CH_3 + HBr \quad (9)$$

The constancy of $G(H_2)$ and $G(CH_4)$ yields observed further suggests that there is no appreciable contribution to the hydrogen yield from thermal H atoms.

Previous work[1] has shown that in the presence of less than 1 per cent benzene the hydrogen yield is half that observed in pure methanol. It was suggested that benzene both scavenges H atoms and quenches excited methanol molecules. It was pointed out, however, that the latter mechanism would require a long-lived excited state of methanol and, in the light of our experiments with methyl bromide, the former mechanism appears less attractive. Because of the low ionization potential of benzene the following charge transfer reaction may also be important in the radiolysis of methanol–benzene mixtures:

$$CH_3OH^+ + C_6H_6 \rightarrow CH_3OH + C_6H_6^+ \quad (10)$$

This reaction is exothermic to the extent of 1·6 eV (ref. 9), and in order to compete favourably with reaction (4) it requires a very high rate constant.

We have found that propylene also reduces the hydrogen yield. In the presence of 0·03 per cent v/v propylene $G(H_2) = 5{\cdot}6$, that is, the efficiency of propylene is approximately 40 times higher than that of benzene. At 1 per cent v/v and 2 per cent v/v propylene $G(H_2) = 1{\cdot}9$, in agreement with the limiting or 'molecular' yield found using benzene[1]. The corresponding charge transfer reaction between C_3H_6 and CH_3OH^+ is also exothermic to the extent of 1·1 eV (ref. 9). In contrast, the charge transfer reactions involving nitrous oxide and methyl bromide are respectively endothermic to the extent of 2 eV and approximately energetically neutral[8].

By extending this work in the methanol system to a wide variety of additives, we intend to test this apparent correlation between ionization potential and the ability of the solute to affect the radiolytic yields.

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BIOCHEMISTRY

Electron Microscope Investigation of Lactic Dehydrogenase Agent

The lactic dehydrogenase (LDH) agent, detectable by increased levels of plasma or serum LDH in inoculated test mice, was found in association with a variety of transplanted murine tumours by Riley *et al.*[1]. Various estimations of its size by filtration methods have been suggested. These include diameters of 2 mμ, approximately 45 mμ, and 55 mμ (refs. 2, 3 and 4, respectively). In an attempt to resolve these conflicting observations, electron microscopic investigations of the LDH agent were initiated, and this report describes preliminary findings.

Three pools of heparinized blood were obtained from *C57BL/Fg* mice 24–36 h after an intraperitoneal injection of 0·1 ml. of a preparation containing $10^{3 \cdot 5}ID_{50}$/ml. of the LDH agent. Plasma was collected by centrifugation at 2,000 r.p.m. for 20 min, diluted with an equal volume of Eagle's basal medium, and centrifuged for 5 min at 9,000 r.p.m. The supernatants were then spun at 105,000*g* for 2 h in a Spinco 'Model L' ultracentrifuge and the pellets removed. For controls, plasma was obtained from non-treated *C57BL/Fg* mice and a pellet prepared as described. A pellet was also prepared from a filtrate (100 mμ 'Millipore' filter) of a human carcinoma of the breast which does not contain the LDH agent[5]. Ice was used throughout and each centrifugation was at 0° C. One pellet, prepared from infected mouse plasma, was resuspended to its original volume; the infective titre was determined to be $10^{10}ID_{50}$/ml. Pieces cut from the remaining four pellets were fixed in 1 per cent osmic acid with veronal buffer (*p*H 7·4), dehydrated, and embedded in methacrylate. Thin sections were cut with glass knives on a Porter–Blum microtome and mounted on 'Formvar'-coated grids. These were stained for 5–10 min in lead citrate and examined in an RCA '*EMU-3C*' electron microscope.

Fig. 1 illustrates the typical appearance of particles found in thin sections from pellets of infected mouse plasma. They are oval-shaped and measure approximately 15 mμ in width and 45 mμ in length. As evidence that these particles are the LDH agent, it should be noted that: (*a*) their length, 45 mμ, is identical with the diameter calculated by Rowson *et al.*[3] from filtration data; and (*b*) similar particles were not seen in preparations of control pellets.

The above observations, and other findings derived from investigations of its epizootiology and properties[4,6], suggest that the LDH agent is a virus. Since this communication was completed, Bladen and Notkins[7] have reported an electron microscope investigation which demonstrates that the LDH agent is an essentially spheroidal particle measuring 69 mμ × 76 mμ in diameter. The techniques used by these investigators were similar to ours with the exception that their material was examined by negative staining. This variable could account in part for these discrepancies, but it is of interest to consider the possibility that the confusion which now exists as to the size of the LDH agent may be due to a failure to recognize that there is more than one agent.

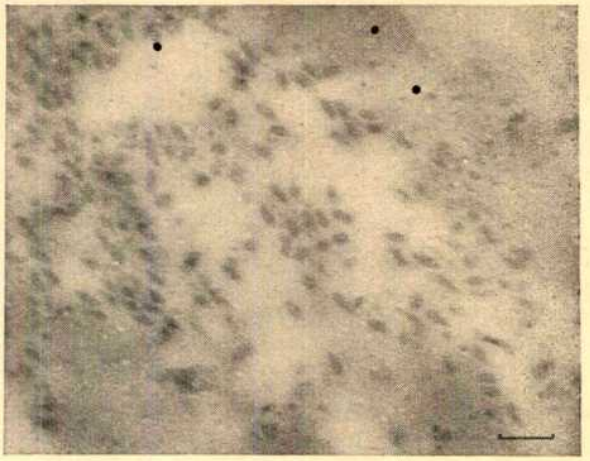


Fig. 1. Electron micrograph of pellet prepared from mouse plasma infected with the LDH agent showing oval-shaped particles (× *c.* 22,700). Line at lower right represents 100 mμ

This investigation was supported in part by grants *CA*–05742–03 and *C*–3501 from the National Cancer Institute, U.S.P.H.S., and a grant from the Maryland Division of the American Cancer Society. We thank Miss Hazel Monserrate for her assistance.

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Interaction of Cytochrome *c* with Yeast D(−) Lactic Oxidase

In 1961 Gregolin and Singer[1] reported that yeast respiratory particles, prepared by mechanical disruption of cells in 1 per cent NaCl, do not oxidize D(−) lactate with oxygen as the terminal electron acceptor. However, the particles exhibit strong D(−) lactic dehydrogenase activity, and oxidize D(−) lactate with oxygen uptake on addition of external cytochrome *c*. More recently, Gregolin and D'Alberton observed[2] that D(−) lactate is oxidized at high rate by oxygen when respiratory particles are prepared by disintegration of the cells in sucrose solution. When tested in a medium known to give maximal oxidase activity, the oxidation is not stimulated by addition of cytochrome *c*. In the presence of the same mixture (plus NaN_3), D(−) lactate is not oxidized by this preparation with cytochrome *c* as artificial electron acceptor.

In simultaneous and independent work, Roy[3] noticed an analogous ineffectiveness of cytochrome *c* on a D(−)

lactic oxidase system, obtained by using a French pressure cell. He presented two hypotheses to explain the different behaviour of his preparation as compared with that by Gregolin and Singer, namely, 'duality' of species or sites of action of cytochrome *c* and solubilization of cytochrome *c* by NaCl. The hypotheses were not substantiated and the problem remained unanswered in Roy's report.

The present communication proposes an alternative explanation, namely, that the inactivity of cytochrome *c* on D(−) lactic oxidase preparations is mainly due to limitations of assay. Indeed, the best conditions to show full D(−) lactic oxidase activity are not those in which cytochrome *c* interacts with the system. Cytochrome *c* does react with the D(−) lactic oxidase obtained from mechanically broken cells, if proper assay conditions are chosen. The same is possibly true for particles prepared by the pressure cell. However, in the latter case it is also possible that cell disruption by pressure, rather than by mechanical treatment, leads to a preparation of more intact respiratory particles, inaccessible to cytochrome *c*.

The following experiments, which support this suggestion, were performed with particles prepared by mechanical breakage and isolated in 0·25 M sucrose with 0·001 M EDTA and 0·02 M potassium phosphate, *p*H 7·3, according to the procedure previously described[2].

When tested under optimal conditions for full D(−) lactic oxidase activity, that is, at *p*H 6 and in the presence of 0·035 M Mg^{2+}, the particles do not exhibit any particular response to cytochrome *c* (Fig. 1, exp. *A*). When Mg^{2+} is absent, the activity with D(−) lactate is much slower, and cytochrome *c* exerts a stimulatory effect, but not sufficient to raise the rate of oxidation to the level obtained in the former case (exp. *B*). That this effect is not due to restoration of a normal level of cytochrome *c* in particles is suggested by the fact that succinic oxidase activity is not stimulated by cytochrome *c* (exp. *C*).

Exp. *D* was performed in the reaction medium reported by Roy[3]. In this case the oxidation of D(−) lactate was less efficient than in the medium used in exp. *A* and cytochrome *c* showed only a very modest stimulatory effect.

It would appear that the inactivity of cytochrome *c*, in exp. *A*, is a consequence of the presence of Mg^{2+}, due either to the high ionic strength of the medium, or because Mg^{2+} restores the normal flow of electrons from the primary dehydrogenase to oxygen.

Table 1. D(−) LACTATE OXIDATION BY YEAST RESPIRATORY PARTICLES WITH VARIOUS ELECTRON ACCEPTORS AND UNDER DIFFERENT ASSAY CONDITIONS

Electron acceptor	μMoles D(−) lactate oxid./min/mg protein: In the reaction medium for oxidase assay	Under optimal conditions for each test	In the medium described in ref. 3
Oxygen		0·10	0·07
Phenazine methosulphate	0·30	0·39	0·32
Cytochrome *c*	0·007	0·1	0·015

Oxygen uptake was measured polarographically, phenazine methosulphate and cytochrome *c* reduction spectrophotometrically. The reaction medium for D(−) lactic oxidase was: 0·05 M potassium phosphate, *p*H 6, 0·035 M $MgCl_2$, 0·01 M D(−) sodium lactate. The medium for reduction of cytochrome *c* and phenazine methosulphate was: 0·05 *M tris*-HCl, *p*H 7·5, 0·01 M D(−) sodium lactate, according to Gregolin and Singer[4]. Sodium azide (5×10^{-3} M) was added in spectrophotometric assays. Activities with cytochrome *c* and phenazine methosulphate are expressed at V_{max} for the electron acceptors. Temperature, 22° C.

Table 2. OXIDATION OF D(−) LACTATE BY YEAST PARTICLES PREPARED BY DIFFERENT PERIODS OF CELL DISINTEGRATION

Time of breakage	μMoles D(−) lactate oxid./min/mg protein: Oxygen	Phenazine	Cytochrome *c*	Ratios of activities: Oxygen / Phenazine	Oxygen / Cyt. *c*	Cyt. *c* / Phenazine
25 sec	0·000	0·34	0·044	0·26	2·04	0·13
40 sec	0·120	0·42	0·080	0·28	1·50	0·19
75 sec	0·142	0·51	0·130	0·28	1·10	0·25
2 min	0·070	0·32	0·075	0·22	0·93	0·23
4 min	0·063	0·31	0·070	0·20	0·90	0·23
6 min	0·046	0·30	0·068	0·15	0·67	0·23
8 min	0·031	0·22	0·065	0·14	0·48	0·29

Particles were prepared as described in the text, with the exception of the disintegration step, which lasted as indicated. Activities were determined as in Table 1.

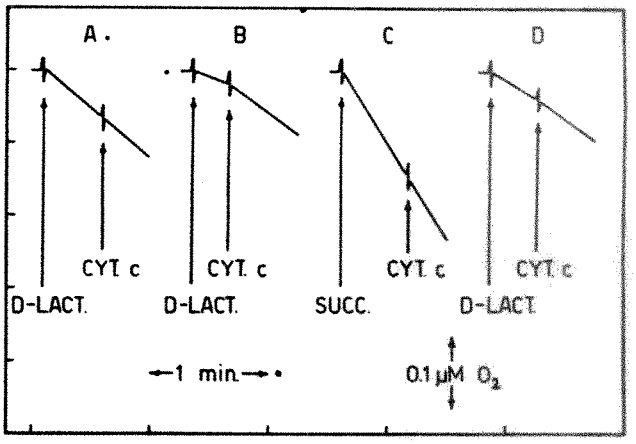


Fig. 1. Polarographic determinations of D(−) lactate and succinate oxidation rates by yeast respiratory particles. Experiments *A* and *B* were performed in 0·05 M potassium phosphate, *p*H 6, 0·035 M $MgCl_2$; exp. *C* in 0·05 M *tris*-HCl, *p*H 7·5; exp. *D* in 0·3 M mannitol, 0·01 M potassium phosphate, *p*H 7·2, 0·01 M potassium chloride and 0·005 M magnesium chloride, according to Roy[3]. D(−) sodium lactate was 0·01 M, sodium succinate 0·02 M, cytochrome *c* 2×10^{-4} M. Amounts of particles corresponding to 4 mg protein were added, suspended in 0·2 ml. of 0·25 M sucrose. Total volume, 2·0 ml. Temperature, 22° C

The influence of the assay conditions on the phenomenon is made clear by spectrophotometric measurements of the rate of reduction of cytochrome *c*. The activity was assayed under optimal conditions for this test, and compared with the rate in the media used for oxidase activity (plus NaN_3). Table 1, where D(−) lactate oxidation rates with oxygen and with phenazine are included for comparison, shows that cytochrome *c* reductase activity is normal in particles prepared in 0·25 M sucrose. However, either at *p*H 6 in the presence of Mg^{2+}, or in the medium used by Roy, it is almost completely suppressed, while phenazine activity is only slightly affected. It must be emphasized that under no conditions is the rate of cytochrome *c* reduction stimulated by Mg^{2+}, meaning that no reduction of 'external' cytochrome *c* takes place through a pathway which requires Mg^{2+} and includes 'internal' cytochrome *c*.

When the time of exposure of the yeast cells to the breakage treatment was varied, the respiratory particles showed different ratios between D(−) lactic dehydrogenase and D(−) lactic oxidase activities (Table 2). Cytochrome *c* reductase activity accompanies oxidase activity. However, particles obtained in very short periods of disruption are proportionately less active with cytochrome *c* than with phenazine. This finding suggests that cytochrome *c* is hindered in its approach to the active site by permeability or accessibility barriers. Depending on the time of cell breakage, the specific activity of the oxidase in the particles falls more quickly than that of the dehydrogenase. This is possibly due to disintegration of the complex structure of the oxidase system under mechanical treatment.

Finally, it must be added that the reactivity of external cytochrome *c* with the D(−) lactic dehydrogenase does not constitute proof that this carrier is the natural electron acceptor of the enzyme.

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Enzymatic Oxidation of Galactose-6-phosphate

Galactose-6-phosphate (gal-6-P) has been shown to be produced from galactose-1-phosphate (gal-1-P) in the presence of phosphoglucomutase[1], demonstrated in erythrocytes of patients with galactosæmia[2], and oxidized in the presence of purified glucose-6-phosphate dehydrogenase (G-6-PD) and NADP as indicated by changes in absorbance at 340 mμ (ref. 3). This communication shows that G-6-PD purified from either yeast or human erythrocytes in the presence of NADP converts gal-6-P to 6-phosphogalactonic acid (6-P-gal A).

For the work described here, the G-6-PD from human erythrocytes was isolated by fractionation on DEAE-cellulose and precipitation with ammonium sulphate resulting in about a 200-fold increase in specific activity from the original hæmolysate[4]. This preparation was free of 6-phosphogluconate dehydrogenase (6PGD) activity. The G-6-PD from yeast was obtained from Sigma Co. Incubation was carried out using 5 mmoles of gal-6-P, 0·5 m.moles of NADP, 0·375 m.moles of magnesium chloride, 10 mg of methylene blue and 700 units (one unit equals reduction of 1·0 μM NADP/min at *p*H 7·4 and 25° C) in a final volume of 1 l. of 0·06 M *tris* buffer, *p*H 7·5. The reaction was maintained at 37° C in the case of enzyme from erythrocytes and at 30° C when using enzyme from yeast. After incubation for 15 h, the reaction was stopped by the addition of cation exchange resin ('Dow 50 × 8, H^+', 60–100 mesh) and the nucleotides were removed with activated carbon ('Darco *G*-60') (ref. 4). The oxidation product was isolated by using a column of anion exchange resin ('Dow 1 × 8, Cl^-', 200–400 mesh), eluting with 4 l. of hydrochloric acid over a linear *p*H gradient of from 4·0 to 1·5 and collecting 20-ml. fractions. Fig. 1 shows that after incubation for 15 h, approximately 50 per cent of the reducing sugar remained in reaction mixtures using enzymes prepared from either yeast or erythrocytes; all remained when enzymes were not added. On fractionation of the incubation mixture on an anion exchange resin column, the material eluted at *p*H 1·7 was non-reducing and positive for glyoxalate and glycolaldehyde after periodate oxidation. After crystallization at *p*H 5·7 as the barium salt, the final yields were 257 and 107 mg using enzymes prepared from yeast and erythrocytes, respectively.

The preparations of phosphorylated aldonic acids were compared with 6-P-gal A. The 6-P-gal A used as the reference compared was synthesized by bromine oxidation of gal-6-P (Nutritional Biochemicals, Ohio, Batch No. 6450, $[\alpha]_D^{25} = +24{\cdot}4°$) (ref. 5), the product isolated by fractionation on a column of anion exchange resin ('Dow 1 × 8, Cl^-', 200–400 mesh), and crystallized as the barium salt. Comparison was carried out using a descending system of ethanol–ammonium acetate (1·0 M, *p*H 7·5) (5 : 2 v : v) developed for 48 h (ref. 6) and of ethyl acetate–pyridine–water (2 : 1 : 1 v : v : v) developed for 18 h and visualized with the periodate permanganate reaction[7]. Neither preparation showed either fluorescence or absorption with ultra-violet light, and after staining with periodate permanganate yielded single densities having mobilities corresponding to authentic 6-P-gal A.

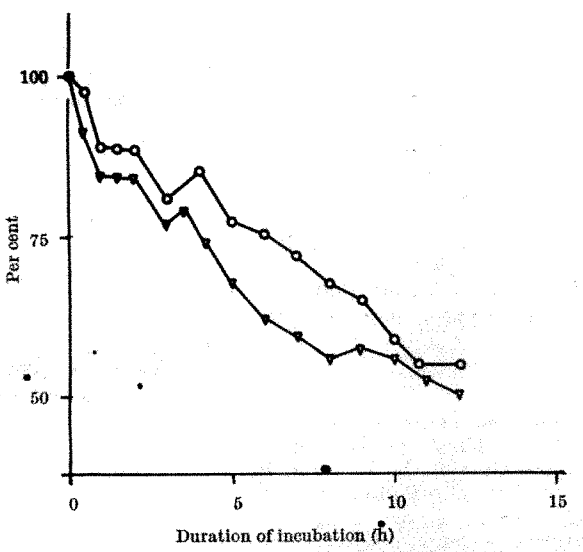


Fig. 1. Results showing the conversion of gal-6-P to 6-P-gal A. The reaction was followed by determining the reducing sugar and expressing remaining reduced sugar as per cent of the initial amount. Results from human erythrocytes are shown as triangles and from yeast as circles

Table 1

	6-P-GA*	Br_2 oxid.	6-P-Gal A G-6PD/ RBC	G-6PD/ Yeast
Yield		222	107	257
Organic phosphate	176	221	185	218
Barium	272	268	269	244
Glyoxalate/DPA†	176	218	187	194
Glycolaldehyde/DPA	176	198	174	191
Periodate uptake	570	620	570	623
Absorbance, 260 mμ‡	0	0·09	0·16	0·07
Free phosphate	3	3	62	3
Total phosphate	179	224	247	221
Glyoxalate/phosphate	1·00	0·99	1·01	0·89
Glyoxalate/glycolaldehyde	1·00	1·10	1·07	1·02
Periodate uptake/glyoxalate	3·24	2·85	3·06	3·21

* 6-Phosphogluconic acid.
† Glyoxalate produced by periodate oxidation and reacted with diphenylamine.
‡ Absorbance at 260 mμ is converted to quantity using the coefficient of $18{\cdot}0 \times 10^3$.

When the phosphorylated aldonic acids were subjected to periodate oxidation and reacted with diphenylamine[8], bluish-green solutions were obtained. Their absorption spectra had two maxima, one at 440 mμ attributed to the reaction of glyoxalate with diphenylamine and another at 660 mμ resulting from the reaction of glycolaldehyde with diphenylamine. The spectrum from 350 to 700 mμ for the two preparations was identical, and similar to that produced by authentic 6-P-gal A and 6-phosphogluconic acid.

The analyses of the two preparations are summarized in Table 1. The results are compared with those for 6-P-gal A synthesized by bromine oxidation and 6-phosphogluconic acid (6-P-GA) obtained commercially. The data are expressed in mμmoles/100 μg of preparation. There is an equimolar relationship among the organic phosphate, the glyoxalate and the glycolaldehyde groups. The positive glycolaldehyde reaction after periodate oxidation indicates substitution of the primary alcohol of the aldonic acid; the positive glyoxalate reaction indicates the presence of a carboxyl group. The ratio of about 3 for periodate uptake to glyoxalate suggests three pairs of vincinal, unsubstituted hydroxyl groups. These results indicate that the carboxyl group has replaced the reducing group and that the carbon structure of gal-6-P is otherwise intact.

The phosphorylated aldonic acids were hydrolysed in the presence of alkaline phosphatase[9] and the resulting aldonic acids were isolated by fractionation on a column of anion exchange resin. When the products were chromatographed on paper using a system of ethyl acetate–pyridine–water, densities corresponding to authentic galactonic acid were observed after staining with periodate permanganate. The products were also acetylated in the presence of cadmium hydroxide, acetic anhydride and hydrogen chloride, crystallized from ethyl alcohol–chloroform and twice re-crystallized from toluene. All the preparations had melting points of 131° C compared with the literature value of 131°–132° C for D-galactonic acid pentaacetate.

The preparations of 6-P-gal A were tested for oxidation in the presence of NADP and hæmolysate as the source of 6-PGD. The change of absorbance at 340 mμ as a function of time was compared with that observed using 6-phos-

phogluconate as substrate. The rate of the former was about 65 per cent of the latter.

Recently, results have been published suggesting that some patients with galactosæmia show variability in the manifestations of the condition on both a clinical[9,10] and biochemical[11–13] basis. The possibility that this oxidation pathway may play a part in determining these differences is being investigated.

This work was supported by grants from the Illinois Mental Health Fund and the Kettering Foundation.

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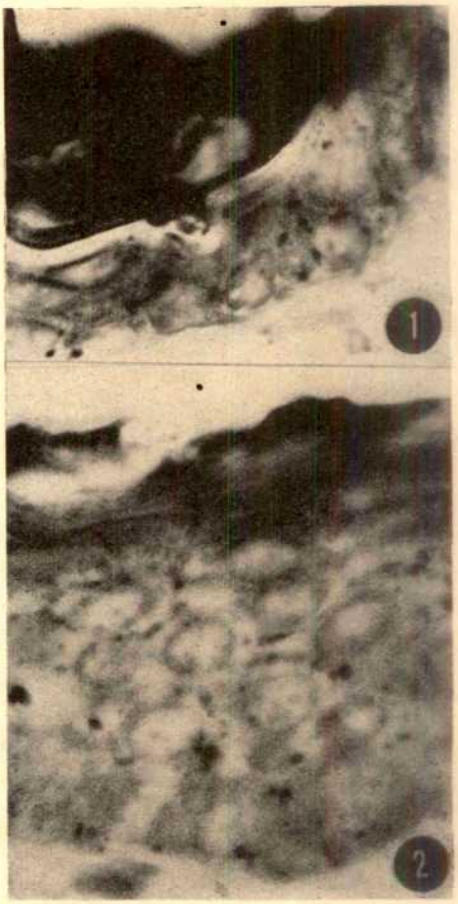


Fig. 1. Epidermis of a normally-fed *BUB* female mouse stained with sudan black *B* (× 870)

Fig. 2. Epidermis of *BUB* female mouse deficient in essential fatty acid, coloured with sudan black *B* (× 870)

Lipids in the Skin of Mice Deficient in Essential Fatty Acids

THE lipids of the skin are affected markedly by the dietary condition of the animal[1,2]. In rats kept on a fat-free diet the skin retained more fat than any of the other organs including, for example, the liver and muscle[3,4]. The epidermis of fatty-acid deficient rats is thicker and more differentiated than that of normal rats, the stratum granulosum is distinct and the stratum corneum is thick[5]. In this communication the lipid content of the skin of mice which were deficient in essential fatty acids is compared with that of normal mice.

Two groups of *BUB* female mice were used. One group consisted of essential fatty-acid deficient mice kept on a fat-free diet for 11 weeks. The diet was composed of Fenton's casein No. 4, 5 g; vitamin-free test casein, 15 g; dextrose, 72 g; salt mix No. 2, 4 g; non-nutritive fibre, 4 g. The other group was of mice kept on an ordinary diet and served as controls. Pieces of skin from the back, the ear and the upper lip were fixed in formal-calcium, post-chromed and embedded in gelatin according to Baker's[6] acid hæmatin routine. Frozen sections, 5–10μ thick, were cut on a cryostat, and some of them were coloured with sudan black *B*[7] and the others were stained with acid hæmatin[6].

The skin of mice deficient in essential fatty acid is thicker than that of normal ones. However, the most striking difference between the two groups is in the epidermis. In all the regions examined, the epidermis of mice deficient in essential fatty acids is nearly three times thicker than the epidermis of normal mice (Figs. 1 and 2). Three different zones are detected in the stratum malpighii: the stratum basal, the stratum spinosum and the stratum granulosum. In normal mice, the stratum malpighii consists of a layer, only 1–2 cells thick. In the experimental animals, the stratum corneum is thicker and more compressed than in normal animals. Sudan black colouring shows that cells of the stratum malpighii contains many more lipid granules in the experimental than in the control animals. Lipid is especially abundant in the stratum granulosum which is laden with sudanophilic granules (Fig. 2). The stratum corneum is coloured to the same degree with sudan black in both groups, and there is no obvious difference between the two groups of animals in the morphology or the stainable lipid of the sebaceous glands. Sections stained with acid hæmatin did not reveal any significant difference in the phospholipid contents of the epidermis in normal mice and those deficient in essential fatty acids.

Accordingly, the excess lipid granules observed in the skin of experimental animals are not phospholipids and they cannot be sterols, since the latter do not usually stain with sudan black at room temperature[8]. It is likely, therefore, that the additional lipids in the epidermis of deficient mice are mainly in the form of glycerides and free fatty acids. The increase in skin lipids found by Smedley-MacLean and Hume[4] in rats fed a fat-free diet was attributed to the synthesis of essential fatty acids by sebaceous glands[9]. According to the observations recorded here, the epidermis is the only site of lipid accumulation in the skin. By definition, essential fatty acids cannot be synthesized in the skin since these fatty acids are a dietary requirement of the animal. It is more likely that this fat is stored in the skin, and that the skin acts as a reservoir for the essential fatty acids sufficient to keep the animals alive. The increase in the thickness of the epidermis can be due to either or both the following two factors: (1) an increase in the mitotic activity of the basal layer;

(2) a decrease in the rate at which the epidermal cells keratinize. Since there is no significant increase in the mitotic activity of the basal cells[10], it seems more probable that the epidermal cells of mice deficient in fatty acid are retarded in their rate of keratinization by their involvement.

I thank Dr. Richard A. Ellis of Brown University and Dr. William Montagna of the Oregon Regional Primate Research Center for their advice, and Dr. Elizabeth Leduc of Brown University for providing the animals. This investigation was supported in part by a fellowship from the Government of the United Arab Republic and in part by U.S. Public Health Service traineeship 5-*TI-GM*-582-04 and research grant *GM*-08380-04 from the Division of General Medical Sciences.

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Influence of Basic Antibiotics on Serum- and Liver-cholesterol Concentrations in Chicks

It has been reported[1] that orally administered neomycin lowers the serum-cholesterol level in man, and these results were confirmed in other studies[2-4]. Neamine and kanamycin have only a moderate effect, whereas streptomycin and dihydrostreptomycin are inactive[3,4].

As neomycin is very poorly absorbed from the gut, its action is probably restricted to the intestinal lumen. The modifications of the intestinal flora, induced by the antibiotic, are generally considered to be responsible for the cholesterol-lowering effect of this drug. Nevertheless, the observation that streptomycin, a substance with a closely related antibiotic spectrum, has no influence on serum-cholesterol concentration casts some doubt on this explanation.

An incidental observation that neomycin precipitates solutions of bile acids led us to suppose that the activity of this product might be due to its ability to form salts which interfere with the absorption of lipids from the intestine.

To examine this hypothesis, we have prepared and tested two types of neomycin derivatives which are devoid of antibiotic activity: *N*-methylated neomycin (Vanderhaeghe and Claes, unpublished work), which is basic, and *N*-acetylated neomycin[5], which is a neutral substance.

Five groups of 15 neonatal broiler chicks were fed a basic casein-sucrose diet containing 6 per cent corn-oil as the sole source of fat[6]. To this basic diet were added 0·2 per cent cholesterol and 0·15 per cent of the different substances under investigation, as indicated in Table 1. The experimental diets were fed for 2 weeks. After fasting overnight, the birds were bled by cardiac puncture and the serum- and liver-cholesterol concentrations were determined by the method of Abell *et al.*[7]. Fæcal fat excretion was determined daily according to Van de Kamer *et al.*[8].

As shown in Table 1, feeding 0·15 per cent neomycin or 0·15 per cent methylated neomycin reduced serum-cholesterol concentrations by 26 per cent and 17 per cent respectively. The *N*-acetylated neomycin was ineffective, and streptomycin rather tended to increase cholesterol levels. Table 1 also shows that the administration of neomycin or methylated neomycin resulted in an increased fæcal fat excretion; streptomycin and *N*-acetylated neomycin had no such effect.

Table 1. Effect of Neomycin, Streptomycin, and Two Neomycin Derivatives on Serum- and Liver-cholesterol and on Fæcal Fat Excretion in Chicks

	—	Neomycin 0·15 %	Strepto-mycin 0·15 %	Methyl-neomycin 0·15 %	*N*-acetyl. neomycin 0·15 %
Average serum-cholesterol, (mg/100 ml.)	267	198	284	222	270
Average total liver-cholesterol, (mg/100 g liver)	762	502	771	589	765
Fæcal fat, (mg/g dried fæces)	57	104	49	92	52
Body weight (g)	143	127	148	140	141

Our results indicate that the cholesterol-lowering effect of neomycin is due not to its antimicrobial activity but to the presence of basic groups in the molecule. This hypothesis could also explain the results obtained in human experiments by Samuel and Waithe[4]. Streptomycin, which does not lower serum-cholesterol, has only three basic groups. Kanamycin and neamine, with four amino groups, present a weak activity. Neomycin, an antibiotic with six amino groups, is the most active of the four drugs.

Further experiments in this laboratory have shown that both neomycin and methylated neomycin, but neither streptomycin nor acetylated neomycin, can break emulsions stabilized by lecithin (to be published). A similar effect on the emulsification of lipids in the intestine could explain the increase in fæcal fat output observed in the present work.

During these studies it was also found that neomycin and methylated neomycin when added to human or to chick bile cause precipitation of bile acids and phospholipids, and a correlation was observed between the bile-acid-precipitating activity of different compounds and their effect on the serum-cholesterol level in chicks. Moreover, feeding chicks with 0·25–0·5 per cent of methylated neomycin caused a five-fold to ten-fold increase in the excretion of fæcal bile acids, and addition of neomycin or methylated neomycin to a diet containing lithocholic acid protected chicks from the hepatotoxic effects of lithocholic acid[9], thus indicating interference with the absorption of bile acids from the intestine.

These experiments support the hypothesis that basic substances such as neomycin and methylated neomycin interfere with the intestinal absorption of fats, cholesterol, and bile acids by disturbing the emulsification of lipids in the intestine or by complexing bile acids. The cholesterol-lowering effect could then be explained by an impaired intestinal absorption of cholesterol and an increased degradation of cholesterol to bile acids in the liver.

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Shrinkage Temperature of Eye Collagen

During a study of the shrinkage temperature of skin collagen, normal human eye collagen was included in the control material. A measure of difference was found between the sclera, which behaved as ordinary mammalian collagen[1–5], and the cornea, which showed a lower hydrothermal stability, in the sense that it shortened at a lower temperature than sclera or skin collagen.

Although the structure of the transparent cornea is well documented[6,7], and descriptions exist of the analytical differences between it and the sclera[8], there seems to be no ready reference to a lower shrinkage temperature.

Using a modified Theis apparatus[9] the results were as shown in Table 1.

Table 1

Specimen	Human eye	Sex	Age	Shrinkage temperature (° C) 55	56	57	58	59	60	61	62	63	64	65
314	Normal cornea	F	66				1							
314	Normal sclera	F	66								1			
318	Normal cornea	M	84			1								
318	Normal sclera	M	84								1			
321	Normal cornea	F	81			1								
321	Normal sclera	F	81								1			
323	Normal cornea	M	62		1									
323	Normal sclera	M	62								1			
324	Normal cornea	M		1										
324	Normal sclera	M									1			
325	Normal cornea	F	60			1								
325	Normal sclera	F	60									1		
330	Normal cornea	M	18								1 } Astro-			
330	Normal sclera	M	18								1 } cytoma			
333	Normal cornea	M		1										
333	Normal sclera	M										1		
291	Normal cornea	M					1							
272	Normal cornea	F	86		1									
STB1	Normal cornea	M	69	1										
STB2	Normal cornea	M	58				1							
STB3	Normal cornea	F	82	1										
STB3	Normal sclera	F	82							1				
STB4	Normal cornea	M	65				1							
STB4	Normal sclera	M	65							1				

These findings relate only to the translucent area of normal eyes; investigation of pathological specimens is pending.

This work was supported by the Medical Research Council of New Zealand.

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Biochemical Chlorination of *Streptomyces aureofaciens*

Earlier work on the chlorination mechanism of *Streptomyces aureofaciens* established that chlorine of chlorinated fatty acids was incorporated into the molecule of 7-chlorotetracycline (CTC)[1,2].

Recently, Sekizawa has found that the chlorine of chlorpropanediols was also incorporated into CTC and, moreover, this compound was able to overcome the effect of known chlorination inhibitors[3–5]. Sekizawa also reported that after the addition of chlorpropanediols to the media of oxytetracycline-producing strains, CTC could be detected[6]. He therefore supposed chlorpropanediols to enter directly into the tetracycline (TC) molecule during the course of biosynthesis preceding the chlorination reaction.

The conditions of chlorine incorporation were investigated by the application of $Na^{36}Cl$, using 2,5-dimercaptothiadiazole[1,3,4] as chlorination inhibitor. Regarding organic chloro-compounds, the incorporation of chlorine from 1-^{14}C-acetate (MCA), 2-^{14}C-MCA and 3-chlorpropanediol[1,2] was investigated. Incorporation of the carbon of monochloracetates into TC was also detected.

Strains of CTC-producing *Str. aureofaciens* 'B-28' (ref. 7) and the tetracycline producer *Str. aureofaciens* 'CDSD-314' (ref. 8) were used for the present experiments. The synthetic medium and experimental conditions were the same as reported earlier[7,9].

Using isotopically labelled NaCl it was shown that TDA exerts its inhibitory action in the first oxidative stage of chlorination. $Na^{36}Cl$ could be detected in the broth of chlortetracycline fermentations inhibited by TDA. Similarly, chloride could be detected about the end of fermentations of TC-producing 'CDSD-314' strain. These facts support the assumption that inhibition of chlorination was effected in the first phase of the biosynthetic chain, genetically manifest during the course of strain production[8]. But, in contrast to the inhibition of TDA, in the case of strain 'CDSD-314' copper did not suspend the inhibition of CTC production; the genetic block has been found to be irreversible (Table 1).

Table 1 shows that, in the case of *Streptomyces aureofaciens* 'CDSD-314', the 'residual reaction' supposed by Sekizawa[6] to be general in chlorination reactions could not be found. In our opinion the appearance of a 'residual reaction' in the case of predominantly TC-producing strains depends on the localization of the genetic block. We suppose, however, that at the very first stage of chlorination there is no alternative pathway in the biogenesis of CTC; later, there may be a partial separation of the reaction routes.

By the use of 1-^{14}C-MCA and 2-^{14}C-MCA it could be shown that, in the case of strain 'B-28', the chlorine of MCA, decomposed enzymatically, was incorporated into the CTC molecule. It was found that TDA did not inhibit the incorporation of carbon from MCA 1-C and 2-C into the tetracycline molecule. Similarly, in the case of strain 'CDSD-314', independently from the genetic block, carbon atoms of MCA were incorporated into TC (Table 2). We suppose that the first step of chlorine utilization is an enzymatic cleavage of the chlorine of MCA to inorganic chloride during the metabolism of both strains. Afterwards, evidently, chloride will be incorporated into CTC only in the case of the CTC-producing strain 'B-28'.

The possibility reported by Sekizawa that propanediole may be a direct precursor for CTC would have been confirmed if the production of CTC could have been detected at the end of the fermentation, after giving chlorpropanediole to the culture of the exclusively TC-producing strain 'CDSD-314'.

Experimental results obtained in our laboratory did not support the assumption of Sekizawa. CTC could not be detected in the fermentation broth of strain 'CDSD-314', thus excluding the possibility that chlorpropanediol acts as a direct precursor of CTC, if it is assumed that, in general, the biosynthesis is similar in TC- and CTC-producing

Table 1

Strain	Materials*	Total antib. activity mcg/ml.	Breaking per mg of antibiotics	CTC (%)*	Chloride det. at the end of fermentation
B-28	—	1,080	1,512	100	32
B-28	0·2 mM TDA	1,040	396	26	80
B-28	0·2 mM TDA + 0·3 mM $CuSO_4$	825	1,180	74	38
CDSD-314	—	1,370	38	0	98
CDSD-314	0·2 mM TDA	1,280	32	0	96
CDSD-314	0·2 mM TDA + 0·2 mM $CuSO_4$	1,010	36	0	99
—	—	—	40†	—	96

* As an isotope source 0·4 μc./90 ml., $Na^{36}Cl$ was added at 0 h for each experiment.
† Activity was related to an equal quantity of butanolic extract. The small activity observed in the control flask may be due to a small amount of ^{36}Cl dissolved in butanol.

Table 2. Incorporation of the Carbons of 1-^{14}C-MCA and 2-^{14}C-MCA into CTC – TC Molecules

Strain	Materials	Total antibiotic activity * mcg/ml.	Breaking in relation to 1 mg CTC + TC
B-28	2-^{14}C-MCA *	1,050	3,170
CDSD-314	2-^{14}C-MCA	1,170	3,470
B-28	2-^{14}C-MCA + 0·2 mM TDA	1,030	2,970
CDSD-314	2-^{14}C-MCA + 0·2 mM TDA	1,200	3,380
B-28	1-^{14}C-MCA	1,130	2,060
CDSD-314	1-^{14}C-MCA	1,330	2,190
B-28	1-^{14}C-MCA + 0·2 mM TDA	1,180	2,110
CDSD-314	1-^{14}C-MCA + 0·2 mM TDA	1,310	2,120

* Monochloracetates (MCA) were used in 1 μc./80 ml. concentration.

Table 3. Effect of Chlorpropanediol on the Biosynthesis of CTC in Cultures of CTC-producing and Non-producing Strains

Strain	Time of addition	Materials added	Fermented CTC + TC mcg/ml.	CTC (%)	Appearance of chloride at the end of fermentation
B-28	—	—	1,140	2	–
B-28	0	4 mg/ml. chlorpropanediol	870	100	+
B-28	0	4 mg/ml. chlorpropanediol + 0·2 mM TDA	1,070	37	+
B-28	24	4 mg/ml. chlorpropanediol	790	85	+
CDSD-314	—	—	1,490	0	–
CDSD-314	0	4 mg/ml. chlorpropanediol	960	0	+
CDSD-314	0	4 mg/ml. chlorpropanediol + 0·2 mM TDA	1,030	0	+
CDSD-314	24	4 mg/ml. chlorpropanediol	760	0	+

micro-organisms. However, by the sensitive radioactive test it was possible to detect inorganic chloride at the end of TC fermentations (supplied with chlorpropanediol), thus furnishing evidence that decomposition of chlorpropanediol takes place during the course of fermentation. This fact strongly supports the evidence that in the case of strain 'B-28' inorganic chloride originated from an enzymatic decomposition of chlorpropanediol was incorporated into CTC, as in the case of chloracetates (Table 3).

After our experiments had been completed[10], it was reported by Goodman *et al.* that the reversing effect of chlorpropanediols on chlorination inhibitors can be explained by the alkylation of the inhibitors[11]; in their roles as chlorine sources in the metabolism of CTC-producing micro-organisms a close parallelism can be drawn between chlorpropanediols and chlorinated fatty acids.

From the results of our experiments we suppose that at least two stages can be distinguished at the early phase of chlorination. Presumably the first step is the oxidation of chloride to chlorinium ion, as was reported by Shaw *et al.* in the case of *Cald. fumago*[12]. A reaction of chlorinium ion with a common intermediary of TC and CTC may represent the second step. At present we have no exact idea about the nature of this intermediate, but it may be pretetramide (1,3,10,11,12-pentahydroxy-naphthacene-2 carboxamide), which is an intermediate in the formation of 6-demethyl-CTC as shown by the recent excellent work of McCormick *et al.*[13]. Presumably TDA inhibition extends only to the first oxidative step and is ineffective. The genetic block of strain 'CDSD-314' naturally can be spread over both steps, but exact elucidation of this question requires further experiments.

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Auxin and Gibberellin-like Substances in Peanut (*Arachis hypogaea*)

The results here presented are part of an investigation on dormancy in seeds of the peanut, variety 'Virginia Bunch'. This variety requires 'after ripening' in dry storage for about 4 months in natural conditions in Israel.

While experimenting with the dormant seeds in order to find a practical method of overcoming dormancy in the laboratory, it was found that seeds (intact and naked), leached overnight at about 25° C, will germinate satisfactorily. Keeping in mind that inhibitors may be leached out, we carried on experiments with the leachates.

Leachates in several concentrations could not prevent germination, but slowed it down and inhibited radicle growth of seedlings, as well as cress-root growth. As no significant difference could be found between dormant and non-dormant seed, more detailed examination of the leachates was made.

Wheat coleoptile section growth (variety 'Atle'), oat mesocotyl section growth (variety 'Victory') and cress-root bioassays were carried out after leachates had been freeze-dried, taken up in 80 per cent methanol and chromatographed in a descending solvent system consisting of isopropanol 1/100 × 0·88 sp. gr. ammonium hydroxide (4 : 1).

No clear results could be obtained with the coleoptile test. In the mesocotyl test, a growth promoter with a peak at R_F 0·2–0·3 was found and the cress-root test showed strong inhibition at about the same R_F. In the leachates from non-dormant seed, a somewhat higher growth-promoting activity was found in the last two bioassays, but no significant difference could be shown. These results suggest that an auxin-like substance is leached out, the R_F of which is lower than that of synthetic indolyl-3-acetic acid (IAA) which was run as a marker. However, no direct relation could be shown between the observed growth promotion and seed dormancy, nor could a growth inhibitor be demonstrated by these methods. The next step was an examination of the growth substances of the seeds themselves.

Seeds were crushed by means of mortar and pestle and extracted with 80 per cent methanol for 24 h at 0° C. The methanol was changed once after 22 h. The combined methanol extract was distilled under vacuum at 30°–35° C and the aqueous residue was freeze-dried. The dry residue was then dissolved in a small volume of 80 per cent methanol ('crude extract'). For chromatographic separation, the same solvent and the same bioassay as with the leachates were used. The equivalent of 5 g dry weight of seed was used for each bioassay.

With the wheat coleoptile section test, again no significant differences were obtained. When the oat mesocotyl test was used with dormant and non-dormant seed extracts, growth-promotion was found at R_F 0·3–0·6. IAA and gibberellic acid (GA) markers respectively corresponded with these R_Fs. In some cases, a second zone of activity was found at R_F 0·8–1·0 in the dormant seed extracts. Cress-root tests made on these extracts showed pronounced inhibition at R_F 0·2–0·4, followed by a very marked decrease in inhibitor. At R_F 0·8–1·0, a slight promotion was found in the dormant seed extract.

For gibberellin investigations, seeds were extracted as mentioned above. The procedure of purification was based on that of Radley[1] with slight modifications[2]. The 'pure' extract was then chromatographed on paper, using as a developing solvent isopropanol/ammonia (as described formerly), or *n*-butanol, saturated with 1/100 × 0·88 s.g. ammonium hydroxide.

Three bioassay techniques were used: (1) Dwarf-maize leaf section test, based on that of Boyarkin and Dmitrieva[3], using dwarf-1 mutant instead of normal maize. (2) Lettuce hypocotyl test[4] with the 'Gotte-A-Forcer' variety. (3) Oat mesocotyl test.

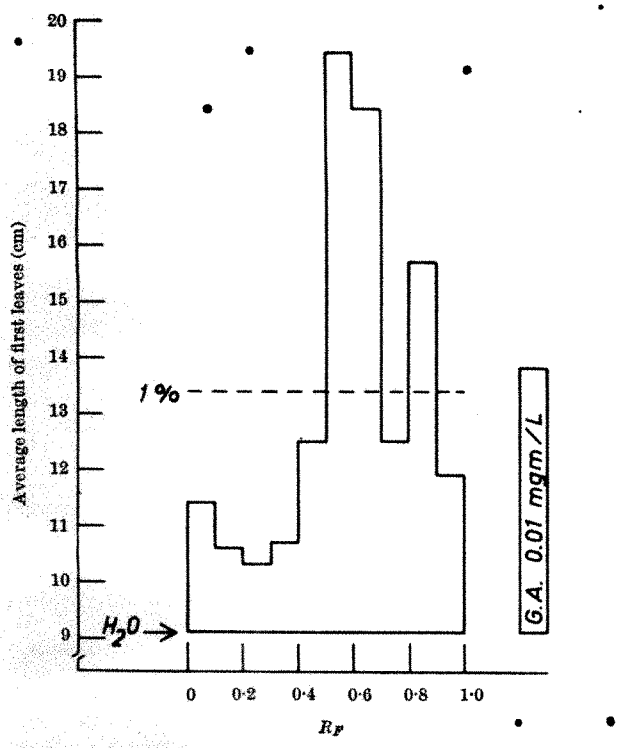


Fig. 1. Maize leaf section bioassay of purified extracts of dormant seed. Ordinates indicate the final length of first leaves. Broken line indicates 1 per cent level of significance

The equivalent of 50 g was used for the bioassays Nos. 1–2 and of 5 g for No. 3. The results obtained suggested the existence of a gibberellin-like promoter in both dormant and non-dormant seed extracts. (When isopropanol/ammonia was used as the solvent system, a peak occurred at R_F 0·5–0·6, and with *n*-butanol/ammonia, a peak was found at R_F 0·2–0·3.)

In dormant seed extracts another zone of promotion occurred. Its R_F (0·8–1·0) with the maize leaf and oat mesocotyl assays corresponded to that obtained with the mesocotyl test on 'crude' extracts of dormant seed (Fig. 1). In our isopropanol/ammonia solvent, IAN occurs at about R_F 0·9. When several concentrations (0·01, 0·1, 1 p.p.m.) of β-indolylacetonitrile (IAN) were used in the dwarf-maize test, no promotion was obtained. These results would suggest that IAN is not responsible for the activity found in dormant seed extracts at R_F 0·8–1·0. The fact that in a cress-root test of the dormant extract a slight promotion was found in the above R_F, while with IAN an inhibition would be expected, may be further evidence for this assumption.

The results of the bioassays with extracted seeds suggest, therefore, that auxin and gibberellin-like substances are found in dormant and non-dormant peanut seeds. In the isopropanol/ammonium hydroxide solvent, IAA appears at about R_F 0·4, GA at R_F 0·5–0·6, and in the dormant seed a second promoting zone is found at R_F 0·8–1·0.

Koshimizu *et al.*[5] obtained in bamboo shoots gibberellin-like substances with R_F values of 0·8–1·0 in the solvent system isopropanol/water (4 : 1). They state that the activity on the *d*-1 maize mutant was very low compared with that on the *d*-3 and *d*-5 mutants. In our dormant seed extract we could get very pronounced promotion with the *d*-1 mutant in the maize leaf section test, but we did not try other mutants for relative activity.

The nearest R_F to that stated here for a known gibberellin is that of gibberellin A_9 which runs at R_F 0·77 in isopropanol/water 4 : 1 (ref. 6). From an investigation on relative activity of the gibberellins[7] it is known that the activity of A_9 on the *d*-1 mutant is practically nil. This fact and the difference in R_F values suggest that the promoting substance found in the dormant peanut seed extract is not gibberellin A_9.

Whether this promoter has any bearing on dormancy and after-ripening in peanut seed should be examined by further investigations.

The experiments described here were carried out while one of us (A. G.-S.) was the holder of a British Council Scholarship.

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PHYSIOLOGY

Effect of Locally Applied Hemicholinium on the Acetylcholine Content of the Caudate Nucleus

ALPHA, alpha-dimethylethanolamino-4, 4′-biacetophenone (hemicholinium-3 or HC-3) is known to inhibit acetylcholine synthesis both in peripheral structures[1] and in the brain[2]. Since it causes death from respiratory failure when given parenterally, detailed investigations of its actions have so far been confined to isolated, perfused structures such as the superior cervical ganglion, while its effects on brain centres, like the caudate nucleus, which have the ability to synthesize acetylcholine at a high rate, have not been analysed. In this report we describe experiments in which we have observed the effects of HC-3, injected directly into the caudate nucleus of the rat, on the acetylcholine content of the nucleus, and on its ability to convert radioactive choline to acetylcholine.

Cannulæ (internal diam. 0·5 mm) were implanted by methods previously described[3] in the caudate nuclei of rats weighing 250 g under stereotaxic guidance and six days allowed for recovery from the operation. 3–5 μl. of saline, 3 μl. of HC-3 (3·6 μg/μl.), or 5 μl. of choline methyl-^{14}C (specific activity 3·6 mc./m.mole, concentration 7·8 μg/μl.), alone or in succession, were injected into the cannulæ from a μl. syringe, and, after appropriate intervals, the animals were killed by lightly anæsthetizing with pentobarbital and slitting the large vessels of the neck.

The head of each nucleus was immediately removed and its acetylcholine extracted in acid ethanol by a modification of the method of Crossland[4]. If the extracts were to be assayed for dopamine, a known quantity of tritiated noradrenaline (specific activity 7·59 c./m.mole) was added to permit estimation of recovery. The total volume of extractant and water used for washing was 4 ml. for each nucleus, on average 40 mg in weight. To this 1 drop of B.D.H. indicator was added, the solution brought to *p*H 4 with 0·2 N NaOH, and the volume then reduced to 0·5 ml. by blowing air over it while it was kept at 40°–50° C.

Chromatography. Extracts to be asayed for radioactive acetylcholine were spotted at this stage on Whatman No. 1 chromatographic paper. Descending chromatograms were run in *n*-propanol–formic acid–water (8 : 1 : 1) for 24 h. The solvent was allowed to run off the end so that acetylcholine achieved an apparent R_F of 0·90 and choline 0·74. A third, unidentified, radioactive spot always appeared at R_F 0·24. No other radioactive area was found. Acetyl-

choline and choline were located by adding cold carrier to the extracts and spraying duplicate chromatograms with Dragendorf's reagent. Areas on the unsprayed chromatogram corresponding to acetylcholine and choline were cut out, placed in vials with liquid scintillation phosphor, and counted in a liquid scintillation spectrometer[5]. The presence of acetylcholine was proved by combining eluants from the appropriate areas of several chromatograms, adding acetylcholine bromide and recrystallizing to constant specific activity from alcohol–ether.

Assay. Extracts to be assayed on a leech strip were diluted with 2 ml. of leech Ringer's solution; 2 drops of 0·4 N $HClO_4$, which produced a considerable precipitate, were then added. The precipitate was discarded, and the neutralized extract made up with leech Ringer to 5 ml. for assay.

The assay was conducted on a 2 mm × 30 mm eserinized strip of the dorsal muscle of *Hirudo medicinalis*. In all cases, the activity assayed as acetylcholine was shown to disappear on treatment with alkali (N NaOH). In most cases, the activity was matched against standard acetylcholine solutions to which an equivalent amount of neutralized alkali-treated extract was added. A few of the extracts shown to be active on eserinized leech muscle were tested on uneserinized preparations and found to have no apparent activity. Extracts of tissue to which HC-3 had been added sometimes depressed the leech muscle and the assays were then repeated on fresh muscle. With a good strip, however, standard acetylcholine solutions to which the appropriate amount of HC-3 was added, as well as caudates to which HC-3 was added at the commencement of the extraction, yielded the values to be expected in the absence of HC-3. In some preparations, HC-3 itself caused a slow contraction of the leech muscle. Such effects were controlled by assay against the usual combination of alkali-treated extract with acetylcholine as well as assay against acetylcholine solutions to which appropriate amounts of HC-3 were added.

About half the extract was treated with alumina at alkaline *p*H, the alumina eluted with 1·5 ml. of 0·5 N acetic acid and the eluant analysed for dopamine[6]. An aliquot of the final column eluant was then counted in a liquid scintillation spectrometer to determine the recovery of tritiated noradrenaline; recoveries averaged about 90 per cent when corrected for the aliquot of extract used in this procedure.

Measurements made on a control series of 12 animals, of which 5 had no operation, 3 had a cannula implanted in one caudate, and 4 had cannulæ in both caudates, established that whether cannulæ had been implanted or not, and whether in one or both nuclei, the acetylcholine concentration of the two caudates of the same animal did not differ from each other by more than 15 per cent, which is within the assay error, and usually by less than 10 per cent. For the 12 control animals the range of values expressed as μg/g tissue for the right and left nuclei was from 3·3 and 3·2 respectively in one animal to 7·4 and 8·6 in another. The mean of the total of 24 values was 4·9 ± 1·27 (*S.D.*).

It can be seen from Table 1 that HC-3 injected into one or other nucleus has a great effect on its acetylcholine content and reduces it significantly within a few minutes. The response to the drug varied somewhat from rat to rat, so that a smooth time curve was not obtained.

The values for dopamine in Table 1 showed considerably more variation than those for acetylcholine, but there was clearly no consistent change due to the HC-3. This would indicate that the effect on acetylcholine content is not due to non-specific destruction or inhibition of the metabolism of the caudate cells.

Preliminary results on the *in vivo* synthesis of acetylcholine from radioactive choline shown in Table 2 are a further indication that the effect of HC-3 is due to inhibition of acetylcholine synthesis.

Table 1. Effect of 3 μl. of HC-3 injected into one caudate nucleus

Time of death after injection of HC-3	Conc. ACh μg/g tissue Control	Test	Test as % of control	Dopamine μg/g tissue Control	Test	Test as % of control
0 min	5·2	6·0	(115)	10·8	13·5	(125)
2½ ,,	4·7	3·1	(66)	10·3	6·5	(64)
5 ,,	7·5	4·8	(64)	23·0	19·0	(83)
10 ,,	7·1	2·7	(52)	23·7	25·8	(109)
10 ,,	6·0	3·7	(45)	24·6	26·7	(108)
15 ,,	7·6	0·6	(8)	9·2	11·0	(120)
15 ,,	8·4	5·3	(63)			
20 ,,	3·7	1·4	(38)	21·2	23·3	(110)
30 ,,	4·9	0·5	(11)	12·9	9·4	(73)
30 ,,	6·2	0·8	(12)	28·0	22·9	(82)
60 ,,	6·3	0·7	(10)	15·8	12·8	(81)
24 h	2·5	1·8	(72)	24·5	21·0	(86)
36 ,,	4·2	5·1	(121)			
48 ,,	5·9	6·2	(105)			

Table 2. Conversion of choline-^{14}C to ACh-^{14}C in caudate nucleus

Time from HC-3 injection to choline-^{14}C inj.	Time from choline-^{14}C injection to death	$\frac{\text{ACh-}^{14}\text{C}}{\text{Choline-}^{14}\text{C}} \times 1{,}000$ HC-3 caudate	Contralateral caudate
1 h	0·5 h	11	34
1 h	1 h	20	44

$\frac{\text{ACh-}^{14}\text{C}}{\text{Choline-}^{14}\text{C}}$ is ratio of counts per min in the two fractions isolated by paper chromatography.

In these experiments, HC-3 was injected into one caudate, and 1 h later choline–methyl-^{14}C was injected simultaneously into both caudates. The effect of the HC-3 was judged by comparing the ratio of acetylcholine–^{14}C to choline–^{14}C in the caudate receiving HC-3 with that in the control caudate. The results show more net synthesis of acetylcholine 1 h after choline–^{14}C than after 0·5 h, and in each case the acetylcholine synthesis was considerably less on the HC-3 side.

Only about 5 per cent of the injected radioactivity was recovered, and only 1–2 per cent of this was as acetylcholine. Choline and the unidentified spot accounted for most of the radioactivity recovered.

The lowest values for acetylcholine in the treated nucleus were 8–12 per cent of the control nucleus. Since this appears to be the minimum level to which the acetylcholine could be reduced by HC-3, the balance of 88–92 per cent may be taken to correspond to the depot acetylcholine of the caudate nucleus as defined by Birks and MacIntosh[7] for the superior cervical ganglion. In the case of the ganglion, this fraction is about 85 per cent of the total acetylcholine. In the case of pons and medulla in the investigation by Metz[2] where HC-3 was given intravenously in minimal doses, the acetylcholine content fell to 4–25 per cent of the control levels during severe depression and respiratory failure. There is, therefore, a reasonable measure of agreement in the dimensions of the response of these various tissues.

The values observed after 24 h and later indicate that the HC-3 effect is reversible, though probably rather slowly so. The variation in response, which is most obvious in the very different values found for the two animals tested 15 min after HC-3, may reflect efficiency of diffusion, individual susceptibility to the drug, or the state of activity in the nucleus. If it is accepted that the reduction of acetylcholine is due to the effect of HC-3 on synthesis only, possibly indirectly by filling up storage spaces, it follows that the rate at which the drug reduces the level of stored acetylcholine will depend on how fast the pre-existing acetylcholine is being released. In these experiments it appears that it required a minimum of 10 min to discharge 50 per cent of the acetylcholine, but this time includes that required for diffusion and penetration of the drug. The true turnover time for acetylcholine in the caudate is therefore probably much less than the 20 min indicated by these experiments. The situation may be analogous to that encountered by Birks and MacIntosh[1,7] in the perfused superior cervical ganglion. In an untreated ganglion the resting content of acetylcholine could be turned over with about 10 min of stimulation, but more than 20 min of stimulation was required to reduce the acetylcholine to the minimal level in a ganglion treated

with HC-3. The difference in time presumably represents the time required for HC-3 to achieve its full effect in this system.

The results on synthesis of radioactive acetylcholine are only preliminary since little is known about how most of the injected choline was metabolized. Alcohol extracts of blood and the various organs showed heavy labelling, indicating wide distribution of the radioactivity. Radioactivity was also detected in the precipitate from the acetylcholine extraction, suggesting the possibility that some injected choline was incorporated into brain lipids. With several routes competing for the injected choline, and with the possibility of HC-3 influencing any of them, there must be some doubt that the influence of HC-3 on the radioactive acetylcholine/choline ratio is solely due to interference with acetylcholine synthesis.

Approximately 0·03–0·06 μg of radioactive acetylcholine were detected, indicating a net synthesis rate of 1–2 μg/g/h. Considering that diffusion of the choline-^{14}C and mixing with the cold pool had to take place before radioactive acetylcholine synthesis, and that breakdown of the synthesized radioactive acetylcholine was undoubtedly taking place *in vivo* before death, the observed net radioactive synthesis rate is obviously far below the true endogenous synthetic rate. A rate closer to 30 μg/g/h would be indicated by the HC-3 experiments, but again this is probably low. The acetylcholine-synthesizing capacity of the caudate of the rat, as indicated by the choline acetylase activity *in vitro*, is of the order of 8,000–10,000 μg/g tissue/h (ref. 8). Ordinarily, synthesizing enzymes are present in generous excess, so that this figure is undoubtedly far above the true rate. Even so, it is probable that our experiments with radioactive choline grossly underestimate the turnover rate of ACh in this tissue. Their positive value is that they provide further evidence that HC-3 interferes with ACh synthesis *in vivo*.

We thank Profs. F. C. MacIntosh and F. Schueler for gifts of samples of HC-3.

The cost of this research was defrayed by grants from the Medical Research Council of Canada (to C. O. H. as visiting scientist), a Canadian Federal Provincial Mental Health Grant (609–5–141) and the U.S. National Science Foundation (*GB*–827).

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Thermogenesis and Thyroid Action

FOR some time[1,2] it has been realized that thyroid hormone raises metabolic rates, increases activity of the hexose monophosphate pathway, decreases apparent TPNH–DPN transhydrogenase activity and stimulates 5 α-steroid reductase activity. More recently thyroxin has been shown to increase by a factor of ten or twenty the activity of the intra-mitochondrial α-glycerophosphate dehydrogenase with no concurrent effect on the 'soluble' counterpart[3,4].

The net result of the latter action is to drive the reduction of dihydroxyacetone phosphate (DHAP) toward alpha-glycerophosphate (α-G-P) by a DPNH-requiring enzyme in the extra-mitochondrial phase, and conversely to facilitate the reverse reaction within the mitochondrial compartment, wherein the oxidation of α-G-P is coupled to cytochrome *c*; the resultant is to give rise to only one phosphorylation during the course of transfer of energy from DPNH to acceptor, with the formation of water. Thus we retain in the potential energy pool the energy equivalent to only one high-energy phosphate bond (~P). This is in contrast to the usual electron transport sequence by which intra-mitochondrial oxidation of beta-hydroxybutyrate, for example, passes through DPNH to water with formation of 3~P.

Hence the latter reaction gives three times the work function that is derived from oxidation of α-G-P, and in lieu of data to the contrary one must conclude that the DPNH oxidation via the α-G-P pathway proceeds at one-third the efficiency of that via the full complement of the electron transport chain. Thus it follows also that this pathway must yield heat in an amount equivalent to at least two of the phosphorylative steps normally associated with the electron transport chain.

In view of the differential stimulatory effect of thyroxine on the α-G-P 'shuttle' as described above, it is proposed that possibly the major thermogenic effect of that hormone is mediated through preferential use of this pathway. This would not be inconsistent with the fact that 3-phospho-glyceraldehyde is an important end product of the thyroid-stimulated hexose monophosphate pathway (HMP) and that extra-mitochondrial equilibrium conditions heavily favour the reduction of DHAP to α-G-P (ref. 5).

This proposal also implies that DPNH of the extra-mitochondrial phase is not directly transferred into the mitochondrion, but rather that its hydrogen is transported by small, readily diffusible components such as α-G-P and β-hydroxybutyrate. Documentation in support of this dates from earlier work[6] showing that DPNH is not readily transferred into the intact mitochondrion *in vitro*, though it may be depleted from these sites by treatments which probably increase the normal permeability of the confining lattice[7].

The extent to which thyroid activity directs triose phosphate into the α-G-P shuttle would appear to influence adversely the synthesis of fats relative to their degradation. According to the line of reasoning outlined, this influence would, in effect, favour the α-G-P pathway over that of β-hydroxybutyrate, with correspondingly greater oxidation relative to phosphorylation, that is, with greater heat production relative to work potential.

The net thermogenic resultant of TPNH generation by HMP activation appears both in non-phosphorylating extra-mitochondrial pathways, coupling probably through cytochrome b_5 with molecular oxygen[2], and also in the extent to which rising levels of TPNH in liver of rats[8] appear during cold-acclimation along with the microsomal TPNH cytochrome *c*-reductase[9,10]. In thyroxinized rats the latter rises as both the DPNH cytochrome *c*-reductase and total DPN fall[10–12] with increasing dosage levels. As no uncoupling of mitochondrial oxidative phosphorylation occurs during such treatment unless microsomal elements are also present[2,10,11], it is concluded[2] that apparent 'uncoupling' (reduction of P/O ratio) in the latter instance implies a shunting of oxidative activity via extra-mitochondrial, poorly phosphorylative pathways, with attending thermogenesis.

Finally, the normal reductive distribution of H via TPNH into the fat depots leaves open the relative thermogenic contribution of the respective degradative pathways

of the fatty acids. Considering only initial and final states between TPNH and H_2O, it is clear that thermogenesis will vary as the work function (potential energy pool) generated along the oxidative pathway. Hence, excluding that portion metabolized via mitochondrial DPNH-dependent pathways, the more thermogenic routes will be those passing by way of the acyl dehydrogenases to the electron transport flavins (ETF) (ref. 13); the latter, coupling through FAD, will exhibit phosphorylative ratios (P/O) of the order of 2, that is, a work function of 2/3 of that of β-hydroxybutyrate and thus thermogenesis in equivalence of $1 \sim P$.

Whether additional thermogenesis is derived through subsequent utilization of the potential energy ('high-energy') bond pool formed in these reactions is essentially irrelevant to the present argument, since the thermal yield from this source will depend on the efficiency of the mechano-chemical or other coupling and the external work performed by the system. It is, of course, acknowledged that in all transfers, both to and from the potential energy pool, heat losses (thermogenesis) may also be expected, so that in this sense total thermogenesis tends to increase as a function of turnover rate whatever the pathways used.

It is concluded, therefore, that thermogenesis from thyroid action is facilitated by recourse not to uncoupling of mitochondrial oxidative phosphorylation, but rather by emphasis of alternative pathways wherein both DPNH and TPNH are oxidized either by extra-mitochondrial, poorly phosphorylating transfers, or by indirect couplings at lower potential levels of the electron transport chain. Important mediators of this appear as the α-G-P shuttle and a quasi-trans-hydrogenation via the fat depots[14] and the acyl dehydrogenases.

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Trigeminus Reflex in Young Infants

A weak electrical stimulus on the receptive field of N. trigeminus (parts II and III) in infants between a few weeks and 12 months old is followed by a discharge in the nerves of the muscles, biceps and triceps brachii, and biceps and quadriceps femori, without any visible movement. The activity of these muscles is synchronized. No reciprocal pattern could be detected. We found two groups of latencies—one with a maximum at 140 msec and a second between 300 and 400 msec (Fig. 1). Stimulation of regions other than that supplied by the trigeminal nerve is without effect, unless the stimulus is very strong —a fact which is in accordance with the work of Hooker[1] on human fœtuses. We suppose that the myographically-

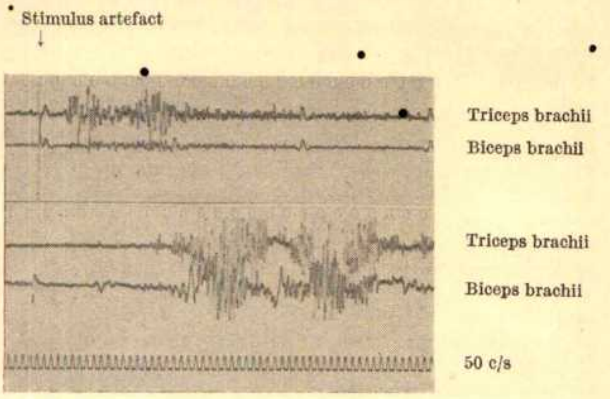


Fig. 1

tested reactions of muscles of the extremities have nothing to do with the Moro-reflex, or common fright reaction, which is often seen after very strong visual, acoustic or tactile stimulation.

This work was supported by a grant from the Deutsche Forschungsgemeinschaft.

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Potassium-free Effect in Squid Axons

Previous work[1] has shown that the sodium efflux from squid giant axons is sensitive to the external concentration of potassium ions; the Na efflux falls to about 1/3 of control values when the sea water surrounding the axon is made free from potassium. Similar observations have been made with frog sartorius muscle[2], but these showed further that when $[Na]_i$ in muscle was low, Na efflux was no longer sensitive to external [K]. The following experiments were undertaken to see if the Na efflux from squid axons would be similarly insensitive to $[K_0]$ if $[Na]_i$ were low.

Preliminary trials showed that two different methods yielded giant axons with a low $[Na]_i$. In the first, axons were immersed for 6 h in 400 mM KCl + 50 mM $CaCl_2$ solution with the *p*H adjusted to 8·0 with *tris*. Such axons had, on analysis, $[Na]_i$ of about 35 mM. In a second method, axons were stimulated in normal sea water with all Na^+ replaced by Li^+ for 0·5 h at 60/sec. An analysis of the axoplasm of 7 axons subjected to stimulation in Li-sea water showed the following mean values: $[Na]_i = 36$, $[K]_i = 158$, $[Li]_i = 117$ m.mole/l. Other (unpublished) measurements from our laboratory for fresh axoplasm showed a value for $[Na]_i$ of about 70 mM so that both experimental treatments reduced $[Na]_i$ to about half that of fresh axoplasm. Because the experimental procedures involved much time and axon survival was judged to be a critical factor in their success or failure, the stimulation in Li method was chosen for all the measurements to be reported. The $[Li]_i$ found above can be compared with an entry of Li of 43 μmole/g calculated on the basis of a resting influx of 50 pmole/cm²sec for the 3 h that the axon was isolated and using a factor of 80 cm^{-1} for the surface : volume ratio of a 500μ axon. To this must be added 69 μmole/g resulting from stimulation at 60/sec for 30 min and using a value of net Li flux of 6 pmole/cm² impulse as appropriate to a temperature of 10° C. The sum of the stimulated and resting entry yields a final $[Li]_i$ of 112 mM, which is quite close to the value obtained from analysis. It might

appear that the most direct method of obtaining axons with a low $[Na]_i$ is to use perfused axons with zero $[Na]_i$ in the perfusion fluid; such axons, however, have an unstable Na efflux and are not suitable for the measurements to be described.

Squid axons used in this work were dissected from *Loligo pealii* and the axon immersed in Li-sea water, where it was cleaned. The axon was then transferred to a 'Teflon' chamber, where it was stimulated in Li-sea water containing about 1 mM ^{24}NaCl. After stimulation, the axon was washed in several changes of Li-sea water and was finally left for 1·5 h in fresh Li-sea water. The axon was then mounted in a 'Teflon' chamber with a groove through which various fluids were circulated past the axon with a motor-driven syringe. The fluid coming from such a chamber was collected every 3–5 min and the samples dried in planchets, and these were monitored for radioactivity by counting under a flow-gas Geiger tube with anticoincidence guard. The sequence of external solution changes was generally: Li-sea water, K-free Li-sea water, and a return to Li-sea water. In some cases, measurements were made of the change in Na efflux when an axon with a normal $[Na]_i$ was transferred from Li-sea water to K-free Li-sea water, and, in other cases, axons were transferred to Na-sea water (K-free) at the end of a long experiment in Li-sea water. In some experiments, 'Vaseline' seals were used to keep the ends of an axon from coming into contact with radioactive loading and efflux solutions; in other experiments this technique was abandoned in favour of radioactive loading of an axon before cleaning and afterwards cutting off about 5 mm from each end of the axon after cleaning. As the results were not different with the two techniques, it was concluded that extensive washing of the axon clears sufficient radioactivity from the ends of the axon so that the measurement of Na efflux is not greatly in error. All axons used were tested for excitability throughout the experiments and axons were discarded if they became inexcitable. Axons were loaded with Li^+ at 10° C, but efflux was measured at 20° C.

The results obtained are summarized in Table 1. In going from Li-sea water to K-free Li-sea water there is no change in Na efflux, while in going back to Li-sea water there is a 6 per cent increase in efflux but this is not statistically significant. The changes in Na efflux are based on c.p.m.^{2} of Na efflux evaluated over sampling times of 3 or 5 min. At least 3 efflux samples (and more usually 5) were collected under each experimental condition. The loss of Na from a fibre as large as a squid axon is so slow that the concentration cannot be expected to change by more than 10 per cent over a sample collection period of 90 min. Efflux as c.p.m.^{2}, rather than rate constant of Na loss, has been used in these measurements. A clear change in Na efflux is evident, however, when the axon is transferred from Li- to Na-sea water; the Na efflux then increases by about 50 per cent over values in Li-sea water and this change is independent of whether the Na-sea water has K^+. This finding is also new because previous measurements of the effect of Na-free solutions on squid axons have shown that Na efflux decreases on going from Na-free to Na-containing sea water[1,3]. Axons No. 2 and 10 (with a normal $[Na]_i$) were loaded with ^{24}Na by stimulation for 5–10 min in Na-sea water and were washed for 1·5 h in Li-sea water. They were then tested for a K-free effect. For axon No. 2, Na efflux in K-free Li-sea water was found to be 0·5 that for Li-sea water, while axon No. 10 showed an efflux in K-free Li-sea water that was 0·4 that of Li-sea water. Both axons were afterwards stimulated in Li-sea water to reduce $[Na]_i$, and the further results with these axons are shown in Table 1.

The results of this investigation may be summarized by saying that when the internal Na concentration of squid axons is decreased to about one-half of normal values (by stimulation of the axon in Li-sea water) the Na efflux from the axon becomes insensitive to the presence of K^+ in the sea water bathing the axon. Furthermore, the onset of lack of sensitivity to external K^+ appears to coincide with the appearance of a changed sensitivity of the Na efflux to the presence of Na^+ in the bathing sea water.

Table 1. Na Efflux Changes in Squid Axons (Mean $[Na]_i$ = 36 mM) when External Solution is changed to that indicated below. Efflux in Li-sea water ([K] = 10 mM) is Unity

Axon No.	On transfer from Li-sea water to K-free Li-sea water	On transfer from K-free Li-sea water to Li-sea water	On transfer from Li-sea water to Na-sea water
2	0·95	1·09	—
3	1·15	1·00	—
7	0·93	1·00	—
8	0·87	1·15	1·4
9	—	1·09	1·4
10	0·99	—	1·6*
11	1·10	—	—
Mean	1·00	1·06	1·5

*K-free Na-sea water

These experiments were performed at the Marine Biological Laboratory, Woods Hole, Massachusetts. We thank the Director for the facilities placed at our disposal. The investigation was aided by a grant (*NB* 03389) from the U.S. National Institute of Neurological Diseases and Blindness, Bethesda, Md.

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Effect of Pituitary Thyrotrophin on the Diglyceride/Triglyceride Interrelationships in Thyroid Tissue

In surviving cellular preparations of thyroid tissue, intermediary metabolism is altered profoundly when 'work' is induced by the addition of pituitary thyrotrophin (TSH)[1]. Heightened phospholipid metabolism is one of the basic features. Thus, TSH augments the incorporation of orthophosphate-^{32}P into certain phospholipids of thyroid slices even in the absence of exogenous organic substrates[2,3]. In the presence of substrate quantities of glucose-^{14}C or albumin-complexed ^{14}C-fatty acids (as potential carbon donors for the glycerophosphate or acyl residues of thyroidal lipids), TSH preferentially channelizes radioactivity into phospholipids rather than neutral lipids[1,4]. Since diglycerides may constitute communal precursors for both phospholipids and triglycerides[5], investigations were instituted to assess whether the disparate activation of phospholipogenesis is accompanied by detectable changes in the diglyceride fraction. The experiments have indicated that the incorporation of carbon atoms into the individual neutral lipid components of thyroid slices during 1–4 h of incubation is altered by TSH. Although TSH does not systematically change the total labelling of neutral lipids from glucose-^{14}C, the triglyceride-^{14}C/diglyceride-^{14}C ratio is reduced, possibly connoting a new 'steady-state' favouring phospholipogenesis.

Individual vessels were filled with 150–200 mg (wet wt.) of canine thyroid slices and incubated at 38° in an

Table 1. Effect of TSH on the Incorporation of Glucose U-^{14}C into the Neutral Lipids (NL) of Dog Thyroid Slices

Exp.* No.	Incubation (h)	−TSH				+TSH			
		Total† NL (c.p.m./g)	% NL counts: TRI‡	DI‡	TRI/DI	Total NL (c.p.m./g)	% NL counts: TRI	DI	TRI/DI
1	1	7,158	70·5	23·0	3·07	5,772	66·9	29·7	2·25
2	2	35,092	84·9	11·6	7·32	36,258	80·3	13·8	5·82
3		19,337	61·8	24·2	2·55	16,513	55·2	31·9	1·73
4		8,202	70·4	24·7	2·85	16,084	73·9	23·9	3·09
5	3	10,322	74·9	18·6	4·03	14,096	65·6	27·5	2·39
6		26,036	67·2	23·2	2·90	26,282	44·4	36·8	1·21
7		37,626	63·9	25·1	2·55	37,886	61·7	26·0	2·37
8		17,429	72·5	15·7	4·62	27,876	72·5	16·3	4·45
9		31,270	81·3	14·1	5·77	38,383	77·2	17·8	4·34
10	4	20,448	79·2	15·3	5·18	18,879	66·2	24·4	2·71
11		38,372	60·4	24·6	2·46	45,652	46·5	34·6	1·34
12		36,503	58·9	24·8	2·38	32,278	44·9	33·3	1·35
8*a*	3	99,649	92·7	4·7	19·7	97,595	91·7	6·6	13·9
9*a*		74,005	91·6	7·1	12·9	93,881	90·6	8·5	10·7

* Suspending media consisted of Krebs–Ringer–bicarbonate containing 2 mg/ml. glucose U-^{14}C (4–5 μc./ml.) ± 0·1 U.S.P. units TSH/ml. in experiments 1–12. The media were further supplemented with 1 μmole/ml. oleate complexed to 10 mg/ml. fat-free albumin in Exps. 8*a* and 9*a*.

† Total neutral lipid (NL) radioactivity is expressed as c.p.m./g of slices (that is, initial wet weight of slices).

‡ TRI and DI refer to the percentages of the total neutral lipid radioactivity that were recovered in the triglyceride and diglyceride fractions respectively. By this convention, 100%—(TRI + DI) represents the fraction of total neutral lipid radioactivity containing monoglyceride, cholesterol, cholesterol esters, free fatty acids, etc.

atmosphere of 95 per cent oxygen + 5 per cent carbon dioxide using diluents and techniques similar to those described earlier[6]. Uniformly labelled glucose-^{14}C (glucose-U-^{14}C; New England Nuclear Corp., Boston, Mass.) 4–5 μc., 2 mg/ml. was added to the suspending medium. Under such conditions, all the phosphoglycerides become labelled *in vitro*, and TSH invariably increases the incorporation of glucose carbons into phospholipids[7]. For the present work, bovine TSH containing 3·52 U.S.P. units/mg was obtained from National Institutes of Health (TSH-B_2 Endocrine Study Section preparations). The hormone was dissolved in Krebs–Ringer–bicarbonate (*p*H 7·4) containing gelatin (2 mg/ml.) to prevent glassware absorption. Sufficient TSH to yield 0·1 U.S.P. unit-ml. incubation medium was added at the beginning of incubation. After defined intervals, the slices were removed and frozen immediately in liquid nitrogen. The tissue was homogenized with chloroform – methanol (2 : 1 v/v) and the crude lipid extract purified using the procedure of Folch *et al.*[8]. Neutral lipids were separated from phospholipids by column chromatography with silicic acid (Bio-Rad). Aliquots were taken for radioactive assay and expressed as c.p.m. of neutral lipid/g initial wet weight of slices. Individual neutral lipid components were separated by thin-layer chromatography (silica-gel *G*) with either diethyl ether–iso-octane (1 : 1 v/v) or petroleum ether–acetic acid–diethyl ether (90 : 1 : 10 v/v/v) as the solvent systems and the inclusion of 0·1 per cent (w/v) butylated hydroxy-toluene to prevent auto-oxidation. The thin-layer plates were sprayed lightly with 0·2 per cent (w/v) 2,7-dichloro-fluorescein in ethanol and the areas of gel corresponding to diglyceride (1,2 and 1,3) and triglycerides were aspirated[9] into counting vials for radioactivity assay by liquid scintillation counting. The counts in diglycerides and triglycerides were expressed as a percentage of the total neutral lipid radioactivity. For statistical analysis, paired *t* tests were performed on the differences of the logarithms of the absolute values observed in the absence (that is, −TSH) and in the presence (+TSH) of TSH. This is equivalent to testing whether the average ratio of values (that is, +TSH/−TSH) is greater than unity[10].

Serial analyses of slices from any one animal indicated that the labelling of neutral lipids from glucose-U-^{14}C progressively increased throughout incubation. In confirmation of our experiences with other species[11], most of this neutral lipid radioactivity was present as glyceride–glycerol at all intervals. The labelling was quite variable in different animals, and the effects of TSH on the incorporation of radioactivity into the total neutral lipids followed no consistent pattern ($P > 0·1$ for Exps. 1–12, Table 1). In other species, we have previously observed comparable variability in the control and TSH-stimulated labelling of thyroidal neutral lipids *in vitro*, and have ascribed it to differences in the prior exposure to TSH and in the availability of endogenous fatty acids for glycerol esterification[1,7]. However, in the work recorded here, regardless of the effects of added TSH on total neutral lipid radioactivity, a striking change in the distribution of radioactivity in the individual neutral lipid components was evident. In the presence of TSH a higher proportion of the neutral lipid radioactivity was recovered in the diglycerides so that the ratio of TRI/DI was reduced significantly ($P < 0·001$ for Exps. 1–12, Table 1).

To assess whether the relative accumulation of labelled diglycerides was due to an impairment of triglyceride synthesis by TSH, added vessels in two experiments were supplemented with 1 μmole/ml. of albumin-complexed oleate to enhance total neutral lipogenesis (Exps. 8*a* and 9*a*, Table 1). The conjoint availability of exogenous glucose and fatty acids caused a three- to five-fold increase in neutral lipid radioactivity in control vessels (as we have previously reported[11]), and an even greater increase in the triglycerides (Table 1). Despite this augmentation in the net labelling of triglycerides, the TRI/DI ratio was still lowered by TSH.

Ongoing investigations with glycerol 1,3-^{14}C afford further corroboration that the TSH-induced relocations in the individual neutral lipids can be dissociated from the availability and biogenesis of fatty acids. In surviving thyroid slices, the incorporation of glycerol radioactivity into fatty acids is negligible although all the glycerides become labelled via esterification of the glycerol-^{14}C. In such circumstances, the ratio of radioactive TRI/DI is consistently lowered by TSH. Work is in progress to characterize the diglycerides on the basis of the position of the acyl residues and to see if more pronounced TRI/DI changes can be unmasked by appropriate subcellular fractionation.

However, even in the absence of such information, the present results indicate that the qualitative pattern of glyceride–glycerol labelling in neutral lipids is altered during the induction of 'work' in thyroid tissue with TSH. Since similar changes were observed during 1–4 h of exposure to the hormone, the phenomenon may reflect a new lipogenic equilibrium which selectively favours cytostructural renewal (that is, phospholipogenesis) and constitutes an integral component of the 'work-hyperplasia' response. Whether it is unique for the thyroid or whether it represents a more generalized phenomenon will depend on the findings in other tissues in which the 'working state' is also attended by an increased turnover of phospholipids[12,13].

This work was supported in part by research grant *A*–1571 and training grant 2 *A*–5060 from the National

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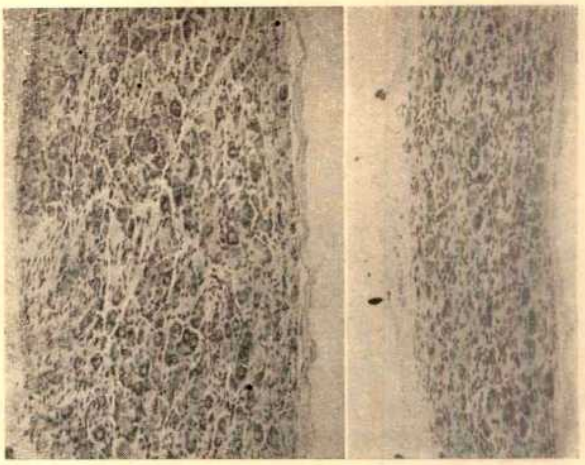
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PHARMACOLOGY

Sources of Noradrenaline in the 'Immunosympathectomized' Rat

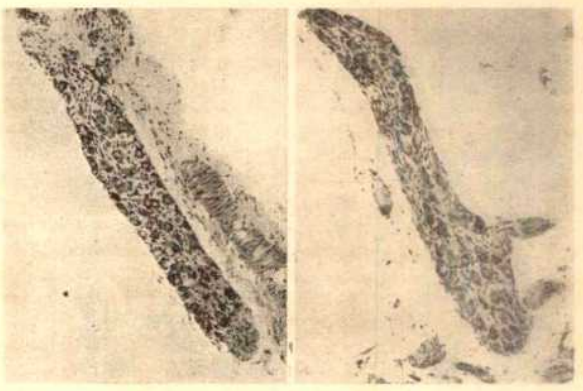
THE good state of health of cats deprived of the whole length of their paravertebral sympathetic chains and a part of the prevertebral (cœliac and mesenteric) ganglia is well known since the work of Cannon[1]. In contrast, removal of the prevertebral ganglia is not well tolerated in dogs, which do not survive this operation because of intractable diarrhœa[2]. 'Immunosympathectomized' rats, in which extensive irreversible atrophy of the peripheral sympathetic system is produced by injecting, during the first few days of life, an antiserum to the nerve growth factor described by Levi-Montalcini and Booker[3], appear healthy, grow, reproduce and have normal gastro-intestinal function[4].

It was, therefore, of interest to know whether these animals, in addition to having lost most of the neurones of the paravertebral sympathetic chains, had been deprived of their prevertebral abdominal sympathetic ganglia. Hooded rats were injected for the first five days after birth either with antiserum (reconstituted freeze-dried material prepared and assayed by Profs. R. Levi-Montalcini and P. Angeletti), or with 0·9 per cent sodium chloride solution. The rats injected with antiserum had narrow palpebral fissures. One experimental rat and one control were killed at 11 weeks, another pair at 18 weeks of age. The regions of the inferior mesenteric ganglia, of the superior mesenteric and cœliac ganglia, and of the stellate or the superior cervical ganglia were dissected, fixed with bichromate-formaldehyde, sectioned serially and stained with cresyl violet. Whereas stellate and superior cervical ganglia of the rats given antiserum showed the expected atrophy to a thin, mainly fibrous structure containing but few ganglion cells, the prevertebral ganglia were, so far as could be ascertained in structures which show a large degree of individual variation, normal in size and undoubtedly normal in nerve cell density. Fig. 1*b* shows the atrophied superior cervical ganglion of the rat injected with antiserum and killed at 18 weeks, and Fig. 1*a* the corresponding ganglion of its normal control. Figs. 2*a* and *b* are inferior mesenteric ganglia taken from the same pair of rats and, in contrast to the superior cervical ganglia, are indistinguishable in size and fine structure.



a *b*

Fig. 1. Longitudinal sections through superior cervical ganglia. (*a*) control rat; (*b*) immunosympathectomized rat. (Cresyl violet; × *c.* 85)



a *b*

Fig. 2. Longitudinal sections through inferior mesenteric ganglia. (*a*) Control rat; (*b*) immunosympathectomized rat. (Cresyl violet; × *c.* 66)

The preservation of the prevertebral sympathetic ganglia in the immunosympathectomized rat may well account for the fact that gastrointestinal and reproductive functions are not noticeably impaired. Furthermore, this finding shows that the action of the antibody formed against the nerve growth factor is of even greater specificity than hitherto believed; within the adrenergic neurones it singles out those of the sympathetic chain while sparing those of cœliac and mesenteric ganglia. This might be due to chemical differences between the two types of ganglia, or to the fact that the prevertebral ganglia might be more mature at birth than the paravertebral ganglia.

Finally, a recent observation by Carpi and Oliverio[5] is readily explained by the presence of intact sympathetic ganglia. These authors have found that, under resting conditions, immunosympathectomized rats do not excrete detectable amounts of noradrenaline, but noradrenaline is excreted in normal or supernormal quantities after an injection of amphetamine or after adrenal demedullation. The source of the excreted noradrenaline was tentatively suggested to be extramedullary chromaffin tissue. However, in contrast to the abundance of such tissue in the adult dog, cat and rabbit, very little of it exists in the adult rat[6], and scarcely any was found in the vicinity of the rat's prevertebral ganglia in the present work. Impulses travelling along the neurones of the prevertebral ganglia, on the other hand, are an obvious potential way of releasing noradrenaline in the immunosympathectomized,

adrenalectomized rat. The question which remains open is whether these neurones discharge as a result of neural or in response to some humoral stimuli.

I thank Prof. Eleanor Zaimis for the gift of injected rats, and Mr. B. S. C. Hollands for preparing the sections.

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Tumour of the Thymus in Magnesium-deficient Rats

DURING the course of examinations on the effects of magnesium deficiency in rats[1–4], tumours of the thymus developed in a few animals after prolonged periods of deficiency. This incidental finding stimulated a special investigation. Twelve male Holtzman Sprague–Dawley rats (110–125 g body-weight) were fed a magnesium-deficient diet[3] for 95 days; twenty controls received the same diet to which was added 0·6 g of magnesium sulphate per 100 g of diet. Only five deficient rats survived until the end of the experiment. Convulsive seizures accounted for most deaths during the first weeks. Animals dying later showed extensive pleural effusion and ascitis; chronic lung disease with bronchiectatic abscesses was also observed. One rat died with a large thymus weighing 1·6 g after 75 days of magnesium deficiency. Microscopic examination revealed a total replacement of the normal architecture of the gland by sheets of large lymphoid cells. Another abnormal thymus was found at autopsy at the end of the experiment. In this case, thymic tissue had extended in all directions, infiltrating the base of the heart as well as the wall of the œsophagus; the same tissue had also grown retrosternally, reaching the diaphragm. Histologically the appearance was again characterized by a predominance of large lymphocytes or lymphoblasts with numerous mitotic figures (Fig. 1). Limited infiltration with similar cells was also seen in the portal spaces of the liver, in the kidney and in the adrenal cortex. The spleen and lymph nodes were not prominently enlarged and the blood was aleukæmic in both cases. The tumour was transplanted subcutaneously in Sprague–Dawley rats and grew rapidly, killing its host in 20–25 days. No tumour of the thymus was found in rats that were not magnesium-deficient.

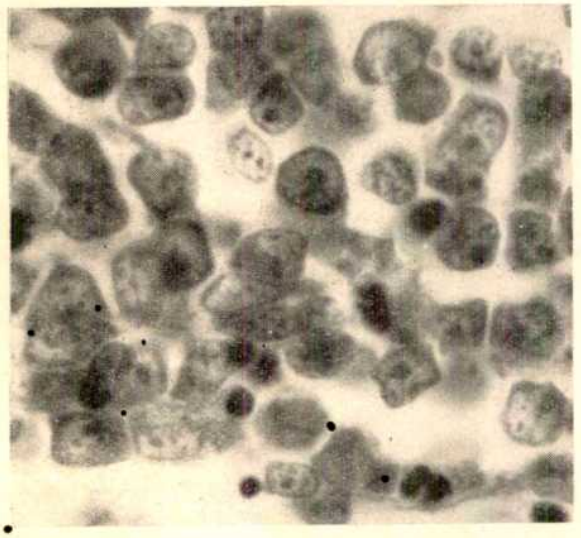


Fig. 1. Typical high-power field of the tumour. Predominance of lymphoblasts in active multiplication (Dominici, × 800)

This tumour is unusual because of its site in this species; spontaneous tumours of the thymus, with or without generalized leukæmia, are uncommon in rats[5–7]. It is also of interest that the tumour was apparently induced by a deficiency in magnesium. The chronic lack of magnesium at the cellular level might increase the removal of magnesium out of the nucleus into the cytoplasm, thus causing chromosomal aberrations and cell mutation, as suggested by Jayson[8]. However, this would not explain the electivity of the thymus. So far, six tumours of the thymus have been observed among 138 magnesium-deficient rats after 75–105 days. The occurrence of lymphoblastic adenoma of the thymus in chronically magnesium-deficient rats was also recorded recently by Jasmin[9].

Previous investigations have shown that magnesium deficiency is conducive to histamine liberation with a reduction of the number of mast cells[1,2,3,10]; it is difficult, so far, to associate these findings with the production of tumours of the thymus.

This work was supported by a grant from the National Cancer Institute of Canada.

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Rapid Induction by Acetylcholine, Bradykinin and Potassium of a Nociceptive Response in Mice and its Selective Antagonism by Aspirin

HOW aspirin exerts its analgesic action has been much discussed. The findings here reported suggest that it depresses certain chemonociceptive responses at or near their peripheral source, rather than in the central nervous system, and that it acts on these directly, rather than indirectly through suppressing œdema formation.

An apparently nociceptive response of mice to intraperitoneal injection of phenylbenzoquinone or acetic acid has been much used in testing analgesic drugs[1–5]. In this response, which has been described as 'writhing', 'squirming', 'stretching' or 'cramping', the dominant feature is a wave of constriction passing caudally along the abdominal wall, followed by extension of the hind limbs. It will here be called the constriction response. Whittle[6] has proposed that acetic acid and other noxious agents do not elicit this response directly, but by releasing a kinin.

Since substances acting directly are likely to have less latency than those acting indirectly, we have measured the latency of response in mice (T/O strain) after intraperitoneal injection of several endogenous substances known to elicit pain in man. Table 1 compares the speed

Table 1. Speed of Onset of the Abdominal Constriction Response after Intraperitoneal Injection of Various Substances into Mice (T/O Strain)

Substance	Dose mg/kg	No. of mice treated	Cumulative % mice responding by					
			2 sec	5 sec	30 sec	2 min	10 min	30 min
Acetic acid	50·0	65	0	0	0	12	74	82
Acetylcholine bromide	0·8	110	0	3	26	74	74	—
	3·2	250	7	20	24	88	96	—
Bradykinin	0·06*	215	0	0	31	54	54	—
	0·125†	20	5	5	45	55	60	75
	0·4*	85	0	5	78	86	89	95
Chlorbutol	6·25	85	0	0	0	0	15	22
	50·0	20	0	0	0	15	55	75
Histamine acid phosphate	2·5	40	0	0	0	5	20	35
5-Hydroxytryptamine creatinine sulphate	0·01	20	0	0	0	5	10	35
	2·5	30	0	0	0	10	10	—
Phenylbenzoquinone	1·0	30	0	0	0	10	80	83
Potassium chloride	65·0	90	1	3	42	61	61	—
	130·0	205	3	11	46	68	71	—
0·9% w/v sodium chloride in distilled water	—	85	0	0	0	3	13	21
1·8% w/v sodium chloride in distilled water	—	20	0	0	0	0	25	35
Distilled water	—	30	0	0	10	43	43	47

Acetic acid, phenylbenzoquinone and potassium chloride were dissolved in distilled water; other substances in 0·9 per cent sodium chloride in distilled water. The injection volume in all treatments was 10 ml./kg. Weights of salts are given as active acid or base.

* Bradykinin used was synthetic material prepared by the method of Nicolaides and De Wald[15].

† Bradykinin used was synthetic material from ampoules containing chlorbutol, and a sodium acetate–acetic acid buffer, kindly supplied by Sandoz Products, Ltd.

of onset of response to 5 endogenous pain-producing substances with that to acetic acid and phenylbenzoquinone. Acetylcholine bromide, bradykinin and potassium chloride elicited the response in some mice within 5 sec and in most mice within 2 min. No other substance had this rapid time-course of action.

Emele and Shanaman[6] and Whittle[8], using bradykinin from ampoules which contained chlorbutol and sodium acetate–acetic acid buffer, have reported a much slower onset of response to bradykinin than we observed with solid synthetic bradykinin freshly dissolved in 0·9 per cent sodium chloride solution. We therefore tested some ampoules containing 100 μg/ml. of bradykinin and 5 mg/ml. of chlorbutol and acetate buffer. In our hands, the onset of response to this treatment was as rapid as that to bradykinin in saline (Table 1). We also tested chlorbutol, which was effective at 50 mg/kg. Chlorbutol resembled acetic acid in time-course of action, both substances eliciting responses from a few mice within 2 min and from most mice within 30 min of injection (Table 1). After 2 min also, more mice responded to the control injection of 0·9 per cent sodium chloride solution. We conclude that the proportion of mice responding within 2 min of intraperitoneal injection is likely to be a more reliable measure of the activity of bradykinin than that recorded after this period. This conclusion applies also to acetylcholine and potassium.

The question arises of how these agents elicit this response. Its rapid onset after acetylcholine, bradykinin and potassium argues that they cause nociception independently of fluid exudation from blood vessels. The following considerations suggest that these three agents probably stimulate chemonociceptors directly, rather than mechanical nociceptors indirectly, by contracting the gut musculature. (1) Each substance elicits pain from an exposed blister base in man[9]; (2) the spasmolytic drug papaverine (50 mg/kg subcutaneous) did not inhibit the acetylcholine-induced response; (3) 5-hydroxytryptamine induced little or no response, although it causes defæcation in mice[10]; (4) bradykinin in dog, cat and rabbit initially inhibits gut movement[11,12]. Since atropine (0·2 mg/kg subcutaneous) inhibited the response to acetylcholine but at a much larger dose (25 mg/kg subcutaneous) it did not reduce that to bradykinin, these two noxious agents probably excite different chemonociceptors.

Morphine, aspirin and other analgesic drugs inhibit the constriction response to phenylbenzoquinone[1,2,4,5], to acetic acid[3,7] and to bradykinin[6]. These actions were shown in tests in which responses were counted from 10 or more min after injection of noxious agent. Anti-nociceptive tests involving a much shorter duration of noxious stimulation are less likely to be associated with inflammation, as well as being ethically and economically preferable. We have, therefore, measured the potencies of morphine and of aspirin in inhibiting constriction induced by acetylcholine, bradykinin and potassium, over a period of 2 min from the time of injection of noxious agent. At the end of this period, the response to these agents had declined.

Table 2 gives the subcutaneous doses, with fiducial limits, of morphine sulphate, calcium aspirin and sodium salicylate which reduced by 50 per cent the proportion of mice responding to acetylcholine. Since bradykinin and potassium induced constriction in much less than 100 per cent of mice, the lowest doses of drug, which reduced by at least half the proportion of mice responding to these agents, are given in Table 2 without fiducial limits. At all finite doses in Table 2, the effect was significant ($P < 0·01$). When aspirin was given by mouth, it also inhibited acetylcholine-induced constriction, the ED_{50} being 56 (limits 36–88) mg/kg.

Table 2. Potency of Morphine, Aspirin and Salicylate against the Constriction Responses of Mice to Acetylcholine, Bradykinin and Potassium

Constricting agent		Median effective dose (mg/kg)		
Substance	Dose (mg/kg)	Morphine sulphate	Calcium aspirin	Sodium salicylate
Acetylcholine bromide	3·2	0·43 (0·29–0·64)	26 (17–40)	151 (97–235)
Bradykinin	0·4	0·3	200	*N.T.*
Potassium chloride	130	0·3	> 400	*N.T.*

Analgesic drugs were administered subcutaneously 20 min before the constricting agent was injected intraperitoneally. An effective dose was that which reduced by 50 per cent the proportion of mice responding. Fiducial limits ($P = 0·95$) are given where these could be calculated. At all finite doses in the table the effect was significant ($P < 0·01$). *N.T.*, not tested. Weights of salts are those of active acid or base.

Although aspirin did not reduce the proportion of mice responding to potassium to less than half that in the control group, it none the less reduced this proportion significantly. The lowest doses of aspirin, in mg/kg subcutaneously, significantly ($P < 0·05$) inhibiting the response to the noxious agents were: to acetylcholine, 12·5; to bradykinin, 25; to potassium, 50. These values, coupled with the median effective doses in Table 2, show that the order of susceptibility to aspirin was acetylcholine > bradykinin > potassium, whereas morphine was about equiactive against all three agents.

Three considerations support the view that inhibition by aspirin and morphine of the constriction response to acetylcholine, bradykinin and potassium is antinociceptive. (1) The response probably arises from stimulation of nociceptors; (2) aspirin and morphine are known to have analgesic action in man; (3) aspirin does not show other forms of anticholinergic action, such as antagonism of physostigmine toxicity. The differences in potency of aspirin against constriction induced by acetylcholine, bradykinin and potassium suggest that it is acting peripherally on different chemonociceptors, in contrast to morphine which is equally effective against all three noxious agents and is believed to act centrally. These views are consistent with the conclusion of Lim, Guzman, Goto, Braun and Rodgers[13], from cross-circulation experiments, that in dogs aspirin acts peripherally and morphine centrally against nociception induced by bradykinin. In anti-inflammatory tests in mouse peritonitis, aspirin is little more potent than sodium salicylate[7,14]. Its different potency from salicylate against acetylcholine-induced constriction, together with the small latency of the response inhibited, suggest that aspirin may exert its antinociceptive action directly, rather than by reducing fluid exudation from blood vessels. The higher potency of aspirin than sodium salicylate implies that this action does not depend on conversion of aspirin to salicylate.

An alternative, but less likely, explanation of the different effectiveness of aspirin against abdominal constriction in mice induced by acetylcholine, bradykinin and potassium is that aspirin distinguishes in the central nervous system between nociception induced by these three substances.

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Inhibition of Uptake of Tritiated-noradrenaline in the Intact Rat Brain by Imipramine and Structurally Related Compounds

Imipramine is one of the most effective drugs for the alleviation of mental depression. There is little information concerning the mechanism of action of this drug at the biochemical level. An impressive body of evidence has been accumulating over the past few years indicating that noradrenaline is involved in behaviour. Although other antidepressant drugs such as monoamine oxidase inhibitors[1], and amphetamine[2], affect cerebral noradrenaline concentration, imipramine was found to have no measurable effect on the level of the neurohumour in the brain[3]. It has been shown that imipramine blocks the uptake of noradrenaline in peripheral tissues[4] and brain slices[5]; however, many other drugs including the tranquillizer chlorpromazine have the same action[5,6]. Because noradrenaline cannot cross the blood-brain barrier, it was virtually impossible to examine directly the action of imipramine or other drugs on the uptake of noradrenaline by the intact brain. We have recently described a procedure which made it possible to introduce tritiated-noradrenaline of high specific activity into the rat brain by an intraventricular injection. The tritiated-noradrenaline is taken up and retained by nerve endings in the brain and then shows the essentially same biochemical behaviour as the endogenous neurohumour[7]. This technique enabled us to investigate the effect of imipramine on the uptake of tritiated-noradrenaline by the intact rat brain. It will be shown that imipramine and structurally related antidepressant drugs but not chlorpromazine block the uptake of tritiated-noradrenaline in the brain.

Sprague–Dawley male rats weighing 300 g received imipramine 1 h before the injection of tritiated-noradrenaline (0·07 μg) into the lateral ventricle of the brain. Two hours after the intraventricular administration of the catecholamine the rats were killed and the brain assayed for tritiated-noradrenaline[8].

Table 1. Inhibition of Tritiated-noradrenaline Uptake in the Rat Brain by Antidepressant Drugs

Treatment	Amount given (mg/kg)	Clinical antidepressant action‖	% of control value
Imipramine	20	Yes	63 ± 3·1*
Desmethylimipramine	20	Yes	64 ± 3·2*
Amitriptyline	10	Yes	77 ± 7·6†
Compound II‡	20	No	99 ± 4·2
Compound III§	20	No	93 ± 3·5
Chlorpromazine	20	No	107 ± 8·6
Chlorpromazine	40	No	91 ± 6·7

* $P < 0.001$. † $P < 0.05$.

Groups of six rats were given the drugs intraperitoneally 1 h before the intraventricular administration of 0·07 μg of *d,l* 7-^{3}H-noradrenaline (5·8 c./m.mole from New England Nuclear Corporation, Boston). Rats were killed 2 h later and the brain assayed for tritiated-noradrenaline. Brain from untreated rats had about 30,000 c.p.m. per g of tissue. Results are expressed in percentage of the control brain tritiated-noradrenaline ± *S.E.M.*

‡ Compound II has the same structure as imipramine except that a dimethylaminoisopropyl side chain is substituted for a dimethylaminopropyl side chain.

§ Compound III has the same structure as chlorpromazine except that a dimethylaminoethyl, ethyl ether side chain is substituted for a dimethylaminopropyl side chain.

‖ Information concerning the antidepressant activity of the various compounds was kindly provided by Dr. Murray Weiner, Geigy Research Laboratories, Yonkers, N.Y.

The uptake of tritiated-noradrenaline by the brain of imipramine-treated animals was markedly reduced (Table 1). The effect of congeners of imipramine and other compounds related in structure, such as amitriptyline and the tranquillizer chlorpromazine, on the uptake of tritiated-noradrenaline was also examined. Desmethylimipramine and amitriptyline reduced the uptake of tritiated-noradrenaline; both these drugs have been found clinically active as antidepressants. A closely related derivative of imipramine with a dimethylaminoisopropyl side chain instead of the dimethylaminopropyl side chain which was found to be clinically inactive as antidepressant had no effect on the uptake of tritiated-noradrenaline in the brain. Chlorpromazine, a tranquillizing drug, which can block the uptake of tritiated-noradrenaline in peripheral tissues and brain slices, had no effect in the intact rat brain.

The uptake of noradrenaline into storage granules of peripheral nerve endings is a major mechanism for the inactivation of noradrenaline[8]. In the periphery it has been shown that drugs which block the uptake of noradrenaline enhanced the physiological actions of the neurohumour[9]. Noradrenaline has been shown to be liberated from nerve endings, react with adrenergic recep-

tors, and then return in part to the stores in the sympathetic nerves[10]. It is likely that a similar mechanism of noradrenaline release and re-uptake is operating in the brain. The experiments described here show that imipramine, desmethylimipramine and amitryptyline block the uptake and binding of noradrenaline by the brain. On the other hand, these structurally related compounds which are devoid of clinical antidepressant activity have no effect on the uptake of tritiated-noradrenaline in the brain. The ability of imipramine to prevent the rebinding of noradrenaline by cerebral tissues may be a mechanism for the antidepressant action of this drug. Such an action of the drug would allow more free physiologically active noradrenaline liberated from the central sympathetic neurones to react with the central adrenergic receptors. The observations that imipramine potentiates the peripheral action of administered noradrenaline[11], that desmethylimipramine will cause hyperactivity after a rapid release of noradrenaline produced by reserpine[12], and that both will potentiate the stimulating action of amphetamine[12,13], a drug that releases noradrenaline in the brain[2], support the mechanism of action of the antidepressant drugs proposed in this communication.

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IMMUNOLOGY

Breaking of Immunological Paralysis by Injection of a Specific Depolymerase

THE elaboration by the soil organism *Bacillus palustris* of a depolymerase (*D*3) specific for the long-chained capsular polysaccharide of type III *Diplococcus pneumoniae* (*S*III) (ref. 1) provides an opportunity to explore some of the mechanisms of immunological paralysis induced by *S*III. The polymerase when incubated *in vitro* for prolonged periods with *S*III cleaves it to hexasaccharides and smaller units[2]. It could readily be shown that when the products of this reaction are injected into adult mice they do not elicit an immune response. Thus, 5/5 mice injected with 0·5 ml. of a 21-h reaction mixture containing 40 μg *S*III and 50 viscosity units of *D*3 failed to form antibodies. When these mice were later injected with 0·1 μg *S*III they all formed antibodies against the *S*III. When *D*3 is injected intravenously into mice which 12 h previously had received 100 μg *S*III it rapidly clears the antigen from the circulation so that within 12 h *S*III is no longer detectable by either the micro-precipitin or the complement fixation tests. The former test detects 0·1 μg and the latter 0·01 μg antigen. Although no *a priori* evidence existed it seemed just possible that the enzyme might even enter the cells containing the *S*III and relieve the immunological paralysis caused by the injection of relatively large amounts of *S*III.

Table 1. EFFECT OF TYPE III DEPOLYMERASE ON IMMUNOLOGICAL PARALYSIS

*S*III (μg)	Interval between *S*III and *D*3 (days)	Bled after *D*3 (days)	Titres*	No. mice released from paralysis on basis of h.a.	challenge†
0·1	—	7	32 64 64 64		
0·1	2	7	8 64 64 32		
100	1/4	7	8 16 8 8	4/4	2/4
100	1	7	8 16 16 8 4 0 8 0	6/8	5/8
100	2	6	4 8 0 32 8 16 4 8 4 4 4 8	11/12	3/4
100	3	6	16 16 16 8 16 8 8 8 8 4 8 8 4	13/13	4/8
100	4	5	0 4 0 8 32 16 32 16	6/8	5/8
100	5	5	0 0 0 0	0/4	2/4
100	6	7	8 0 0 0	1/4	0/4
100	7	7	8 4, 7×0	2/9	
100	8	5	0 0 0 0	0/4	2/4
100	18	7	4 4 4 4, *14*×0	4/18	3/14
100	69	7	*10*×0	0/10	
100	92	7	*13*×0	0/13	
2,000	1	7	9, 32 16	3/3	
2,000	2	7	16 16 16 8 8 4	6/6	
2,000	3	7	4 16 4 4 4 4	5/5	
2,000	4	7	8 8 8 0	3/4	
2,000	5	7	4 0 4 4	3/4	
2,000	6	7	8 4 4 4	4/4	
2,000	7	7	4, 4, 16, 4, 0, 0 0	4/7	
2 000	10	7	4 0 4 0	2/4	
2,000	69	7	*14*×0	0/14	

* Titrations were commenced with a serum dilution of 1 in 4. The figures are the reciprocals of the serum dilution at which an end-point was noted.
† Live/total. Not all mice were challenged.

Four separate experiments were done over 2 years, but for convenience the results are presented collectively. Two measures of immunity were used. Mice were bled from the retro-orbital sinus and their sera, after absorption with sheep cells, titrated for antibodies to *S*III using as antigen a filtrate of type III *D. pneumoniae* coated to sheep erythrocytes[3]. In some experiments some mice were challenged about one month after *D*3-treatment with 100–200 lethal doses of type III *D. pneumoniae*.

The intraperitoneal injection of 0·1 μg *S*III into 12–14-week-old male *CAF* mice results, whether or not it is followed 2 days later by the intravenous injection of *D*3, in the formation of relatively high titres of antibodies to *S*III (Table 1). On the other hand, when mice were injected with 100 μg or 2·0 mg *S*III and bled at intervals over the next 24 weeks *S*III antibodies were never detected. Entirely different results were noted when *D*3 was injected into the paralysed mice. If the enzyme was injected 6 h to 4 days after the paralysing dose of *S*III, then the mice were no longer paralysed and indeed 40/45 animals formed detectable—albeit relatively low-titred—antibodies against *S*III. As the interval between the injection of antigen and enzyme was increased fewer mice were relieved of their paralysis and they formed only low titres of antibodies against *S*III. Thus, only 7/39 paralysed mice injected with *D*3 5–18 days after *S*III and 0/23 injected 2–3 months after *S*III formed detectable antibodies. The results of the challenge test essentially confirm these findings and the slightly less impressive results obtained with this method (the figures are 19/32 for the period 6 h–4 days; 7/26 for the period 5–18 days and 0/5 for the 2–3-month period) might be attributed to the waning of immunity in the interval between the injection of *D*3 and the subsequent challenge. As an additional control some of the paralysed mice were injected intravenously with saline at the same time as other mice were injected with *D*3. They were still paralysed when bled one week later.

Similar results were obtained with those mice injected with 2·0 mg *S*III. Seventeen of 18 mice injected with *D*3 during the 4 days after the *S*III injection were freed from their paralysis: 13/19 injected with *D*3 between 5 and 10 days after *S*III were also freed of paralysis whereas 0/14 injected with *D*3 69 days after *S*III were able to produce detectable antibodies to *S*III.

It must be stressed that the injection of enzyme into paralysed mice not only removes the paralysis but also results in the formation of antibodies against *S*III. Thus, in no instance did a mouse which had failed to form antibodies after the injection of *D*3 then form antibodies if injected with 0·1 μg *S*III, even if this injection was delayed for several weeks.

That immunological paralysis can be broken by *D*3 and that there is a critical period during which the enzyme is operative suggests the possibility that the *S*III or part of it is integrated into a specific cell-type before paralysis can occur. The cell type may not evolve until some time after the injection of antigen or integration may not occur until after several cell divisions.

An observation which failed to agree with the results recorded here is that in some rare instances mice injected 9 months previously with paralysing doses of *S*III, either spontaneously or after the injection of 0·1 μg *S*III, formed low titres of *S*III antibodies. It will be of interest to determine the effect of *D*3 at this time. It will also be of interest to determine by means of fluorescent staining if the cells of mice injected with *D*3 after *S*III still contain specific staining material.

Towards the end of this work a note appeared by Salzman and MacLeod[4] reporting failure to break immunological paralysis when *D*3 was injected into mice which had received 25 μg *S*III. The abstract contained no further details, and the many possible variabilities probably explain the difference between their results and those reported here.

I thank Dr. J. Campbell for supplies of *S*III and *D*3. This work was supported by a grant from the U.S. National Institutes of Health (*CA*–05175).

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Antibody Production by Leucocytes in Peripheral Blood

The immune response to foreign antigens is focused in lymphoid tissue, notably spleen and lymph nodes; cells from these sites manifest the entire spectrum of immunological capabilities. Despite the fact that a considerable proportion of leucocytes in peripheral blood are of lymphoid origin and have been used to transfer hypersensitivity states, their role in the production of humoral antibody has been uncertain. It was recently shown by Hullinger and Sorkin[1] that circulating leucocytes (buffy coat) from rabbits hyperimmunized with human serum could produce significant amounts of specific antibody. By a quite different means we have obtained direct evidence for the elaboration of specific antibody *in vitro* by such cells, thus confirming and extending their findings.

NIH rabbits, 4–5 months of age, were injected once with 5 μg of *Salmonella enteritidis* somatic polysaccharide[2] given intravenously. At appropriate intervals the animals were bled by cardiac puncture and the blood collected in heparin. To facilitate separation of leucocytes, 20 ml. of heparinized blood was added to two volumes of 3 per cent dextran (mol. wt. 188,000), mixed for 10 min and then allowed to stand for 20 min, after which the leucocyte-rich plasma was drawn off and centrifuged for 10 sec at 250*g* to further remove erythrocytes. The supernatant was then centrifuged at 150*g* and the sedimented leucocytes were resuspended in Eagle's medium. The total number of leucocytes usually obtained was approximately 5×10^7; contaminating erythrocytes averaged about 10 per leucocyte. For enumeration of antibody-forming cells the localized hæmolysis technique of Jerne[3] was used with sheep erythrocytes which had been coated with *S. enteritidis* endotoxin previously treated so as to facilitate its uptake by red cells[4]. When this polysaccharide is used to modify the antigenicity of erythrocytes, lymphoid cells of normal rabbits yield few or no zones of hæmolysis (plaques) (< 1 per 10^6 lymphoid cells). When the erythrocyte itself serves as both antigen and indicator, there is a major element of uncertainty in assessing data such as that obtained for the early inductive phase of antibody formation, inasmuch as cells secreting natural hæmolysin are almost invariably present in significant numbers. As antigens, the somatic polysaccharides offer a number of important advantages, one being that in normal rabbits the number of plaque-forming cells discerned with the *S. enteritidis* coated red cells, for example, are essentially the same as those obtained with uncoated erythrocytes. Thus, even a modest increase in numbers of plaque-forming cells specific for this somatic polysaccharide can be detected.

Five days after rabbits are injected with 5 μg of the *S. enteritidis* polysaccharide an average of 2,000 plaques per 10^6 spleen cells are seen when these cells are plated on coated erythrocytes[5]. In contrast, plating these spleen cells on normal sheep erythrocytes disclosed no significant increase in plaque formation (< 1 per 10^6 cells). Moreover, similar low 'background' activity was seen when these spleen cells were plated on erythrocytes coated with immunologically unrelated somatic polysaccharides. These findings attest to the specificity of the antibody detected in this system.

In an investigation of the characteristics of the immune response to these somatic polysaccharides, it was soon evident that while most of the antibody-forming cells were present in spleen[5], appreciable numbers of plaque-forming cells were also discerned in leucocyte suspensions from peripheral blood when these were mixed with polysaccharide-coated sheep erythrocytes, plated, incubated and then exposed to guinea-pig complement. The findings on 44 rabbits are summarized in Table 1. It is evident that within 3 days after the single antigenic stimulus large numbers of antibody-producing cells are found in the circulation. It was possible to process the leucocyte suspensions so as to remove the granulocytes, leaving a population consisting predominantly of small lymphocytes, which nevertheless retained the same level of plaque-forming activity. There was, however, no apparent correlation between the percentage of lymphocytes in the leucocyte suspensions and the numbers of plaques obtained. The maximum number of plaque-forming cells appeared in blood at about day 4; in spleen, on the the other hand, the peak was attained consistently on day 5. It is also noteworthy that whereas in spleen there is a progressive decline in the number of plaque-forming cells between days 5–30, followed by the persistence of

Table 1. Production of Anti-polysaccharide Plaques by Peripheral Leucocytes of Rabbits injected with *S. enteritidis* Somatic Antigen

Time post-immunization (days)	No. of plaques per 10^6 leucocytes * (values are for individual animals)	Incidence of reactors	Mean values for reactors (PFC†/10^6 cells)
< 2	1, 10, 0, 0, 0, 0	2/6	5
2	0, 40, 1, 0	2/4	20
3	0, 130, 80, 10, 18	4/5	60
4	400, 400, 0, 30, 0, 15, 76	5/7	184
5	0, 0, 120, 120, 1, 20, 8, 20, 0	6/9	48
6	100, 120, 0, 0, 0	2/5	110
7–49		0/8	

* Peripheral cells from each rabbit were also plated on uncoated (normal) sheep erythrocytes. Generally these values were in the range of 1 plaque-forming cell per 10^6 leucocytes, or less. In the few instances where higher control values were obtained these were used to adjust the results obtained with polysaccharide-coated erythrocytes.
† PFC, plaque-forming cells.

significant numbers of these cells for many weeks, in peripheral blood plaque-forming cells are suddenly no longer evident after about 1 week. Again, in contrast to the findings with spleen cells, the response of the peripheral cells was rather erratic, varying considerably from animal to animal. Since the animals were killed for parallel investigations on other lymphoid organs, it was not feasible to obtain serial blood samples from individual rabbits. This could, in part, account for the variable results.

Despite the less consistent appearance of antibody-producing cells in blood, as compared with the uniformity of response in spleen, the fact remains that during the productive phase of antibody synthesis the general pattern of response by peripheral cells is not unlike that of spleen. Consequently the appearance of plaque-forming cells in blood is not likely to be a fortuitous occurrence, but rather a meaningful facet of the host's response to this antigen.

It remains to be determined whether the plaque-forming cells found in peripheral blood are derived from other lymphoid sites, such as spleen, thymus, or lymph nodes, or whether blood leucocytes *per se* are directly stimulated by this antigen. Since it is believed that lymphocytes continually migrate through lymph nodes and other lymphoid structures into lymph and through the blood[6], it is entirely possible that these cells originate in sites traditionally associated with antibody production. The aforementioned variability in numbers of plaque-forming cells in peripheral leucocytes may also be an indication that these circulating cells have emerged from other lymphoid sites.

Although our findings affirm the report by Hullinger and Sorkin on antibody production by peripheral blood leucocytes, there are also noteworthy differences. Since these workers hyperimmunized rabbits with a soluble protein the ensuing immune response probably consisted of 7*S* γ-globulin antibody production. In contrast, the present work was based on an antigenic stimulus consisting of a single injection of 5 μg of polysaccharide which has been shown[7] to lead to the production of macroglobulin antibody exclusively. The considerable difference in proportion of reactive cells in our respective investigations is understandable in view of the major differences in the type of antigen used, the immunization schedule utilized and the ensuing immunoglobulin produced.

While by no means comparable to the population of antibody-forming cells in other lymphoid organs (spleen with intravenous injection and draining lymph nodes with subcutaneous administration) the magnitude of the contribution by peripheral cells to the response is none the less appreciable. The contribution of these peripheral cells to the overall immune response can be approximated by considering the blood volume to be 13 per cent of the body-weight, and the concentration of lymphocytes in blood as averaging 35 per cent of the total leucocytes. On this basis, the total population of blood lymphocytes in the rabbit is estimated to be 600 million; of these an average maximum of 110,000 are antibody-producing cells. It is useful to compare these figures with those for the major organ of antibody production in this system, namely spleen; here the number of cells also average about 600 million; of this total about 1·2 million are antibody-producing cells at the peak of response. Accordingly, it is estimated that of the cells in the rabbit which are committed to the production of antibody specific for this polysaccharide, as much as 10 per cent may be present in peripheral blood.

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A Continuous-flow Micro Double Diffusion Technique with Cellulose Acetate

The value of the Ouchterlony double diffusion test in the analysis of antigen-antibody reactions is well established. In the micro modification, introduced by Wadsworth[1] and developed by Crowle[2], a continuous-flow diffusion system was achieved by using wells made in 'Perspex' templates. Relatively large volumes of reactants diffuse under hydrostatic pressure into a thin layer of agar, greatly increasing the sensitivity and resolution of the test while economizing on reagents[3]. However, the use of agar in the micro-technique has certain disadvantages. Primarily, there is the considerable risk of complete loss of the reaction due either to tearing of the fragile agar layer when the template is removed, or to its loosening and washing off during the staining procedures. Splitting and

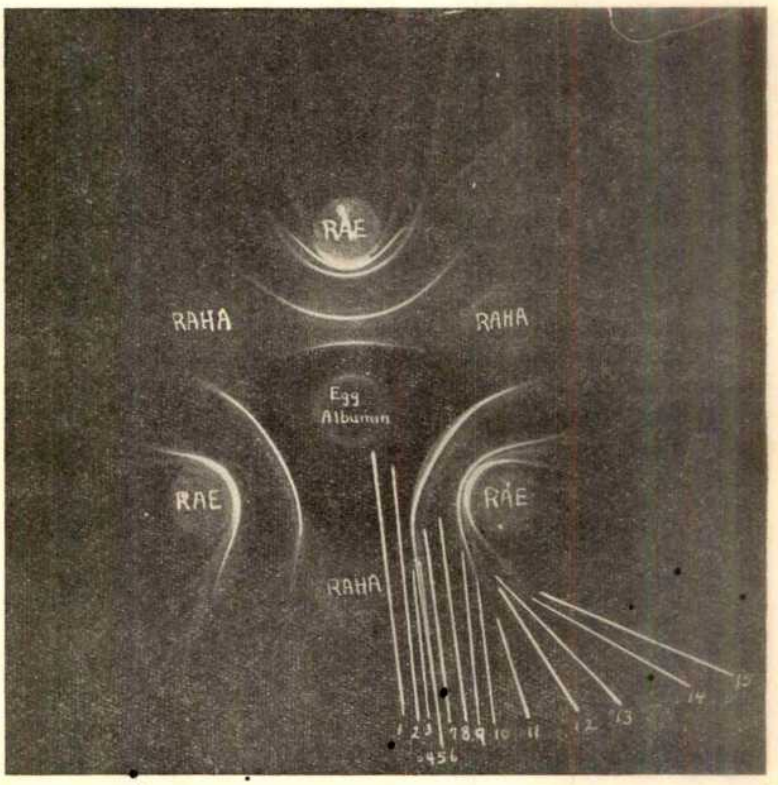


Fig. 1. Micro double diffusion reaction in cellulose acetate between egg albumen, rabbit anti-egg albumen serum (*RAE*), and rabbit anti-horse albumen serum (*RAHA*) stained with Ponceau *S*

distortion of the agar layer during drying may also occur. Secondly, the test is relatively inflexible in that the *p*H and electrolyte concentration of the agar cannot easily be varied, and, finally, variations are encountered with different batches of agar both as regards their mechanical stability and the reactions developing in them[3].

For these reasons, we have developed a technique designed to combine the advantages of the continuous-flow system with those offered by the use of cellulose acetate as a medium for immunodiffusion[4].

Cellulose acetate strips ('S and S Cellulose Acetate Strips for Electrophoresis', Consolidated Laboratories, Canada, Ltd.) are cut transversely into pieces approximately 2·3 cm × 5 cm. The strips are soaked by floating them on the buffer to be used and are then immersed until required. 'Perspex' templates of the type described by Crowle[2,3] are used, and care must be taken to keep the under surface of each template perfectly smooth and polished. To mount each template a wet cellulose acetate strip is laid lengthwise on a clean glass slide and the template is placed at one end of the strip. Then the template is slipped into a central position along the wet surface, eliminating air bubbles in the process. Excess buffer is blotted from the ends of the cellulose acetate strip which should extend beyond both ends of the template. Blotting is continued until the paper visible at the bottom of the wells just changes from a shiny to a matt appearance. The prepared slides are placed, in pairs, into small moisture chambers (square Petri dishes, containing damp filter paper).

The test materials are applied with finely drawn-out glass pipettes, and all the wells of the individual templates should be charged as quickly as possible. If the preparation is satisfactory there will be no leakage between the template, paper and slide interfaces, and there will be no change in the appearance of the cellulose acetate at the bottom of the unfilled wells as the neighbouring wells are filled.

The individual moisture chambers are placed together into a larger container which is sealed more efficiently and preserves a moist atmosphere throughout incubation. The precipitin reaction develops at approximately the same rate as in agar. With the reagents tested, 40–48 h at room temperature was most satisfactory.

After incubation, the templates are removed by holding the slides under running tap water. The strips are washed in physiological saline for a minimum of 4 h and then for 10 min in distilled water. Precipitin lines are stained with 0·2 per cent Ponceau *S* (Consolidated Laboratory) in 3 per cent trichloroacetic acid or 0·1 per cent thiazine red *R* ('*CI* 14780') in 1 per cent acetic acid for a minimum of 10 min. Background differentiation is effected with 3–5 per cent acetic acid baths. After a distilled water wash, the strips are drained and dried at room temperature.

When the cellulose acetate strips are completely dry, they are immersed in 'Whitemor Oil No. 120' (Consolidated Laboratory), which immediately makes them transparent. The oil-soaked strip is layered on a slide and a coverslip placed over the reaction area. After the excess oil is blotted with filter paper and the cellulose acetate is trimmed to coverslip size, clear varnish (nail enamel) is used to seal the edges, making a permanent mount. To allow photographic evaluation as recommended by Crowle[3], and because very faint lines sometimes become visible after clearing where none had previously been evident, clearing and mounting the precipitin reaction are especially important parts of this micro-immunodiffusion technique and should be routinely performed.

Fig. 1 is an enlargement of a micro double diffusion reaction in cellulose acetate between 0·7 per cent egg albumen in physiological saline (Nutritional Biochemicals, crystalline egg albumen, lot 6081), rabbit anti-egg albumen serum (*RAE*) and rabbit anti-horse albumen serum (*RAHA*) (commercial preparation). A total of 15 separate component lines can be counted in the precipitin reaction between the egg albumen and its specific antiserum (*RAE*). It has been found, by direct comparison of this reaction in agar and cellulose acetate, that a more complex system of component lines with better separation and resolution was revealed using cellulose acetate.

In a titration of horse antitoxin (diphtheria antitoxin, globulin modified 40,000 I.U., Connaught Medical Research Laboratories) with diphtheria toxoid ('Diphtheria Toxoid', Connaught Medical Research Laboratories, lot No. 1975–1) 0·26 units and 0·13 units antitoxin were readily demonstrated using a 1 Lf/ml. dilution of toxoid. These dilutions failed to give a positive ring test.

A variety of cellulose polyacetate membranes are available for filtration and electrophoresis and a comparative examination of different membranes is planned having special interest in the effect of different porosities in achieving separation of components. Further investigations will involve the application of scanning and elution techniques in the development of qualitative and quantitative analyses.

This work was supported by the Medical Research Council of Canada.

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HÆMATOLOGY

Blood Group Antigen Xg^a in Gibbons

The antigen Xg^a, which in man is an X-linked dominant character[1], was looked for in various mammals but was found only in gibbons. It was not found in the following animals: 67 chimpanzees (*Pan satyrus*), 2 gorillas (*Gorilla gorilla gorilla*), 20 orangutans (*Pongo pygmaeus*), 60 baboons (*Papio cynocephalus*), 31 Celebes black apes (*Cynopithecus niger*), 4 drills (*Mandrillus leucophaeus*), 10 rhesus monkeys (*Macaca mulatta*), 5 pigtailed monkeys (*Macaca nemestrina*), 1 marmoset, 20 mice and 37 dogs.

In testing the cells of some of these animals heteroagglutinins in the anti-Xg^a plasma had to be circumvented: heteroagglutinins in the antiglobulin serum caused difficulty in testing the orangutans, which therefore had to be grouped by absorption tests. All the primates listed above have been tested for other blood groups[2–5].

Thirteen gibbons were tested, with the following results:

	Antigen Xg^a	
	present	absent
Males	3	4
Females	4	2

These gibbons were not known to be related to each other; they are maintained at the Delta Regional Primate Research Center. Details of their other blood groups are available[2–6]. The thirteen may be identified as follows: males Xg(a+) Britt, 404 and 406; males Xg(a−) Charlie, Viet, 76 and 78; females Xg(a+) Kerry, Thai, Suma and Burma; females Xg(a−) Indo and 403. Indo is the one *Hylobates pileatus* member, the other twelve belong to *Hylobates lar lar*.

Two examples of human anti-Xg^a were used for the tests on the gibbons[1,7]. In man this antibody reacts only by the antiglobulin method and it was so used on the animal red cells. With the gibbon cells and by the antiglobulin method heteroagglutinins caused but little

trouble: however, the results were confirmed by absorption tests.

How alike the gibbon Xg^a antigen is to that of human beings may ultimately be seen from chemical analysis, but at present it is indistinguishable: absorption of the two available examples of anti-Xg^a by Xg(a+) gibbon cells removed the antibody for human and for gibbon Xg(a+) cells, as did absorption by human Xg(a+) cells. Absorption by gibbon or human Xg(a−) cells did not remove the antibody for Xg(a+) members of either species.

The distribution of the antigen in the two sexes suggests that it may be X-linked in the gibbon as it is in man. If the antigen were an autosomal character in the gibbon the chance of observing such a distribution would be 1 in 3. It is hoped that more gibbons will be available in 1965 and that the question of X-linkage will then be settled.

That the gibbon Xg^a antigen may be X-linked is perhaps the less surprising since hæmophilia is X-linked in the dog[8] as it is in man, and the clotting factor deficiency appears to be the same[9]. The presence of Xg^a in the gibbon can scarcely be considered taxonomically important, though X-linkage, if confirmed, would suggest that there is some powerful evolutionary reason why the gene for this antigen should be located on the X chromosome.

We acknowledge the following help. Dr. J. Moor-Jankowski, chief, Division of Experimental Immunogenetics and Oncology, Yerkes Primate Research Center, Emory University, Atlanta, Georgia, stimulated us to test primates and sent us the samples: ten of the chimpanzees and all the gibbons were obtained by Dr. Moor-Jankowski through the kind collaboration of Dr. A. J. Riopelle, director, Delta Regional Primate Research Center, Tulane University, New Orleans, Louisiana. Advice on the taxonomy of the primates was given by Dr. W. C. Osman Hill, associate director, Yerkes Regional Primate Research Center. Dr. L. H. Collier, Medical Research Council, Trachoma Research Unit, Lister Institute, London, provided some of the samples from baboons, Dr. N. A. Mitchison, National Institute for Medical Research, Mill Hill, the samples from mice, and Dr. H. C. Rowsell, Ontario Veterinary College, Guelph, Ontario, the samples from dogs.

Dr. J. D. Mann, Butterworth Hospital, Grand Rapids, first began the search for the antigen Xg^a in animals, Dr. Victor McKusick, Moore Clinic, Johns Hopkins Hospital, Baltimore, suggested the testing of dogs, and Dr. Kurt Benirschke, Dartmouth Medical School, Hanover, New Hampshire, the testing of apes.

We thank Dr. A. Cahan, Knickerbocker Biologics, New York, and Dr. I. A. Cook, North Scotland Blood Transfusion Service, Inverness, for supplies of the anti-Xg^a plasma.

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PATHOLOGY

Serum Enzymes in the Jaundiced Homozygous Gunn Rat

In the course of investigations using jaundiced Gunn[1] rats, serum enzyme examinations were performed on these rats and on those of a Wistar strain. Jaundiced homozygous Gunn rats are lacking in hepatic glucuronyl transferase using bilirubin as a substrate. Many workers have established that hepatic cellular damage is associated with elevation of certain serum enzymes, and an investigation was undertaken to compare the serum enzyme levels in jaundiced Gunn rats and those of a Wistar strain to determine whether the jaundice was associated with raised serum enzyme activities.

Blood specimens were obtained from the tail vein of male rats and the serum was analysed for glutamic-oxalacetic and glutamic-pyruvic transaminase[2], lactic dehydrogenase[3], cholinesterase[4] and aldolase[5]. Normal values for the activities of the five serum enzymes were established by analysing random blood samples in 25 Wistar strain rats. The serum enzyme activities in jaundiced Gunn rats were established by analysing random blood samples in 16 rats.

Table 1. Serum Enzyme Activities in 16 Jaundiced Gunn Rats and 25 Rats of a Wistar Strain

Results (units/ml.) given as mean and standard deviations

	Gunn rats	Wistar rats
Glutamic oxalacetic transaminase	117 ± 24	124 ± 53
Glutamic pyruvic transaminase	36 ± 9	41 ± 13
Lactic dehydrogenase	2,540 ± 560	2,408 ± 662
Aldolase	133 ± 34	128 ± 36
Cholinesterase	51 ± 17	55 ± 24

There was no significant difference between the enzyme levels in jaundiced Gunn rats and those of the Wistar strain (Table 1). The transaminase levels in these rats were lower than those found in male Sprague–Dawley rats by Altland and Highman[6]. They found levels of 261 ± 25 units and 63 ± 3 units for glutamic-oxalacetic and glutamic-pyruvic transaminase, respectively. Lactic dehydrogenase and aldolase activity was greater than that found by Altland and Highman, who found levels of 1,082 ± 72 units and 68 ± 3 units for lactic dehydrogenase and aldolase, respectively. Sibley and Lehninger[5] found a range of aldolase activity of 30–100 units/ml. in male Sprague–Dawley rats.

The results show that lack of glucuronyl transferase in jaundiced Gunn rats is not associated with increased serum enzyme activity for those enzymes tested.

We thank Dr. Barbara Billing for a gift of Gunn rats and the British Empire Cancer Campaign for Research for financial assistance.

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Demonstration of Hydroxyindole-O-methyl Transferase, Melatonin, and Serotonin in a Metastatic Parenchymatous Pinealoma

Pinealomas have been associated with two endocrine syndromes: destructive, non-parenchymal lesions frequently produce precocious puberty in young boys; true parenchymatous tumours have been found in children with delayed pubescence[1]. Little is known about the biochemistry of these tumours, or about possible mechanisms by which they exert their pathological effects on gonad function.

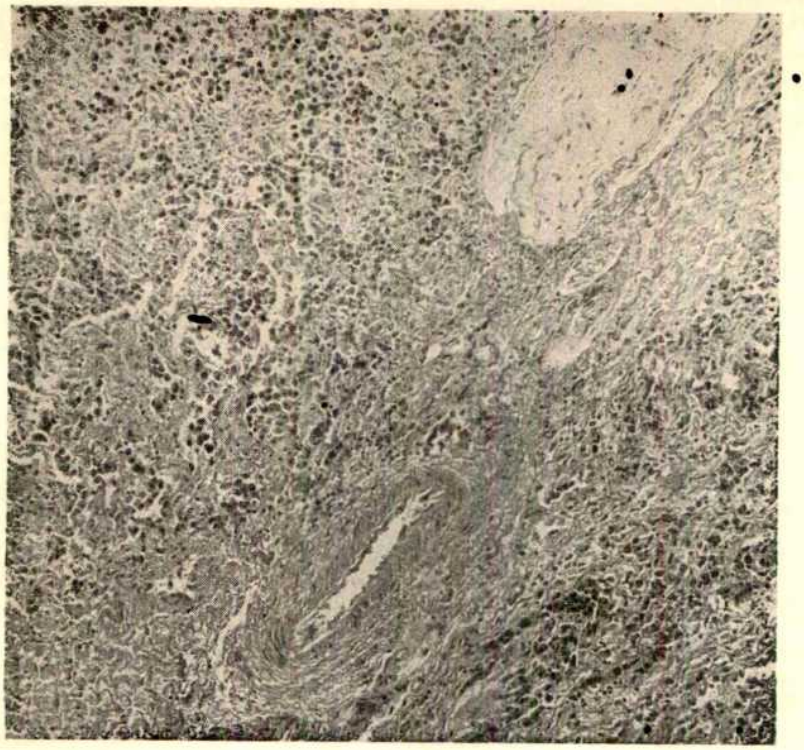


Fig. 1. Section of subcutaneous metastasis of parenchymatous pinealoma (haematoxylin and eosin; × c 52)

Recently, considerable information has become available concerning the formation and actions of certain indoles in the pineal gland[2]. Of particular interest has been the finding of high concentrations of melatonin (5-methoxy *N*-acetyltryptamine) in this gland[3], and the observation that only the pineal has been shown to contain the enzyme (hydroxyindole-*O*-methyl transferase) required for its synthesis[4]. Melatonin[5] and another pineal methoxyindole, methoxytryptophol[6], have both been shown to exert an inhibitory effect on gonad growth and the œstrous cycle in rats.

We have had an opportunity to examine a specimen of metastatic parenchymatous pinealoma from a 14-year-old boy with symptoms of delayed pubescence. The patient was first seen at the age of 9·5 years because of symptoms of increased intracranial pressure. A pneumoencephalogram revealed evidence of a mass in the pineal region; this was treated with irradiation with lasting remission of the intracranial symptoms. Two years later, the patient was seen for symptoms arising from an osteolytic lesion in the left femur, which was consistent histologically with metastatic pinealoma. This lesion also responded well to irradiation. Recently, the patient has developed subcutaneous nodules; one of these was biopsied and examined histologically (Fig. 1), and a 4-g specimen was utilized for the investigations which are described. A detailed report of the clinical findings in this patient will be given elsewhere. At the present time, he shows no evidence of penile or testicular growth, or axillary or pubic hair.

Giarman *et al.* have found that the human pineal gland contains relatively large amounts of serotonin[7] (0·36–22·82 μg/g). The serotonin content of this tumour was examined by the fluorometric method of Bogdanski *et al.*[8] and by the ninhydrin method of Vanable[9]. The content of this amine was found to be 8 μg/g by the former method and 3 μg/g by the latter. These differences are probably related to the unequal distribution of the various tissue components in the tumour samples (Fig. 1).

The presence of pineal methoxyindoles in the tumour was examined by thin-layer chromatography of a chloroform extract of the tissue. The solvent system used was chloroform–methanol, 94 : 6. Using 30 mg of tissue, a compound having the same R_F and colour reactions as authentic melatonin was clearly demonstrable. Since 10 mμg of authentic melatonin would probably be visible as a single spot in these circumstances, the tumour tissue was estimated to contain 0·3 μg/g of melatonin, or about as much as is found in bovine pineals.

Another sample of tumour tissue was assayed for hydroxyindole-*O*-methyl transferase activity by a method described before[10]. The tumour was capable of synthesizing at least 10 μg of melatonin (from *N*-acetylserotonin and ^{14}C-*S*-adenosylmethionine)/g tissue/h. The formation of ^{14}C-melatonin was confirmed by cochromatography with the authentic compound in solvent systems described before[11].

These observations provide the first demonstration of hydroxyindole-*O*-methyl transferase in tissue other than normal pineal. The presence of this enzyme, along with a high concentration of serotonin (in a non-carcinoid tumour), may be useful tools in the diagnosis of this rare lesion. Melatonin has been shown to depress gonad growth and some aspects of gonad function in the rat[5]. The activity of the melatonin-synthesizing enzyme varies with a diurnal cycle, which is controlled by environmental lighting. It is possible that the sustained synthesis of melatonin or related substances by this tumour has contributed to the delayed pubescence seen in this patient.

We thank Drs. John Daly, Kenneth Melmon, and Solomon Snyder for help with some of the indole assays.

This work was supported by U.S. Public Health Service grant *AM* 4501.

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RADIOBIOLOGY

Adenosine-triphosphatase and Potassium Retention in X-irradiated Erythrocytes

The total adenosine-triphosphatase (ATP-ase) activity of erythrocytes can be divided into two portions, one of which is inactive in the absence of sodium and potassium ions, or in the presence of ouabain; the potassium-

dependent fraction is associated with active ion transport in the cell[1,2]. Recent reports have suggested that X-irradiation (0·9–4·5 kr.) of erythrocytes inhibits both the active ion transport and the specific ATP-ase associated with this process[3,4]. We have re-investigated this problem to determine whether ATP-ase inhibition could account for the loss of potassium ion from X-irradiated erythrocytes.

The potassium-dependent ATP-ase was inhibited only by 1 mM ouabain in rat erythrocyte ghosts (Fig. 1) or hæmolysates. The high concentration of ouabain required to inhibit this ATP-ase corresponds well with the high concentrations required to inhibit active ion transport in rat thyroid or uterus[5,6]. Surprisingly, rat brain appeared to contain another ATP-ase with 200-fold greater sensitivity to ouabain (Fig. 1); it is noteworthy that the metabolism of rat brain is also affected by very low concentrations of ouabain[7]. In both cases, the inhibitory effect of ouabain was rapidly reversible on dilution.

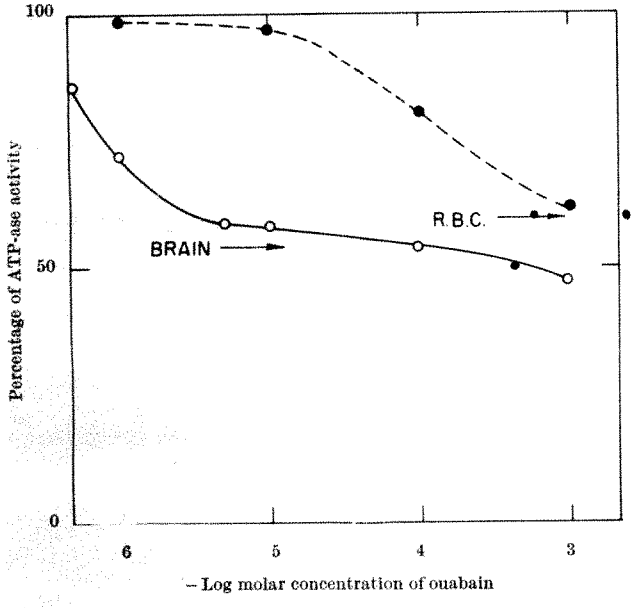


Fig. 1. Inhibition of the ATP-ase activity of rat brain homogenate and of rat erythrocyte ghosts by ouabain. The horizontal arrow in each case indicates ATP-ase activity in the absence of potassium ion. Erythrocytes from 7-week female rats were washed several times with 0·15 M NaCl and were then lysed in 5 mM *tris*-hydroxymethylaminomethane (*tris*) buffer plus 1 mM ethylenediaminetetraacetate (EDTA) at *p*H 8; the erythrocyte ghosts were recovered by centrifugation at 25,000*g* and 0° C for 30 min and washed free of hæmoglobin in the same *tris*-EDTA solution. Rat brains were homogenized with 100 volumes *tris*-EDTA solution, 0·1 ml. homogenate being diluted to 3 ml. in the enzyme assay mixture. ATP-ase activity was estimated from the rate of liberation of inorganic phosphate by the enzyme preparation at *p*H 7·4 and 37° C in the presence of 100 mM NaCl, 20 mM KCl, 6 mM $MgSO_4$, 1 mM EDTA, 10 mM *tris* buffer and 3 mM adenosine triphosphate

The concentrations of ouabain required to inhibit the potassium-dependent ATP-ase in rat liver, kidney and thymus were found to be similar to those shown for rat erythrocytes. The importance of this ouabain-sensitive enzyme for the maintenance of intracellular potassium-levels could readily be demonstrated with liver slices. When incubated for 1 h at *p*H 7·4 and 37° C in a Ringer–glucose–phosphate medium, control liver slices retained their potassium, while the potassium-level in slices with 1 mM ouabain dropped to 52 per cent of the control value. This effect of ouabain was reversible; when the liver slices were rinsed free of ouabain and incubated at 37° C in fresh Ringer medium for another hour, their potassium content increased again to 85 per cent of the control value. Incubation of control liver slices for 1 h at 0° C instead of 37° C resulted in a loss of 61 per cent of their potassium; the subsequent addition of 1 mM ouabain completely prevented the normal re-accumulation

Table 1. Effect of Ouabain and X-irradiation on Potassium-levels in Rat Erythrocytes

Incubation h	procedure ° C	Concentration of ouabain (mM)	Intracellular K^+ (% initial value) Control	10 kr.	60 kr.
20	37	0	98	97	75
20	37	1	74	75	55
4	4	0	103	102	73
20	4	0	100	72	26
20	4°	1	99	74	27
44	4	0	91	43	17
1	37, then	0	98	80	35
20	4				
4	37, then	0	99	91	50
20	4				
20	4, then	0	99	84	41
1	37				
20	4, then	0	99	93	66
4	37				
20	4, then	1	97	74	28
1	37				
20	4, then	1	92	70	29
4	37				

Intact, washed erythrocytes were suspended in 15–20 volumes of a modified Krebs–Ringer solution containing 5 mM glucose, 10 mM phosphate buffer at *p*H 7·4, two antibiotics (penicillin and streptomycin) and 20 per cent calf serum; the serum was not essential but did reduce erythrocyte fragility and hæmolysis. After incubation, the cells were centrifuged down and washed once with 0·15 M NaCl. Potassium concentrations in the cells were measured on a flame photometer with an internal lithium standard. X-ray exposures (1–12 kr./min) were measured with a ferrous sulphate dosimeter[13].

of this potassium when the potassium-depleted slices were incubated at 37° C during the second hour. Similar results have been reported recently by Judah and Ahmed[8].

Active ion transport did not seem to be essential for potassium retention in rat erythrocytes. Neither low temperature nor low temperature plus 1 mM ouabain produced any appreciable loss of intracellular potassium within 20 h (Table 1). Ouabain did produce some loss of potassium from control erythrocytes at 37° C, but this loss was very slow. Active ion transport across the cell membrane might be responsible for the high concentrations of potassium and low concentrations of sodium inside the normal erythrocyte, and might also compensate for any slow leakage of potassium from the cell. However, these results suggest that potassium ion, once transported to the interior of the erythrocyte, must be held there in the cellular structures, and that a cessation of active transport does not necessarily result in a rapid equilibration of ion concentrations across the external cell membrane. The same conclusion appears to hold for intact thymus lymphocytes[9], but not for liver cells. The latter, in contrast to erythrocytes, not only lose and regain potassium rapidly *in vitro* but also equilibrate rapidly with extracellular potassium *in vivo*[10].

No evidence could be obtained for the immediate inhibition of active ion transport or of the associated ATP-ase by moderate doses of X-irradiation. Exposure of the liver slices to 60-kr. X-rays at 0° C did not affect the normal re-accumulation of potassium during subsequent incubation at 37° C. Direct measurements of the ouabain-sensitive ATP-ase activity of erythrocytes also showed very little change following X-ray doses from 1 kr. up to 60 kr., either with intact rat cells, with rat erythrocyte hæmolysates or with ghost preparations from rat and human erythrocytes. (The total ATP-ase activity also remained constant in these experiments.) Despite the absence of any appreciable effect on ouabain-sensitive ATP-ase, X-irradiation does increase the loss of potassium from erythrocytes during prolonged incubation (Table 1). In agreement with previous reports on human cells[11], the potassium loss from irradiated rat erythrocytes was accelerated at low temperatures (Table 1), where active ion transport is much reduced[12]. However, addition of 1 mM ouabain (to inhibit active transport) failed to alter the effects of X-irradiation on potassium retention, either at 4° or at 37° C (Table 1).

The loss of potassium from irradiated erythrocytes at low temperatures remains unexplained. It was not accompanied either by hæmolysis or by loss of acid-soluble ribonucleotide bases. We have found that this loss is reversible, for when irradiated cells (60 kr.) were pre-incubated at 4° C for 20 h and then incubated at 37° C for another 4 h, their potassium content increased from 26 to 66 per cent of the original content. This re-accumulation was completely inhibited by 1 mM ouabain (Table 1). It was also noted that pre-incubation at 37° C for 4 h was sufficient to prevent excess loss of potassium from irradiated erythrocytes during subsequent incubation at 4° C. X-irradiation apparently destroys some component of the erythrocytes which they can repair or replace during incubation at 37° C but not at 4° C. However, the nature of this component has not yet been identified.

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Influence of Soil Organic Matter on the Entry of Cæsium-137 into Plants

Interest in the behaviour of cæsium in soils has been aroused by the presence of the long-lived nuclide, cæsium-137, in world-wide fall-out. It is well established that in temperate climates the fixation of cæsium in clay minerals causes it to be little absorbed by plants from mineral soils[1], although it has been shown that in some tropical soils the degree of fixation is much less[2]. Thus, in general, contamination of the Western diet with cæsium-137 has been considered to depend predominantly on the levels of the recent deposition of world-wide fall-out[3].

However, when the rate of fall-out decreased after the middle of 1959 the activity of cæsium-137 in milk from many areas decreased to a lesser extent than would be expected on this basis[4–6]. These observations suggest that in such pastures cæsium-137 may remain available to plants for considerable periods after deposition. This may be due to the retention of cæsium-137 in the plant base zone[7] or to fixation in the soil under these conditions being considerably less than had hitherto been thought. As part of an investigation of this question we have examined the uptake of cæsium-137 by ryegrass from pasture soils.

Soil samples to a depth of 10 cm were collected from permanent pastures at five sites in Britain where varying levels of cæsium-137 had been observed in milk. A tropical soil was included in the experiment for comparison. Some characteristics of the soils are shown in Table 1; the cation exchange capacities of clay and organic matter were determined from measurements made before and after the organic matter had been destroyed by hydrogen peroxide.

The soils were uniformly contaminated with cæsium-137 and sown with perennial ryegrass, the foliage of which was gathered and analysed at regular intervals. The uptake of cæsium-137 between 10 and 26 weeks after sowing for two experiments is given in Table 1. The results are shown both in terms of the amount of cæsium-137 absorbed per pot and the uptake per g of plant tissue, since there were considerable differences in the yields on different soils. These were especially marked in the second experiment, which was conducted largely during the winter in a greenhouse with limited heating and supplementary illumination. Between soils the uptake of cæsium-137 varied by factors of up to about 20, when the results are expressed on either basis.

Previous work[8,9] suggested that the availability of cæsium is governed by the content of clay and exchangeable potassium. However, no simple relationships were apparent in the present results; the correlation coefficients (9 d.f.) were: ^{137}Cs uptake and percentage clay, $r = -0{\cdot}47$; ^{137}Cs uptake and exchangeable potassium, $r = -0{\cdot}51$.

The content of organic matter varied widely between the soils, and there is evidence of a greater availability of cæsium-137 in peaty soils[10]. The effect both of the percentage organic matter content and the cation exchange capacity (C.E.C.) of the organic matter on the uptake of cæsium-137 was therefore examined. The following correlation coefficients were obtained: ^{137}Cs uptake and percentage organic matter, $r = 0{\cdot}94$; ^{137}Cs uptake and C.E.C. on organic matter, $r = 0{\cdot}95$.

Both coefficients are significant at the 0·1 per cent level. It is suggested that these relationships reflect the fact that, when organic matter is responsible for a large fraction of the total exchange capacity of the soil, the adsorption and subsequent fixation of cæsium-137 on the clay minerals are much reduced. Eagle[11] has demonstrated a similar relationship between the organic matter content of soil and the availability of potassium, such that the greater the organic matter content the higher the level of exchangeable potassium.

Table 1. Uptake of Cæsium-137 by Perennial Ryegrass from Different Soils
(Values adjusted to a level of 1 μc./g soil)

Soil: Dominant clay mineral	(1) Mica	(2) Vermiculite	(3) Mica	(4) Vermiculite	(5) Montmorillonite	(6) Kaolin
Clay %	12·8	18·0	22·2	11·8	15·2	57·8
Organic matter %	9·95	4·62	2·74	1·95	1·66	1·30
Cation exchange capacity (m.equiv./100 g soil)						
Clay	7·7	12·0	11·2	9·4	19·7	30·5
Organic matter	27·1	15·2	11·0	8·4	0·7	1·0
Exchangeable potassium (m.equiv./100 g soil)	0·19	0·20	0·28	0·15	0·32	3·00
Ryegrass: 10–26 weeks after sowing						
Experiment I						
Yield per pot (g dry weight)	9·27	7·77	12·67	5·71	13·41	31·26
^{137}Cs uptake (mμc. g dry weight)	1,983	1,077	621	1,003	89	89
^{137}Cs uptake (mμc./pot)	18,380	8,340	7,850	5,720	1,200	2,770
Experiment II						
Yield per pot (g dry weight)	5·48	3·62	7·18	—	5·52	13·80
^{137}Cs uptake (mμc./g dry weight)	2,930	1,000	718	—	83	77
^{137}Cs uptake (mμc./pot)	16,060	3,630	5,160	—	460	1,050

Because of the slow downward movement of cæsium-137 in soil[12] it is, therefore, to be expected that shallow-rooted grasses will absorb the nuclide relatively freely from the cumulative deposit when the organic matter content of the upper soil layers is high. This frequently occurs in permanent pastures of cool temperature regions.

I thank Dr. R. Scott Russell for his advice during the course of this work.

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BIOLOGY

Cyclical Behavioural Activity in the Pregnant Golden Hamster (*Mesocricetus auratus* Waterhouse)

In an examination of the behaviour of pregnant hamsters, cycles of activity have been found which are comparable to those seen in the non-pregnant female during the œstrous cycle. Animals were watched for an hour each day in their home cages. Observations were made under red lights half an hour after the lights went out on a 12 h/12 h reversed light cycle. At each minute during the watch, the activity in which the animal was engaged at that time and also its position in the cage were recorded. The vaginal cycles of the females were followed by a modified smear technique which depends on changes in the viscosity of the vaginal mucus[1].

The most commonly occurring categories were sleeping and presence in the nest, which are not wholly dependent on one another, for though sleeping only occurs in the nest other activities, such as grooming, are also commonly performed there. Sleeping also increases significantly during pregnancy, but the time spent in the nest does not. Both these measures show significant variation in the non-pregnant animal during the four-day œstrous cycle. Similar cycles have been obtained with activity wheels[2,3].

These measures also show cycle variations during pregnancy with significant falls from days 4 to 5 and 9 to 10, but not on other days (conception takes place on day 1). Preliminary results from activity wheel studies also suggest that peaks of activity occur on these days[3].

The sleeping scores for days 1, 5 and 10 were also compared with the means for the intervening or succeeding days. The probabilities are as follows:

Day 1, v. mean of days 2–4	$P = < 0{\cdot}01$
Day 5, v. mean of days 2–4	$P = < 0{\cdot}01$
Day 5, v. mean of days 6–9	$P = < 0{\cdot}01$
Day 10, v. mean of days 6–9	$P = < 0{\cdot}05$
Day 10, v. mean of days 11–13	$P = < 0{\cdot}05$

(2-tailed Wilcoxon matched pairs signed-ranks test)

Another day of high activity might be expected about day 13 or 14. However, by this time the placental hormone output may be relatively more important. Ovariectomy between the ninth and thirteenth days of pregnancy induces abortion in the hamster, but pregnancy can be maintained in spayed females with œstrogen and progesterone[4]. Further, the mean time spent sleeping per watch is higher in the latter half of pregnancy than in the first 8 days (P, 0·03, two-tailed sign test).

Mating during pregnancy has been reported in the hamster[2,5] and the frequency of occurrence tends to show a 4–5 day period[5]. Similar observations have been made on the rat[6,7] and mouse[8–10].

Cycles of vaginal cornification have been reported in a pregnant rat[6]. Though smears were followed through pregnancy in the present investigation no cyclic changes

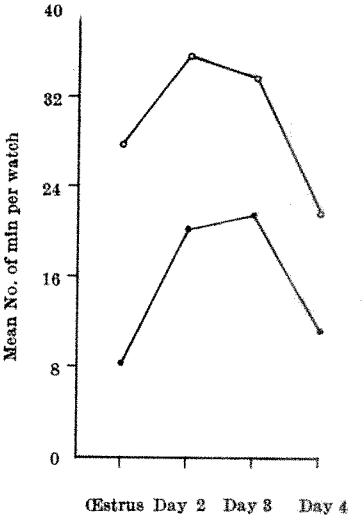


Fig. 1. Mean time spent sleeping (solid circles) and in the nest (hollow circles) for 20 non-pregnant females followed through the œstrous cycle. For both measures, days 1 and 2 and days 3 and 4 differ significantly. In each case $P = < 0{\cdot}01$ (2-tailed Wilcoxon matched pairs signed-ranks test)

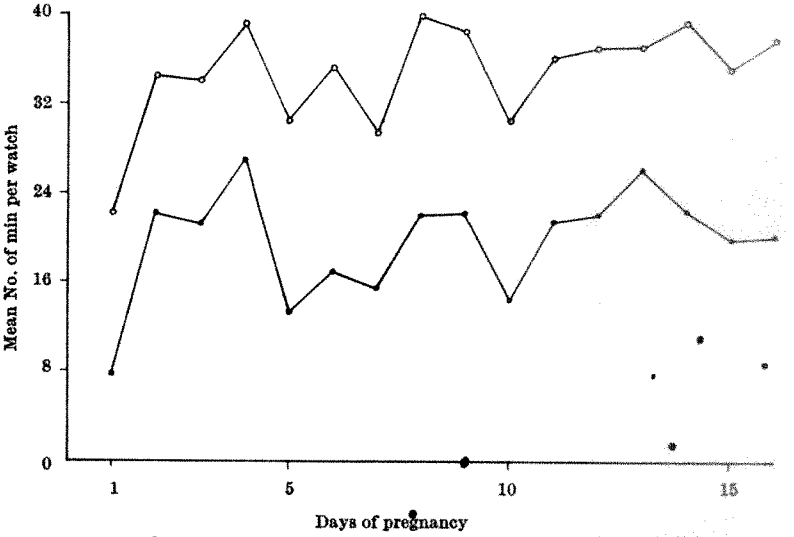


Fig. 2. Mean time spent sleeping (solid circles) and in the nest (hollow circles) for 16 animals followed through pregnancy. The fall in both measures from days 4 to 5 and 9 to 10 is significant ($P = < 0{\cdot}05$)

were seen, but the method used may not be sensitive to minor changes.

In 1930 Swezy and Evans[11] reported that cycles of oogenesis in the rat ovary were not interrupted by pregnancy and that small corpora lutea were formed on the 5th, 10th, 14th and 18th days of pregnancy. Apparently this observation has never been confirmed. However, more recently Zeiner[12] has shown that the gonadotrophic content of the pituitary of the pregnant rat tends to show peaks on the days when œstrus would be expected had mating not taken place.

In the light of Harris and Levine's suggestion[13] that the female central nervous system, and therefore the endocrine system, is inherently cyclic, it is interesting to note that cycles are only partly suppressed during pregnancy.

I thank Prof. A. S. Parkes, Prof. R. A. Hinde and Dr. R. V. Short for much helpful advice and discussion, and acknowledge a research studentship from the Department of Scientific and Industrial Research.

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Chloroform Sensitivity of Mice

We, and many of our colleagues, did not appreciate the danger of chloroform to adult male mice until the following accident happened in our animal house. Our mouse population included two pure strains, one albino (*A.SW*), and one brown (*CBA-p*). There were breeding pairs with litters and non-mated adult stock mice, all in cages kept close together. We found two adult male brown mice dead one morning, and several others apparently very ill. Throughout the following week other mice were affected until our entire stock of adult male brown mice had died.

At first some infection was suspected, but bacteriological examination was negative, and only the adult male brown mice were affected. Post-mortem examination showed that the kidneys were pale and had a granular appearance. Histological sections showed that the renal tubules were necrotic and filled with eosinophilic material.

Shortly after recording these findings we learned that chloroform is a specific poison to adult male mice in very low concentrations, although albino males are somewhat more resistant. Rats had been killed with chloroform in the same room, about 20 ft. from where our mice were kept. This pattern is essentially the same as that described by Hewitt[1], that is, the production of severe renal damage in adult male non-albino mice with the sparing of all albino mice, and female and unweaned non-albino mice (both male and female).

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Effect of Gibberellic Acid on the Growth and Multiplication of Some Soil Micro-organisms and Unicellular Green Algae

Since gibberellin has been isolated, results of its effect on the growth of higher plants have accumulated. However, little is known with regard to its influence on the growth and reproduction of lower plants.

Experiments utilizing bacteria have shown that no stimulation of growth or multiplication was found on introducing gibberellic acid into the culture media[1–3]. However, on applying the acid or 'Gibrel' (potassium salt of the acid) in the soil certain biochemical processes become more active, such as ammonification, nitrification, denitrification, sulphate reduction, nitrogen fixation, and the decomposition of other organic compounds[4–6]. No stimulation was observed when gibberellic acid was introduced into a pure culture of *Rhizobium trifolii*, strain 157 (ref. 7), but a significant increase in the growth of inoculated leguminous plants has been obtained when the acid was applied[8,9].

In most cases gibberellic acid has a neutral effect on fungal growth and reproduction[3,10,11], except that it inhibited spore formation of *Claviceps purpurea*[13] and some species of Basidiomycetes[12].

In concentrations of 0·1–10 mg/l. the acid has a stimulating effect on the growth of species of green alga[3,14–17], while concentrations of 100–400 mg/l. stimulate the growth of protonemata of some mosses[18–20]. The effect decreases with the increase of the acid concentration.

A normalization of growth was noted when plants infected by certain viral diseases were treated with gibberellic acid[21], but the virus itself was not affected by the acid[22].

Table 1. Effect of Gibberellic Acid on the Growth and Reproduction of Some Species of Unicellular Green Alga as shown in Numbers (millions) of Cells per ml. of Solution

Species	Concentration of gibberellic acid (mg/l.)					
	0	1·0	5·0	10·0	15·0	20·0
Chlorella vulgaris	7·1	8·4	6·9	3·5	4·2	4·5
Chlorella pyrenoidosa	9·4	11·2	9·9	7·2	6·8	6·4
Scenedesmus obliquus	4·3	5·1	4·2	6·6	3·7	4·2
Scenedesmus quadricauda	2·1	4·3	—	5·0	3·3	3·0

The experiment was conducted in Knop's solution, on a shaker, at 27° C, for 10 days, under constant illumination of daylight lamps of 3,000 lux.

With the view of finding microbial test objects to be utilized in the identification of natural growth stimulators the effect of gibberellic acid was tested on the growth and multiplication of 52 strains of soil microbes comprising 31 species of bacteria, 7 species of actinomycetes and 4 species of unicellular green algae.

Experiments with bacteria and actinomycetes were carried out by means of the cup-diffusion technique. In the case of algae, a liquid solution under constant illumination was used.

The acid was tested in the following concentrations: 0, 0·5, 1·0, 2·0, 5·0, 10·0, 15·0, 20·0, and 50·0 mg/l.

With the exception of two strains of *Azotobacter chroococcum* and *Actinomyces coelicolor*, the tested microbes did not react to the acid. Stimulation of growth and pigmentation was noted with *Az. chroococcum* strain 12 and strain 22; but this does not depend on the concentration of the acid. In the case of *Act. coelicolor* an increase of pigmentation with no stimulation of growth was observed.

Low concentrations of gibberellic acid stimulated the multiplication of all species of alga tested (Table 1). The maximum effect on *Chlorella* was obtained in a concentration of 1 mg/l., which then declined with the increase of the acid concentration. In the case of *Scenedesmus*, the maximum growth was noted in a concentration of 10 mg/l., and again it decreased with the in-

crease of the acid concentration. It appears that both species of *Scenedesmus* are more resistant to higher concentrations of the acid than those of *Chlorella*.

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Toxicity of Captan Analogues to Oat Powdery Mildew

The fungicides captan (*N*-(trichloromethylthio)tetrahydrophthalimide) and phaltan (*N*-(trichloromethylthio)-phthalimide) are about equally toxic to *Alternaria tenuis* Nees and *Neurospora crassa* Shear and Dodge[1], but differ in toxicity to powdery mildews. Captan, for example, fails to control apple mildew (*Podosphaera leucotricha* (Ell. and Everh.) Salm.), but phaltan has been reported[2,3] to be effective against rose mildew (*Sphaerotheca pannosa* (Fr.) Jaczew.). This difference in effect is surprising because both fungicides contain the —$SCCl_3$ group which is generally considered to be responsible for the toxicity of captan[1], and the —CO.NX.CO— grouping which has also been suggested as a toxophore[4]. Powdery mildew spores have a high lipid content[5] and phaltan is more fat-soluble than captan[1]. The greater toxicity of phaltan to powdery mildews may therefore be due to easier penetration into the spores.

The importance of lipid solubility for powdery mildew fungicides was examined by testing captan, phaltan and five structurally similar analogues against *Erysiphe graminis* DC. ex Mérat on oats under glasshouse conditions. The analogues showed a wide range of lipid solubility; their purification and physical properties have been described elsewhere[1]. The compounds used were:

(*A*) *N*-(trichloromethylthio)tetrahydrophthalimide (captan)

(*B*) *N*-(trichloromethylthio)phthalimide (phaltan)

(*C*) *N*-(trichloromethylthio)hexahydrophthalimide

(*D*) *N*-(trichloromethylthio)-4-methylhexahydrophthalimide

(*E*) *N*-(trichloromethylthio)-4,5-dimethyltetrahydrophthalimide

(*F*) *N*-(trichloromethylthio)-3,6-endoxohexahydrophthalimide

(*G*) *N*-(trichloromethylthio)-4,5-epoxyhexahydrophthalimide

The compounds were prepared for test by dissolving in acetone then adding an aqueous solution of 'Ethylan CP', a non-ionic wetting agent (concentration of compound between $2{\cdot}5 \times 10^{-4}$ and $1{\cdot}0 \times 10^{-3}$ M; wetting agent, 0·0025 per cent; acetone, 2 per cent). The first fully expanded leaves of oat seedlings (var. 'Black Supreme') were dipped in the resulting solutions or suspensions, maintained at 30° C to ensure complete wetting. It was found that the chemically determined[1] deposits on the leaves, after drying, varied considerably according to the physical properties of the compounds. Thus at concentrations of 10^{-3} M, the solution of (*C*) gave a deposit of 0·35 μg/cm² of leaf surface, the suspension of (*A*) gave 4·2 μg/cm², and the suspension of (*D*), which contained large platelets, gave up to 31 μg/cm². This effect illustrates the error of assuming equivalent leaf retention after dipping in formulations of different compounds at the same tank concentration. Deposits of the same order of magnitude (up to 2 μg/cm²) were obtained by adjusting the concentrations of the compounds in the solutions or suspensions.

The compounds were tested for eradicant properties as follows. First, fully expanded oat leaves were inoculated by a standardized procedure of dusting with conidia of *E. graminis* from sporing infections maintained on oat seedlings. The compounds were applied by dipping the leaves 48 h after inoculation. The plants were left on the glasshouse bench arranged in randomized blocks and the mildew lesions which developed on both surfaces were counted 10–14 days later. Each treatment was replicated eight times and there was no evidence of phytotoxicity. For comparison, an emulsifiable concentrate of dinocap (2-(1-methyl-*n*-heptyl)-4,6-dinitrophenyl crotonate) was applied; deposits were determined by the method of Skerrett and Baker[6]. Inoculated untreated leaves and inoculated leaves dipped in acetone-wetter solutions were included in each test. Eradicant action was assessed from the reduction in the number of lesions per 10 cm of length of leaf upper and lower surface.

The lipid solubility of each compound was assessed from its oleyl alcohol/water partition coefficient determined previously[1].

The levels of deposit, the reductions in infection, and the water solubilities and partition coefficients of the compounds are shown in Table 1. Only compounds (*C*), (*F*) and (*G*), which had the highest water solubilities, showed significant eradicant effects. Compound (*C*) gave an excellent control, a deposit of 0·21 μg/cm² showing a toxic action not significantly different ($P = 0{\cdot}05$) from that of a deposit of dinocap of 0·75 μg/cm². Preliminary trials have shown compound (*C*) to be similarly effective as an eradicant of *Oidium* sp. on marrow.

Table 1. Eradicant Control of Oat Powdery Mildew by Analogues of Captan

Compound	Deposit (μg/cm² leaf)	Reduction of infection %	log $(x+1)$*	Water solubility (moles $\times 10^{-6}$/l.)	Oleyl alcohol–water partition coefficient
A	1·1	12	2·00	30	140
B	1·8	5	2·04	11	1,350
C	0·21	98	0·56	354	250
D	0·21	3	2·05	28	700
E	1·7	24	1·94	33	850
F	0·27	74	1·49	663	45
G	0·56	52	1·75	348	7
Dinocap	0·75	95	0·79	—	—
Inoculated leaves dipped in acetone/wetter solution			2·06		
Inoculated untreated leaves			2·07		
Least significant differences ($P = 0{\cdot}05$)			0·24		
($P = 0{\cdot}01$)			0·32		
($P = 0{\cdot}001$)			0·42		

* Where x is the number of lesions per 10 cm length of leaf.

The compounds were also tested as protectant fungicides by dipping oat leaves and allowing the deposit to dry before inoculation with *E. graminis*. At the concentrations of (*A*)–(*G*) shown in Table 1, none of the compounds gave a significant reduction in infection; dinocap at 0·75 μg/cm² gave 96 per cent reduction in infection. At higher levels of deposit, compound (*C*) gave control comparable with dinocap, deposits of 0·34 and 0·43 μg/cm² giving reductions in infection of 74 and 96 per cent respectively.

The toxicities of compounds (*A*)–(*G*) either as eradicants or as protectants to *E. graminis* follow a different pattern from their fungistatic activities to *N. crassa*[1]. There was no evidence that lipid solubility, as assessed from the oleyl alcohol/water partition coefficient, was directly correlated with either protectant or eradicant toxicity to *E. graminis*; thus phaltan (compound (*B*)), though tested at a higher level of deposit than the other compounds, was no more effective than captan either as an eradicant or as a protectant oat mildew fungicide. In fact the most effective eradicants ((*C*), (*F*) and (*G*)) had high water solubilities though wetting injury to the mildew[7] may have contributed to the effects obtained.

On the assumption that captan and its analogues have the same mode of action, it would seem that a ratio of water solubility to lipid solubility similar to that shown by (*C*) is necessary before toxicity is achieved.

We thank Dr. A. R. Kittleson, Esso Research and Engineering Co., New Jersey, for samples of the captan analogues.

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Sensitivity to Dinitrophenol of Potassium Absorption in Carrot Root Tissue Cultures

In 1954, Steward and Millar[1] reported that the absorption of cæsium ions by carrot root tissue was inhibited by dinitrocresol, and that after tissues had been stimulated to rapid growth in the presence of coconut milk they were more sensitive to the inhibitor than were slowly growing cultures. Steward concluded that the rapidly growing tissues consisted mainly of meristematic cells while the slowly growing tissues comprised mainly vacuolated cells. On this assumption he explained the differing sensitivity to uncoupling agents of the tissue in its two conditions by the suggestion that salt absorption into the cytoplasm of plant cells is more dependent on oxidative phosphorylation than is the subsequent transfer of ions into vacuoles. The experimental data provided by Steward and Millar[1] and by Millar[2] in support of this important conclusion are, however, rather complicated and difficult to interpret. Furthermore, Arisz[3] claimed that chloride absorption into the cytoplasm of *Vallisneria* leaf cells is less sensitive to dinitrophenol than is its accumulation into vacuoles. In view of these considerations, it seemed desirable to examine the situation further and we wish to report some observations on the sensitivity to dinitrophenol of potassium absorption in rapidly and slowly growing carrot tissue cultures.

The cultures were grown in liquid media in the manner described by Caplin and Steward[4] except that in our experiments the coconut milk used to stimulate growth had previously been desalted. The advantage of this modification is that it makes possible the examination of absorption from media of known ionic composition. Sutcliffe and Counter[5] directed attention to the importance of this in interpreting the results of observations on the effects of salt concentration on uptake by carrot tissue cultures. The growth-promoting qualities of desalted coconut milk when added to a basal medium of adequate salt composition are 10–20 per cent less than those of whole coconut milk, but cell counts and anatomical investigations have indicated that the tissue cultures obtained in this way are essentially similar to those used by Steward and his collaborators in their investigations.

Various concentrations of dinitrophenol were added to the nutrient medium and the effect on fresh weight increase and potassium content of slowly and rapidly growing carrot tissues is shown in Table 1.

Table 1. Potassium content and fresh weight of 5 replicate batches of carrot root explants grown for 21 days at 23° C in a nutrient medium ± desalted coconut milk (D.C.M.) containing a series of concentrations of 2,4-dinitrophenol. Initial fresh weight 2·43 mg and initial potassium content = 0·04 μequiv. per explant

Treatment	Fresh weight mg per explant (± *S.E.*)	Increase in wt. (mg)	Potassium content μequiv. per explant (± *S.E.*)	Increase in potassium content (μequiv)	μequiv. per g fresh wt.
Basal					
Control	11·82 ± 0·39	9·39	1·45 ± 0·07	1·41	123
10^{-7} M DNP	11·77 ± 0·28	9·34	1·43 ± 0·04	1·39	122
10^{-6} M DNP	12·15 ± 0·51	9·72	1·44 ± 0·02	1·40	114
5×10^{-6} M DNP	10·60 ± 0·24	8·17	1·20 ± 0·05	1·16	113
10^{-5} M DNP	9·55 ± 0·18	7·12	0·91 ± 0·05	0·87	95
10^{-4} M DNP	Toxic				
× D.C.M.					
Control	65·51 ± 1·49	63·08	4·20 ± 0·24	4·16	64
10^{-7} M DNP	62·26 ± 1·37	59·83	4·15 ± 0·28	4·11	67
10^{-6} M DNP	62·19 ± 1·20	59·76	3·95 ± 0·17	3·91	64
5×10^{-6} M DNP	51·52 ± 1·03	49·09	3·45 ± 0·01	3·41	67
10^{-5} M DNP	40·64 ± 1·41	38·21	2·21 ± 0·14	2·17	54
10^{-4} M DNP	Toxic				

The results of this experiment show that tissues cultured for 21 days in the presence of coconut milk increased in fresh weight about twenty-seven times, while those cultured in a basal medium had increased by only four to five times. Although the rapidly growing cultures absorbed about three times as much potassium per explant as did the slowly growing cultures, the amount, when expressed on a fresh weight basis, was only about one-half owing to the more rapid absorption of water. This effect of rapid growth in lowering the apparent concentration of salt in tissue-cultured cells has previously been noted by Steward and Millar[1], and by Sutcliffe and Counter[5], but the magnitude of the effect seems to vary considerably from one experiment to another.

Concentrations of 10^{-7} M and 10^{-6} M dinitrophenol did not significantly affect growth or potassium content in either rapidly or slowly growing tissues under the conditions of the present experiment. Concentrations of 5×10^{-6} M and 10^{-5} M caused significant inhibition of both growth and potassium content. There appeared to be a slightly more pronounced effect on potassium content than on growth, particularly at the higher concentration of dinitrophenol. The rapidly growing cultures seemed to be slightly more sensitive to the inhibitor than the slowly growing ones, but the difference was not statistically significant. 10^{-4} M dinitrophenol was toxic to both rapidly and slowly growing cultures.

The sensitivity of carrot tissues to dinitrophenol has been found to vary somewhat on different occasions. In another experiment, for example, 10^{-5} M dinitrophenol was found to be toxic to both rapidly and slowly growing cultures. Caplin[6] has reported in some detail the variability in growth of carrot tissue cultures, and we have some evidence that variation in sensitivity to dinitrophenol may be related to growth potentialities.

The experiments described here differ in design from those of Steward and Millar[1] in that in our experiments the inhibitor was supplied for the whole of the culture

Table 2. Potassium content and fresh weight of 4 replicate batches of carrot root explants grown for 11 days at 23° C in nutrient media ± desalted coconut milk to which a series of concentrations of 2,4-dinitrophenol was added. Explants were then collected after 14 days. Initial fresh weight 2·63 mg, and initial potassium content 0·05 μequiv. per explant

Treatment	Fresh weight		Potassium content		
	Mg per explant (± S.E.)	Increase in wt. (mg)	μequiv. per explant (± S.E.)	Change in potassium content (μequiv.)	μequiv. per g. fresh wt.
Basal					
11 day control	6·10 ± 0·25	—	0·50 ± 0·03	—	82
14 day control	6·56 ± 0·01	0·46	0·55 ± 0·04	+0·05	84
10^{-6} M DNP	6·51 ± 0·10	0·41	0·55 ± 0·04	+0·05	85
10^{-5} M DNP	6·42 ± 0·17	0·32	0·53 ± 0·02	+0·03	83
10^{-4} M DNP	6·11 ± 0·12	0·01	0·45 ± 0·005	−0·05	74
D.C.M.					
11 day control	25·98 ± 0·04	—	1·91 ± 0·05	—	74
14 day control	33·67 ± 0·83	7·69	2·68 ± 0·15	+0·77	80
10^{-6} M DNP	33·22 ± 0·54	7·24	2·57 ± 0·05	+0·66	77
10^{-5} M DNP	30·29 ± 0·08	4·31	2·31 ± 0·06	+0·40	76
10^{-4} M DNP	26·37 ± 0·69	0·39	1·69 ± 0·06	−0·22	64

period. In other experiments, following Steward and Millar[1], we have supplied dinitrophenol for only a part of the growth period. In one experiment, the results of which are presented in Table 2, tissues were allowed to grow for eleven days in a nutrient medium in the presence or absence of coconut milk and then dinitrophenol was added at a series of concentrations. Fresh weight and potassium content were determined (*a*) initially, (*b*) after the preliminary eleven days of culture, and (*c*) at the end of the fourteen-day experimental period. The growth of the control tissues in both the basal and coconut-milk-supplemented media was much less than in the first experiment described above. This can be partly, but not entirely, accounted for by the shorter growth period employed. As in the earlier experiment (Table 1), the fresh weight attained in the rapidly growing cultures was about five times that attained in the basal medium. The potassium content per explant in the control cultures after fourteen days was lower than that attained after twenty-one days in the first experiment. The amount of potassium expressed per unit of fresh weight was also lower in the case of the basal cultures, but in the rapidly growing cultures it was somewhat higher.

Dinitrophenol at 10^{-6} M was again without significant effect on the growth and potassium content of either type of culture. 10^{-5} M DNP inhibited growth and potassium uptake to a comparable extent, and the two tissues showed similar sensitivity to the inhibitor. As in the first experiment, however, slightly greater inhibitions occurred in the rapidly growing cultures. 10^{-4} M DNP prevented any significant increase in the fresh weight of both types of culture, and there was a decrease in potassium content during the three days in which the cultures were exposed to the inhibitor.

There seems to be little evidence from our experiments to support Steward and Millar's[1] contention that the absorption mechanisms themselves exhibit differing sensitivity to uncoupling agents in rapidly growing and slowly growing cultures. Growth itself is slightly more sensitive to inhibition in the rapidly growing than in the slowly growing cultures and this may account for the small differences in sensitivity of salt uptake observed in our experiments. At lower concentrations of DNP, growth and potassium content are reduced proportionately as dinitrophenol concentration increases.

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MICROBIOLOGY

Sphæroplasts of *E. coli* obtained by the Lytic Enzyme isolated from Phage T_2

Sphæroplasts of *E. coli B*, obtained by treatment of cultures with 'ghosts' of phages, present an incomplete medium for the reproduction of the virus[1].

This incompleteness of sphæroplasts is determined by the disturbance of the synthetic processes of intact cells[1–3], which are probably connected with the increased permeability of 'ghost' sphæroplasts[4]. However, it is not clear which structures of the phage membranes provoke the disturbance of cellular permeability.

Our work was aimed at elucidating the following: (*a*) characterization of sphæroplasts, obtained by the treatment of *E. coli* cells with the lytic enzyme of the phage, as a system for virus reproduction; (*b*) investigation of the permeability of the cells during treatment with the lytic enzyme of the phage.

For the isolation of the lytic enzyme of bacteriophages concentrated phage T_2 ($2–3 \times 10^{13}$ particles/ml.) was freeze-thawed 15 times (−78° C and 37° C) in the presence of DNase (renal) 35 μg/ml. The preparation was purified from intact particles and sub-structures of phages by two rounds of centrifugation: 1 h at 18,000*g* (centrifuge *CLR*–1) and a second centrifugation of the supernatant fluid 1 h at 105,000*g* (Spinco model *L*50).

As the lytic phage enzyme the supernatant fluid obtained after the second centrifugation was used. The residual phage in this fraction was $2–5 \times 10^5$ particles/ml.

In the experiments a 2-h culture of *E. coli B* or *B*/2 was used, grown with shaking in phosphate-buffer-broth (*p*H 7·2) to $2–3 \times 10^8$ cells/ml. The lytic activity was determined by measurement of the optical density of the mixture, consisting of 0·9 ml. of the culture washed in medium *M*9 and treated with chloroform, and 0·9 ml. of the corresponding dilution of the lytic enzyme. Measurements were made using a photoelectric colorimeter at E_{418} or on plates[7].

Sphæroplasts of *E. coli* were obtained in the *tris*-buffer (*p*H 8·2) with the addition of 0·03 per cent versene (EDTA) and 40 per cent of sucrose: the culture of *E. coli B*/2 washed in the *tris*-buffer was resuspended in one volume of the aforementioned mixture and 2 volumes of the lytic enzyme of phage T_2 were added. 15–20 min later the sphæroplasts were formed. The lysozyme sphæroplasts were obtained by Repaske's[8] technique. The method of 'ghost' sphæroplast production and infection of sphæroplasts by the π-factor has been reported before[1].

The permeability of sphæroplasts was determined by the yield of aldolase. The suspension of sphæroplasts was centrifuged for 10 min at 5,000 r.p.m. and in the supernatant fluid the aldolase activity was determined[6].

The lytic enzyme of the phage did not dissolve the living cells of *E. coli B* or *B*/2. The activity of the enzyme was revealed only after preliminary treatment of the cultures with chloroform or in the presence of EDTA (Fig. 1). In the presence of EDTA and sucrose the lytic enzyme of phage T_2 induced the conversion of cells of *E. coli B* or *B*/2 into spherical forms morphologically similar to lysozyme sphæroplasts.

It was found that sphæroplasts of *E. coli B*/2 induced by the lytic enzyme of phage T_2 were able to provide a medium for the reproduction of the virus after infection with the π-factor—obtained from phage T_2h (Table 1).

The yield of phages in these sphæroplasts was identical to the yield of phages demonstrated in the lysozyme sphæroplasts infected by the same factor.

In experiments on measurements of the yield of aldolase it was demonstrated that in sphæroplasts obtained from *E. coli B*/2 the permeability barrier for this enzyme is not disturbed and in this respect they are similar to the lysozyme sphæroplasts (Table 2).

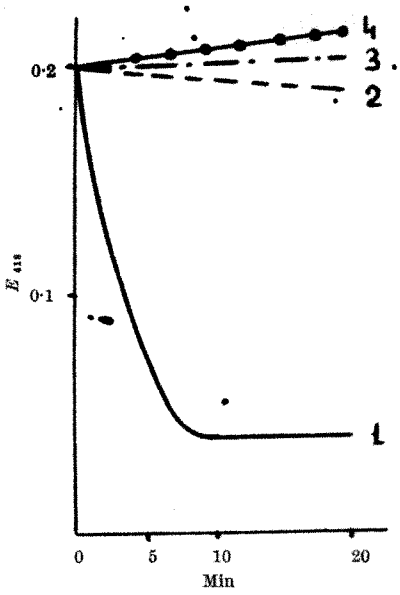


Fig. 1. Action of the lytic enzyme, isolated from phage T_2 on a living culture of *E. coli* B/2 and a culture treated by chloroform. (1) Culture treated with chloroform + enzyme; (2) culture treated with chloroform + medium; (3) living culture + medium; (4) living culture + enzyme

Hence the sphæroplasts obtained by the action of the lytic enzyme of the phage and by egg-white lysozyme are similar, and differ from the 'ghost' sphæroplasts which in these experiments, as in earlier experiments[1,4], were found to be unable to provide the reproduction of the virus, and this inability is related with the disturbance of the cytoplasmic membrane.

On the basis of these results and those published previously[1,4] it can be concluded that the so-called 'killing-effect' of phage T_2 is related to the lytic enzyme, and is unable in isolated form to induce disturbances of the permeability barrier. Probably the protein membrane of phage T_2 maintains besides the lytic enzyme a factor, acting similarly to EDTA or chloroform. This inference is a sequence to the fact that the ability of the 'ghosts' to dissolve the cell wall or to convert the bacteria in a sphæroplast in hypertonic media is realized in media without EDTA or chloroform. The isolated lytic enzyme of the phage is capable of producing similar effects only by the 'sensibilization' of cells with the aforementioned co-factors. On the other hand, it is logical to admit that the protein membrane of phage T_2, besides the lytic enzymes, is maintaining a factor which provides the 'killing-effect' by a sharp disturbance in the permeability of the cell. This factor may be a specific enzyme. The possibility that disturbance of the cell permeability barrier is produced by a mechanical perforation of the membrane in the irreversible phase of the adsorption of the phage particles on the bacterial cell must not be excluded.

Table 1. Reproduction of the Phage in Sphæroplasts obtained by Lytic Enzyme isolated from Phage Particles, Egg-white Lysozyme and Phage 'Ghosts'

$0{\cdot}8–1\times10^9$ bacteria treated with	Sphæroplast production (by the quantity of surviving bacterial cells) (%)	Phage titre after 30 min incubation of the mixture 1 ml. of sphæroplasts + 1 ml. broth	Phage filter after 30 min incubation of the mixture: 1 ml. of sphæroplasts suspension + 1 ml. π-factor
Lytic enzyme of the phage T_2	95·4	0	$2{\cdot}1\times10^4$
Egg-white lysozyme	99·9	0	$5{\cdot}5\times10^4$
Phage 'ghosts'	99·9	$1{\cdot}1\times10^2$	$6{\cdot}1\times10^2$

Table 2. Permeability of Sphæroplasts, obtained by Lytic Enzyme isolated from Phage Particles, Egg-white Lysozyme and Phage 'Ghosts'

$0{\cdot}8-1{\cdot}0\times10^9$ of cells treated with	Production of sphæroplasts (measured by the quantity of surviving cells) (%)	Release of aldolase in incubation media (in units of activity)
'Ghosts' of phages	99·9	288
Lysozyme of egg-white	99·9	0
Lytic enzyme of phage T_2	97·4	0

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ENTOMOLOGY

High-intensity Flash Discharge as a Source of Radiant Energy for Sterilizing Insects

The possibility of using sterilized male insects to reduce the numbers of a pest species or even to eliminate it within a restricted area has assumed considerable importance since the successful application of this method by Baumhover *et al.*[1] against the screw-worm fly. Gamma-radiation is most frequently used to sterilize males, though lately chemo-sterilants are receiving increasing attention. Both these methods have drawbacks, especially in regard to safety aspects.

A paper by Nelson[2] on the chemical reactions which can be initiated by the heat from a high-intensity flash indicated that the possibility of using this form of radiant energy to sterilize insects was worth investigating, particularly since short-wave radiation in the ultra-violet region is known to damage proteins and nucleic acids and has been shown by Luria and Latarjet[3] to produce lethal mutations in bacteriophage.

Preliminary experiments were conducted with two species of insects: a small chalcid *Dahlbominus fuscipennis* Zett. that had been used previously in heat-sterilization studies[4], and the mosquito *Aëdes aegypti* (L.). The flash apparatus was an ordinary photographer's photo-flash that has a nominal output of 100 joules at 2,500 V. The insects were confined in cylindrical glass jackets encircling the envelope of the flash-tube so that the *D. fuscipennis* were held within 3 mm of the envelope and the *A. aegypti* within 7 mm.

Immediate mortality in the *D. fuscipennis* males was not recorded, but propagation of the survivors showed that 82–87 per cent were sterilized by a single flash. Two flashes killed or sterilized 100 per cent. Twenty-six per cent of the *A. aegypti* males were killed by a single flash and all the survivors were sterile.

Following the results a more refined flash apparatus was built to permit precise positioning of the insects in relation to the flash and in which the light output can be controlled. Experiments are now in progress to establish dose/effect relationships in *A. aegypti* and also to determine the longevity and competitiveness of the males that are sterilized. Similar experiments are planned with insect pests of stored food products.

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Nervous Control of Flight Orientation in a Beetle

In a recent publication about the nervous control of dipteran flight, Wilson and Wyman[1] confirmed and extended previous observations of the absence of phase relationship between the movements of the wings and the nervous input to the muscles of insects possessing a fibrillar flight-motor. Their records show, however, that an increase in wing-beat frequency is accompanied by an increase in the frequency of nervous stimulation to the power muscles. In a concluding sentence, Wilson and Wyman suggest that frequency control may operate in the regulation of parameters like direction of locomotion.

In an investigation of flight orientation in the rhinoceros beetle, *Oryctes boas*, I have found that change of direction about the yaw axis is accompanied by a unilateral increase in frequency of the nervous input to the fibrillar flight muscles. Extracellular recordings have been obtained from the left and right members of all six pairs of fibrillar flight muscles during straight flight and yawing rotations of tethered beetles. The traces in Fig. 1 show the marked increase in frequency of the nervous input to the left muscle of a pair during yaw to the right induced by rotation of a striped cylinder.



Fig. 1. Extracellular recordings from left (upper trace) and right (lower trace) dorsal oblique muscles of *Oryctes boas*. (*a*) Straight flight; (*b*) yaw to the right

Stroboscopic photographs of tethered beetles executing yawing rotations show that yaw is achieved chiefly by a unilateral increase in amplitude of the wing stroke. Machin and Pringle[2] observed that an increased frequency of electrical stimulation to the basalar muscle of *Oryctes rhinoceros* resulted in an increase in the amplitude of oscillation of the muscle.

It is concluded that, during yawing rotations, the observed increase in frequency of the nervous input to the flight muscles of the appropriate side brings about a greater amplitude of oscillation of these muscles and so leads to a unilateral increase in amplitude of the wing stroke.

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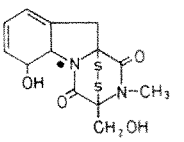
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VIROLOGY

Antiviral Activity of Gliotoxin and Gliotoxin Acetate

During a search for antiviral materials, we found several *Penicillium* culture filtrates that inhibited the cytopathic effect of type 2 poliovirus MEF_1 in monkey kidney primary cell cultures. The antipolio activity of these filtrates was found to be due to gliotoxin and gliotoxin acetate.

Gliotoxin (I) (ref. 1) was first isolated in 1936 by Weindling and Emerson[2] and afterwards by others from culture filtrates of species of *Gliocladium* (?), *Trichoderma*, *Aspergillus* and *Penicillium*[3]. Its inhibitory activity towards bacteria, fungi and transplantable neoplasms is well known[4]. There has been no report of antiviral activity for gliotoxin. It is highly toxic for rodents[4] and as a result has no practical value as a therapeutic agent. For this reason we have not investigated its antiviral effect or mode of action, but we present this communication: (1) to demonstrate that methods are available for detecting potentially useful antiviral materials; (2) to report that this long-known compound exhibits antiviral activity; and thereby (3) to provide another tool for virus and chemotherapy research.



I

One monoverticillate *Penicillium*, strain *F*1072, had been isolated from a soil sample from Bath, Maine, U.S.A., and fermented aerobically at 26° C for 4 days in a medium containing glucose, acid-hydrolysed casein, enzymatically hydrolysed yeast, and sodium chloride. Crystalline material was first obtained from such culture filtrates by *n*-butanol extraction followed by alumina chromatography and countercurrent distribution. By modifying fermentation media and conditions, more potent culture filtrates were prepared. These filtrates were extracted with ethylene dichloride; and, after partitioning the dried extract between methanol and heptane, the active material was crystallized from the methanol fraction. The identity of this material with gliotoxin was established by physical and chemical comparison with an authentic sample.

Another monoverticillate *Penicillium*, *F*642, isolated from soil collected in Anvers, Belgium, yielded both gliotoxin and its monoacetate, which were differentiated chromatographically and separated by fractional crystallization. Both were active against type 1 (Mahoney) poliovirus.

Fermentation and isolation were assisted by agar diffusion assays and bio-autography with poliovirus type 1 (Mahoney) in *H.Ep.* 2 cells, using methods described by Herrmann and by Siminoff[5]. It was found that assays with *Micrococcus lysodeikticus* reflect poliovirus-inhibitory activity and that similar R_F values are obtained with samples developed in the same solvent system. Hence, agar diffusion with *M. lysodeikticus* was used to help guide fermentation, fractionation, and isolation because of its greater precision, speed and convenience. Poliovirus assays and bio-autograms were limited to confirmation of key samples.

Table 1 gives the antiviral spectrum of gliotoxin when tested at half-log serial dilutions with *H.Ep.* 2 and *KB* cell suspensions in plastic panels and with monkey kidney primary cell and *KB* cell monolayers in tubes. The test procedures in plastic panels have been described[6]. Similar methods were used for tests in tubes except that virus and drug were added simultaneously to 3–5 day monolayer cultures containing 1·0 ml. of maintenance medium (synthetic medium 199 for monkey kidney, Eagle's plus 10 per cent horse serum for *KB* cells). Gliotoxin was active against poliovirus, herpes and Asian influenza; but the end-points were more definite with poliovirus. Gliotoxin was inactive against rabies when added to a CVS infected mouse brain homogenate, and this mixture inoculated intracerebrally into healthy adult mice.

Chemotherapy trials were limited because of the poor solubility of gliotoxin and its toxicity for animals. The maximum tolerated single dose for mice was found to be 7·5 mg/kg intravenously (*N*,*N*-dimethylacetamide solution) and < 19 mg/kg intraperitoneally (sodium carboxymethyl cellulose emulsion). When gliotoxin was admini-

Table 1. Activity of Gliotoxin against Viruses in Cell Culture

Virus	Strain	Host cell	Gliotoxin (μg/ml.) Toxic*	Active†
Poliovirus type 2	MEF_1	Monkey kidney	80	0·80
Poliovirus type 2	MEF_1	*KB*	0·45	< 0·014‡
Poliovirus type 2	MEF_1	*H.Ep.* 2	64	< 2·0‡
Herpes simplex	*HF*	*H.Ep.* 2	64	< 2·0‡
Influenza, A_2, Asian	Jap. 305	Monkey kidney	250	8·0
			Toxic	Inactive§
Para-influenza 1	Sendai	Monkey kidney	250	80
Para-influenza 3	*HA*–1 (Huebner *C*203)	*KB*	25	8
'*B*' virus	Yale	Monkey kidney	250	80
Measles	Edmonston	*H.Ep.* 2	64	20
Vaccinia	Lederle *CA*	*H.Ep.* 2	64	20
ECHO 9	Bourn *LR*–451	Monkey kidney	80	25
Coxsackie B_1	Conn 5	Monkey kidney	250	80

* Toxic: minimal concentration of compound that produces a microscopically evident alteration in host cell morphology. Differences in toxicity may reflect the fact that tests were performed in two laboratories on various lots of cells.

† Active: minimal concentration of compound that inhibits the cytopathic effect of virus on host cells as indicated by microscopic examination.

‡ Lowest concentration tested with *KB* cells was 0·014 μg/ml.; with *H.Ep.* 2 cells, 2·0 μg/ml.

§ Inactive at highest concentration tested.

stered orally for twenty-one days, mice tolerated 10 but not 20 mg/kg/day (gum acacia suspension). Mice were challenged intracerebrally with 10 or 32 LD_{50} type 2 poliovirus (MEF_1), then treated orally with 5 or 10 mg/kg/day for 21 days starting 24 h after challenge. This treatment failed to protect the animals; it is not known whether virus-inhibitory gliotoxin-levels were achieved systemically with the suspension used.

We thank Lucia E. Anderson, Gussie Arnett, John D. Douros, Salvatore A. Fusari, Robert J. Hans, Arthur B. Hillegas, Ann Holcomb, John D. Howells, Eugene C. Loomis (deceased), H. E. Machamer, I. W. McLean, jun., Robert F. Pittillo, Frank M. Schabel, jun., Howard E. Skipper and Dorothy L. Vogler for their assistance.

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CYTOLOGY

Chromosomes of X-ray-induced Giant Cells in Chinese Hamster *in vitro*

The description of giant cells in biological and medical reports is limited to morphology[1,2], biosynthesis[3,4] and cellular growth[5,6]. This communication describes the chromosome status of the giant cells induced by X-irradiation *in vitro*.

A 'near-tetraploid' cell sub-line of Chinese hamster (*Cricetulus griseus*)[7] with doubled stemline chromosome number (46) (ref. 8) was used for this work. Cells were grown on HUT–15 medium and incubated at 37° C in a humid atmosphere of 2 per cent carbon dioxide and air. They were X-irradiated at a dose of 1,500 r. with 250 kVp. X-rays (half-value-layer of the order of 0·9 mm copper) at a dose rate of about 110 r./min. Observations of chromosomes were made on each sample at intervals 24–144 h after irradiation. Cytological preparation involved colchicinization of cells at a concentration of 2 μg/ml. culture medium for 2 h, hypotonic treatment with 0·9 per cent sodium citrate for 30 min, fixation in acetic alcohol (1 : 3) for 1 h, air-dry technique, and staining with 2 per cent aceto-orcein.

The giant cells described here have a large number of chromosomes at metaphase. They were found to have approximately 92, 184, 368 or 736 chromosomes which appeared to be $8n$, $16n$, $32n$ or $64n$, respectively, since the cell sub-line was designated as 'near-tetraploid' (46 chromosomes). The percentages of giant cells obtained at different intervals after X-irradiation are listed in Table 1. The $8n$ cells in this culture reached 64 per cent by 48 h after X-irradiation. After 48 h, the $8n$ cells decreased and cells with higher ploidies ($16n$, $32n$, etc.) predominated. The $8n$–$64n$ cells accounted for approximately 80 per cent (74–84 per cent) of mitoses observed at each interval of 48–144 h after irradiation.

Table 1. Per cent Giant Cells induced by X-rays (1,500 r.) in Chinese Hamster *in vitro*

Hours after irradiation	Giant cells in polyploid forms* 4*n*	8*n*	16*n*	32*n*	64*n*
Control	99	1			
24	84	16			
30	76	24			
48	26	64	10		
72	20	54	18	8	
96	18	46	26	10	
120	18	40	26	14	2
144	16	30	28	20	6

* The values $4n$, $8n$, etc., are approximate only.

The formation of higher ploidies due to chromosome doubling of lower ploidies is known as endo-reduplication (endo-mitosis). Yu and Sinclair[9] pointed out that in the Chinese hamster cells the X-ray-induced polyploidization was due primarily to endo-reduplication. All the $8n$ cells

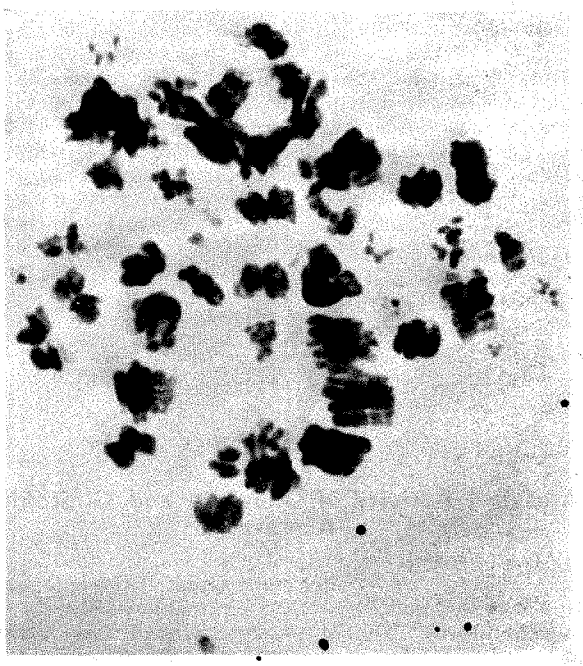


Fig. 1. An endo-reduplication cell. Each bundle consists of eight chromosomes

observed by them 24 h after irradiation were endo-reduplication cells with diplochromosomes. Levan and Hauschka[10] believed that the configuration of diplochromosomes at metaphase was the result of endo-reduplication. Endo-reduplication may occur several times without division of the cells, therefore each centromere observed at metaphase is associated with four chromatids (single endo-reduplication), eight chromatids (two endo-reduplications)[11], or even 16 chromatids (three endo-reduplications). For example, in the work recorded here, a giant cell observed 72 h after irradiation had bundles of eight chromosomes (16 chromatids), which indicated that endo-reduplication had occurred three times in succession, thus giving rise to $32n$ (Fig. 1).

In general, the X-ray-induced chromosome aberrations found in the giant cells were the same as those described in the literature. However, there is a type of aberration, the polycentric chromosome, found in the giant cells which has not yet been reported. Some fragments of the broken chromosomes resulting from X-irradiation may join at their broken ends to form a di- or poly-centric chromosome. Such a chromosome may duplicate itself through endo-reduplication resulting in two identical chromosomes (in this case, they are sister diplochromosomes). These two identical chromosomes may again join together at their same broken ends to form a bilaterally identically-symmetrical (BIS) chromosome; its right and left halves are, therefore, mirror images of each other. If a second endo-reduplication occurs before the cell enters mitosis, these BIS chromosomes will be reduplicated. Therefore, two identical BIS chromosomes will have been formed and will be seen at the next metaphase. Furthermore, BIS chromosomes may replicate themselves several times, for example, four identical BIS chromosomes may result from three endo-reduplications and be seen at the following metaphase (Fig. 2). Almost every giant cell ($16n$–$64n$) was found to have one or more BIS chromosomes. However, they may be di- or tetra-centric.

It is possible to interpret, particularly in this case, the meaning of nuclear growth of giant cells in relation to the occurrence of BIS chromosomes, and the appearance of diplochromosomes. When X-rays cause endomitosis, the cells may continue biosynthesis without division being recognized by their large size due to nuclear growth. The length of the nuclear growth period may be dependent on the time needed to complete endo-reduplication. If endo-reduplication should recur, the mitosis may appear much later. For example, cells with two and four identical BIS chromosomes have been found at 96 and 120 h, respectively, after X-irradiation. This suggests that induction of BIS chromosomes in giant cells by X-rays is not a spontaneous but a complex process involving a series of cytological mechanisms.

This work was supported by the U.S. Atomic Energy Commission.

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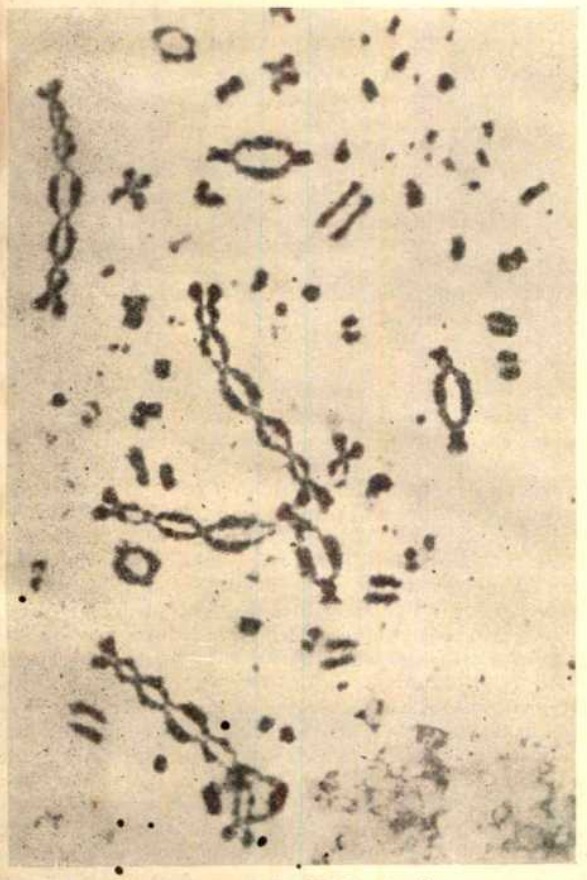


Fig. 2. A portion of metaphase cell with 4 identical BIS chromosomes of each type

GENETICS

Blood Culture Method for the Study of Bovine Chromosomes

THE development of leucocyte culture techniques[1–3] has greatly facilitated the investigation of chromosome anomalies associated with various human disorders[4–9]. Chromosome investigations on domestic animals with similar disorders have been relatively rare, probably because the techniques used for the delineation of human chromosomes are often inapplicable. The most popular procedure for the cultivation of leucocytes involves the use of navy bean extracts both as a hæmagglutinating agent[1,3] and as a mitogenic agent[10,11]. Reports from various laboratories have indicated that phytohæmagglutinin M used for the separation of human leucocytes is ineffective on the erythrocytes of several domestic and wild animals[12–14]. The dextran method developed by Nichols and Levan for the separation of leucocytes from laboratory animals[15] has been extended successfully to some domestic and wild animals when the phytohæmagglutinin method has failed to provide adequate amounts of leucocytes for culture[12]. Neither the phytohæmagglutinin method nor the dextran method has been efficient enough for the separation of bovine leucocytes; consequently, investigators concerned with the investigation of bovine chromosomes have resorted to various agents such as fibrinogen[16] and ficoll[13] or to prolonged centrifugation[14,17] for obtaining leucocytes. In the course of our work on the chromosomes of cattle with lymphosarcoma we have developed a simple method which provides good preparations of bovine chromosomes. The method has also proved efficient for the investigation of somatic chromosomes of other domestic animals.

Venous blood, drawn aseptically into a syringe previously rinsed with 0·2 ml. of 1 per cent sodium heparin solution, was used in all experiments. The growth medium used for the cultivation of blood cells was Connaught's '*H*597', supplemented with 20 per cent inactivated calf

serum, 0·08 per cent sodium bicarbonate and potassium penicillin G at a concentration of 100 I.U. per ml.

9 ml. of growth medium, 0·5 ml. of phytohæmagglutinin M (Difco) and 1·0 ml. of whole blood were introduced into each 30 ml. capacity McCartney bottle. The blood was mixed with the medium by gently shaking the bottle before placing in the incubator at 38° C.

On the third day after setting up the cultures, 1·0 ml. of phosphate buffered saline (PBS) containing 100 μg of colchicine was released into each culture bottle in order to accumulate metaphase stages. After 6 h incubation with colchicine, the bottles were left at room temperature for 10 min after adding 20 ml. of distilled water to each bottle. The diluted medium was spun at 300 r.p.m. for 2 min in narrow 15 ml. capacity centrifuge tubes and the sedimented cells were fixed in fresh carnoy solution for 10 min. The sediment was dispersed by gentle aspiration with a Pasteur pipette and the suspension was centrifuged again as described before. The sedimented cells were re-suspended in 0·5 ml. of fresh Carnoy solution and two or three drops of this suspension were placed on each clean slide. The slides were air-dried before being stained with 2 per cent natural orcein or carbol fuchsin. The procedure for making permanent mounts was the same as that for human leucocytes.

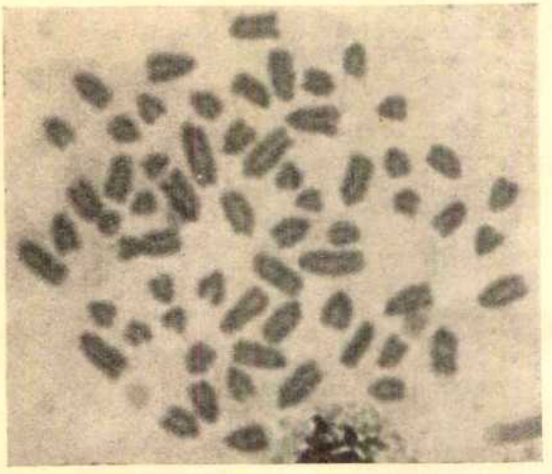


Fig. 1. Metaphase chromosomes of a normal cow. Air dried. Orcein stain. (× 2,000)

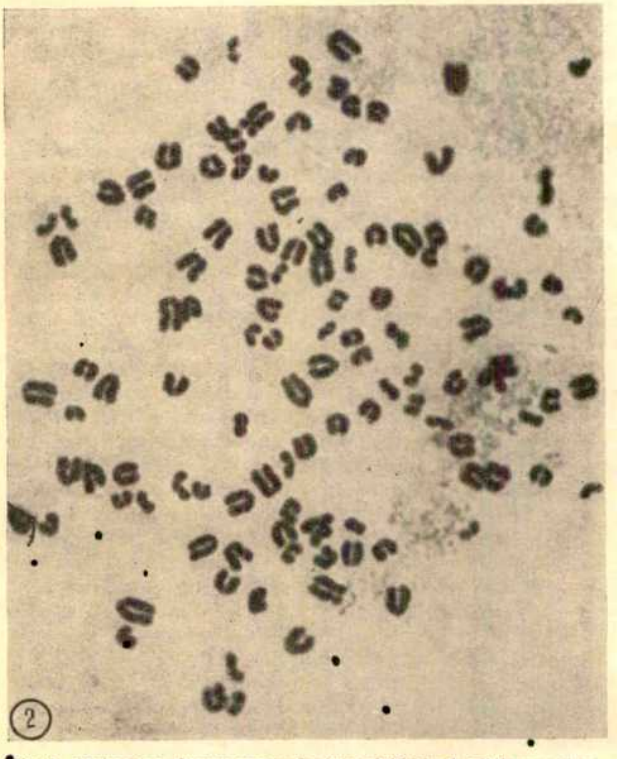


Fig. 2. Metaphase chromosomes of a tetraploid blood cell from a steer with lymphosarcoma. Air dried. Orcein stain. (× 2,000)

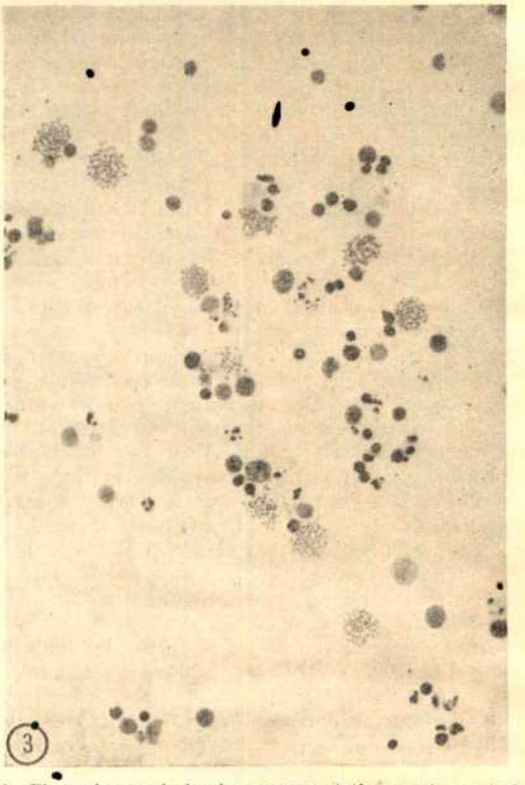


Fig. 3. Photomicrograph showing a representative area in an air-dried preparation from a blood culture of a normal cow. The blood was mixed with the medium and stored at room temperature for 6 h before incubation. Note the abundance of metaphase plates with well spread chromosomes. Orcein stain. (× 600)

Good chromosome preparations have been consistently obtained with this method from blood samples of normal as well as lymphosarcoma cattle (Figs. 1 and 2). Under our laboratory conditions a single culture of 1·0 ml. yields 30–50 well-spread metaphase plates for chromosome analysis (Fig. 3). Storing heparinized whole blood or blood samples mixed with the culture medium at room temperature, for up to 6 h before incubation, does not seem to affect the quality of chromosome preparations or the percentage of mitoses in the culture.

The addition of 2 volumes of distilled water to the medium before fixing the cells has proved adequate to lyse the erythrocytes in the culture. The ghosts of these erythrocytes are eliminated along with the discarded centrifugates before and after the addition of the fixative. We have tried both M and P fractions of phytohæmagglutinin obtained from Difco Laboratories as well as the crude extract produced by Wellcome Laboratories. Both Wellcome's phytohæmagglutinin and Difco's phytohæmagglutinin M have been equally satisfactory for metaphase spreads, although the latter in our hands tended to yield more mitotic stages per culture. We have also noticed that cultures incubated for 4 days are as good as those used after 3 days incubation, although incubation for more than 4 days has tended to reduce the percentage of mitoses.

This method of cultivation has also provided well-spread metaphase plates from blood samples of goats (Fig. 4), sheep and mink, although the fixed cells of sheep and mink had to be squashed in a drop of acetic acid in order to get good metaphase spreads (Fig. 5).

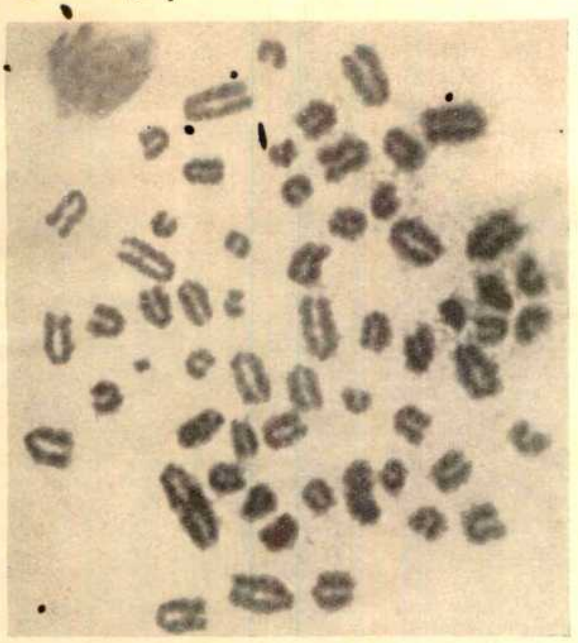


Fig. 4. Metaphase chromosomes of a male goat. Air dried. Orcein stain. (× 2,000)

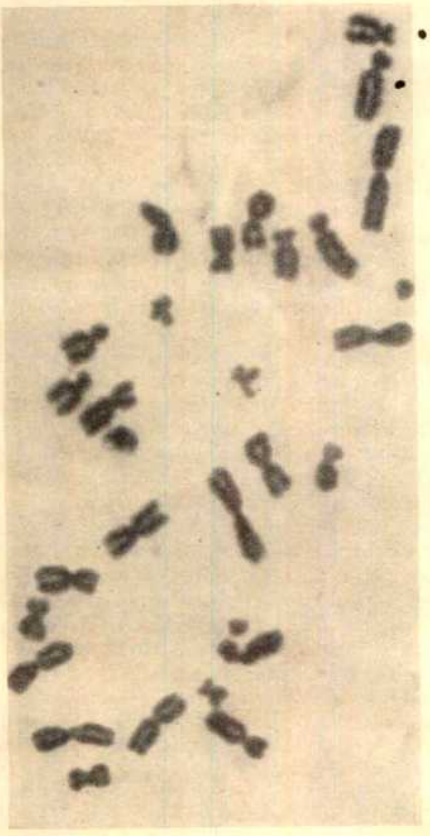


Fig. 5. Metaphase chromosomes of a female mink. Acetic acid squash. Carbol fuchsin stain. (× 2,000)

This culture is especially suitable for chromosome examinations on a large number of animals in a herd, since very little time and effort are required to set up cultures from the blood sample of each animal, and the results are comparable with those obtained by more involved techniques (see refs. 13, 14, 16, 17, 20). Furthermore, persons untrained in blood culture technique can release the blood samples into previously prepared bottles for shipment to the investigator for chromosome analysis.

This work was supported in part by the National Cancer Institute of Canada. We thank Dr. Lynette Keur and Mrs. Agnes Anderson for their assistance.

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PSYCHOLOGY

Dream Deprivation: an Experimental Reappraisal

Dement[1], using the rapid eye movement method of dream detection, prevented subjects from dreaming on five successive nights by awakening them at the onset of each rapid eye movement period (REMP). The results included a steady increase in the forced awakenings necessary during the deprivation period, a marked increase in the percentage of dream time when subjects were allowed to sleep undisturbed and certain behavioural changes summarized as "anxiety, irritability, and difficulty in concentration". From a psychiatric point of view we felt the implications of this apparent 'need to dream' were significant. We therefore elected to carry out a dream curtailment of our own to assess any quantitative or qualitative psychic changes through continual observation and psychometric testing.

Two male graduate student subjects were screened for emotional and physical health. A large room equipped for normal daytime activities was provided and the subjects were continually observed by hospital volunteers in order to prevent any sleeping. The subjects were taken to the EEG laboratory at 10 p.m. nightly and allowed to sleep in adjacent, air-conditioned and sound-attenuated rooms. Cortical EEG and eye movements were recorded continuously on two 16-channel Grass electroencephalographs.

The subjects were allowed to sleep undisturbed for several nights while baseline recordings were obtained. During the following six nights, subjects were fully awakened at the onset of each rapid eye movement period. Usually awakenings occurred within 15–40 sec after the first eye movement burst. Following this dream (REMP) curtailment, the subjects were allowed to sleep undisturbed for two nights. As a control study, the same subjects returned to the laboratory and were awakened an equal number of times during non-REMPs for six nights. During both experiments, measurements were made nightly of total sleep, total dream and percentage dream times using the usual scoring method[2]. Psychometric studies, including one or more of the following tests, were carried out daily for both the deprivation and control experiments: M.M.P.I., Nowlis adjective checklist, Clyde mood scale, Stroop colour word test and digit span.

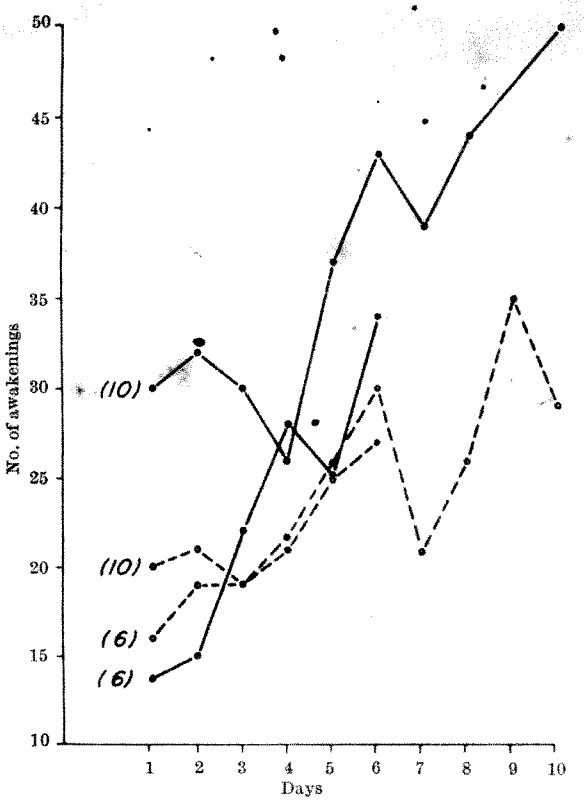


Fig. 1. Dream deprivation. ——, Subject No. 1; — — —, Subject No. 2. In brackets, No. of dream deprivation nights

Our results confirmed the previous findings of an unequivocal progressive increase in so-called 'attempts to dream' during the deprivation period. No such tendency occurred during the control awakenings. Our results also confirmed the finding of an increase in the percentage dream time during the recovery nights following the deprivation period. However, psychological testing and subject observation revealed only minimal psychic changes. These changes were as characteristic for the control as well as the deprivation period so that they could not be attributed to the dream curtailment itself.

We were then interested in increasing the number of deprivation nights from 6 to 10 to determine if the objective findings would increase proportionately and to again evaluate any psychic changes. In order to make the deprivation more complete, we decided to use a decrease in muscle tonus as a signal for awakening, as this decrease characteristically occurs just prior to, or concomitant with, the onset of a REMP[3,4]. Electromyographic leads were attached to the sub-mental region and the decrease in muscle tonus from this area was utilized as the awakening signal. Psychometrics were administered more often during the 10-day study, but otherwise the procedure was as before. The same two subjects were again used in order to (1) avoid the variable of comparing the effects of 10 nights versus six in different persons, and (2) because it was felt that a new set of control awakenings were not mandatory if the same subjects were used and the findings were similar.

As can be seen in Fig. 1, the number of awakenings required to prevent dreaming on the first and sixth nights is greater in the 10-night series than in the previous six-night study. Also, the total number of awakenings greatly increased, 51 awakenings being required with Subject No. 1 on night No. 10. In Table 2, the percentage dream time and percentage increase on recovery nights are listed. The increase in both these objective findings is attributed to the more complete deprivation afforded by the use of the EMG signal for awakening, and the increase in the length of the deprivation period. Psychological testing again revealed few significant changes and, although the testing and observation indicate that subjects were somewhat more affected by the longer experiment, there is little evidence that these changes are anything more than the result of sleep deprivation and the irritating accompaniments of the experimental situation.

Table 1. Six Night Deprivation Period

	Subject	No. of dreams	Dream time (per cent)	Increase dream time (per cent)
Baseline recording (av. of 3 nights)	1	4	20·3	
	2	4	22·7	
Recovery night No. 1	1	5	34·9	72·5
	2	4	31·8	40·0
Recovery night No. 2	1	4	30·8	52·0
	2	4	30·5	34·3
Control recovery nights	1	5	23·8	17·5
	2	4	19·6	−13·6

Table 2. Ten Night Deprivation Period

	Subject	No. of dreams	Dream time (per cent)	Increase dream time (per cent)
Baseline recording	1	6	20·0	
	2	5	20·1	
Recovery night No. 1	1	5	40·6	102·5
	2	4	36·0	78·6
Recovery night No. 2	1	5	32·6	62·4
	2	4	39·9	98·3

Subjects' dream time was normal when measured again one week after deprivation.

Subsequent to our work, Dement[5] deprived several additional subjects of dreams. All the subjects again showed the same objective findings of increased dream attempts during deprivation nights and percentage dream time on recovery nights. In addition, two of the subjects exhibited personality changes of a non-psychotic nature. Dement, himself, has questioned these results on the basis of 'Dexedrine' used in both of these subjects, a lack of controls, and a pre-existing schizoid behaviour pattern in one of these subjects.

In summary, our results agree with the previously reported findings of an increase in the number of awakenings necessary to prevent dreaming and an increase in the dream time percentage on the first recovery night, both findings being proportional to the amount of dream curtailment. Our results do not substantiate the hypothesis that psychic changes occur with deprivation of REMPs; however, in interpreting these results we realize there may be large variations in response from individual to individual. Subjects are permitted to experience only a few seconds of dreaming by using the EMG signal to indicate when awakening is required. One of the subjects consistently reported bizarre, vivid dreams of approximately 1-min duration when awakened after this signal. The other subject often reported fragmentary mentation and occasionally a brief, vivid dream when awakened. It may be possible that there is a compensatory acceleration of the dreaming process under conditions of deprivation so that within a few seconds there is substantial content. Thus this dreaming, brief as it is, may be sufficient to prevent significant psychic changes. This possibility is at present being investigated.

We thank Drs. William C. Dement, Frederick Snyder and Norman Q. Brill for advice and Mrs. Kathryn S. Carter for assistance.

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[1] Dement, W., *Science*, **131**, 1705 (1960).
[2] Dement, W., paper presented at the annual meeting, Assoc. Psychophysical Study of Sleep, Chicago (March 1962).
[3] Berger, R. J., *Science*, **134**, 840 (1961).
[4] Jacobson, A., Kales, A., Lehmann, D., and Hoedemaker, F. S., *Exp. Neurol.*, **10**, 418 (1964).
[5] Dement, W., *Science and Psychoanalysis*, 7, edit. by Masserman, J. (Grune Stratton, Inc., New York, 1964).

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Tuesday, December 29

INSTITUTE OF BIOLOGY (in the Lecture Hall of the British Museum (Natural History), Cromwell Road, London, S.W.7), at 11.30 a.m.—Dr. L. Broadbent: "Being a Biologist" (Christmas Lecture for Children).

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 2.30 p.m.—Mr. Peter Hall: "London 2000" (Christmas Lecture for Children).

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 3 p.m.—Dr. Desmond Morris: "Animal Behaviour". (The one hundred and thirty-fifth course of six lectures adapted to a juvenile auditory. Further lectures on December 31, January 2, 5, 7, 9.)

Wednesday, December 30

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 2.30 p.m.—Mr. Peter Scott: "Wild Life in Danger" (Christmas Lecture for Children).

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 2.30 p.m.—Prof. S. Tolansky, F.R.S.: "The Curiosities of Light" (Dr. Mann Juvenile Lecture).

Thursday, December 31

LINNEAN SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 2.30 p.m.—Meeting on "Fossil Spores" with contributions by Dr. A. R. H. Martin, Dr. W. G. Chaloner, Mr. J. M. Pettitt, Dr. G. A. Leisman, Dr. J. Richardson and Dr. K. L. Alvin.

Friday, January 1

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 3 p.m.—Prof. E. W. Gilbert: "Andrew John Herbertson, 1865–1915".

Friday, January 1—Monday, January 4

GEOGRAPHICAL ASSOCIATION (at the London School of Economics, Houghton Street, London, W.C.2)—Annual Conference.

Monday, January 4

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. J. R. Knight and Mr. K. Hambleton: "The Present State of Gallium Arsenide Technology".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 6 p.m.—Geographical Films.

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Mr. B. E. Moody: "Recent Developments in Container Glass".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

PHYSICIST or ELECTRICAL ENGINEER, ASSISTANT EXPERIMENTAL OFFICER grade (with a degree, Dip. Tech. or H.N.C. in an appropriate subject) for design work on lighting and air conditioning facilities needed for the study of plant growth—The Secretary, National Institute of Agricultural Engineering, Wrest Park, Silsoe, Bedford, quoting Ref. N/64/ECD/19 (December 31).

VICE-PRINCIPAL (graduate with good industrial and teaching experience, and administrative experience in the field of further education, for example, as head of a department in a technical college) of the South-West Essex Technical College and School of Art, Forest Road, Walthamstow—Chief Education Officer, County Hall, Chelmsford, Essex (January 4).

LECTURER IN THE DEPARTMENT OF GEOGRAPHY, University of Tasmania—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 5).

LECTURER (with special interests in meteorology and climatology and qualifications which include a knowledge of physical meteorology) IN THE DEPARTMENT OF ENVIRONMENTAL STUDIES—The Secretary, University of Lancaster, Bailrigg House, Lancaster (January 7).

LECTURER or ASSISTANT LECTURER IN PHILOSOPHY—The Registrar, University of York, Heslington, York (January 8).

MICROBIOLOGIST (with an honours degree in science majoring in bacteriology, or dairy science or equivalent, and preferably some postgraduate bacteriology research experience) IN THE DAIRY BACTERIOLOGY DEPARTMENT—The Director, An Foras Taluntais (The Agricultural Institute), 33 Merrion Road, Dublin 4, Republic of Ireland (January 11).

ASSISTANT LECTURER (with a good honours degree) IN GEOGRAPHY—The Registrar, Room (T), The University, Hull (January 12).

LECTURER or ASSISTANT LECTURER (graduate in economics, statistics or mathematics, and an interest in the application of statistics and mathematics in economics and other social sciences) IN STATISTICS with special reference to econometrics—The Assistant Secretary, The London School of Economics and Political Science, Houghton Street, London, W.C.2 (January 12).

LECTURER IN THE DEPARTMENT OF THEORETICAL PHYSICS—The Registrar, The University, Liverpool, quoting Ref. CV/377 (January 15).

RESEARCH FELLOW IN PURE MATHEMATICS—The Master, Clare College, Cambridge (January 15).

SENIOR TECHNICIAN or TECHNICIAN IN THE DEPARTMENT OF VETERINARY PHYSIOLOGY AND BIOCHEMISTRY, University of Khartoum—The Registrar, University of Khartoum, c/o The Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (January 15).

EXPERIENCED MICROBIOLOGIST (with veterinary qualifications) to assist in the expansion of work on avian respiratory infections; an EXPERIENCED PHYSIOLOGIST or BIOCHEMIST (preferably with a veterinary qualification) to assist in the expansion of the Physiology/Biochemistry Department; and a VIROLOGIST (experienced in tissue culture techniques) to assist in work on avian respiratory infections—The Secretary, Houghton Poultry Research Station, Houghton, Huntingdon (January 15).

LECTURER IN BIOCHEMISTRY—The Principal, Cambridgeshire College of Arts and Technology, Collier Road, Cambridge (January 20).

LECTURER or ASSISTANT LECTURER (preferably with recent industrial experience) IN AIRCRAFT DESIGN—The Deputy Secretary, The University, Southampton (January 20).

LECTURER (preferably with an interest in applied nuclear physics), and an ASSISTANT LECTURER IN THE DEPARTMENT OF APPLIED PHYSICS—The Registrar, Room (X), The University, Hull (January 20).

ASSISTANT LECTURER IN THE DEPARTMENT OF PSYCHOLOGY—The Registrar, University College of North Wales, Bangor, North Wales (January 20).

LECTURERS or ASSISTANT LECTURERS (2) IN THE DEPARTMENT OF APPLIED MATHEMATICS (a research interest in fluid dynamics is desirable for one of these posts)—The Registrar, University College of North Wales, Bangor, North Wales (January 20).

ASSISTANT LECTURERS/LECTURERS IN THE DEPARTMENT OF GEOGRAPHY in the following fields: polar or marine studies; geography of Asia or Mediterranean Lands; climatology and meteorology; history of cartography—The Secretary, The University, Aberdeen (January 22).

LECTURER IN PREHISTORIC ARCHAEOLOGY (primarily in the Palaeolithic and Mesolithic)—The Secretary of Faculties, University Registry, Oxford (January 23).

READER, and LECTURERS or ASSISTANT LECTURERS (3) IN THE DEPARTMENT OF ZOOLOGY—The Deputy Secretary, The University, Southampton (January 23).

SENIOR LECTURER or LECTURER (preferably with considerable experience in the fields of ecology, nature conservation and/or ethology) IN THE DEPARTMENT OF ZOOLOGY, University of Ibadan, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (January 23).

LECTURERS or ASSISTANT LECTURERS IN MATHEMATICS at the University of Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (January 25).

CHAIR OF PHYSICAL CHEMISTRY—The Registrary, University Registry, The Old Schools, Cambridge, marking envelope "Confidential" (January 26).

CHAIR OF ZOOLOGY—The Registrary, University Registry, The Old Schools, Cambridge, marking envelope "Confidential" (January 26).

SENIOR LECTURER (with a degree in science or engineering, preferably a doctorate, experience in the maintenance and operation of electron microscopes, and a wide knowledge of electron microscopy techniques, including application to biological, mineralic and metallurgical materials) IN ELECTRON MICROSCOPY at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, January 29).

LECTURER IN CLINICAL BACTERIOLOGY IN THE UNIVERSITY DEPARTMENT OF BACTERIOLOGY with an honorary appointment as Senior Registrar in the United Bristol Hospitals—The Registrar, The University, Senate House, Bristol, 2 (January 31).

LECTURERS (with postgraduate experience in experimental or theoretical solid-state physics) IN PHYSICS—The Secretary, University of Lancaster, Bailrigg House, Lancaster (January 31).

CHAIR OF SOCIOLOGY—The Secretary, The University, Edinburgh (January 31).

LECTURER or ASSISTANT LECTURER (with an honours degree in a biological subject or in physiology) IN BIOLOGICAL OCEANOGRAPHY; and a LECTURER or ASSISTANT LECTURER (with an honours degree in mathematics, electronics or a physical science) IN PHYSICAL OCEANOGRAPHY—The Deputy Secretary, The University, Southampton (February 1).

LECTURER or ASSISTANT LECTURER (with an honours degree in engineering or physics and experience in industrial acoustics) IN INDUSTRIAL ACOUSTICS at the Institute of Sound and Vibration Research—The Deputy Secretary, The University, Southampton (February 1).

PRINCIPAL (with high qualifications and wide administrative experience)—The Clerk and Treasurer, Paisley College of Technology, High Street, Paisley, Renfrewshire, Scotland (February 1).

RESEARCH FELLOW IN PURE MATHEMATICS—The Master, Corpus Christi College, Cambridge (February 1).

LECTURER (preferably with experience and interests in general psychology together with competence in the field of experimental psychology) IN PSYCHOLOGY at Victoria University of Wellington, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, February 5).

POST-DOCTORAL RESEARCH FELLOWS (2) IN CHEMICAL CRYSTALLOGRAPHY, to join a Group which is carrying out systematic X-ray crystallographic studies of biochemically interesting metal-peptide complexes—The Registrar, University of Sydney, Sydney, New South Wales, Australia (February 8).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOWS IN PHYSICS, CHEMISTRY, BIOCHEMISTRY, ENGINEERING, METALLURGY AND PHARMACOLOGY or any related subjects—The Registrar, The University, Liverpool, quoting Ref. CV/375/N (February 13).

ISAAC WOLFSON PROFESSOR OF METALLURGY—The Registrar, University Registry, Oxford (February 13).

PROFESSOR OF ELEMENTARY PARTICLE PHYSICS—The Registrar, University Registry, Oxford (February 13).

CHAIR OF GEOGRAPHY—The Registrary, University Registry, The Old Schools, Cambridge, marking envelope "Confidential" (February 18).

SENIOR LECTURER (with a higher degree in physics with suitable research and teaching experience, and preferably with experimental experience in one of the following fields: solid state physics, X-ray crystallography, infra-red spectroscopy, polymer physics, or low-temperature physics) IN PHYSICS—The Registrar, New Asia College, Chinese University of Hong Kong, Farm Road, Kowloon, Hong Kong (February 28).

DIRECTOR OF THE COMPUTER LABORATORY, University of Auckland, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, March 1).

ASSISTANT LECTURER and a COLLEGE LECTURER (with the degree of M.B. and Ph.D. or equivalent) IN THE DEPARTMENT OF PHYSIOLOGY—The Secretary and Bursar, University College, Dublin, Republic of Ireland.

ASSISTANT LECTURER (with a good class honours degree, and preferably research or industrial experience) IN PHYSICAL ORGANIC CHEMISTRY—The Academic Registrar, Hatfield College of Technology, Hatfield, Hertfordshire.

DIRECTOR OF RESEARCH FOR THE FISHERIES RESEARCH DIVISION, Wellington—The Secretary, Marine Department, P.O. Box 2395, Wellington, New Zealand.

ELECTRON-MICROSCOPE TECHNICIAN (aged between 25 and 35 with experience in physical and biological work and with a sound knowledge of replica, embedding and sectioning techniques) to take charge of the operation and maintenance of a Siemens Elmiskop Mk. I and ancillary equipment—

The Director of Research, The Natural Rubber Producers' Research Association, Welwyn Garden City, Hertfordshire.

EXPERIMENTAL OFFICER (preferably with experience of chromatographic, electrophoretic and radioactive tracer techniques as applied to nucleic acids and proteins) IN THE BIOCHEMISTRY DEPARTMENT, for work concerned with the structure and mechanism of replication of animal viruses—The Secretary, Animal Virus Research Institute, Pirbright, Surrey.

IMMUNOLOGIST (with general experience in microbial immunology, and preferably with a medical qualification) at the Mill Hill Laboratories of the Imperial Cancer Research Fund—The Secretary, Imperial Cancer Research Fund, Lincoln's Inn Fields, London, W.C.2.

INVESTIGATOR (graduate or equivalent) to join research staff initially for work on the evaluation of dust-sampling instruments for industrial use which will involve laboratory investigations and practical trials in industry—The Director, The British Cast Iron Research Association, Bordesley Hall, Alvechurch, Birmingham.

LECTURER IN CIVIL ENGINEERING—The Academic Registrar, Loughborough College of Technology, Loughborough, Leicestershire, quoting Ref. 28/G.

ORGANIC CHEMIST (Ph.D. with experience of the phosphines, phthalocyanines and/or similar substances) to investigate the reactions of their metal complexes—Prof. J. Chatt, Agricultural Research Council, Unit of Nitrogen Fixation, University of Sussex, Brighton, Sussex.

PLANT PATHOLOGIST (national of the United Kingdom or the Republic of Ireland, with a degree in botany of natural science, and preferably specialist training or postgraduate experience in mycology, virology or plant pathology) in Zambia, to investigate major diseases of crops of economic importance and to make recommendations based on experimental results—The Appointments Officer, Room 301, Ministry of Overseas Development, Eland House, Stag Place, London, S.W.1, quoting RC213/132/07.

PRINCIPAL LECTURER IN PSYCHOLOGY—The Director, Huddersfield Training College for Technical Teachers, Holly Bank Road, Lindley, Huddersfield.

PRINCIPAL SCIENTIFIC OFFICER (physicist or physical chemist) to work on the structure and physical properties of animal viruses and the intracellular processes involved in their replication—The Secretary, Animal Virus Research Institute, Pirbright, Surrey.

PROFESSOR OF POLYMER CHEMISTRY; a PROFESSOR OR ASSOCIATE PROFESSOR (with established research interests in mass spectroscopy) OF PHYSICAL CHEMISTRY; and ASSISTANT PROFESSORS OF ANALYTICAL, INORGANIC AND PHYSICAL CHEMISTRY—The Chairman, Department of Chemistry, McGill University, Montreal, Quebec, Canada.

RESEARCH ASSISTANT (graduate with an honours degree in botany or agricultural botany) to assist with an investigation of the factors which determine the weed seed content of crop seeds—The Secretary, National Institute of Agricultural Botany, Huntingdon Road, Cambridge.

RESEARCH STUDENT (with a good honours degree or holder of G.R.I.C. at an equivalent level) IN THE DEPARTMENT OF CHEMISTRY, for research in the field of the synthesis of heterocyclic compounds or potential pharmacological activity—The Registrar, The University, Hull.

RESEARCH STUDENTS (with a good honours degree or a higher degree or expecting to obtain such a degree by next July) in both theoretical and experimental physics—Prof. L. R. B. Elton, Head of the Department of Physics, Battersea College of Technology, London, S.W.11.

SCIENTIFIC OFFICER OR SENIOR SCIENTIFIC OFFICER (with a first- or good second-class honours degree in chemistry, metallurgy or physics, and preferably experience in the field of oxidation) to join a team interested in basic studies related to the oxidation of metals and ceramics—The Personnel Department, Atomic Energy Research Establishment, Harwell, Didcot, Berkshire.

SCIENTIST (graduate in chemistry or biochemistry) in the Radioisotope Unit, to be concerned with developing new diagnostic radioisotope procedures, and to assist in clinical research involving the use of radioactive isotopes—The Administrator, Royal Marsden Hospital, Surrey Branch, Downs Road, Sutton, Surrey.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Commonwealth Mycological Institute. Mycological Papers. No. 95: A Supplementary List of Plant Diseases in Nyasaland. By D. C. M. Corbett. Pp. 16. 7*s*. 6*d*. No. 96: Revision of British Gymnoascaceae. By A. E. Apinis. Pp. 56+3 plates. 17*s*. 6*d*. Miscellaneous Publications. No. 17: The Genus *Sclerospora*: Diagnoses (or Descriptions) from the Original Papers and a Key. Compiled by Grace M. Waterhouse. Pp. 30. 7*s*. 6*d*. No. 18: Virus Diseases of Cotton. By Dr. S. A. J. Tarr. Pp. 23. 7*s*. 6*d*. (Kew: Commonwealth Mycological Institute, 1964.) [3010

Department of Scientific and Industrial Research: National Engineering Laboratory. N.E.L. Report No. 165: Rheological Research in Britain 1964—a Survey made jointly by the British Society of Rheology and the National Engineering Laboratory. By A. Y. McLean and A. T. J. Hayward. Pp. 104. (East Kilbride: National Engineering Laboratory, 1964.) [3010

Imperial College of Science and Technology. Calendar 1964–65. Pp. xi+506. (London: Imperial College of Science and Technology, 1964.) [3010

Central Youth Employment Executive. Choice of Careers, No. 111: Veterinary Science. Second edition. Pp. 28+9 photographs. (London: H.M. Stationery Office, 1964.) 1*s*. 3*d*. net. [3010

Imperial College of Science and Technology. Prospectus 1965–66. Pp. viii+74+5 photographs. (London: Imperial College of Science and Technology, 1964.) [3010

The Society for Analytical Chemistry. Monograph No. 2: The Determination of Sterols. (Six Papers originally presented at a Meeting of the Society held on May 2nd, 1962.) Pp. iv+72. (London: The Society for Analytical Chemistry, 1964.) 15*s*.; 2 dollars. [3010

Ministry of Agriculture, Fisheries and Food. Review of the Persistent Organochlorine Pesticides: Supplementary Report by the Advisory Committee on Pesticides and other Toxic Chemicals. Pp. iv+8. (London: H.M. Stationery Office, 1964.) 1*s*. 3*d*. net. [3010

The British Cast Iron Research Association. Forty-Third Annual Report for the year ending 30th June 1964. Pp. 110. (Alvechurch, Birmingham: The British Cast Iron Research Association, 1964.) [3010

A Few Ideas: a Series of Talks broadcast in the B.B.C. Third Programme on Six Scientific Concepts. By John Maynard Smith, Prof. Rayner Hilla Stoyle, Prof. C. A. Coulson, S. A. Barnett, Prof. Donald Mackay and Prof. O. R. Frisch. Pp. 56. 4*s*. 6*d*. A New Kind of Physics: a Broadcast Symposium. By Dr. Dennis Sciama, Dr. C. W. Kilmister, Prof. Fred Hoyle and Prof. Hermann Bondi. Pp. 32. 3*s*. (London: British Broadcasting Corporation, 1964.) [3010

National Council for Quality and Reliability. Third Annual Report 1963–64. Pp. 19. (London: National Council for Quality and Reliability, 1964.) [3010

Institute of Biology. Biology as a Career. Pp. 44. (London: Institute of Biology, 1964.) 3*s*. 6*d*. [3010

An Annotated Bibliography of Memoirs and Papers on the Soils of the British Isles. Part 1: The Classification, Morphology, Distribution and Reclamation of British Soils. Edited and Compiled by B. T. Bunting. Pp. iv+100. (London: Geomorphological Abstracts, Department of Geography, London School of Economics, 1964.) 10*s*. [3010

Society for the Promotion of Nature Reserves. Handbook 1964: Forty-Seventh Annual Report, year ending 31st March, 1964. Pp. 56+2 plates. (London: Society for the Promotion of Nature Reserves, c/o British Museum (Natural History), 1964.) 10*s*. [3010

The International Nickel Company (Mond), Ltd. Nickel-Alkaline Accumulators. Pp. 24. (London: The International Nickel Company (Mond), Ltd., 1964.) [3010

Year Book of the Royal Society of Edinburgh, 1964 (Session 1962–1963). Pp. 136. (Edinburgh: Royal Society of Edinburgh, 1964.) 20*s*.; 3.50 dollars. [3010

The Chemistry of Proteins. By R. J. Taylor. (A Unilever Educational Booklet—Advanced Series.) Pp. 43. (London: Unilever, Ltd., 1964.) [3010

University College of Wales. Memorandum No. 7: Synopsis of Discussions held on March 18th, 1964, on the subject of Major Weather Hazards Affecting British Agriculture. Edited by James A. Taylor. Pp. 55. (Aberystwyth: University College of Wales, 1964.) [3010

Bulletin of the British Museum (Natural History). Entomology. Vol. 15, No. 7: Revisional Notes on African *Charaxes* (Lepidoptera: Nymphalidae). Part 2. By V. G. L. van Someren. Pp. 181–235+23 plates. (London: British Museum (Natural History), 1964.) 63*s*. [211

Imperial College of Science and Technology (University of London). Research Report of the City and Guilds College, 1961–64. Pp. 108. (London: City and Guilds College, 1964.) [211

Other Countries

North Atlantic Treaty Organization: Advisory Group for Aeronautical Research and Development. AGARD—General Information 1964. Pp. 21. (Paris: Advisory Group for Aeronautical Research and Development, NATO, 1964.) [311

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